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Use of biodegradable materials as alternative packaging of typical Calabrian Provola cheese

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Abstract

Calabrian Provola cheese is typically manufactured in the Southern Italy. The request of a more suitable expansion in the national market has promoted this research, based on the evaluation of biodegradable packaging on its qualitative characteristics as alternative of the conventional plastic multilayer film. The tested materials were: Polyethylene/Ethylene vinyl alcohol/Polyamide/Polyethylene (PE/EVOH/PA/PE), Polylactic acid (PLA), coated with a silicon oxide barrier, and Cellophane, coated with resins. The results of this study evidenced that the material based on PLA can be considered a valid alternative packaging because of the quality maintenance of Calabrian Provola cheese and its sustainable characteristics.

Key words: Biodegradable materials, Calabrian Provola cheese, Cellophane, Packaging, Polylactic acid

1. Introduction

The dairy industry is the first food product sector in Italy. The cheese production has increased in the last decade and today it counts more than one millions of tons with a growth in the export, so much so one of three cheeses produced in Italy is sold abroad (Eurostat, 2015). About 48 Italian cheeses possess the Protected Denomination of Origin (PDO) and, in addition, the Calabrian Provola cheese has received the quality denomination of PAT (Prodotto Agroalimentare Tipico - Typical Food and Agricultural Product) by the Ministry of Agricultural, Food and Forestry Politics among other typical food products (Official Gazette of the Italian Republic, 2014). The Provola is a 'pasta filata cheese', generally cow's milk-based, characterized by a stretched curd, and a compact and smooth rind. Its colour varies from bright white to light yellow, influenced by the length of ripening. It owes the name to the Italian term "prova" which means "test", that is the amount of curd taken during manufacturing to control the stretching rate. The Provola cheese is generally waxed by microcrystalline wax after ripening, to preserve the product by moisture loss and external agents. Calabrian Provola cheese (CPc) is produced in the Calabria region (South Italy), and is traditionally from the Crotone's province. Local producers bond Provola cheese with a natural fibre string and sold it fresh or after few days by production. In particular pasta filata cheese needed to be packaged to limit the water evaporation and prevent oxidation reaction that which causes colour changes, off-flavour production and loss of nutrients. The textural food properties are linked to the breakdown of the proteins and in successive free amino acid release. Many studies evidenced the influence of packaging on the quality preservation of dairy products (Pintado & Malcata, 2000, Mortensen, Bertelsen, Mortensen, & Stapelfel, 2004; Di Marzo et al., 2006; Favati, Galgano, & Pace, 2007; Papaioannou, Chouliara, Karatapanis, Kontominas, & Savvaidis, 2007; Cakmakci, Gurses, & Gundogdu, 2011; Del Caro et al., 2012). A proper storage is fundamental to preserve the original nutrients and hygienic characteristics of food products, as well as their typicality. The transparent packaging is greatly demanded by consumers, to better appraise the cheese prior to purchase. The use of some plastic materials, as PET (Polyethylene terephthalate), PVC (Polyvinylchloride), PE (Polyethylene), PP (Polypropylene), PS (Polystyrene), PA (Polyamide) has been increased in food packaging, often combined in multilayer structures, because they are largely available, low-cost and show good physical properties, among which the heat sealability, the structural strength, the barrier to gas and volatile compounds. They are not totally recyclable or biodegradable, so their use has to be restricted (Siracusa, Rocculi, Romani, & Dalla Rosa, 2008). Different polymers are present in the market used principally as films or trays: some of these originate from petrochemical monomers, like some types of polyester, polyester amides and polyvinyl alcohol. The bio-based polymers are represented by starch materials, cellulose-based materials, polylactic acid (Polyester, PLA), polyhydroxy acid (polyester, PHA) and other ones. Cellophane is a cellulose-based biopolymer, obtained from natural resources like cotton, wood, wheat and corn. The cellophane is composed by regenerated cellulose, a softening agent, and water as plasticizer. It offers a good gas barrier when kept dry and further surface (friction, antistatic and antifog) properties can be showed when it coated. Despite some positive properties of cellophane (heat resistance, strength, clarity, and barrier to gas), this bio-based material shows a limited shelf life for a loss of volatile plasticizer that make brittle it and reducing the shelf life of food contained in (Yam, 2010). The PLA is produced by depolymerization of the monomer of lactic acid deriving from fermented and biodegradable substances of different origin (Cabedo, Luis Feijoo, Pilar Villanueva, Lagarón, & Giménez, 2006). Literature reports that the choice of PLA as 'green' packaging material is growing because it often shows a better performance than the conventional plastic materials, for example the oriented polystyrene and polyethylentherephtalate (Auras, Singh, & Singh, 2005). Moreover, PLA is recyclable and compostable, shows good optical properties, processability and water solubility resistance. Several studies proved the PLA to be suitable for packaging of some cheeses in modified atmosphere (Ahmed &Varshney, 2011; Holm, Mortensen, & Risbo, 2006; Dukalska et al., 2011; Plackett et al., 2006; Dmytrów, Szczepanik, Kryża, Mituniewicz-Małek, & Lisiecki (2011). The use of biopolymers shows some critical issues, regarding the cost, the performance and the processing. Concerning these last two, the problems are more evident in materials derived from bio-mass, such as cellulose or starch (Petersen et al., 1999).

In a previous study, Piscopo, Zappia, De Bruno, & Poiana (2015) investigated the improvement of quality of CPc, under different types of packaging: vacuum and modified atmosphere in two forms: thermoformed tray and pouch. Results suggested that the packaging in pouch with MAP (70:30 N_2:CO_2), using high barrier material (Polyethylene/Ethylene vinyl alcohol/Polyamide/Polyethylene) was the best solution to preserve these dairy products. Based on these results, in this work the use of biodegradable materials with different permeabilities to gas and water vapour was studied as an alternative to the high barrier petrochemical-based material currently used for the preservation of the quality of Provola cheese produced in Calabria.

2. Materials and methods

2.1 Experimental procedure

Fresh Calabrian Provola cheeses (CPc) were produced in Cimino & Ioppoli s.r.l. company located in Crotone (Italy) using the procedures and showing the characteristics described by Piscopo et al. (2015). Twenty-four hours after cheesemaking, the cheeses (mean weight of 0.5 kg) were packed in pouches (20 x 28 cm^2) of three different packaging materials, namely: one multilayer co-extruded film (A) and two bio-based polymers (B and C). The sealing of pouches was performed by a Tecnovac S100 DGT chamber machine (Bergamo, Italy) in modified atmosphere constituted of 70% N_2 and 30% CO_2. The film A was a coextruded multilayers of Polyethylene/Ethylene vinyl alcohol/Polyamide/Polyethylene (PE/EVOH/PA/PE), 70 μm (Krehalon, USA), the film B consisted of PLA, coated with a silicon oxide barrier (Ceramis Amcor, Italy) 42 μm, and the material C was a cellophane film coated with a proprietary barrier and sealable layer (NVS coated Natureflex™, Carton pack, Italy), 28 μm.

Qualitative changes were monitored up to 65 days (beyond the commercial expiry date of 45 days without packaging) on cheeses stored in refrigerated incubators (mod. Foc 225 I Velp Scientifica, Italy) at two different temperatures: 4 °C as optimal temperature for cheese storage and 7 °C as the temperature to which food are generally stored in exhibiting refrigerators of supermarkets.

2.2 Standards and reagents

All chemicals were of analytical reagent grade. Methanol, chloroform, methylene chloride were of HPLC grade from Carlo Erba reagents (Italy). Pure standards of triglicerides (trimyristin, tripalmitin, triolein), diglicerides (dimyristin, dipalmitin, diolein) and monoglicerides (monomyristin, monopalmitin, monolein) were obtained by Sigma-Aldrich Chemicals Co. (S. Louis, Missouri).

2.3 Gas and water vapour permeability measurements

The oxygen transmission rate (OTR), carbon dioxide transmission rate (CO_2TR) and water vapour transmission rate (WVTR) of packaging materials were performed in accordance with ASTM D3985 for oxygen, ASTM F2476 for carbon dioxide and ASTM F1249 for water vapour by an iso-static permeabilimeter at 23 °C and different values of relative humidity (mod. MultiPerm, ExtraSolution®, TotalPerm, Italy).

2.4 Physical, microbiological, and chemical analyses of Calabrian Provola cheeses

The gas composition inside the packaging, expressed as O_2 and CO_2 percentages, was detected by a gas analyser (CheckPoint; PBI Dansensor, Ringstedt, Denmark). The volume taken from the package headspace was of 15 cm^3. The measurement was replicated in three packages for each sample.

The Total Bacterial Count (TBC) was performed by inoculation at 25 °C for 48 h of diluted minced cheeses in Plate Count Agar (PCA) medium (Oxoid, Milan, Italy) according to ISO 4833:2003 method and expressed as colony forming units (cfu)/g of CPc. Analysis was made in three replicates.

The colour coordinates of the CIELAB space (L*, a* and b*) were randomly monitored in five points of the CPc surface by a tristimulus colorimeter (Konica Minolta CM-700d, Osaka, Japan) referred to the D65 illuminant. Measurements were performed in three replicates. Total colour difference (ΔE)

in surface and inner layer of Provola cheeses before the packaging and after 65 days of storage was obtained by the following formula (Thompson, 2004):

$$CD = \sqrt{((L^*-L^*_0)^2 + (a^*-a^*_0)^2 + (b^*-b^*_0)^2}$$

where L^*_0, a^*_0, and b^*_0 are the initial values, obtained before packaging.

Moisture (%), pH and total acidity of cheeses were calculated according to AOAC methods (AOAC, 1980a; 1980b; 1990); water activity (a_w) of minced Provola cheeses was measured by Aqualab LITE hygrometer (Decagon devices Inc., Washington USA). The Peroxide values were quantified according European Union Commission (1991) in the cheese lipids, previously extracted following the method reported by Folch, Lees, & Sloane-Stanley (1957).

For the mono-, di- and tri-glycerides content analysis, the samples were obtained according to Gomes & Caponio (1999) and injected (20 µL) into a HPLC (mod. Smartline Pump 1000 Knauer, Berlin, Germany), provided of RI Detector 2300 (Knauer). Two-column series PLgel 5 µm (column length 300 mm, ID 7.5 mm, 100 Å) (Polymer Laboratories (United Kingdom) fitted with a guard column PLgel 5 µm (column length 50 mm, ID 7.5 mm) (Polymer Laboratories (United Kingdom) were used. The flow rate of solvent was 1 mL min^{-1} and analysis was carried out at ambient temperature. The used mobile phase was CH_2Cl_2. Pure standards were used to identify the compounds which were after quantified by derivative areas (%). The results were also elaborated to achieve the Lipolysis Index (LI) by the following formula:

$$LI\ \% = FFA/L * 100$$

where FFA: Free Fatty acids (g/100 g) and L: Lipid content (g/100 g).

All the reported analyses were performed in triplicate taking samples from the six cheeses.

For textural analysis of CPc authors followed the method, equipment and instrumental parameters reported in a previous published work on Provola cheeses (Piscopo, Zappia, De Bruno, & Poiana, 2015). The Total Nitrogen (TN) of samples was quantified as described by Lynch & Barbano (2002). For the Water Soluble Nitrogen (WSN) Kjeldahl's method was applied, as reported by Christensen, Bech, & Werner (1991).

The Proteolysis Index (PI) % was calculated following the formula:

PI % = WSN/TN *100

where WSN: Water Soluble Nitrogen (%) and TN: Total Nitrogen content (%)

2.3 Statistical analysis

The data statistical elaboration was performed by One-way and multivariate analysis of variance by using of SPSS software (Version 15.0, SPSS Inc., Chicago, IL, USA). Tukey Post-hoc test was conducted to evidence significant differences among samples.

3. Results and discussion

3.1 Gas and vapour transmission rates of the packaging materials

Comparisons of tested biodegradable polymers, B and C, and conventional plastic material, A, revealed that OTR measured at 23 °C and 50% RH of A is three and five times lower than B and C respectively. These results are related to the presence in the film A of EVOH, one of the lowest oxygen permeability reported among polymers commonly used in packaging. It provided better barrier properties than those observed in PLA with SiOx and in cellophane coated with resins. Moreover, a progressive higher oxygen permeability was observed in A and C materials with the increasing of relative humidity: it can be explained by the EVOH and cellophane high-sensitivity to moisture, as illustrated in literature (Zhang, Britt, & Tung, 2001; Muramatsu et al., 2003; Hernandez & Giacin, 1998, Del Nobile, Buonocore, Dainelli, Battaglia, & Nicolais, 2002). The CO_2TR of A film was one and two order of magnitude lower than B and C respectively, confirming that the presence of EVOH gives a good CO_2 barrier (Maes et al., 2017). Despite the molecular diameter of carbon dioxide is bigger than the molecule of oxygen, the CO_2TR through the PLA polymer was very higher than the OTR, as also observed by Siracusa, Dalla Rosa, & Iordanskii (2017). This is due to the different behaviour of CO_2 in terms of solubility and diffusion through the polymeric chain. Regarding the water vapour, the highest barrier was opposed by PLA film (0.8 g/m^2 24h), similar to the result for A film (1.3 g/m^2 24h), whereas cellophane film manifested the greatest WVTR, despite the presence of resins in its surface.

3.2 Microbiological, physical and chemical analyses of Calabrian Provola cheeses

Confirming the diffusional properties of materials reported in Table 1, the O_2 percentages of the atmosphere inside the biodegradable packaging (B and C) were higher with respect those measured in plastic packages (A) from the initial time of storage to 35 days (Fig.1). After this period, the same concentration of O_2 was observed in all pouches.

Perceptual increases of CO_2 were observed in packaging A and B. Concerning the first packaging, the increase of CO_2 was due to the highest barrier properties of the EVOH layer that involved a higher CO_2 retention inside the internal atmosphere respect to the B samples. The trend of CO_2 during storage in B pouches resulted from both the permeability of film and the active microbial metabolism, as evidenced in total microbial counts at both temperatures (Figure 2). The total microbial growth did not produce an increase of CO_2 percentage in the internal atmosphere of C samples because of the greatest transmission rate of the cellophane material. That physical characteristic of cellophane film involved a leaving of CO_2 outside the package, naturally formed during cheese storage.

The multivariate analysis of data evidenced a strong effect of packaging type ($p=0.000$), and no influence ($P>0.05$) by the storage temperature and times in that qualitative parameter ($p = 0.078$ and $p= 0.206$).

The physical and chemical parameters possessed by CPc after 65 days of storage are reported in Table 2. Regarding the ΔE measured in samples from the initial time and after 65 days of storage at both temperatures, just noticeable values greater than 2 were often observed with the only exception of the core of A sample. Similar values were evidenced in A and B, and the C sample manifested the highest colour differences in both layers (about 30 in the surface and 15 in the core) probably due to the submitted dehydration at the end of storage, as confirmed by the lowest values of a_w and moisture reported in the same table. From the initial percentage of 48.52±1.75, the moisture content remained constant in A and B samples ($p = 0.061$ and $p= 0.052$) during the monitoring times, whereas it decreased in C sample, in particular after 21 days at 7 °C and after 35 days at 4 °C (data not shown), due to the higher WVTR of the cellophane material. The pH value of cheeses before the storage (5.86±0.02) decreased with significance ($p<0.01$) during the storage in all samples. The total acidity of CPc was 0.30 ± 0.01g of lactic acid/100 g of dry matter after manufacturing; in particular, the total acidity of Provola cheeses packaged in PLA increased after 65 days of storage at both temperatures (0.53±0.05 g of lactic acid/100 g of dry matter). The Peroxide value measured in CPc after manufacturing was of 1.90±0.50 mEq O_2/kg. This parameter after 65 days significantly varied on the samples, in particular the highest number was observed in A sample stored at 4 °C, whereas at 7 °C it was observed a higher amount in the C sample. Positive Pearson's correlation coefficients resulted for cheese PV and O_2 % in the packages after 65 days at both temperatures ($r = 0.920$ $P<0.01$; $r = 0.910$ $P<0.01$).

Textural analysis showed a higher hardness in sample packaged in cellophane, with the following order at both temperatures: C>B>A (Table 2). The increase of the penetration force on the sample was due to a variation of moisture, as reported by several studies (Delgado, Gonzàles-Crespo, Cava, Ramìrez, 2011; Dmytròw, Mituniewicz-Małek, Dmytròw, & Antonowicz, 2009; Bonczar & Walczycka, 2001). Positive correlations were in fact evidenced by the Pearson correlation coefficients between hardness and dry matter in A ($r = 0.665$ $P<0.05$), B ($r = 0.854$ $P<0.05$), and C ($r = 0.656$ $P<0.05$). The expected consistence of CPc by a habitual consumer is certainly soft and not excessively hard, as other "pasta filata" cheeses that are subjected to a ripening. So, the results of textural analysis evidenced no positive characteristics in C sample, that is the Provola packaged in cellophane film at both storage temperatures.

The influence on physical and chemical parameters of the variables 'Packaging' and 'Temperature' obtained by Two ways-ANOVA are illustrated in Table 3. Packaging materials influenced the various qualitative parameters more than the applied storage temperatures which affected only a_w, pH, and peroxide values.

The Figure 3 reports the lipolysis and proteolysis indices in CPc during the storage: they were obtained by elaboration of the mean data for both storage temperatures because no significant differences were observed. The LI% is determined by the analysis of mono-, di- and tri-glycerides content. In cheeses packaged in biodegradable materials (B and C) their measured content did not vary during the storage from 0 to 65 days at both temperatures (data not shown), so in graph a) the LI% of B and C samples remained constant at the three monitoring times (about 8%). An increase of diglycerides and free fatty acids was instead observed in A samples during the storage, with higher values respect those observed in literature for higher aging-cheeses (Malacarne, Formaggione, Franceschi, Summer, & Mariani, 2006) and it is reported in graph a) as the highest observed lipolysis index (26%). The A sample packaged in conventional plastic material, manifested the highest proteolysis index at the end of storage and at both temperatures (26-27%) (Figure 4, graph b). This is probably correlated to their higher a_w respect the other cheeses that promoted the extension of reaction. Texture analysis of cheeses also confirmed that A sample showed after 65 days of storage the lowest force in Hardness measurement (3.48 N at 4 °C and 4.01 N at 7 °C), expression of proteolysis progress. The cheeses packaged in biodegradable materials (B and C samples) manifested instead the lowest percentage of proteolysis at the end of storage, ranging from 16 to 18%). In particular, the cheese packaged in Cellophane denoted a negative correlation between proteolysis index and dry matter percentage, expressed by Pearson coefficient ($r = -0.992$ $P < 0.01$).

4. Conclusions

Considering all experimentally obtained results, it can be affirmed that the packaging based on PLA film in modified atmosphere can be suggested for Calabrian Provola cheese. Specifically, its shelf life could be prolonged to 65 days with some characteristic comparable to the multilayer film (colour difference during the storage and moisture). That material can be also considered a valid alternative to the conventional plastic one because of a good performance in the Provola cheese storage, in particular for the observed lower proteolysis and lipolysis indexes, and peroxide values. The tested Cellophane film was not useful for this purpose because of the final physical and microbial parameters measured on cheeses. This type of local dairy product can be thus packaged in biodegradable film with barrier properties similar to those reported in this paper without losing its specific and safety characteristics, and to gain a higher added value for the sustainability of its manufacturing.

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Table 1 Physical characteristics of materials used for packaging of Provola cheeses

Packaging[§]	Thickness (µm)	Total Composition and layer ratio	Layer number	OTR (cc/m^2 day)	CO_2TR (cc/m^2 day)	WVTR (g/m^2 day)
A	70±4	PE/EVOH/PE/PA 5/1/2/1	4	2.3 (23°C RH 50%) 3.3 (23°C RH 75%) 3.6 (23°C RH 90%)	9.5 (23°C RH 65%)	1.3 (23°C RH 65%)
B	42±2	PLA-SiOx/PLA 1/1	2	6.8 (23°C RH 50%) 8.3 (23°C RH 75%) 6.3 (23°C RH 90%)	95 (23°C RH 65%)	0.8 (23°C RH 65%)
C	28	CP 1/1	2	11.5 (23°C RH 50%) 52.3 (23°C RH 75%) 48.3 (23°C RH 90%)	646.1 (23°C RH 65%)	30.8 (23°C RH 65%)

[§]A: multilayer co-extruded film (PE/EVOH/PA/PE); B: biobased film (PLA, coated with a silicon oxide barrier); C: biobased film (cellophane coated with resins)

Table 2 Physical and chemical parameters of Calabrian Provola cheeses after 65 days of storage

Temperature (°C)		4 °C				7 °C			
Samples[§]		A	B	C	Sign	A	B	C	Sign.
ΔE	surface	18.83±1.44b	18.28±1.52b	30.61±2.03a	*	18.30±1.02b	18.81±1.72b	32.04±2.85a	*
	core	1.96±0.36c	11.18±1.62b	15.32±2.06a	**	2.64±0.77c	13.09±2.54b	15.75±1.11a	**
a_w		0.975±0.002a	0.969±0.000b	0.943±0.002c	**	0.975±0.000a	0.964±0.001b	0.943±0.000c	**
Moisture (%)		47.22±1.40a	49.22±0.19a	38.36±8.57b	**	47.41±0.97a	48.60±0.37a	35.04±8.42b	**
pH		5.50±0.03c	5.70±0.04a	5.60±0.02b	**	5.51±0.06b	5.72±0.05a	5.57±0.21ab	*
Total acidity (g % lactic acid/d.m.)		0.47±0.03ab	0.53±0.05a	0.43±0.12b	*	0.51±0.01a	0.53±0.02a	0.40±0.11b	**
Peroxide value (mEq O_2/Kg)		6.22±0.12a	2.08±0.12c	2.66±0.09b	**.	3.86±0.09b	2.02±0.06c	6.16±0.22a	**
Hardness (N)		3.48±0.15c	8.94±0.24b	12.45±1.19a	**	4.01±0.27c	9.02±1.69b	12.42±0.82a	**

[§] For A, B and C see Table 1. Values are Means ± Standard Deviation (n=15 for ΔE; n=3 for a_w, moisture, pH, total acidity and peroxide value; n=9 for Hardness.) *Significance at $P<0.05$;**Significance at $P<0.01$. Data followed by different letters are significantly different by Tukey's multiple range test.

Table 3. Influence of packaging and storage temperature on physical and chemical parameters of Calabrian Provola cheeses

	Moisture		pH	Total acidity	Peroxide value	Hardness
Packaging (P)	**	**	**	**	**	**
Temperature (T)	n.s.	*	**	n.s.	**	n.s.
P x T	*	*	*	n.s.	**	n.s.

n.s., not significant; * Significance at $P < 0.05$; ** Significance at $P < 0.01$.

Fig. 1 Gas composition of different Provola cheeses samples during the storage at 4 and 7 °C (A: PE/EVOH/PA/PE; B: PLA-SiOx, C: Cellophane coated with resins)

Fig. 2 Total microbial count in Provola cheeses stored with different packaging (A: PE/EVOH/PA/PE; B: PLA-SiOx, C: cellophane coated with resins)

Fig. 3 Hydrolysis of principal components on Provola cheeses stored with different packaging: (A: PE/EVOH/PA/PE; B: PLA-SiOx, C: cellophane coated with resins)- a) Lipolysis index % and b) Proteolysis index %. Different letters denoted significant differences by Tukey's multiple range test ($P<0.05$).

Fig. 1

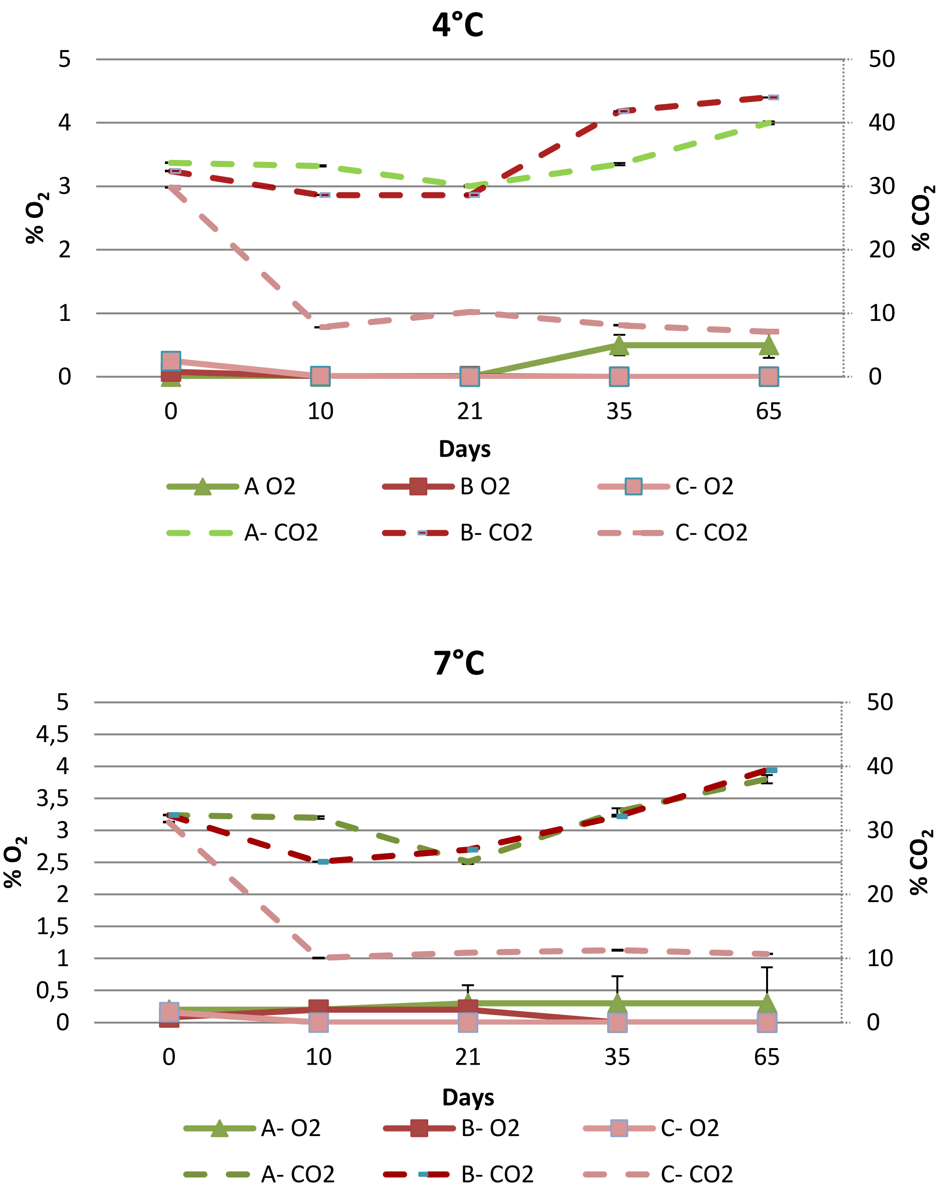

4°C
% O2
% CO2
Days
A O2
B O2
C- O2
A- CO2
B- CO2
C- CO2
7°C
% O2
% CO2
Days
A- O2
B- O2
C- O2
A- CO2
B- CO2
C- CO2

Fig. 2

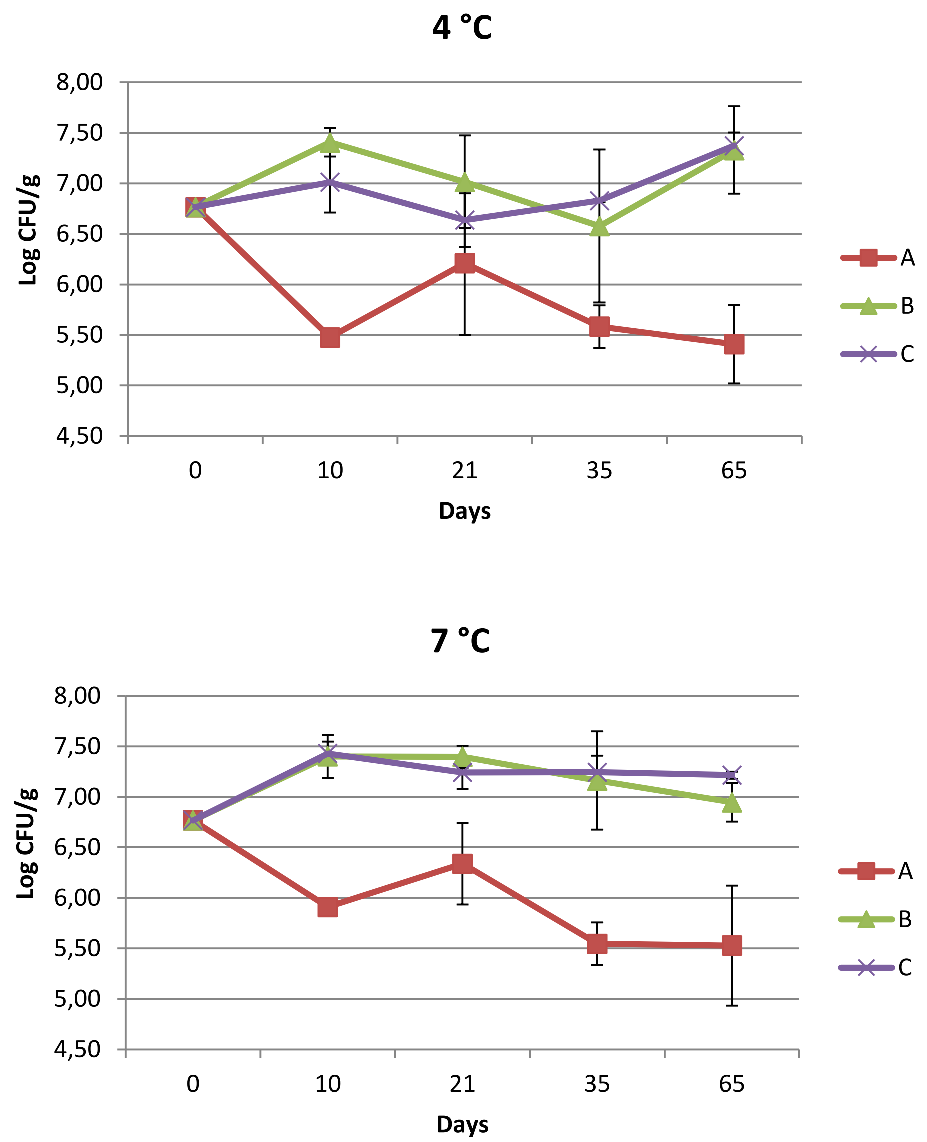
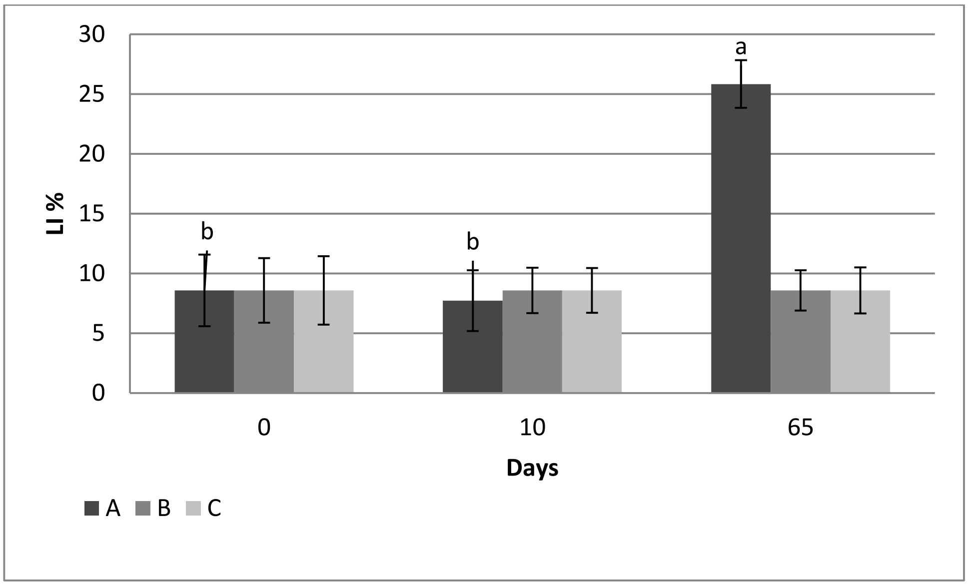
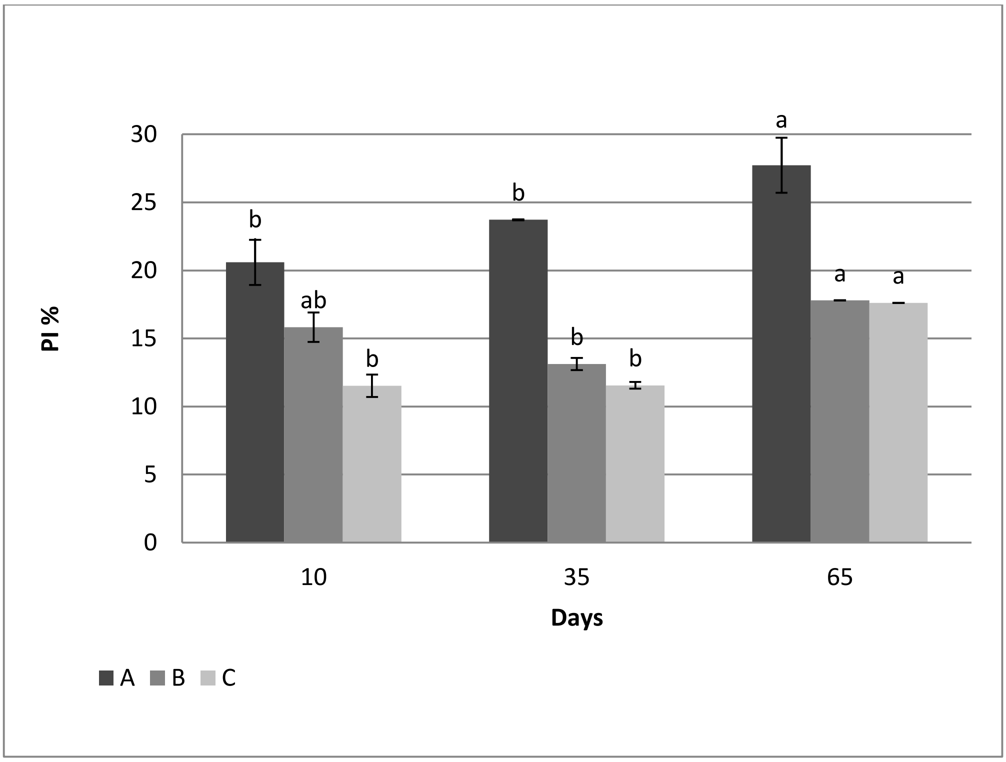

4 °C
8,00
7,50
7,00
6,50
6,00
5,50
5,00
4,50
Log CFU/g
0
10
21
35
65
Days
A
B
C
7 °C
8,00
7,50
7,00
6,50
6,00
5,50
5,00
4,50
Log CFU/g
0
10
21
35
65
Days
A
B
C

Fig. 3

a)


30
25
20
15
10
5
0
LI %
a
b
b
0
10
65
Days
A B C

b)


30
25
20
15
10
5
0
PI %
b
ab
b
b
b
b
a
a
a
10
35
65
Days
A B C