



Use of natural antimicrobials to improve the quality characteristics of fresh “Phyllo” – A dough-based wheat product – Shelf life assessment



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ABSTRACT

This study explores the effects of chitosan and natamycin on the quality of fresh “Phyllo” – a dough-based wheat product, by monitoring the microbiological, physicochemical and sensory parameters. Four different lots of phyllo samples stored under aerobic packaging conditions, in the absence or presence of the aforementioned antimicrobials, were prepared and stored at 4 °C. Microbiological data suggested that, the combination of chitosan and natamycin resulted in significant reductions (1–3 log cfu/g) of the microbial species examined (mesophilic total viable counts; TVC), yeasts/molds, psychrotrophic and lactic acid bacteria (LAB), *Enterobacteriaceae* and coliforms) by day 10. The pH values of treated phyllo samples were lower on final day 10, as compared to the untreated phyllo, and of the Hunter color parameters (L^* , b^* and a^*) that were evaluated, mostly the combined treatment of chitosan and natamycin maintained the original lightness (L^*) and color (yellowness) stability (b^*) of phyllo product during the storage period. Sensory data, based on overall acceptability (mean values of appearance and odor) scores confirmed the superiority of combined treatment of chitosan and natamycin, resulting in almost a doubling of the shelf-life of fresh phyllo, while retaining excellent sensorial characteristics (appearance and odor) even on final storage day (10).

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1. Introduction

Dough is a nutrient-rich ecosystem. The microbial population of dough reflects that of the flour, consisting of LAB, Gram-positive (e.g., *Bacillus* spp.) and Gram-negative (e.g., *Pseudomonas* spp.) aerobic bacteria, *Enterobacteriaceae*, yeasts and molds. Due to both its popularity and simplicity, dough is usually made with all-purpose (mostly wheat) flour, into which oil, vinegar (optional) salt and warm water are added. The dough is then processed; either by hand, or by automatic machines, introduced in the last years by the bakery processing industries, to produce the “Phyllo” product, usually available fresh in thin sheets at small retail shops or in

supermarkets, aerobically packaged and stored under refrigeration. Phyllo consists of paper-thin sheets of wheat dough, which are stacked together, and separated from one another with a thin plastic membrane.

Phyllo usually (a dough-based wheat product) has been traditionally used for making savory pies (e.g. spinach, cheese, meat, chicken, etc.) as well as sweet pastry delicacies such as baklava etc., in Eastern Mediterranean (Greece, Turkey, Lebanon etc.) countries, whereas in the last decade, phyllo has also become a popular food commodity among consumers in other countries (US, Europe etc.). Due to its relatively high water activity (a_w) ranging from 0.96 to 0.98, phyllo is mostly prone to spoilage by aerobic microorganisms such as *Pseudomonas* spp. and molds, limiting its shelf-life to approximately 3–5 days, when stored under aerobic packaging and refrigeration (4 °C).

With today's growing interest in the so called “organic” or “green” additives, research has focused on naturally occurring antimicrobials that could potentially extend the shelf-life of

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perishable food products, whilst guaranteeing their safety (Burt, 2004). Additionally, packaging technologies (Kotsianis et al., 2002; Gutiérrez et al., 2009) that could be combined with 'natural' antimicrobials, such as nisin (Settanni and Corsetti, 2008) etc., have been suggested as alternative hurdles to existing processes, relying on the use of potentially harmful conventional/chemical food additives such as benzoate, propionate or sorbate (Guynot et al., 2005).

Chitosan, a deacetylated form of chitin, is a polysaccharide usually found in the shells of crustaceans such as crabs and shrimps, as well as the cell walls of some fungi. Attributable to its antimicrobial activity against a wide range of food-borne filamentous fungi, yeasts and bacteria, chitosan has been used to extend the shelf life of numerous foods including meat, fish, sausage, poultry, fruits, vegetables, cheeses, bread, and cake among others (Gitrakou and Savvaidis, 2012).

Natamycin has a broad spectrum of activity against various fungi (Lule et al., 2016). Based on the Directive 95/2009/EC of the European Commission on food additives other than colors and sweeteners, the use of natamycin (E235) is allowed for surface treatment of hard, semi-hard and semi-soft cheeses, as well as dried cured sausages. Recently, the European Food Safety Authority (EFSA) has published a favorable scientific opinion on the use of natamycin as a food additive (EFSA, 2009). A recent discussion held by a panel of experts of the Food and Drug Administration concluded that natamycin could be added to a maximum level of 5 mg/L in yogurt (FDA, 2014).

The concept of combining several preservation technologies was recognized by Leistner (1995) as the 'hurdle effect'; accordingly, in this study we have explored the use of natural compounds (natamycin, chitosan) in combination with aerobic packaging and low temperature (4 °C) storage, as a means to improve the quality characteristics of phyllo. To the best of the authors' knowledge, no such study has been conducted or reported to date. Therefore, the objectives of the present work were to (1) determine the effects of natural antimicrobials (chitosan, natamycin) on the quality characteristics of fresh "Phyllo", a dough-based wheat product and (2) determine the shelf-life of phyllo, both in the absence and presence of the aforementioned natural antimicrobials, stored under aerobic packaging conditions at 4 °C.

2. Material and methods

2.1. Preparation of natamycin and chitosan solutions

Chitosan of low molecular weight (MW; 340) in powder form from crab shells was purchased from Aldrich Company (Athens, Greece). Moisture content was less than 10% and chitosan had a deacetylation degree of 75–85% (Manufacturer's data). A stock solution of chitosan was prepared by dissolving 1.5 g in 100 ml of 1% (w/v) glacial acetic acid and stirred overnight at room temperature (final chitosan concentration = 1.5% w/v). Natamycin in powder form was purchased from Beste Hellas (Athens, Greece). A stock solution of natamycin was prepared by dissolving 0.1 g in 100 ml of 1% (w/v) glacial acetic acid and stirred overnight at room temperature (final natamycin concentration = 10 mg/L).

2.2. Raw materials, application of the antimicrobials to the phyllo samples

Fresh phyllo sheets (40 cm × 40 cm × 1 mm; length × width × thickness) were provided by a local dough processing company (Havelas, Ioannina, Greece) within 1 h after its preparation, were transported to the laboratory in insulated polystyrene boxes. Fresh phyllo sheets, having a beige color and a

pleasant, aromatic odor (fruity flavor) were provided in a square form, stacked on top of each other with a plastic thin membrane in between every 2 sheets, to prevent sheets from sticking one another, subsequently stored under refrigeration (1 h) in a cooling incubator (2 °C) before the addition of the antimicrobials.

The antimicrobials were added to the phyllo samples, either singly, or sequentially using the following procedure: A portion (ca. 20 g ± 1.0 g, area of 10 × 10 cm²) was aseptically removed from a whole phyllo sheet, and transferred into an open sterile packaging pouch, containing 10 ml of chitosan solution (1.5% w/v). Each phyllo sample was individually dipped and remained in contact with the chitosan solution for 10 s. Immediately, after dipping, the excess solution was drained off on a rack, that was previously sterilized (absolute alcohol) and this procedure was done under aseptic conditions in a sterile cabinet. The above procedure was also repeated for the addition of natamycin to the phyllo samples, using a natamycin solution of 10 mg/L (w/v) concentration. Finally, for the combined antimicrobial treatment, chitosan solution was applied first to the samples, followed by natamycin, both added at concentrations previously applied. It must be noted that, preliminary experiments tested the effect of acetic acid on the quality of phyllo samples. Samples were analyzed sensorially (results not shown). It was concluded that addition of acetic acid to the phyllo samples (dipped in 1% w/v glacial acetic acid, similar to the treatment with chitosan solution in 1% w/v acetic acid) did not negatively affect the sensory parameters of phyllo and did not have any effect on the mesophilic total viable counts (TVC) of the product.

2.3. Packaging of samples and storage conditions

Each phyllo sample (untreated, and treated) was then transferred aseptically into an open low density polyethylene/polyamide/low density polyethylene pouch (VER PACK, Thessaloniki, Greece), 75 µm in thickness having an oxygen permeability of 52.2 cm³/m²/day/atm, at 75% relative humidity (RH), 23 °C, a carbon dioxide permeability of 191 cm³/m²/day/atm at 0% RH, 23 °C and a water vapor permeability of 2.4 g/m²/day at 100% RH, 23 °C. Phyllo samples were subdivided into 4 lots: A (control, no antimicrobials added), AN with natamycin 10 mg/L; AC with chitosan 1.5% w/v and ANC with chitosan 1.5% w/v and natamycin 10 mg/L (w/v). Pouches were heat-sealed using a Minipack-Torre, model MV31 (Minipack-torre SpA/Dalmine-Italy). Aerobically-packaged phyllo samples (control and treated) were stored under refrigeration (4 °C) for a period of 10 days. Sufficient pouches were prepared to permit triplicate destructive sampling every 2nd day up to 10 days.

2.4. Microbiological and physicochemical analyses

The growth of the following groups of microflora was monitored: Mesophilic TVC, yeasts and molds, *Pseudomonas* spp., lactic acid bacteria (LAB), *Enterobacteriaceae*, Coliforms, and enterococci. A phyllo sample (20 g) was removed aseptically from each package, transferred to a stomacher bag (Seward Medical, Worthing, West Sussex, UK), containing 180 ml of sterile quarter strength Ringer's solution, and homogenized using a stomacher (Lab Blender 400, Seward Medical) for 60 s at room temperature. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, quarter-strength Ringer's solution) of phyllo homogenates were spread on the surface of agar plates. Mesophilic TVC were determined using plate count agar (PCA; Merck, Darmstadt, Germany), after incubation for 2–3 days at 30 °C. Yeasts and molds were enumerated on rose bengal chloramphenicol agar (RBC, Merck) by spread plating at 25 °C for 5 days in the dark. *Pseudomonas* spp. were enumerated on ceftrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basingstoke,

UK) and incubated at 25 °C for 2 days. For members of the *Enterobacteriaceae* family, 1.0 ml sample was inoculated into 10 ml of molten (45 °C) violet red bile glucose agar (Oxoid, Basingstoke, UK). After setting, 10 ml overlay of molten medium were added and incubated at 30 °C for 24 h. The large colonies with purple haloes were counted. LAB were determined on de Man Rogosa Sharpe medium (Oxoid code CM1153, Basingstoke, UK) after incubation at 35 °C for 3 days. Coliforms and enterococci were enumerated by using surface spreading on violet red bile agar (VRBA, Merck, Darmstadt, Germany) and kanamycin esculine azide agar (KAA, Merck), respectively, and plates were incubated at 24 and 35 °C, for 1 and 3 days.

The pH value was recorded using a Metrohm (Herisau, Switzerland) model 691 pH meter equipped with a glass electrode that was immersed into the phyllo homogenate (10 g homogenized with 90 ml of distilled water). Color determination was carried out on the surface of each phyllo sample using a Minolta Colourimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D65. L^* (lightness), a^* (redness) and b^* (yellowness) were quantified on each sample using a 10° position of the standard observer. Phyllo color was determined on 7 pre-selected locations. At each sampling day three samples were analyzed per treatment.

2.5. Sensory analysis

A trained panel of 12 individuals, members (4 staff, 5 graduate students of the Laboratory of Chemistry and Food Microbiology and 3 experts, members of the staff of the manufacturing company) was used to evaluate the phyllo quality on the basis of odor and appearance attributes, using a 1–8 point scale (Gutiérrez et al., 2009). A score of 8 implies a product of excellent quality, whereas a score of 1 denotes a product of unacceptable quality, unsuitable for consumption. A score of 4 was considered the as the lowest limit of acceptability, therefore sensory scores <4 demonstrated unacceptable products (Gutiérrez et al., 2009). As a reference, recently just made phyllo products were introduced in the trials. Phyllo samples (20 g each, area of 10 × 10 cm²) were coded with randomly chosen three-digit numbers and presented in random order in plastic disposable dishes with lids.

2.6. Statistical analysis

Experiments were replicated twice on two different occasions with fresh phyllo samples in each session. Analyses were run in triplicate for each replicate ($n = 2 \times 3 = 6$). Results are reported as mean values ± standard deviation (S. D.). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences among means ($P < 0.05$). Microbiological counts were converted to log cfu/g and were subjected to analysis of variance (ANOVA) using the software Stat graphics (Statistical Graphics Corp., Rockville, MD, USA).

3. Results

3.1. Effect of chitosan and natamycin on microbiological changes

Changes in the mesophilic TVC (Fig. 1a), yeasts/molds (Fig. 1b), *Pseudomonas* spp. (Fig. 1c), LAB (Fig. 1d), *Enterobacteriaceae* (Fig. 1e) and Coliforms (Fig. 1f) in the aerobically packaged phyllo samples, both in the absence and presence of chitosan, natamycin and their combination, were monitored during storage at 4 °C. Enterococci were absent in phyllo (data not shown) during storage of 10 days. The initial population density of the mesophilic TVC in the untreated (A) phyllo samples was 4.3 log cfu/g and increased

incrementally with storage time ($P < 0.05$) reaching a final average of 5.3 log cfu/g after 10 days of storage at 4 °C (Fig. 1a). It is noteworthy that, irrespective of treatment, population densities of all microbial species enumerated in our study remained always <6 log cfu/g, even on day 10 (end of the storage period). Of the treatments examined ACN resulted in significant ($P < 0.05$) reductions (1–3 log cfu/g) in the population densities of the mesophilic TVC, yeasts/molds, psychrotrophic and lactic acid bacteria (LAB), *Enterobacteriaceae* and coliforms by day 10.

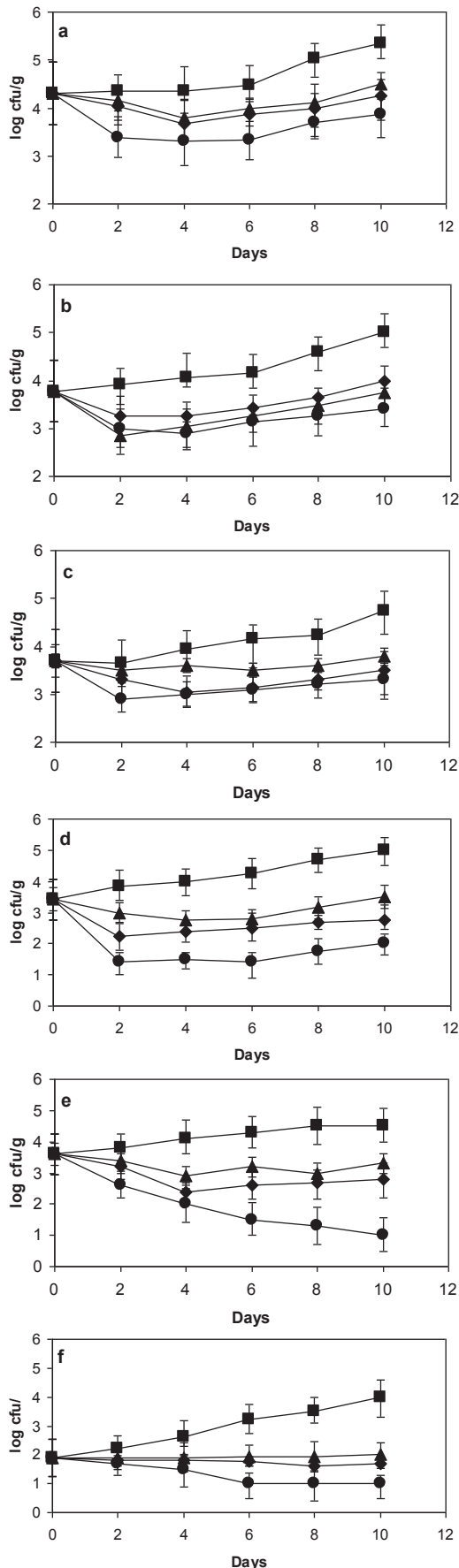
3.2. Effect of chitosan and natamycin on physicochemical changes

The initial pH of the phyllo was 6.4. Values of pH of untreated (A) phyllo samples were in the range 6.3–6.6 showing no major variations ($P < 0.05$) during storage (Fig. 2). In contrast the pH of the treated (AC, AN, ACN) phyllo samples reached a mean pH of 4.9–5.5, significantly lower ($P > 0.05$) on final day 10, as compared to the untreated phyllo. Among AC, AN and ACN treatments, no significant differences ($P > 0.05$) were noted in their pH values during the storage period.

Hunter L^* , b^* and a^* values of control (A) and treated (AC, AN, ACN) phyllo are shown in (Fig. 3a–c). Lightness (L^*) values of A and AN, AC, ACN samples on average did not show significant differences ($P > 0.05$) during the first 4 days of storage. After day 4 and up to the end of the storage period, L^* values of ACN samples were significantly higher ($P < 0.05$) among treatments (Fig. 3a). It is apparent that L^* values of untreated (A) and treated (AN, AC) samples showed major declines after days 4, and 6, respectively, of storage dropping steeply on final day 10 (Fig. 3a). Hunter b^* (yellowness, Fig. 3b), and a^* (redness, Fig. 3c) values of untreated (A) and treated (AN, AC, ACN) phyllo samples followed a similar to the L^* values pattern, with b^* and a^* values of ACN samples, being the highest ($P < 0.05$) among all the treatments during the entire storage period (Fig. 3b and c).

3.3. Effect of chitosan and natamycin on sensory changes

The results of the sensory evaluation (appearance, odor and overall acceptability) of the untreated (A) and treated (AC, AN, ACN) phyllo samples stored at 4 °C are shown (Fig. 4a,b,c). The sensory scores (appearance, odor and overall acceptability) for both untreated (A) and treated (AC, AN, ACN) phyllo samples showed a similar pattern of decreasing ($P < 0.05$) acceptability with storage time. Appearance and odor scores for the control and treated phyllo samples did not show significant differences ($P > 0.05$) during the entire period of storage (Fig. 4a and b). Based on both appearance and odor scores, and as judged by the trained panelists, untreated (A) and treated (AC, AN, ACN) phyllo samples were acceptable during the first 5 days of storage. Beyond day 5, untreated (A) samples were rejected on the basis of appearance (Fig. 4a) and odor (Fig. 4b) scores, dropping below the sensorial limit value of 4, whereas treated (AN and AC) reached this limit value between days 7 and 8 of storage (Fig. 4a and b). Interestingly, ACN samples reached the sensorial limit value on the final day 10 of storage. Overall acceptability data (mean value of appearance and odor scores) for A and AC, AN ACN phyllo showed similar patterns of decreasing acceptability over time (Fig. 4c). Based on the overall acceptability data and a score of 4, taken as the sensory limit value of acceptability, a shelf-life of 5, 7, 7 ½ and 10 days for A, AN, AC and ACN phyllo, respectively, was obtained. Therefore, the use of natamycin, chitosan and their combination, together with storage under aerobic packaging conditions and low temperature (4 °C), resulted in a shelf-life extension of 2, 2 ½ and 5 days, respectively, for AN, AC and ACN phyllo samples.



4. Discussion

Initial mesophilic TVC of 4.3 logs corresponds to phyllo of good quality, in correlation with the sensory data (appearance and odor attributes) as judged by the panelists. Fresh bakery products such as dough pastries and pasta, because of their high water activity ($a_w > 0.88$) and pH values in the range 5.7–6.5, are prone to growth of diverse microorganisms, with primarily a microbiota, consisting of O_2 depending organisms such as *Pseudomonas* spp., yeasts and molds. Of the species examined, yeasts/molds, LAB and psychrotrophs dominated the microbial flora of the fresh phyllo, stored under air packaging conditions. It is speculated that the aforementioned species, therefore, could be involved as specific spoilage organisms of phyllo, however no isolation and characterization of bacterial or fungal colonies were made at the days of spoilage. Finally, *Enterococci*, their presence usually in high numbers denoting unsatisfactory and poor handling practices during processing, were not detected in phyllo, in agreement with the findings of Rocha and Malcata (2012) for maize and rye flours.

According to the literature growth of aerobic bacteria and fungi (molds) is the most common cause of spoilage in bakery products (Corsetti et al., 1998). Present results suggest that, the combination of chitosan and natamycin inhibited the growth of aerobic spoilage microorganisms, (the *Pseudomonas* spp., and yeasts/molds) on the phyllo product. In another study, it was reported that natamycin applied singly or in combination with a low concentration of potassium sorbate reduced the growth of fungi, according to a survey conducted in agar at pH 5.5 (Mann and Beuchat, 2008). Jespersen et al. (1994) reported that fresh maize dough consisted of fungi (10^5 cfu/g) identifying mainly *Penicillium*, *Aspergillus*, *Fusarium* and yeasts ($<10^3$ cfu/g) namely: *Candida*, *Saccharomyces*, *Trichosporon*, *Kluyveromyces* and *Debaryomyces*. Our results show that the combination of chitosan and natamycin yielded an effective antimicrobial treatment against the pseudomonas, LAB and enterobacteria, organisms, involved in the phyllo microbiota, in agreement with results reporting in relation to the action of chitosan against mesophilic, psychrotrophic bacteria and coliforms in fresh pasta dough packaged in air (Del Nobile et al., 2009a). Of the aforementioned antimicrobials natamycin, as expected, applied singly to phyllo was more effective against yeasts/molds, than the use of chitosan. Natamycin's action and effectiveness against yeasts and molds "in vitro" studies have been well noted (Lule et al., 2016) whereas limited work to date has been done in real food systems. In one recent study, its effectiveness was demonstrated against yeasts/molds in traditional Greek products, such as "Galotyri", a traditional Greek acid-curd soft cheese (Kallinteri et al., 2013) and "Tzatziki" a yogurt-based salad dip appetizer (Tsiraki and Savvaidis, 2014). The effectiveness of natamycin to control the growth of yeasts and molds, in combination with other hurdles, such as low temperature, low pH could mean, that the use of conventional harmful chemical preservatives such as benzoate, propionate and sorbate could be replaced in foods, that are spoiled by molds, including bakery products. In a recent study, modified atmosphere packaging (25–75% CO_2 :75–25% N_2 and 100% CO_2) inhibited the growth of yeasts/molds in part-baked Iranian Sangak bread (Khoshakhlagh et al., 2014).

One of the objectives of the present study was to establish a maximum acceptability limit (MAL) value, based on microbial

Fig. 1. Evolution of mesophilic TVC (a), yeasts/molds (b), *Pseudomonas* spp. (c), LAB (d), *Enterobacteriaceae* (e) and coliforms (f) in phyllo stored under aerobic packaging at 4 °C, in the absence of natural antimicrobials (A; ■) in the presence of chitosan (AC; ◆) in the presence of natamycin (AN; ▲) and in the presence of both chitosan and natamycin (ACN; ●). Data shown represent the average of two independent experiments ($n = 2 \times 3 = 6$) plus the standard deviation (error bars).

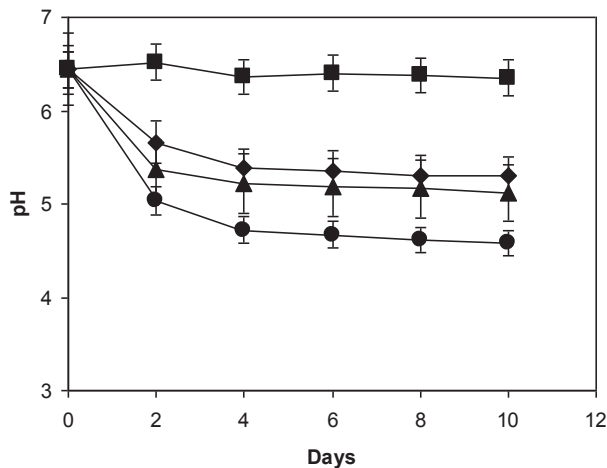


Fig. 2. Changes of pH values in phyllo stored under aerobic packaging at 4 °C, in the absence of natural antimicrobials (A; ■) in the presence of chitosan (AC; ◆) in the presence of natamycin (AN; ▲) and in the presence of both chitosan and natamycin (ACN; ●). Data shown represent the average of two independent experiments ($n = 2 \times 3 = 6$) plus the standard deviation (error bars).

indices of fresh phyllo quality. It must be stated that, currently no MAL value exists for fresh phyllo. Del Nobile et al. (2009a) set MAL values of 10^6 for total mesophilic and psychrotrophic bacterial counts and 10^4 cfu/g for total coliforms and *Staphylococcus* spp. in amaranth-based homemade fresh pasta. Inspection of sensory overall acceptability data (based on appearance attribute) revealed that fresh phyllo was rejected on day 5 under aerobic packaging, respectively, when yeasts/molds populations were on average 4 log cfu/g, a value that could be suggested as a MAL for fresh phyllo. Molds, as judged by the panelists (appearance attributes) could be considered as being a spoilage indicator of phyllo dough.

The initial pH value of fresh phyllo of 6.4, is in agreement with the pH values of fresh pasta (Del Nobile et al., 2009b). Reduced pH values of the treated phyllo may be due to the low pH of the solutions of chitosan and natamycin (4.4 and 3.0, respectively) that were used for the treatment of phyllo. Silveira et al. (2007) reported that the initial pH value (5.9) of confectionery dough decreased to pH 5.0 after 40 days.

Fresh phyllo, a dough-based wheat product, is sold in the form of thin sheets in small retail shops, or in supermarkets (usually aerobically packaged) and stored under refrigeration. Due to its high water activity and handling practices, phyllo has a rather short and limited shelf-life of approximately 3–5 days, depending on its initial microbial quality and subsequent storage conditions. In our study, we decided to assess the sensory quality of the fresh “Phyllo”, by examining its appearance and odor, attributes that consumers usually evaluate when buying fresh phyllo sheets. Therefore, in our study, the appearance/color and odor of the raw product was assessed sensorially by the panelists, depicting the market purchase and home handling practices of the consumer.

Phyllo was judged as sensorially being unacceptable by the panelists after 5 days, showing white discolored areas, fungal growth (colonies) on the surface, and having an unpleasant, sour off-flavor. The color (clear, bright appearance) is considered as a prime key factor for the initial acceptance of bakery products, including dough-based preparations, by the consumers. Therefore, for noodles a bright and light appearance is desired, while a dark color is a negative feature (Li et al., 2012). Hesseltine et al. (1969) reported that spoiled dough (as compared to the fresh sample) was darker and slightly discolored in places. Li et al. (2011) chose

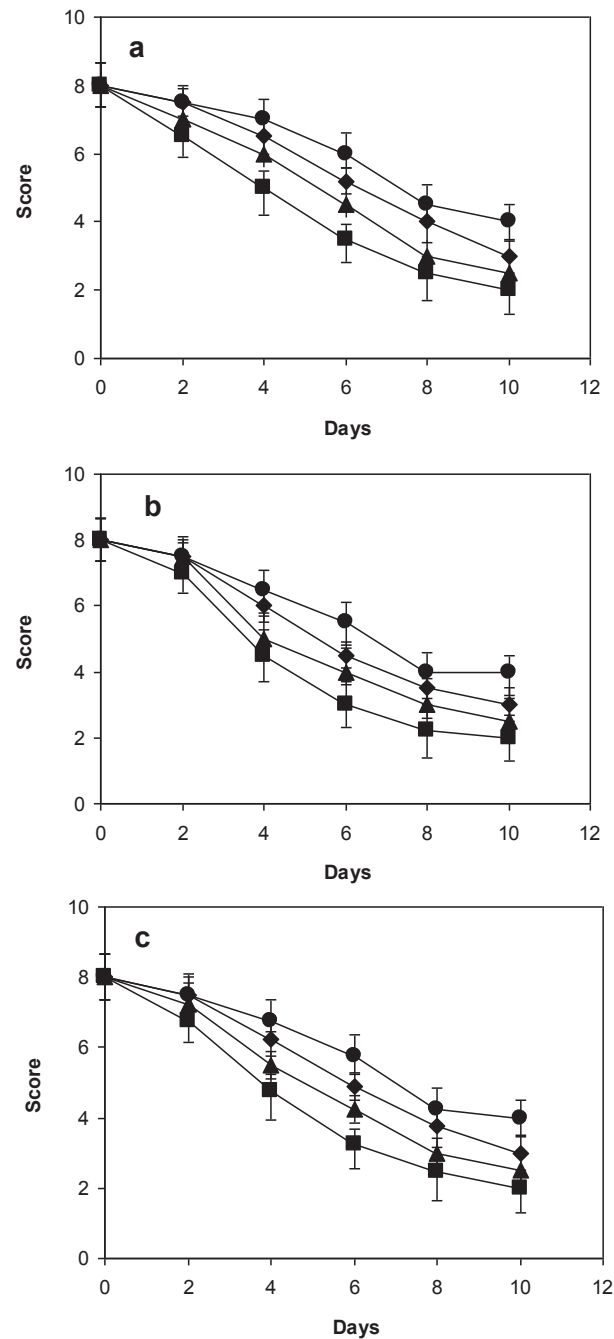


Fig. 3. Changes of L^* (lightness; a), b^* (yellowness; b) and a^* (redness; c) values in phyllo stored under aerobic packaging at 4 °C, in the absence of natural antimicrobials (A; ■) in the presence of chitosan (AC; ◆) in the presence of natamycin (AN; ▲) and in the presence of both chitosan and natamycin (ACN; ●). Data shown represent the average of two independent experiments ($n = 2 \times 3 = 6$) plus the standard deviation (error bars).

the color and odor as indicators of quality/freshness to determine the shelf-life of noodles.

Upon prolonged storage, control samples became darker and progressively lost the initial beige–ecru light color, characteristic of the phyllo appearance/color. Phyllo (untreated) when judged as being unacceptable, by sensory data (appearance and odor) appeared to have a dark color (decrease in L^* values) with local white-discolored areas (decrease in a^* values) and fungal growth appeared on its surface. Silveira et al. (2007) reported that after 13

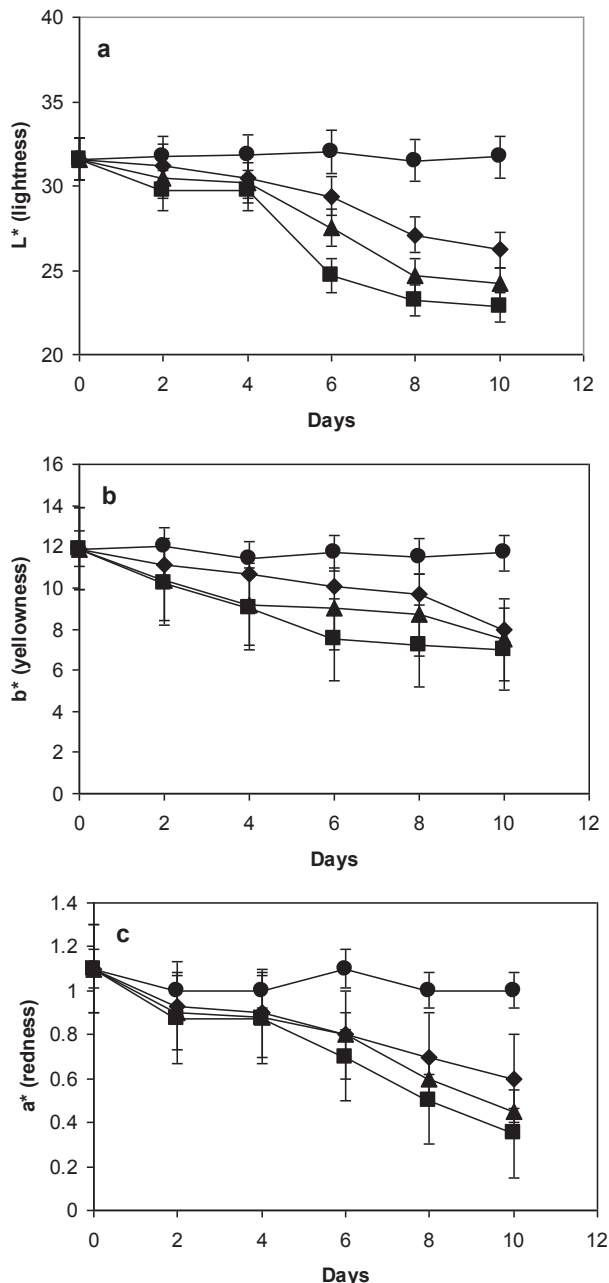


Fig. 4. Changes in appearance (a), odor (b) and overall acceptability scores (c) in phyllo stored under aerobic packaging at 4 °C, in the absence of natural antimicrobials (A; ■) in the presence of chitosan (AC; ◆) in the presence of natamycin (AN; ▲) and in the presence of both chitosan and natamycin (ACN; ●). Data shown represent the average of two independent experiments ($n = 2 \times 3 = 6$) plus the standard deviation (error bars).

days fungi appeared on the surface of pastry dough without preservatives, at 8 °C.

Of the treatments examined in our study, chitosan combined with natamycin maintained phyllo color stability during storage, as shown by the higher lightness L^* and yellowness b^* values. Of the remaining treatments, chitosan (to a larger extent) and natamycin maintained phyllo lightness and yellowness for approximately 7–8 days, in agreement with the sensory data (appearance) obtained in our study, by the trained panelists.

With regard to a^* values, ACN treatment maintained the highest values, showing no apparent discoloration of the phyllo, during

storage. Discoloration may be due to the number of total bacteria on the phyllo surface, suggesting that higher numbers (mesophilic TVC) in phyllo samples stored in air may be responsible for the discoloration (lower a^* values) of phyllo. Aerobic bacteria such as *Pseudomonas aeruginosa*, *Ps. fluorescens* and *Ps. geniculata* or fungi/yeasts may consume O_2 , thereby reducing oxygen tension on the surface of phyllo, causing discoloration.

Present results obtained by the use of packaging in combination with chitosan and natamycin delayed or even inhibited the spoilage of the phyllo, giving acceptable sensorial characteristics, maintaining the original freshness and appearance of the product. In other studies, Huang et al. (2007) reported that the shelf-life of fresh noodles after addition of chitosan was extended by 6 days, at 4 °C, whereas Tan et al. (2009) noted that chitosan applied in sweet potato noodles improved the sensory characteristics of the product.

5. Conclusions

The results of this study indicate that air packaging combined with chitosan and natamycin could be used as an antimicrobial treatment against the microbiota (spoilage) flora of phyllo dough stored under refrigeration, maintaining its freshness and quality (appearance) characteristics. Interestingly, the combination of packaging, chitosan and natamycin resulted in a shelf-life of 10 days (as compared to the controls' shelf life of 5 days) for phyllo maintaining acceptable sensory characteristics. Present results show that air packaging, combined with chitosan and natamycin are effective hurdles over the microbiota of the phyllo dough. However, further studies are needed to support and reinforce our findings involving different bakery (dough based) products. Chitosan and natamycin proved the most powerful natural antimicrobial agents, when added to traditional phyllo, without altering the sensory characteristics of the product.

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References

- Burt, S., 2004. Essential oils and their antibacterial properties and potential applications in foods - a review. *Int. J. Food Microbiol.* 94, 223–253.
- Corsetti, A., Gobetti, M., Rossi, J., Damiani, P., 1998. Antimould activity of sour-dough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Appl. Microbiol. Biotechnol.* 50, 253–256.
- Del Nobile, M.A., Di Benedetto, N., Suriano, N., Conte, A., Lamacchia, C., Corbo, M.R., Sinigaglia, M., 2009a. Use of natural compounds to improve the microbial stability of Amaranth-based homemade fresh pasta. *Food Microbiol.* 26, 151–156.
- Del Nobile, M.A., Di Benedetto, N., Suriano, N., Conte, A., Corbo, M.R., Sinigaglia, M., 2009b. Combined effects of chitosan and MAP to improve the microbial quality of amaranth homemade fresh pasta. *Food Microbiol.* 26, 587–591.
- European Food Safety Authority (EFSA), 2009. Scientific opinion on the use of natamycin (E 235) as a food additive. *EFSA J.* 7, 1412.
- FDA, 2014. <http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm427606.htm>.
- Gitrakou, V., Savvaidis, I.N., 2012. Bioactive packaging technologies with chitosan as a natural preservative agent for extended shelf-life food products. In: Arvanitoyiannis, I. (Ed.), *Modified Atmosphere and Active Packaging Technologies*. Taylor & Francis, Boca Ration, FL, pp. 685–730 (Chapter 16).
- Gutiérrez, L., Sánchez, C., Ramón Batlle, R., Cristina Nerin, C., 2009. New antimicrobial active package for bakery products. *Trends Food Sci. Technol.* 20, 92–99.
- Guynot, M.E., Ramos, A.J., Sanchis, V., Marin, S., 2005. Study of benzoate, propionate, and sorbate salts as mould spoilageinhibitors on intermediate moisture bakery products of low pH (4.5–5.5). *Int. J. Food Microbiol.* 101, 161–168.
- Hesseltine, C.V., Graves, R.R., Rogers, R., Burmeister, H.R., 1969. Aerobic and facultative microflora of fresh and spoiled refrigerated dough products. *Appl. Microbiol.* 18, 848–853.

- Huang, J., Huang, C., Huang, Y., Chen, R., 2007. Shelf-life of fresh noodles as affected by chitosan and its Maillard reaction products. *LWT Food Sci. Technol.* 40, 1287–1291.
- Jespersen, L., Halm, M., Kpodo, K., Jakobsen, M., 1994. Significance of yeasts and molds occurring in maize dough fermentation for 'kenkey' production. *Int. J. Food Microbiol.* 24, 17–248.
- Kallinteri, L.D., Kostoula, O.K., Savvaidis, I.N., 2013. Efficacy of nisin and/or natamycin to improve the shelf-life of Galotyri cheese. *Food Microbiol.* 36, 176–181.
- Khoshkhalagh, K., Hamdami, N., Shahedi, M., Le-Bail, A., 2014. Quality and microbial characteristics of pat-baked Sangak bread packaged in modified atmosphere during storage. *J. Cereal Sci.* 60, 42–47.
- Kotsianis, I.S., Giannou, V., Tzia, C., 2002. Production and packaging of bakery products using MAP technology. *Food Sci. Technol.* 13, 319–324.
- Leistner, L., 1995. Principles and applications of hurdle technology. In: Gould, G.W. (Ed.), *New Methods of Food Preservation*. Blackie Academic and Professional, London, pp. 1–21.
- Li, M., Zhu, K., Guo, X., Peng, W., Zhou, H., 2011. Effect of water activity (a_w) and irradiation on the shelf-life of fresh noodles. *Innov. Food Sci. Emerg. Technol.* 12, 526–530.
- Li, M., Luo, L.J., Zhu, K.X., Guo, X.N., Peng, W., Zhou, H.M., 2012. Effect of vacuum mixing on the quality characteristics of fresh noodles. *J. Food Eng.* 110, 525–530.
- Lule, V.K., Garg, S., Gosewade, S.C., Khedkar, C.D., 2016. Natamycin. *Encyclopedia of Food and Health*. Elsevier, pp. 56–62. <http://dx.doi.org/10.1016/B978-0-12-384947-2.00482-7>.
- Mann, D.A., Beuchat, L.R., 2008. Combinations of antimicrobials to inhibit the growth of molds capable of producing 1,3-pentadiene. *Food Microbiol.* 25, 144–153.
- Rocha, J.M., Malcata, F.X., 2012. Microbiological profile of maize and rye flours, and sourdough used for the manufacture of traditional Portuguese bread. *Food Microbiol.* 31, 72–88.
- Settanni, L., Corsetti, A., 2008. Application of bacteriocins in vegetable food bio-preservation. *Int. J. Food Microbiol.* 121, 123–138.
- Silveira, M.F.A., Soares, N.F.F., Geraldine, R.M., Andrade, N.J., Botrel, D.A., Gongalves, M.P.J., 2007. Active film incorporated with sorbic acid on pastry dough conservation. *Food Control* 18, 1063–1067.
- Tan, H.Z., Li, Z.G., Tan, B., 2009. Starch noodles: history, classification, materials, processing, structure, nutrition, quality evaluating and improving. *Food Res. Int.* 42, 551–576.
- Tsiraki, M.I., Savvaidis, I.N., 2014. Citrus extract or natamycin treatments on "Tzatziki" – a traditional Greek salad. *Food Chem.* 142, 416–422.