

Combination of Process Technology and Packaging Conditions to Improve the Shelf Life of Fresh Pasta

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Abstract

In this work, the effects of Potassium Sorbate (PS) in the dough, antimicrobial air filter after the pasteurization and Modified Atmosphere Packaging (MAP) as combined preservation techniques were studied on shelf life of fresh pasta. In particular, in the first experimental step the influence of an antimicrobial air filter used to cool down pasta temperature after heat treatment and packaging under MAP (70:30 CO₂:N₂) were tested. Subsequently, different concentrations of PS (500, 750 and 1000 mg kg⁻¹), combined with the air filter, were added to the pasta dough to control moulds and bacteria proliferation. In the final trial, PS (1000 mg kg⁻¹), air filter and MAP were combined. In each experimental step, both microbiological and sensory quality was monitored. Results demonstrated that the preservation strategies adopted in each step were effective to control microbial and fungi proliferation and, when all of them were combined; the shelf life was about 40 days against about 8 days of the control sample.

Keywords: Fresh pasta; Potassium sorbate; Antimicrobial air filter; Shelf life

Introduction

Pasta, the main foodstuff in Italian cuisine, is obtained by extrusion or lamination of dough made with durum wheat semolina and water. Pasta is a generic word for a wide range of food products with very different characteristics in terms of shape, composition and moisture content. Water content influenced shelf life and storage temperature. In particular, dry pasta (maximum 12.5% of water) is stored at room temperature and has a shelf life more than one year; differently, fresh pasta (moisture content between 24% and 30%) is stored at temperatures lower than 4°C and is an easily perishable product. Fresh pasta spoilage is principally due to the metabolic activity of bacteria, yeasts and moulds that negatively influence the shelf life and sensorial characteristics of product [1-4]. Therefore, the industrial process of pasta production generally comprises heat treatments to the aim of maintaining product hygiene and quality. In particular, fresh pasta pasteurization reduces mould spores and spoilage microorganisms [5]. Other preservation techniques to be applied before and after packaging, such as microwave, convection heating alone or in combination, can provide extensive and efficient sanitization, thus assuring shelf life periods from 30 to 90 days [6,7]. However, these types of thermal stress generally compromise the sensory characteristics of fresh pasta.

To improve the microbial stability and prolong the shelf life of fresh pasta other strategies than thermal treatments have been suggested in the literature, as the use of active compounds in the dough and modified headspace conditions during packaging [8,9]. Among the antimicrobial agents, potassium sorbate (PS) has received considerable interest for food applications, being able to prevent moulds development without modifying taste, odor or color of food [10,11]. As regard Modified Atmosphere Packaging (MAP), gas mixtures where carbon dioxide ranges from 50% to 80% retard moulds development and microbial proliferation. Interesting results were also recorded in homemade fresh pasta by combining MAP and antimicrobial compounds [3]. Costa et al. [4] also demonstrated that chitosan in combination with MAP assured the acceptability of fresh pasta, produced by a pilot plant without any thermal treatment, for more than two weeks, thus suggesting the use of hurdle technology for food preservation [12].

On the basis of these considerations, in this work a step-by-step

approach was adopted to optimize the processing and packaging conditions of durum semolina-based fresh pasta with the aim to improve the shelf life reducing thermal treatment after packaging that generally compromise the sensory attributes. In particular, PS was added to the dough, an antimicrobial air filter was used to cool down pasta temperature after the pasteurization process to create a cleanroom, and MAP conditions were used for packaging. The effects of combined preservation strategies were assessed on both microbiological and sensory quality decay.

Materials and Methods

The work was carried out in three steps summarized in Table 1. Below the fresh pasta production process, the adopted packaging conditions and the analyses carried out during the storage period are described.

Pasta samples preparation

Fresh pasta was produced by using durum semolina provided by Molino Agostini (Montefiore dell'Aso, Italy). Semolina and tap water (30% w/w) were mixed with a rotary shaft mixer (Namad, Rome, Italy) at 25°C for 20 min to prepare the pasta dough to be extruded. The pilot plant was made of an extruder (60VR; Namad, Rome, Italy) and was equipped with a bronze head to give the pasta dough the shape of macaroni (diameter 1 cm, thickness 1 mm and length 2 cm). After extrusion, pasta was pasteurized with steam for 3 min at 90°C (Namad, Rome, Italy). The pasteurization system, that uses air at room temperature to cool down the pasta after the thermal treatment, was equipped with an antimicrobial air filter (Megalam H14, Camfil spa, Milano, Italy), which was either activated or inactivated on the

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	Sample	Antimicrobial air filter	Potassium sorbate	Headspace atmosphere
Step-1	no-FilterAir-1	OFF	/	Air
	no-FilterMAP-1	OFF	/	MAP (70:30 CO ₂ :N ₂)
	FilterAir-1	ON	/	Air
	FilterMAP-1	ON	/	MAP (70:30 CO ₂ :N ₂)
Step-2	no-FilterAir-2	OFF	/	Air
	FilterAir-2	ON	/	Air
	PS-500Air	ON	500 mg Kg ⁻¹	Air
	PS-750Air	ON	750 mg Kg ⁻¹	Air
Step-3	no-FilterAir-3	OFF	/	Air
	FilterAir-3	ON	/	Air
	FilterMAP-3	ON	/	MAP (70:30 CO ₂ :N ₂)
	Filter-PS-MAP	ON	1000 mg Kg ⁻¹	MAP (70:30 CO ₂ :N ₂)

Table 1: Summary of applied strategies for each experimental step

basis of the type of sample to be prepared. The filter is characterized by a technology able to create a cleanrooms to provide a highest level of protection for product process. In particular, the air filter is characterized by Most Penetrating Particle Size (MPPS) efficiency of 99.995%. Moreover, the filter media are glass fiber paper, the frame is extruded and anodised aluminium and the gasket is polyurethane endless at inlet.

To obtain pasta samples supplemented with different antimicrobial concentrations, active solutions were prepared dissolving potassium sorbate (Farmalabor s.r.l.-Canosa di Puglia, Italy). These solutions were added to the dough, separately, to obtain final concentrations of 500 mg kg⁻¹, 750 mg kg⁻¹ and 1000 mg kg⁻¹ of PS in pasta.

The pasta samples were packaged under air or MAP conditions (70:30 CO₂:N₂) and sealed by means of a thermal sealer (Gandus sealers, Milan, Italy). In particular, 80 g of fresh pasta in a plastic dish were closed in a high-barrier multilayer film made up of Polyethylene Terephthalate (PET), Ethylene-Vinyl Alcohol (EVOH) and polyethylene (PE), (90 µm thickness), kindly provided by Di Mauro Officine Grafiche s.p.a. (Napoli, Italy). All the samples were stored at 4 ± 1 °C.

Headspace gas composition

The changes in headspace O₂ and CO₂ concentration were measured using a PBI Dansensor O₂/CO₂ analyzer (Checkmate 9900, Denmark). The volume taken from the package headspace for gas analysis was about 10 cm³. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single measurement. Two bags per treatment were used at each sampling time.

Microbiological analyses and pH determination

For microbiological analyses of fresh pasta, about 10 g of sample were aseptically removed from each package, placed in a stomacher bag, diluted with NaCl solution (9 g L⁻¹) and homogenized with a stomacher LAB Blender 400 (Pbi International, Milan, Italy). Serial dilutions in sterile saline solution were plated onto appropriate media according to Del Nobile et al. [2]. In particular, Plate Count Agar (PCA) incubated at 30°C for 48 h for aerobic mesophilic bacteria and at 4°C for 7 days for psychrotrophic bacteria; Violet Red Bile Agar (VRBA) incubated at 37°C for 24 h for total coliforms; Baird- Parker Agar, supplemented with egg yolk tellurite emulsion, incubated at 37°C for 48 h for *Staphylococcus* spp.; deMan Rogosa Sharpe agar (MRS), added with 0.17 g L⁻¹ of cycloheximide (Sigma-Aldrich, Milan, Italy) incubated at

30°C for 48 h for lactic acid bacteria; Sabouraud Dextrose Agar, added with 0.1 g L⁻¹ of chloramphenicol (C. Erba, Milan, Italy), incubated at 25°C for 48 h for yeasts. All media and supplements were from Oxoid (Milan, Italy). All microbiological analyses were performed twice on two different batches.

The measurement of pH of the homogenized fresh pasta was performed twice on samples from two different batches by using a pH-meter (Crison, Barcelona, Spain).

Sensory analysis

Both uncooked and cooked fresh pasta were subjected to sensory evaluation. To obtain cooked pasta 30 g of fresh pasta were cooked in 400 ml of tap water at 100°C for 130 seconds. All the uncooked samples were submitted in a single session to a panel of eight tasters. The panelists had at least several years of experience in evaluation of fresh pasta prior to this study. Panelists were asked to estimate color, odor, homogeneity and overall quality on uncooked pasta and, in addition, adhesiveness, bulkiness, firmness, elasticity, taste and overall quality on cooked pasta. A nine-point rating scale, where 5 represented the threshold for product acceptability, was used to perform the panel test. In addition, panelists were also asked to search for visual moulds, thus allowing determining the day between the latest storage time at which moulds were not visible and the earliest storage time at which moulds were visible, hereinafter referred to as VMT (Visual Moulds Time).

Shelf life calculation

For each step, the Microbial Acceptability Limit (MAL), the Sensory Acceptability Limit (SAL) and the VMT values were taken into account to assess pasta shelf life, considered as the lowest value among the three quality indices. To calculate the MAL, i.e. the storage time at which the viable cell concentration of considered bacteria reached the threshold, the re-parameterized Gompertz equation was used [13]. In particular, 106 CFU g⁻¹ was the imposed threshold for mesophilic bacteria (MALmesophilic) and psychrotrophic bacteria (MALpsychrotrophic) and 104 CFU g⁻¹ for coliforms (MALcoliforms) and *Staphylococcus* spp. (MALStaphylococcus) (Ministerial Health Decree 32, 1985). In order to determine the SAL in terms of overall quality for uncooked (SALuncooked) and cooked pasta (SALcooked), the same modified version of the Gompertz equation was used to fit the sensory data. A score equal to 5 represented the threshold for sensory acceptability.

Statistical analysis

The microbial count (Log CFU g⁻¹), the MAL, the SAL and the shelf life values of all the investigated samples were compared by one-way ANOVA analysis. A Duncan's multiple range test, with the option of homogeneous groups (p<0.05), was used to determine significance among differences. To this aim, STATISTICA v. 7.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used.

Results and Discussion

Headspace gas composition

The headspace gas analysis, for the three experimental steps (data not shown), highlights a slight decrease of oxygen concentration and an increase of carbon dioxide concentration (final value recorded for O₂ and CO₂ 18% and 0.6%, respectively) for all samples packaged under air conditions. Change in headspace gas composition in pasta sample packaged under air conditions can be principally due to the metabolic activity of aerobic microorganisms. Differently, for samples packaged under map conditions (70:30 CO₂:N₂) the initial headspace

composition was unchanged during the entire storage period (data not shown). In particular, the tested polymeric film, able to maintain the select active MAP during the experimental period, was the same used in a previous study. In particular, it was observed that the permeability properties of the selected film were able to preserve the initial gas composition (70:30 CO₂:N₂) up to 50 days.

Microbiological quality

In step-1 the efficacy, alone or in combination, of the antimicrobial air filter and packaging conditions (air or MAP) was tested. As example, in Figure 1 the cell loads of mesophylic bacteria as a function of storage time for fresh pasta samples produced with and without air filter, packaged in air or MAP are reported. As can be observed, a rapid increase in microbial population was reported for sample produced without the antimicrobial air filter and packaged in air (no-filterAir-1). Lower cell loads were reported for FilterAir-1 sample compared to no-filterAir-1 pasta. Moreover, packaging in air promoted moulds proliferation and after 10 and 13 days, no-FilterAir-1 and FilterAir-1, respectively, were refused for visible moulds appearance. A gradual increase of mesophylic bacteria was observed for both samples packaged under MAP conditions (no-FilterMAP-1 and FilterMAP-1), even though a reduced cells numbers were counted for FilterMAP-1 sample. Similar trends, like the above described for each tested sample, were also recorded for lactic acid bacteria, psychrotrophic bacteria and *Staphylococcus* spp. (data not shown). According to literature data, air filtration for cooling down temperature during process reduces the number of airborne contaminants [14,15], whereas packaging under MAP conditions improves sample microbial stability. It is worth nothing that all samples packaged under MAP conditions without filter (no-FilterMAP-1) overtook the imposed threshold (106 CFU g⁻¹ for mesophylic and psychrotrophic bacteria and 104 CFU g⁻¹ for *Staphylococcus* spp.) whereas, for FilterMAP-1 the imposed limit was exceeded only in terms of mesophylic bacteria.

In Figure 2 the evolution of yeasts populations of step-1 samples is reported. In particular, an increase in yeasts population from 102 to 104 CFU g⁻¹ during the first 10 days of storage was observed for samples packaged under air conditions. Differently, for no-filterMAP-1 and filterMAP-1 an increase of yeasts population was observed after 12 days. This result is in agreement with the study carried out by Eliot et al. [16], who observed a significant inhibitory effect of carbon dioxide on development of yeasts. Moreover, the application of antimicrobial air filter in pasta processing further slowed down the yeasts development. In particular, reduced cell loads of about 1 logarithmic cycle were recorded at the end of the storage period for filterMAP-1 compared to no-filterMAP-1. Therefore, our experimental findings proved that air filtration reduced number of airborne contaminants, whereas MAP conditions improved the microbial quality.

In step-2 the antimicrobial effects of PS added to dough combined with antimicrobial air filter was evaluated. In Figure 3 the evolution of lactic acid bacteria in pasta produced with and without filter or PS is reported. As can be seen, a rapid increase in microbial population was reported for samples without PS and different efficacy of the antimicrobial compound was found depending on its concentrations. These data are in agreement with results of other researches on PS applied to food [17,18]. Similar microbial trends were observed for *Staphylococcus* spp., psychrotrophic and mesophylic bacteria (data not shown). An increase in yeast population from 103 to 105 CFU g⁻¹ was recorded for the no-FilterAir-2 and the FilterAir-2 samples. On the contrary, no yeasts were counted for samples supplemented with PS (data not shown).

In Table 2 the initial and final cell loads of mesophylic, psychrotrophic, lactic acid bacteria and yeasts in pasta produced in step-3 with combination of PS (1000 mg kg⁻¹), antimicrobial air filter and

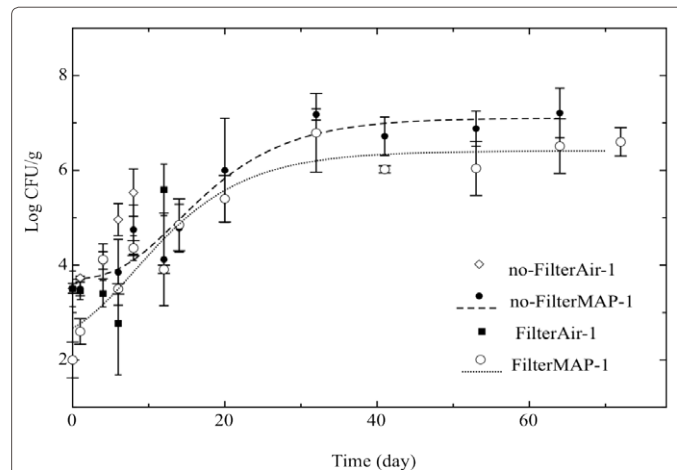


Figure 1: Mesophylic bacteria during storage for fresh pasta samples of step-1. The curves are best fit of the re-parameterized Gompertz equation to experimental data.

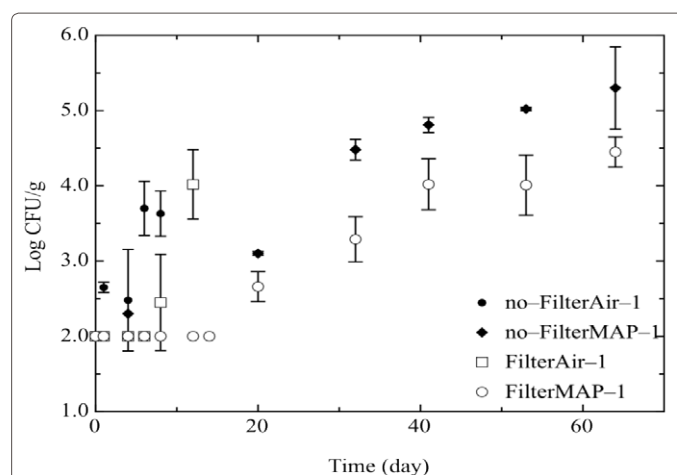


Figure 2: Evolution of yeasts during storage for fresh pasta produced in step-1.

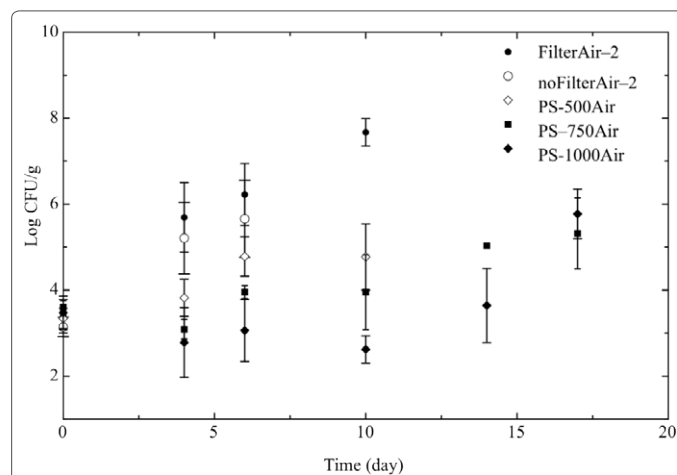


Figure 3: Evolution of lactic acid bacteria during storage for fresh pasta produced in step-2.

	Mesophilic bacteria		Psicrotrophyc bacteria		Lactic acid bacteria		Yeasts	
	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g
no-FilterAir-3	2.94 ± 0.33 ^a	5.36 ± 0.07 ^c	2.00 ± 0.00 ^a	3.58 ± 0.25 ^c	2.79 ± 0.21 ^a	3.82 ± 0.19 ^b	2.60 ± 0.33 ^a	3.08 ± 0.90 ^b
FilterAir-3	2.84 ± 0.51 ^a	2.82 ± 0.32 ^d	2.00 ± 0.00 ^a	3.44 ± 0.49 ^c	2.40 ± 0.19 ^a	3.26 ± 1.80 ^b	2.00 ± 0.00 ^b	2.00 ± 0.00 ^c
FilterMAP-3	2.84 ± 0.51 ^a	7.10 ± 0.14 ^a	2.00 ± 0.00 ^a	6.34 ± 0.20 ^a	2.40 ± 0.19 ^a	6.25 ± 0.52 ^a	2.00 ± 0.00 ^b	4.74 ± 0.17 ^a
Filter-PS-MAP	2.00 ± 0.01 ^b	4.98 ± 0.02 ^b	2.00 ± 0.00 ^a	4.66 ± 0.04 ^b	1.48 ± 0.21 ^b	4.69 ± 0.08 ^b	2.00 ± 0.00 ^b	2.00 ± 0.00 ^c

Table 2: Initial (Log₁₀ CFU/g) and final (Log₁₀ CFU/g) cell loads of mesophilic bacteria, psicrotrophyc, acid lact bacteria and yeasts in fresh pasta samples analyzed in the final step. The analyses were conducted for no-FilterAir-3 and FilterAir-3 up 13 and 17 days, respectively due to moulds development. FilterMAP-3 and Filter-PS-MAP were monitored up to 45 and 50 days, respectively.

^{a-d} Means in the same column followed by different superscript lower cases are significantly different (P<0.05).

MAP are reported. As can be observed in the table, for each microbial group the antimicrobial effect can be immediately observed after pasta production. In particular, low cell loads were detected for sample supplemented with PS; in addition, for mesophilic bacteria, lactic acid bacteria and yeasts, statistically significant differences between no-FilterAir-3 and FilterAir-3 samples were detected. Data confirmed the antimicrobial activity of PS and the improvement of microbial quality due to air filter application. It is worth nothing that no-FilterAir-3 and FilterAir-3 were monitored for 13 and 17 days, respectively due to moulds appearance. On the contrary, FilterMAP-3 and Filter-PS-MAP were monitored for 45 and 50 days, respectively. Data reported in Table 2 highlight that the application of antimicrobial air filter reduced microbial development compared to sample produced without filter and MAP improved pasta microbial stability. The lowest cell loads were obtained by combining MAP and PS.

During the entire storage period of all the investigated trials, no coliforms bacteria were counted.

Small oscillations around the initial pH value of fresh pasta (6.09 ± 0.06) were recorded in all tested sample (data not shown). No differences in pH value were detected among different samples of each trial.

Sensory characteristics

In Table 3 the sensorial acceptability limits in terms of overall quality for uncooked (SALuncooked) and cooked pasta (SALcooked) and the main parameters that influenced the overall quality of fresh pasta are reported. Results highlighted that while the overall quality of uncooked pasta was mainly influenced by the odor, change in taste and physical characteristics are the main factors responsible for the unacceptability of cooked pasta. The structural change and the development of off-odours in pasta samples during the storage period are principally due to the microbial growth.

As can be observed in Table 3, samples of step-1 packaged under MAP conditions were acceptable for a longer period from a sensorial point of view compared to samples packaged under air conditions. Moreover, among samples packaged under MAP (no-FilterMAP-1 and FilterMAP-1) best results were obtained for FilterMAP-1 sample. The antimicrobial air filter reduced microbial contaminations and improved also the sensorial characteristics. Samples of step-2 never reached the imposed sensorial limits due to the visible moulds development that break off sensorial analysis. In the step-3 the overall quality was positively influenced by the combination of the selected strategies. In particular, Filter-PS-MAP sample was acceptable for more than 50 days for uncooked pasta and about 40 days for cooked pasta.

As an example, in Figure 4 the overall quality of uncooked pasta produced with the combined preservation techniques is reported. As can be observed, a fast decrease in overall quality was observed for

samples packaged under air conditions (no-FilterAir-3 and FilterAir-3). However, no-FilterAir-3 exceeded the sensory threshold value before FilterAir-3 sample due to change in odor and color characteristics. The interesting results obtained with FilterAir-3 sample compared to no-FilterAir-3 can be ascribed to the use of the antimicrobial air filter that limits the microbial pollution. For samples packaged under MAP a gradual decrease in overall quality was observed. The best results were obtained for the Filter-PS-MAP that never reached the imposed threshold for the tested sensorial parameters. Similar trends in overall quality were found for cooked samples (data not shown).

Shelf life evaluation

As can be noted in Table 3, the shelf life of step-1 samples packaged in air conditions with and without filter (no-FilterAir-1 and FilterAir-1) was influenced by moulds appearance and change in sensory quality, respectively. According to literature data, packaging under MAP conditions increased pasta shelf life. In this case, microbial growth affected the shelf life of samples under MAP. A better microbiological and sensory stability was observed when filter and MAP were combined.

As also observed in Table 3, the use of the antimicrobial air filter combined to PS retarded moulds proliferation. In particular, visual moulds were detected after 12, 17 and 21 days in pasta samples supplemented with 500, 750 and 1000 mg kg⁻¹ of PS. The application of the antimicrobial air filter with PS and MAP conditions further controlled moulds appearance, microbial growth and sensory decay. In particular, shelf life of 7.91 and 11.65 were obtained for no-FilterAir-3 and FilterAir-3, respectively. Moreover, MAP retard microbial development and control moulds appearance in particular, more than 1 month of shelf life was reached for FilterMAP-3 and about 40 days were recorded with all the three preservation strategies (Filter-PS-MAP).

Conclusions

In this study a step-by-step approach was adopted to increase fresh pasta shelf life. In particular, an antimicrobial compound to be added to the initial dough, an antimicrobial air filter applied after heat treatment and MAP conditions were utilized in packaging process. The results obtained in each step allowed evaluating the influence of the selected process variables on microbiological and sensory quality of pasta. In particular, step-1 highlighted that the combination of the antimicrobial filter and MAP can control microbial and moulds growth better than each treatment alone. In the step-2, in combination with the filter, the most effective concentration of PS (1000 mg kg⁻¹) to control moulds appearance was found. In the last step PS (1000 mg kg⁻¹), the antimicrobial filter and MAP were combined and a shelf life of about 40 days was found, compared to the control that remained acceptable for little more than one week.

		MAL (day)				VMT (day)	SAL ^{o,q} (day)		Shelf life (day)
		MAL ^{Mesophilic}	MAL ^{Psychrotrophic}	MAL ^{Coliforms}	MAL ^{Staphylococcus}		SAL ^{o,q} Uncooked	SAL ^{o,q} Cooked	
Step-1	no-FilterAir-1	>8	>8	>8	>8	10	>8	>8	10
	no-FilterMAP-1	21.43 ± 2.63 ^a	26.84 ± 5.70	>50	25.63 ± 1.67	/	28.67 ± 1.16 ^{bs}	28.67 ± 1.16 ^{bs} ^a	21.43 ± 2.63 ^a
	FilterAir-1	>12	>12	>12	>12	13	9.86 ± 0.09 ^{c+}	9.86 ± 0.09 ^{c+}	9.86 ± 0.09 ^b
	FilterMAP-1	25.91 ± 6.13 ^a	>50	>50	>50	/	32.74 ± 0.33 ^{a+}	32.74 ± 0.33 ^a	25.91 ± 6.13 ^a
Step-2	no-FilterAir-2	>6	>6	>6	>6	10	>6	>6	10
	FilterAir-2	>10	>10	>10	>10	12	>10	>10	12
	PS-500Air	>10	>10	>10	>10	12	>10	>10	12
	PS-750Air	>17	>17	>17	>17	17	>17	>17	17
	PS-1000Air	>17	>17	>17	>17	21	>17	>17	21
Step-3	no-FilterAir-3	>13	>13	>13	>13	15	7.91 ± 1.16 ^{cs}	9.54 ± 0.93 ^{ef}	7.91 ± 0.93 ^d
	FilterAir-3	>17	>17	>17	>17	19	11.60 ± 1.09 ^{bs}	11.65 ± 1.69 ^{bt}	11.60 ± 1.09 ^c
	FilterMAP-3	31.46 ± 0.90	>45	>45	>45	/	39.41 ± 1.33 ^{a+}	38.44 ± 1.48 ^{a+}	31.46 ± 0.90 ^b
	Filter-PS-MAP	>50	>50	>50	>50	/	>50	39.60 ± 1.33 ^{af}	39.60 ± 2.45 ^a

Table 3: Microbial acceptability limits (MAL), sensory acceptability limit for overall quality (SAL^{o,q}) and visual moulds time (VMT) of fresh pasta for each experimental steps. Shelf life is assumed as the lowest value between MAL, SAL and VMT.

^{a-d}For each step, means in the same column followed by different superscript lower cases are significantly different (P<0.05). Superscript symbols indicate the parameter that affected the overall quality of uncooked and cooked pasta: *Colour, §Odour, +Homogeneity, #Bulkiness, ^Taste, ¶Firmness. / No detected moulds for all the experimental period

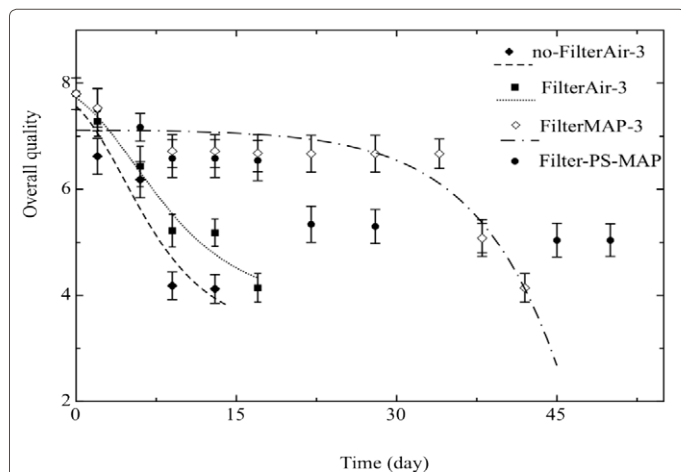


Figure 4: Overall quality of uncooked pasta produced in step-3. The curves are best fit of the re-parameterized Gompertz equation to experimental data.

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