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Influence of an innovative, biodegradable active packaging on the quality of sunflower oil and "pesto" sauce during storage

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ABSTRACT

The aim of this research was to produce an innovative, biodegradable multilayer active packaging solution, with excellent oxygen barrier properties, to extend shelf- life of oily foods. In fact, one of the main drawbacks of biodegradable films is the low barrier they offer against external agents that can thus easily accelerate the foods degradation during shelf-life. In this study, a multilayer material obtained by the adhesion of two polylactic acid (PLA) films with cold plasma application, in place of synthetic adhesives, was realized. Moreover, cold plasma treatment was employed to immobilize the oxygen scavenger agent (ascorbic acid), chosen for the activation of the packaging material. Preliminary studies on activated PLA pouches filled with sunflower oil, used as model system, were performed. Model systems were stored at 35 °C to accelerate oil oxidation phenomenon and analysed for PV and colour during 64 days of storage. After that, different samples of "Genovese pesto", were tested as real food, stored at 25 and 45 °C and analysed for PV, water activity, rheological parameters and microbiological loads during 41 days of storage. Obtained results showed the greater ability of the new active packaging to decrease the oxidation kinetics of "pesto", mainly when stored at 25 °C. Moreover, all samples packed in the activated biodegradable pouches (both sunflower oils stored at 35 °C and pesto stored at 25 °C) showed better and more stable quality characteristics, in terms of colorimetric, microbiological and textural parameters when compared with the respective control samples. Overall obtained results highlighted the potentiality of the new biodegradable material, activated with the oxygen scavenger, to be applied successfully in food industry, to extend food products shelf-life and/or maintain high quality levels during storage.

1. Introduction

Food deterioration can occur during harvesting, food processing and distribution (Malhotra et al., 2015). Packaging is an effective means of protecting food products from external contaminants and prevent chemical, physical and biological changes during storage. Conventional packaging materials cannot actively control deterioration phenomena of food during storage, while offering the requested barrier against oxygen, moisture and light, that can be considered a good protection only for the most sensitive foods (Robertson, 2006; Khaneghah et al., 2018; Alamri et al., 2021). Active packaging is one of the innovative technologies which is being developed to extent the protection function of the packaging materials thus increasing the shelf-life of food products (López-de-Dicastillo et al., 2012; Benito-Pena et al., 2016; Upasen &

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Wattanachai, 2018). These packaging systems are designed to deliberately incorporate components that release or adsorb substances (e.g. oxygen, ethylene, water, etc.) into or from the packaged food or environment surrounding the food, to extend the shelf-life or to maintain or improve the quality of the packaged food (Charles et al., 2006; Flores et al., 2007; Regulation (CE) No. 450/2009)). Active packaging systems can be obtained by the inclusion of sachets or pads containing specific compounds, or by the incorporation of active substances directly into the packaging material itself, by immobilization or surface modification techniques (Bastarrachea et al., 2015). Among different techniques, one of the most promising and innovative seems to be cold plasma atmosphere. Cold plasma is an emerging, green technology that presents various potential applications in the food packaging sector. Notably, the application of cold plasma for surface functionalization of polymers allows the modification of some surface properties giving improved wettability, sealability and printability, promoting the adhesion of selective functional compounds, thus overcoming the problem related to the use of primers and synthetic adhesives (Fazeli, 2019; Bahrami, Delshadi, Assadpour, Jafari, & Williams, 2020; Hoque et al., 2022).

Synthetic polymers are widely used in the food packaging sector because of their low production costs and high technological and functional performances. However, in the last decades the use of bio-renewable materials obtained from natural sources has been proposed as a potential green and eco-friendly alternative solution (Mohamed et al., 2020). In this way, biodegradable active packaging, can satisfy the demand of consumers for both healthy food and better environment protection, if compared to traditional ones (Huang et al., 2021). However, the use of bio-based materials for food packaging is still very limited because most of natural polymers have poor barrier and weak mechanical properties. For this reason, natural polymers are frequently blended with other polymers, added with reinforcing nanoparticles, or preliminarily modified either chemically or enzymatically in order to extend their applications (Cakmak & Sogut, 2020; Mujtaba et al., 2021). Among bio-degradable materials, polylactic acid (PLA) is one of the most widely used as environmentally friendly alternatives to the synthetic ones; recognized as safe (GRAS), proved to be one of the most suitable biopolymer owing to its biodegradability, renewability and superior technological properties (Farah, Anderson & Langer, 2016; Swaroop & Shukla, 2018).

Lipid oxidation is one of the main causes of the quality reduction of many food, being a big challenge for companies. Lipids are susceptible to oxidative processes in the presence of catalysts giving rise to the development of unpleasant flavors in food and the loss of essential amino acids, fat-soluble vitamins and other bioactive molecules (Shahidi & Zhong, 2015; da Costa Monção, Grisi, de Moura Fernandes, Souza, & Souza, 2022). Oxygen scavengers used as active compounds in food packaging materials represent an innovative and effective solution to prolong the shelf-life of many foods sensitive to oxidative degradation, also allowing to reduce the use of antioxidant additives in formulation (Gaikwad, Singh, & Lee, 2017).

Sakanaka and Tachibana (2006) realized an active packaging with oxygen scavenger properties based on activity of egg-yolk protein hydrolysates, to stabilize lipid oxidation in beef and tuna homogenates; Janjarasskul et al. (2011) developed an activated film with whey protein isolate that incorporated ascorbic acid, to retard shelf lives of a wide variety of oxygen-sensitive products; activated films containing α-tocopherol were also used to extend shelf- life of salmon fish by Barbosa-Pereira et al. (2013), observing a reduction of 70% in lipid oxidation, while Gaikwad, Singh, Shin, and Lee (2020) tested a novel polyisoprene based UV-activated oxygen scavenger film to reduce lipid oxidation in beef jerky . The aim of this research was to set-up an innovative, biodegradable active packaging material with excellent oxygen barrier properties, to extend the shelf-life of oily foods. In this study, a multilayer packaging was realized by the adhesion of polylactic acid (PLA) films by cold atmospheric plasma application, used also to promote the immobilization of the oxygen scavenger agent (ascorbic acid) for packaging activation in the surface of the material. The effect of the activated bio-based packaging material was tested on some quality characteristics of both sunflower oil and Genovese pesto sauce during storage.

2. Materials and methods

2.1. Materials

The polymer and the gel use as support for the activation of the material were chosen after preliminary tests and considering their biodegradability; the polymer was polylactic acid (PLA) purchased by Taghleef Industries, S.p.A (San Giorgio di Nogaro, Italy) and the gel sodium alginate purchased by Sigma -Aldrich (Gallarate, MI). Ascorbic acid, used as active compound, was purchased by Sigma–Aldrich (Gallarate, MI, Italy).

A commercial sunflower oil (Conad, Italy) was purchased from a local market and used as model system in order to preliminarily test the performances of activated material; a commercial refrigerated Genovese pesto (Buitoni, Italy) purchased from a local market was subsequently used as "real food" to be packed with the new pack system. The composition of Genovese pesto as reported on the label was: basil 31.5%, sunflower oil, whey powder, extra virgin olive oil 10%, "pecorino" cheese, "grana Padano" cheese 5%, dextrose, pine nuts 1.5%, salt, garlic 0.5% and natural flavors.

2.2. Film activation and pouches production

Pouches were realized by lamination of two PLA film layers by using cold plasma treatment at atmospheric pressure, employing an open-air Dielectric Barrier Discharge (DBD) source, with a peak voltage of 20 kV and 20 kHz of frequency. Plasma treatment had the dual objective of pre-functionalize the films in order to immobilize covalently the oxygen scavenger agent in gel matrix and to promote the adhesion between the two PLA film layers.

PLA films had a thickness of 40 μm, an oxygen transmission rate (OTR) of 540 cm^3/m^2/day evaluated according with ASTM D3985 at 23 °C and 0% RH (ASTM D3985-05 2010); a water transmission rate of 200 cm^3/m^2/day estimated with the standard test ASTM F1249 at 38 °C and 90% RH (ASTM F1249-13 2013) and a tensile strength of respectively 105 N/mm^2 in machine direction (MD) and 205 N/mm^2 in transverse direction (TD), assessed according with ASTM D882-18 2018. A quantity of 25% ascorbic acid, selected after preliminary trials, was dispersed on a sodium alginate (2%)/water supporting gel solution, with a density of 0.3 mg/cm^2, and set down between the two plasma-treated polymeric films, by using a prototype machine realized for this purpose. As control a similar not activated material was produced (double layer PLA).

From the ascorbic acid activated (AA) and not-activated materials (C) a high number of pouches were obtained for the shelf-life test of sunflower oil and pesto sauce.

The dimension of final pouches was 20 × 10 cm. Films were sealed by using a heat-sealer (Impulse Heat Sealer PFS-300) at 100 °C.

2.3. Test on packed sunflower oil samples

Thirty pouches activated with ascorbic acid (AA) and 30 not activated (C) were filled in sterile conditions with 60 mL of sunflower oil, by using an automatic pipette, under a laminar flow cabinet, and then sealed off on the upper part. Samples were stored at 35 °C in climatic chambers at RH of 50% for 64 days and analysed in triplicate at 0, 7, 14, 25, 30, 36, 46, 50, 57 and 64 days.

During storage the following determinations on sunflower oil samples were carried out:

2.3.1. Peroxide value (PV)

Peroxide values (PV) were evaluated according with the official method (AOAC, 2001) for iodometric titration.

2.3.2. Colour

Colour of oil samples was determined by using a tristimulus spectrophotocolorimeter (mod. A60–1010–615 ColorFlex, HunterLab, USA) equipped with a sample holder (12 mm diameter). Colour was measured using the CIE $L^*a^*b^*$ colour space and illuminant D65 and expressed as hue angle (h°) and chroma (C*), calculated as reported by McGuire (1992).

2.4. Test on pesto sauce as real system

Forty pouches activated with ascorbic acid (AA) and 40 not activated (C) were filled with 60 ml of "Genovese pesto", by using an automatic pipette, under a laminar flow cabinet, and then sealed off on the upper part. Samples were stored at two different temperatures: 25 °C and 45 °C in climatic chambers at RH of 50% for 41 and 34 days respectively and analysed in triplicate at 0, 8, 14, 20, 27, 34 and 41 days. During storage the following determinations on pesto sauce samples were carried out:

2.4.1. Peroxide value (PV)

PV were evaluated on oil fraction extracted by centrifugation and filtration from pesto samples as for sunflower oil (Section 2.3.1).

2.4.2. Water activity, textural and colorimetric analysis

The water activity (a_w) was measured by using a dew point hygrometer, AcquaLab-Water Activity Meter (mod. SERIES 3TE. Decagon Device, Inc., Nelson Court, NE).

A TA.HDi 500 Texture analyzer (Stable Micro System Vienna Court, England) was employed to investigate pesto consistency. The measurement was performed by using a back extrusion test with a load cell of 5 kg. The parameters used were: a pre- test speed of 1 mm s^{-1}, a test speed of 1 mm s^{-1}, a post test speed of 1 mm s^{-1} and a distance of 30 mm. From obtained curves, we analyzed the consistency parameter (N. s).

Colour of whole pesto samples was determined as for model system (Section 2.3.2) and expressed in terms of lightness (L*) and hue angle (h°).

2.4.3. Microbiological analysis

The cell load of mesophilic aerobic and anaerobic bacteria and aerobic spore forming bacteria was detected on Plate Count Agar (PCA) (Oxoid, Ltd.), anaerobic spore forming bacteria on Reinforced Clostridial Medium (RCM) (Oxoid, Ltd.). In addition, for samples stored at 45 °C also thermoduric Lactococci were detected on M17 (Oxoid, Ltd.) and Staphylococci on Baird Parker (BP) (Oxoid, Ltd.). For each microbiological analysis, 10 g of each sample were re-suspended in 90 mL of sterile saline solution (9 g/L NaCl) and homogenized using a Stomacher (Lab Blender Seward, London, UK) for 2 min at room temperature; then serial dilutions in sterile saline solution were performed. The search for spore forming microorganisms (aerobic and anaerobic) was performed after heat treatment of the sample at 80 °C for 10 min. Plates were then incubated at 25 °C for 48 h for the detection of mesophilic aerobic and anaerobic bacteria and aerobic spore forming bacteria; at 30 °C for 24 h for anaerobic spore forming bacteria; at 45 °C for 24 h for Lactococci and Staphylococci. In the case of anaerobic spore forming bacteria plates were incubated in anaerobic conditions using anaerobic jar (Oxoid Ltd.) and AnaeroGen sachet (Oxoid Ltd.). Each sample was analysed in triplicate.

2.5. Statistical analysis

At each time of storage, obtained data represent the means of three independents replicates. Analysis of variance (ANOVA) and the test of mean comparisons according to Fisher's least significant difference (LSD) with a 0.05 level of significance were applied to find out significant differences among the different samples. The statistical package STSG Statistica for Windows, version 6.0 (Statsoft Inc., Tulsa, USA) was used.

3. Results and discussions

3.1. Sunflower oil model system

3.1.1. Peroxide values (PV)

In Fig. 1 the PV of sunflower oil packed in both activated (AA) and not activated (C) PLA pouches during storage at 35 °C are showed. The PV were used to monitor the effect of the activated film on lipid oxidation degree of sunflower oil during storage. As shown in Fig. 1, both oil samples showed an increase in the PV starting from the 14th day of storage, although the rate of oxidation differed according to the packaging films used. Oil sample packed in the activated pouches showed significantly lower values than control one until the end of storage. Moreover, the PV of C samples exceeded the threshold of acceptability of the product (10 meq O_2/kg of oil) (FAO/WHO, 1993) after about 25 days of storage while the oil samples in activated material reached this limit after 37 days.

Results demonstrated the potentiality of ascorbic acid to slowing down the oxidation reactions in sunflower oil, underlining the effectiveness of the new active packaging solution in delaying this phenomenon during product storage.

3.1.2. Colour

In Figs. 2 and 3 the hue angle and Chroma values of sunflower oil during storage were showed.

Both AA and C oils had a hue that can be classified as greenish-yellows (h°> 90°) (Popovici et al., 2022). A reduction in the hue angle parameter was observed for both samples during storage, however, even if not significantly differences were always observed between them, activated ones showed lowest h° values compared to control, underlining the retention of a more shine yellow colour (Yin et al., 2022). Moreover, AA samples presented also a better colour saturation expressed in terms of chroma values, highlighting the protective effect promoted by ascorbic acid in maintaining a brighter and glossier colour (Koncsec et al., 2019)

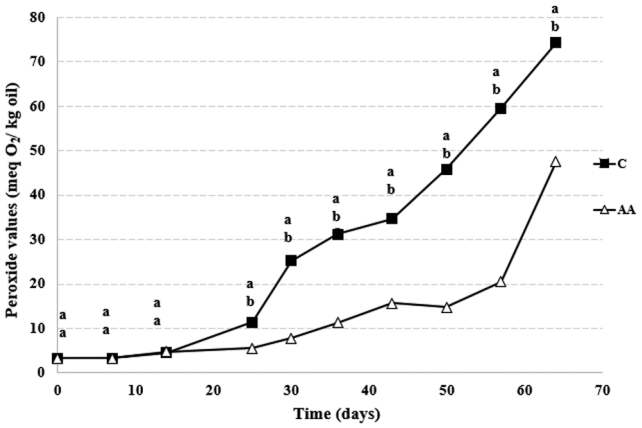

Fig. 1. Peroxide values behavior of sunflower oil samples packed in activated and not activated PLA pouches during storage at 35 °C.
[a–b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature

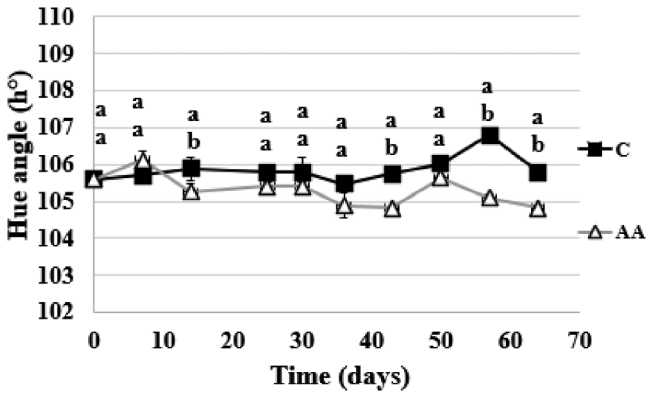

Fig. 2. Hue angle parameters of sunflower oil samples packed in activated and not activated pouches during storage at 35 °C.
[a-b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature

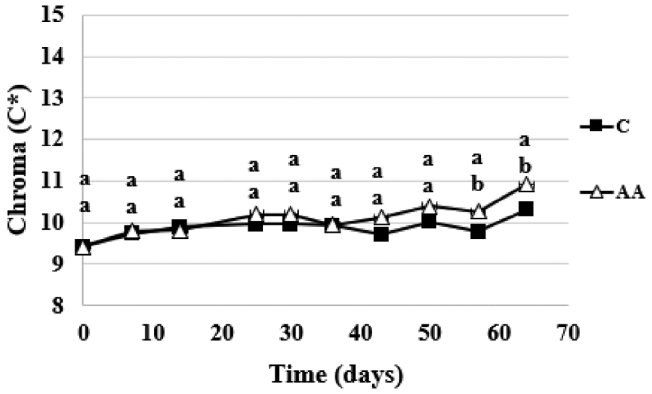

Fig. 3. Chroma parameters of sunflower oil samples packed in activated and not activated pouches during storage at 35 °C.
[a-b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature

3.2. Pesto sauce real system

3.2.1. Peroxide values (PV)

In Fig. 4 the PV of oil samples extracted from Genovese pesto samples packed in both activated (AA) and not activated (C) PLA pouches during storage at 25 °C and 45 °C are showed. From results it is possible to highlight as, also in this case, PV tend to increase during storage times for all considered samples. For what concern storage at 25 °C, oil samples from pesto packed in activated pouches showed lower significantly PV compared to their control, probably due to the oxygen scavenger ability of AA to remove the oxygen inside of the packaging system (Janjarasskul et al., 2011; Tian Fang et al., 2013; Uluata, McClements & Decker, 2015). Moreover, oil from pesto in activated package did not exceed the threshold of acceptability (10 meq O_2/Kg oil) until the 27th day of storage at 25 °C, while in control ones this level was exceeded after less than 10 days, underlined the ability of AA pouches to retard the pesto oxidation level and prolong shelf-life of this product (Frankel, 1997). Generally, no statistical differences in PV were found between oil samples from pesto packed in activated and control packaging materials during storage at 45 °C. These results can be attributed to the higher storage temperature that probably promoted the ascorbic acid degradation thus vanishing its active action. In fact, as known by literature (Ruiz et al., 2018) the ascorbic acid activity is strictly dependent on temperature and tends to decrease with the temperature increase starting from 37 °C especially at high RH percentage, from 45 to 90% (Kim et al., 2015). Moreover, a pro-oxidant effect of ascorbic acid, was also observed around 45–50 °C in accordance with literature (Kitts, 1997; Yen et al., 2002). Among the main degradation products of ascorbic acid there is hydrogen peroxide, which is known to trigger pro-oxidation reactions against ascorbic acid itself, leading to an increase in the number of peroxides, as observed during storage at 45 °C and in particular for AA samples respect C ones at the first days of shelf-life.

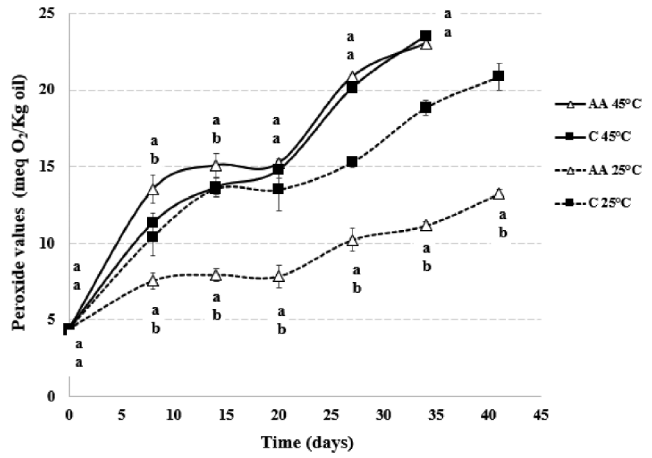

Fig. 4. Peroxide values of oil obtained from pesto samples packed in activated and not activated pouches during storage at 25 °C and 45C°.
[a–b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature

3.2.2. Water activity, textural and colorimetric properties

In Fig. 5 water activity values of pesto samples stored at 25 and 45 °C are presented.

As shown in Fig. 5 water activity values tend to decrease during storage for all considered samples, without showing significantly differences between AA and C samples at each storage temperature. However, samples stored at 45 °C showed a more drastic decrease in a_w compared to samples stored at 25 °C; this behavior can be reasonably attributed to a higher moisture loss of these samples in the environment due to the highest storage temperature, (Forsido et al., 2021), that according with literature (Gorrasi et al., 2013) promoted an increase in the water vapour transmission rate of the employed materials. Several studies (Mrkic et al., 2007; Siracusa, 2012; Gorrasi et al., 2013) highlighted in fact, how in polymeric films such as PLA an increase in water vapor transmission was observed as temperatures rate raised, especially in the range from 40 to 60 °C. Moreover, as observed by Kadir Basha

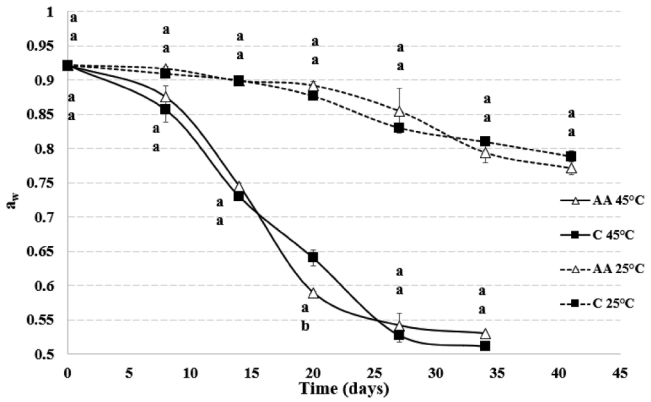

Fig. 5. Water activity behavior of Genovese pesto samples packed in activated and not activated pouches during storage at 25 °C and 45C°.
[a–b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature

et al. (2011) the WVTR rate seems to increase of about 70–85% for a 10 °C rise in temperature.

In Fig. 6 the consistency values of pesto samples stored at 25 and 45 °C are presented. An increase in consistency was observed during storage especially in samples stored at 45 °C, and faster in control sample, probably due to the decrease in water activity, as previous observed, and certainly in total water content. Nevertheless, AA samples at 7 and 14 days of storage showed significantly lowest textural parameters that C ones, despite to the similar water activity values. This behavior can be reasonably attributed to the presence of ascorbic acid in AA pouches, that involving a pH reduction, promote also a decrease in consistency parameter of samples (White et al., 2008; Ahouagi et al., 2021; López-de-Dicastillo et al., 2012). Moreover, after 14th day of storage as previous stated, because of ascorbic acid degradation, an increase of consistency values was highlight, not statistically different from control ones. It is important to underline as the pH reduction it is strictly dependent by temperature, being an inverse relationship between these two parameters (Reineke, Mathys, & Knorr, 2011), that so promoted a more acidification in activated samples stored at 45 °C respect to 25 °C ones. A reduction in water content induces a more aggregate structure, that involve an increase in the solid packed fraction (Agoda-Tandjawa, Dieudé-Fauvel, Girault and Baudez, 2013). It was not possible to analyse the consistency on samples stored at 45 °C beyond the 34th day of storage due to the excessively high values reached by this parameter. According with Alexieva et al., 2014, both samples stored at 25 °C showed very suitable values in relation to this parameter, ranging from 18 to 23 (N.s).

In Table 1 the lightness and hue angle values of pesto samples stored both at 25 and 45 °C are showed. All analysed samples showed a reduction in lightness and hue angle during storage time, or rather a gradual matrix browning, probably due to the degradation of the chlorophyll a and chlorophyll b both present in the basil used in the formulation (Severini et al., 2008). As expected, in pesto samples stored at 45 °C the colour parameters decrease was more drastic. Instead, samples packed in activated pouches, at 25 °C, only at the last days of storage, maintained a better colour retention in terms of lightness and hue angle parameters, than respective controls, probably because of the lower adopted storage temperatures.

3.2.3. Microbiological analysis

In Table 2 the cell loads of mesophilic aerobic bacteria, yeasts and aerobic spore forming bacteria, of samples stored at 25 are reported.

For both mesophilic aerobic bacteria and yeasts, an increase in cell load was observed during storage regardless to the sample. The initial cell load of mesophilic aerobic bacteria was 1.46 log CFU/g, after 14 days at 25 °C the microbial population increased up to 3.58 and 3.91 log CFU/g respectively for C and AA samples. At the end of storage (41 days), the mesophilic aerobic bacteria reached a load of 6.23 and 5.88 log CFU/g. A similar trend was observed for yeasts that starting from 1.30 log CFU/g reached a cell load of about 5 log CFU/g at the end of storage, independently to the considered sample. No significant differences between C and AA samples were observed for all microbial groups except total mesophilic bacteria after 8 and14 days storage. Aerobic spore forming bacteria increased their cell load during storage reaching 3.25 and 2.91 log CFU/g respectively in C and AA samples. On the contrary anaerobic spore forming bacteria resulted below the detection limit (1 log CFU/g) in all the samples for the whole period of storage.

In the case of samples stored at 45 °C, microbiological analyses have included the search for thermoduric microorganisms such as lactococci and staphylococci. Staphylococci resulted below the detection limit (1.0 log CFU/g) for the whole period of storage, independently to the sample,

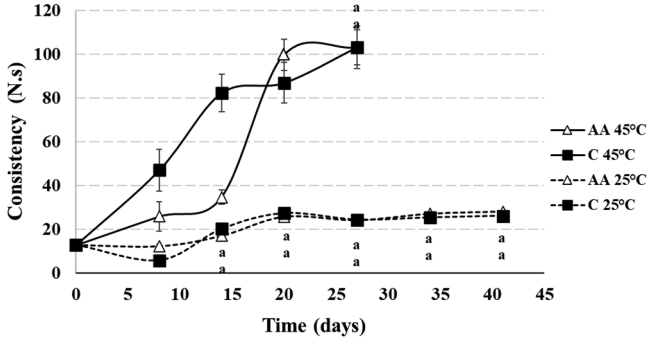

Fig. 6. Consistency values of Genovese pesto samples packed in activated and not pouches during storage at 25 °C and 45C°.
[a-b] Values followed by different letters differ significantly at p at $p<0.05$ level for each temperature

Table 1
Lightness and hue angle parameters of pesto packed in activated and not activated pouches during storage at both 25 and 45 °C.

	T0	T8	T14	T20	T27	T34	T41	
25 °C								
Lightness (L*)								
C	48.69 ±1.60[a]	40.92 ±1.72[a]	36.89 ±2.15[a]	34.99 ±1.90[a]	32.27 ±2.08[a]	26.23 ±0.94[b]	26.73 ±1.81[a]	
AA	48.69 ±1.60[a]	40.22 ±2.10[a]	37.39 ±1.20[a]	33.55 ±1.85[a]	32.81 ±1.19[a]	30.41 ±1.12[a]	32.42 ±0.94[b]	
Hue angle (h°)								
C	108.13 ±3.03[a]	101.35 ±3.22[a]	98.83 ±3.16[a]	94.56 ±4.05[a]	91.89 ±3.41[a]	88.76 ±3.12[a]	89.02 ±3.06[a]	
AA	108.13 ±3.03[a]	102.05 ±3.05[a]	97.96 ±3.34[a]	95.34 ±2.01[a]	94.76 ±2.98[a]	80.54 ±1.69[b]	80.61 ±1.17[b]	
45 °C								
Lightness (L*)								
C	48.69 ±1.60[a]	28.53 ±2.85[a]	19.00 ±1.09[a]	20.05 ±0.78[a]	18.30 ±2.05[a]	21.12 ±1.19[a]	–	
AA	48.69 ±1.60[a]	33.86 ±3.07[a]	20.51 ±1.87[a]	19.91 ±0.59[a]	19.27 ±1.34[a]	21.73 ±0.73[a]	–	
Hue angle (h°)								
C	108.13 ±3.03[a]	88.03 ±2.67[a]	53.01 ±2.72[b]	51.11 ±1.79[a]	37.82 ±3.98[a]	41.79 ±3.27[a]	–	
AA	108.13 ±3.03[a]	92.15 ±1.90[a]	66.35 ±2.98[a]	49.08 ±1.26[a]	44.33 ±3.13[a]	44.48 ±3.34[a]	–	

[a–b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature.

Table 2
Cell loads of mesophilic aerobic bacteria, yeasts and aerobic spore forming bacteria, of C and AA samples during storage at 25 °C.

	T0	T8	T14	T20	T27	T34	T41
	Total mesophilic aerobic bacteria log CFU/g						
C	1.46 ± 0.16[a]	1.81 ± 0.04[a]	3.58 ± 0.18[a]	4.10 ± 0.28[a]	5.54 ± 0.20[a]	6.23 ± 0.22[a]	7.18 ± 0.24[a]
AA	1.46 ± 0.16[a]	2.51 ± 0.06[b]	3.91 ± 0.05 [b]	3.50 ± 0.45[a]	5.11 ± 0.27[a]	5.88 ± 0.17[a]	7.01 ± 0.19[a]
	Yeasts log CFU/g						
	T0	T8	T14	T20	T27	T34	T41
C	1.30 ± 0.30[a]	1.55 ± 0.33[a]	1.88 ± 0.25[a]	3.64 ± 1.24[a]	4.38 ± 0.28[a]	4.89 ± 0.22[a]	5.28 ± 0.19[a]
AA	1.30 ± 0.30[a]	1.50 ± 0.50[a]	2.23 ± 0.41[a]	2.97± 0.26[a]	3.91± 0.34[a]	4.53± 0.20[a]	4.95± 0.27[a]
	Aerobic spore forming bacteria log CFU/g						
	T0	T8	T14	T20	T27	T34	T41
C	1.45 ± 0.02[a]	1.50 ± 0.14[a]	2.79 ± 0.26[a]	2.14 ± 0.16[a]	2.48 ± 0.29[a]	2.79 ± 0.31[a]	3.25 ± 0.23[a]
AA	1.45 ± 0.02[a]	1.66 ± 0.15[a]	2.66 ± 0.01[a]	2.05 ± 0.04[a]	2.56 ± 0.21[a]	2.58 ± 0.26[a]	2.91 ± 0.19[a]

[a–b] Values followed by different letters differ significantly at $P<0.05$ level for each temperature.

while lactococci did not exceed 2.0 log CFU/g regardless to the sample considered (data not shown).

According to FSA (2016), the spoilage threshold for total mesophilic aerobic bacteria is reported to be 6.0 log CFU/g. Control and activated samples exceed this limit respectively after 34 and 41 days. Activation of pouches with ascorbic acid did not lead to a significant increase in antimicrobial activity compared to control. On the other hand, the addition of ascorbic acid in active packaging is mainly aimed to prevent lipid oxidation (Yıldırım & Barutçu Mazı, 2017). Normally the antimicrobial activity of active films containing ascorbic acid is increased when additional compounds with high antimicrobial activity, such as nisin, are included (Popa et al., 2022). For example, Janani et al. (2022) showed that the incorporation of ascorbic acid and zinc oxide nanoparticles into nanocomposite films improved not only antioxidant activity, but also the antimicrobial activity against *S. enterica*, L. *monocytogenes, Y. enterocolitica, P. aeruginosa, E. coli* and *S. aureus*. However, the antimicrobial activity was attributed to zinc oxide nanoparticles in the films.

4. Conclusions

Obtained results showed the greater ability of the new biodegradable active packaging to reduce lipid oxidation kinetics of both sunflower oil and Genovese pesto samples during storage. These samples, in fact, exceeded the PV threshold of acceptability several days later than respective controls packed in not activated packaging. Activated pouches showed the best oxygen scavenger effect in samples stored at 25 °C compared to those stored at 45 °C, probably because ascorbic acid activity tends to decrease at high storage temperatures. Better textural properties, in terms of consistency parameter, were also found in pesto samples stored at 25 °C. Regarding the effect on microbial population, no significant effects compared to the control samples were observed. Obtained results highlighted the potentiality of the new multilayer activated biodegradable packaging to be applied successfully in food industry.

5. Ethical statements

The authors declare that the study does not involve animals and humans

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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