

# **Antimicrobial, sealable and biodegradable packaging to maintain the quality of shredded carrots and pineapple juice during storage**

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Sincerely,

Pr. Fabienne Remize

## la science pour la vie, l'humain, la terre

UMR (+ nom de l'UMR) Code postal et ville Tél. : 00 00 00 00 00







# Antimicrobial, sealable and biodegradable packaging to maintain the quality of shredded carrots and pineapple juice during storage

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## 14 Abstract

2 15 Increasing consumer demand for foods with high nutritional quality, prolonged shelf life and 31 40 32 **Key-words** 35 36 Abbreviations 49 52 37 TA: Titratable Acidity; PS: Potassium Sorbate; SB: Sodium Benzoate; PLA: Poly(Lactic)  $\frac{54}{2}$  38 Acid; PBAT: Poly(Butylene Adipate-co-Terephthalate); TR: TRansparency; TPC: Total Plate  $\frac{56}{57}$  39 Counts; YMC: Yeast and Mold Counts; EC: Enterobacterium Counts 1  $36\,$  30  $\,$  or minimum processed roods.  $37 \quad 21$  $38 \t31$ 39 41  $45$   $\phantom{0}$   $46\degree$  $47 \quad 33$ 48 50 51 53 55 (a) 55  $57$   $57$  counts, rmc. reasonate more c 58 59 60 61 62 63

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65

 $\frac{4}{5}$  16 low environmental impact of the package, is driving innovation towards the development of  $\frac{6}{7}$  17 new packaging. Multifunctional food packaging films, biodegradable, heat-sealable and  $\frac{8}{9}$   $\,$  18  $\,$  antimicrobial, were developed. A PLA coating layer incorporating either sodium benzoate, 19 potassium sorbate, or a combination of them was deposited onto a poly(lactic)  $13$   $20$  acid/poly(butylene adipate-co-terephthalate) substrate film. The effectiveness of the  $\frac{15}{16}$  21 developed systems to preserve the quality of foods was tested in shelf-life experiments  $\frac{17}{18}$   $\,$  22  $\,$   $\,$  performed on shredded carrots and pineapple juice, selected as model processed raw foods. 23 The best performance was observed for the active film containing potassium sorbate: 22 24 microbial populations increased less rapidly and were 0.7 to 1.8 log CFU/g lower at the end  $^{24}_{25}$  25 of storage period in this film than in control packs. Of the two model foods, the pineapple  $\frac{26}{27}$   $\,$  26  $\,$  juice was better preserved: after 7 days in active packaging, color change and microbial 27 counts of juice were below that of control, observed after one day and after 3 days of storage 28 31 28 respectively. Moreover, the incorporation of the active phases did not significantly affect the  $33 - 29$  mechanical, barrier and optical properties of the films, opening new ways to prolong shelf-life  $\frac{35}{36}$  30 of minimally processed foods. 3  $5 \qquad \qquad \blacksquare$  $7<sup>17</sup>$  Tiew packaging. Manifesterial T  $9$  18 anumicropial, were developed.  $\epsilon$ 10 11 19 potassium sorbate, or a combina 12 14  $16$  21 acrosspea systems to processe  $18$   $22$  performed on sine due callots. 19 and the contract of the con 20 23 The best performance was obse 21 23 25 **and 12 and 12 a**  $27 - 20$  July was belief preserved. after  $29$  27  $\,$  counts of juice were below that  $\,$ 30 32 34

<sup>42</sup> 33 Active packaging; biodegradable films; preservative; shelf-life; pineapple; carrot; heat- $\frac{44}{45}$  34 sealability 43 and the contract of the con

### 40 Introduction

2 41 Packaged minimally processed fruit and vegetables are of growing popularity for their high  $\frac{4}{5}$  42 nutritional value and convenience. However, these foods are highly susceptible to microbial  $\frac{6}{7}$  43 growth, which represent the main cause of their spoilage (Ragaert et al., 2007). In fact,  $\frac{8}{9}$   $\,$  44  $\,$  minimal processing operations, such as cutting, shredding, juicing, lead to the disruption of 45 subcellular compartmentalization and the release of cellular nutrients, which promote the  $13\quad 46\quad$  growth of microorganisms (Klaiber et al., 2005) and cause undesirable biochemical and  $^{15}_{16}$   $\,$  47  $\,$  physiological changes. These changes decrease the safety and the nutritional and sensory  $\frac{17}{18}$   $\,$  48  $\,$  quality and thus, shorten food shelf life (Wang et al., 2015). 1 3  $5<sub>1</sub>$  $7 - 7$  grown, which represent the main  $9\frac{44}{9}$  minimal processing operations, 10 11 45 subcellular compartmentalization 12 14  $16$   $\ldots$  priyolological original mode of  $18$  40 yudiity driu trius, silorien lood si

49 Among the minimally processed fruit and vegetables, shredded carrots and pineapple juice  $22$   $50$  are among the most widespread and consumed for their sensory and nutritional properties  $^{24}_{\circ}$  51  $\;$  (Klaiber et al., 2005; Leneveu-Jenvrin et al., 2020). Typically, the shelf-life of fresh pineapple  $\frac{26}{27}$  52  $\;\;$  juice or shredded carrots stored at 4°C is comprised between three and eight days (Leneveu-<sup>28</sup> 53 Jenvrin et al., 2020; Mahendran, 2015; Piscopo et al., 2019). The decrease in quality of fresh 54 pineapple juice is observed particularly through yeast and mold population increase and 31  $\frac{33}{2}$  55 modification of color towards browning, leading to sensory descriptors of "fermented"  $\frac{35}{36}$  56 (Leneveu-Jenvrin et al., 2020). On the contrary, quality decrease of shredded carrots during  $\frac{37}{38}$   $\,$  57  $\,$  storage is mainly due to gram-negative bacteria development, especially Pseudomonaceae, 58 and surface color change (Klaiber et al., 2005; Xylia et al., 2018). Hence, the limitation of 40  $\frac{42}{12}$  59 quality loss in the two products has different microbial targets, fungi for pineapple juice and  $^{44}_{45}$  60 bacteria for shredded carrots. 20 49 Among the minimally processed 21 23 25 and the contract of the con  $27 \,$   $22 \,$  Julye VI shill club value studied  $29$   $33$  Jenvrin et al., 2020; Mahendran 30 32 34  $36 \times 10^{10}$  (Leneved behand of all, 2020).  $38$   $\sqrt{3}$  storage is mainly que to gram-no 39 41 43

 $\frac{46}{47}$   $\,$  61  $\,$  The feasibility of a single treatment to limit microbial growth in these two foods requires 62 investigations. Lowering temperature is the most potent way to decrease the microbial 49  $51$  63 growth rate, but it cannot be enough. The addition of antimicrobial agents is another  $^{53}_{5.6}$  64 approach to limit the growth of microorganisms (Durango et al., 2006). Among the  $^{55}_{56}$   $\,$  65  $\,$  antimicrobials, sorbates and benzoates hold the major share of the market (Kuplennik et al.,  $\,$  ${\frac{57}{58}}$   ${\frac{66}{2015}}$ . Potassium sorbate (PS) is the salt of sorbic acid. It is classified as a food additive 67 (E202) according to EC 1333/2008 and inhibits yeasts and molds and some bacteria, at pH 60  $47$  of the reasibility of a single treatm 48 50 52  $54$  $56<sup>0</sup>$  dritting obtains, solidates and belo  $58$  66 2015). Potassium sorbate (PS) i 59 61

lower than 6.5 (Davidson et al., 2005). Sodium benzoate (SB) is the salt of benzoic acid: it 69 has been classified as a food additive (E211) in EC 1333/2008 and inhibit yeasts, molds and  $^{4}$  70 bacteria at pH lower than 4-4.5 (Musyoka et al., 2018). Synergistic effects between these  $\frac{6}{7}$   $\,$  71  $\,$  compounds have also been reported (Stanojevic et al., 2009). Usually, these antimicrobial  $\frac{8}{9}$   $\,$  72  $\,$  agents are directly added into the food. However, the incorporation of the antimicrobials into a package could be more efficient than their direct addition into the food, because they may  $74$  gradually migrate from the package onto the food surface. Release rate of antimicrobials  $\frac{15}{16}$  75 depends on temperature, pH, and on the food surface for diffusion, as reported by many  $\frac{17}{18}$   $\,76$   $\,$  authors on several foods and packaging systems (Glicerina et al., 2021; T. Z. Jin, 2017; Uz & Altınkaya, 2011; Vasile & Baican, 2021). Pineapple juice and shredded carrots differ by their  $22\quad 78$  liquid or solid nature, which modifies their interaction with packaging film. For that reason and  $^{24}$  79 because of different microbial targets to inhibit, these two minimally-processed products were  $\frac{26}{27}$  80 chosen as model foods for this study.  $5 \quad \overline{\phantom{1}}$  $7 \t 1$  compounds have also been repr  $\beta$  /2 agents are directly added into the  $11 \quad 73$  a package could be more efficie  $\sim$   $\sim$  appends on temperature, pri, and  $10$  authors on several loods and pa 20 77 Altinkaya, 2011; Vasile & Baicar 25 and 25 and 25 and 26 and 26 and 26 and 25 and 25 and 26 an  $80$  chosen as modernoods for this a

 $\frac{28}{29}$   $\,$   $81$   $\,$   $\,$  Different authors reported that the incorporation of PS or SB or their combination at 82 concentration ranging from 10 to 15 wt% into biodegradable and non-biodegradable  $\frac{33}{2}$   $\,$  83  $\,$  polymeric systems was effective to delay the growth of microorganisms (Careli-Gondim et  $\frac{35}{36}$  84 al., 2020; T. Jin et al., 2010; Shen et al., 2010). However, most of the existing investigations  $\frac{37}{38}$   $\,$  85  $\,$  focused on the antimicrobial activity of the films *in vitro*, while a few studies are available on 40 86 *in vivo* tests that demonstrated the effectiveness of these biodegradable systems on real  $\frac{42}{3}$  87 food such as fresh noodles, strawberry puree, berries and avocado (Careli-Gondim et al.,  $^{4.4}_{4.5}$   $\,$   $88$   $\,$   $\,$  2020; T. Jin et al., 2010; Junqueira-Gonçalves et al., 2016; Wangprasertkul et al., 2021).  $\frac{46}{47}$   $\,$   $89$   $\,$  In this work, we intended to develop innovative, multifunctional and environmentally friendly 90 food packaging films able to prolong the shelf-life of minimally processed fruits and 51 91 vegetables. 81 Different authors reported that the or all, 2020, i. on or all, 2010, One  $\delta$   $\delta$  rocused on the antimicropial act  $\frac{60}{2020}$ , i. on ot an, 2010, our que  $89$  in this work, we interided to devi 

 $\frac{53}{5}$  92 To this aim, biodegradable systems consisting of a PLA/PBAT substrate film, coated with an  $^{55}_{56}$   $~93$  amorphous PLA layer incorporating either PS, SB or a combination of them, were prepared.  $_{56}$   $\sigma$  anorphous FLA layer incorporal **a.** 2007 - 2008 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009 - 2009

 $58\quad 94\quad$  The coating layer constituents were selected to ensure a biodegradable film structure,

combined with heat-sealing ability and antimicrobial activity. The ability to increase food

96 shelf-life and the technical suitability of the developed films were determined.  $\frac{1}{2}$  96 shelf-life and the technical suital<br> $\frac{3}{4}$  97 2 96 shelf-life and the technical suital

### 98 Materials and Methods

2 99 Fruit and vegetable sampling and processing

 $^{4}$  100 Carrots (Daucus carota cv. Maestro) and pineapples (Ananas comosus cv. Queen Victoria)  $\frac{6}{7}$  101 were collected from local markets in Reunion island (France). They were transferred to the  $\frac{8}{9}$  102  $-$  laboratory and stored at 10°C until processing, within 24h. All the equipment used was 103 previously rinsed with sodium hypochlorite solution.  $5 \quad \overline{\phantom{1}}$  $7<sup>101</sup>$  were concerted from local market  $9\,$  TV $2\,$  Taboratory and stored at TV  $\,$  U dr 10  $11\,103$  previously rinsed with sodium hy 12

 $13\,104$  Carrots were rinsed into chlorinated water 200 ppm for 3 min, washed with distilled water and  $^{15}_{16}$  105  $\phantom{1}$  then allowed to dry naturally. Then, they were subjected to shredding and put into a tray.  $\frac{17}{18}$   $106$   $\;$  Fresh lemon juice (100 ± 20 mL for 1 kg of carrots) was added in order to obtain a pH equal to  $4.$ 14  $16$   $100$  and allowed to any maturally. The  $18$  TVO FIESH IEIHOH JUICE (TVO ± ZU IIIL  $19 \quad 19$ 

 $22\,108$  Pineapple fruit at a similar stage of ripeness were manually peeled and cut and prepared into  $^{24}_{2}$  109 juice (Extractor Wismer EW-01, CAPAVENIR, France). 23 25 and  $\sum_{i=1}^{n}$ 

Active films  $26_{110}$  Active films  $27 \, \text{110}$  Active  $\text{min}$ 

#### 111 Film composition and production 28 29 III Film composition and pro

31 112 Active films were developed through a coating technique. The support surface used for the  $^{33}_{11}$  113 coatings was a commercial biodegradable film named as Biopolymer (Euromaster, Italy).  $\frac{35}{36}$  114 This film is made by a blend of PLA and PBAT, has a medium thickness of 22.0 ± 1.0 µm, an  $_{38}^{37}$   $115$   $\rm\,$  \, oxygen and water vapor permeability of 5.15 ± 0.35 (cm $^3$ ×cm)/(m $^2$ ×d×bar) and 9.19 ± 0.37 40 116 (g×m)/(m<sup>2</sup>×Pa×s) respectively (Apicella et al., 2019). The material selected as matrix for the  $^{42}$   $117$  coating layer was PLA4060D supplied by NatureWorks™ (Minnetonka, USA). The active  $^{44}_{45}$  118 phases added in the coating layer were potassium sorbate (PS) and sodium benzoate (SB).  $\frac{46}{47}$   $119$   $\;\;$  Acetone and a surfactant (Tween 85) were also used for the coating production. They were 120 all supplied by Sigma Aldrich Co. (Missouri, USA). All organic solvents were analytical grade. 49  $51\,$   $121\quad$  A polymeric solution of consisting of acetone and PLA, in a mass ratio of 80:20, was mixed  $^{53}_{-2}$  122 with an aqueous solution containing the active phases and the surfactant (at a concentration  $^{55}_{56}$   $123$  of 1 wt% of the total solid content) in a volumetric ratio of 100:10. Then, the obtained  ${\frac{57}{58}}$   $124$   $\phantom{1}$  emulsion was applied to the surface of the support, which was the biopolymer film, by means  $60\,125$  of a threaded hand coater. The active phases were PS, SB and a combination of them in a 32 34  $36<sup>114</sup>$  This fill is filled by a bicity of t  $38\,113$  oxygen and water vapor permea 39 41 43  $45$  and phases added in the seating lay  $_{47}$   $_{119}$  Acetone and a surfactant (Twee 48 50  $52$ 54  $56 \,$   $123$  OF FWL/0 OF the total solid correct  $58\,124$  emulsion was applied to the surf 59

126 50% mass ratio and were added at a concentration of 0 wt%, 5 wt%, 10 wt% or 15 wt% of 2 127 the total solid content of the coating layer. A level of 15 wt% was the highest limit for the  $\frac{4}{5}$  128 complete solubilization of SB in water. 1 3

#### $\frac{6}{7}$  129 Film characterization  $7 \t 127$   $\t 7 \t min$  characterization

 $\frac{8}{9}$  130  $\,\,\,$  Mechanical tests were performed on rectangular specimens of the produced films (width = 131 12.7 mm, length = 30 mm) using a SANS dynamometer equipped with a 100 N load cell. The  $13\,$  132 testing speed was set according to the ASTM 882. A minimum of five specimens for each  $\frac{15}{16}$  133 film were tested.  $9\,130$  Mechanical tests were performed 10  $11\,131$  12.7 mm, length = 30 mm) using 12 14  $16 \t{100}$  mm wore tooted.

 $\frac{17}{18}$   $134$   $\;\;\;$  Oxygen permeability measurement on the films was carried out using a permeabilimeter 135 (GDP - C 165 of Brugger), with a manometric operation, connected to a thermo-controlled  $22\,$   $136$  bath (ThermoHaake). Before the test, an evacuation was performed on both the upper and  $^{24}_{2}$  137  $^{\circ}$  lower half-cells to remove the moisture and other residual gases. The test temperature was  $\frac{26}{27}$   $138$   $\phantom{1}$  set at 23°C and the oxygen flow to 80 mL/min, following ISO 15105-1. Sample area was 16  $^{\rm 28}_{\rm 29}$   $\,$  139  $\,$   $\,$  cm<sup>2</sup>. The obtained oxygen transmission rate (OTR) was multiplied by the respective 31 140 thickness of the film to calculate the permeability coefficient (PO<sub>2</sub>). All tests were made in  $33 \overline{)141}$  triplicate and the averaged values are reported (standard deviation < 8%).  $\frac{35}{36}$  142 The transparency of the films was measured following the ASTM D1746 – 03 through a UV- $\frac{37}{38}$   $143$   $\;$  VIS spectrophotometer (Lambda 800, USA). Squared samples of the films (length = 5 cm) 40 144 were placed on the internal side of the spectrophotometer cell and the transmittance was  $42\,$   $145\quad$  measured at 560 nm. Three replicates of each film were tested. The percentage of  $^{44}_{45}$  146 transparency (TR) was calculated according to:  $18$   $134$  Cyygen permeability measurem 19  $20\,135\,$  (GDP - C 165 of Brugger), with a 21 23 25  $27 \,$   $130$  set at 20 G and the Oxygen now  $29\,139$  cm<sup>2</sup>. The obtained oxygen trans 30 32 34  $36T$ <sup>1-12</sup> The admoparency of the films we <sub>38</sub> 143 vis spectrophotometer (Lambda 39 41 43  $45$  and  $\mu$  and  $\mu$  and  $\mu$  and  $\mu$  and  $\mu$  and  $\mu$ 

$$
^{46}_{47} 147 \t\t TR = Tr/T0 × 100 \t\t(1)
$$

 $49\,148$  where T<sub>r</sub> was the transmittance with the specimen in the beam and T<sub>0</sub> was the transmittance 51 149 with no specimen in the beam. 50

 $^{53}_{-2}\,$   $150$   $\;\;$  Hot seal strength (hot-tack) tests were performed with a heat seal tester model HSG-C  $^{55}_{56}$   $151$   $\quad$  (Brugger), according to standard ASTM F1921-98, Method B. Samples were cut into strips  $_{58}$   $152$  (width = 1.5 cm and length = 30 cm) and then were hot pressed at a temperature of 85°C  $54$  $_{56}$  T<sub>2T</sub> (Drugger), according to startuard 57

153 under a pressure of 15 N/cm<sup>2</sup> with a welding time of 0.5 s. The heat-sealing strength was 2 154 measured right after the sealing. A minimum of five replicates was tested for each sample.  $4\overline{155}$  Food packaging 1 3

 $\frac{6}{7}$  156 Films were cut into squared shapes of 11×11 cm<sup>2</sup> and sealed in pairs on three sides using a  $\frac{8}{9}$  157  $\;\;$  Multivac sealing machine. The obtained bags were filled either with 25g of shredded carrots 158 or with 10 ml of fresh pineapple juice, sealed on the remaining side and stored at 4 °C. One  $13\,$  159  $\,\,\,$  pack was prepared for each date of analysis. All the experiments were performed in triplicate  $\frac{15}{16}$  160 Food quality determination  $7^{130}$  mms were out mo squared sha  $9\,137$  Munivac sealing machine. The c 10  $11\,158$  or with 10 ml of fresh pineapple 12 14  $16$   $100$   $100a$  quality dotomination

#### $\frac{17}{18}$  161 Microbiological counting  $18<sup>101</sup>$  Microbiological counting

 $5 \quad 7 \quad 5 \quad$ 

162 For the microbiological analysis, 4.5 ± 0.5 g of shredded carrots or of pineapple juice were  $22\,163$  removed aseptically from each bag and were transferred into a sterile stomacher bag. Then,  $20\,162$  For the microbiological analysis, 21 23

 $^{24}_{2}$  164  $\phantom{1}$  the same weight of saline peptone water was added (SPW, Condalab, Torrejón de Ardoz, 25<sup>25</sup>

 $\frac{26}{27}$   $165$  Madrid, Spain). Carrots and SPW were blended for 60 s by using a stomacher.  $27$  TO TWAGHT DRAIN). Call OIS and OFT

<sup>28</sup> 166 Total aerobic plate counts (TPC), and yeast and <mark>mold</mark> counts (YMC), were determined after  $29\,166$  Total aerobic plate counts (TPC)

31 167 Plating on Plate Count Agar (PCA, Biokar Diagnostics Solabia, Beauvais, France) incubated 32

 $^{33}_{168}$  168 at 30°C for 72h and Sabouraud glucose agar with 100 mg/L chloramphenicol (SGA, Biokar 34

 $\frac{35}{36}$  169 diagnostic, Solabia, Allonne, France) incubated at 30°C for 5 days, respectively.  $36<sup>107</sup>$  algoritostic, colabia, *Ri*lemic, Fie

 $\frac{37}{38}$   $170$   $\;\;$  Enterobacterium plate counts (EC) were determined on VRBG agar  $38\,$  T/0 Enterobacterium plate counts (E

171 (Biokar diagnostic) after 48h of incubation at 37°C. 40

#### $42$  172 pH and titratable acidity 43

 $^{44}_{45}$  173 Shredded carrots (1.5 g) were mixed with 10 mL of distilled water prior to pH and titratable  $_{\rm 47}^{4\,6}$   $\,$  174  $\,$   $\,$  acidity (TA) determination. The pH value was determined by a pH meter (5231 and GLP22, 49 175 Crison Instruments S.A. Barcelona, Spain), and TA was determined by titration with 0.05 M  $51\,176$  NaOH (TitroLine easy, Schott, Mainz, Germany). TA was expressed in citric acid equivalents  $^{53}_{5.4}$  177 in g/100 mL.  $45$   $175$  embadoa banbio (1.0 g) word in  $_{47}$  174 acidity (TA) determination. The p 48 50 52  $54$  and  $\frac{1}{2}$  and  $\$ 

#### $^{55}_{56}$  178  $\hspace{1cm}$  Visual appearance and color determination  $56<sup>17</sup>$   $^{\circ}$  visual appearance and c

 $^{57}_{58}\,$   $179$   $\;\;\;\;$  Pictures of 3.0 ± 0.5 g of shredded carrots were taken using a viewing booth Just Normlicht 60 180 in order to have a constant light source.  $_{58}$  179 Pictures of 3.0  $\pm$  0.5 g of shredd 59

The color of samples (mixed carrots or juice) was assessed with a spectrophotometer CM 2 182 3500d (Minolta®, Carrières-sur-Seine, France). The coordinates L\*, a\* and b\* of the CIELAB  $4\overline{183}$  space were measured. The total colour variation was calculated as follow: 

$$
\frac{6}{7} 184 \qquad \Delta E = \sqrt{(L^*_{a} - L^*_{0})^2 + (a^*_{a} - a^*)^2 + (b^*_{a} - b^*)^2}
$$
 (2)

185 in which L\*a, a\*a and b\*a refer to the assay condition and L\*<sub>0</sub>, a\*<sub>0</sub> and b\*<sub>0</sub> to the initial 186 condition used as a control. 9 185 in which  $L^*$ <sub>a</sub>,  $a^*$ <sub>a</sub> and  $b^*$ <sub>a</sub> refer to 

 $\frac{13}{14}$  187 Statistical analysis To  $\theta$  because to a large side of  $\theta$ 

 $^{\rm 15}_{\rm 16}$   $\rm 188$   $\;$  The statistical analysis of the data was performed with XLSTAT software (Addinsoft, Paris, France). A confidence interval of 95% was used for all analyses. The Fisher (LSD) test was  $20\;\; 190$  applied for ANOVA for carrots. The Bonferroni test was applied for pairwise comparisons for  $\frac{22}{22}$  191 pineapple juice with a p-value of 0.0001.  $16\,$  T<sub>6</sub>  $\,$  The statistical analysis of the da 18 189 France). A confidence interval o 

## 193 Results and Discussion

2 194 The effectiveness of the developed systems was tested on two different foods, shredded  $^{4}$  195 carrots and pineapple juice. The foods were packed in active bags produced from films of  $\frac{6}{7}$  196 different compositions and stored in refrigerated conditions <del>for 10 days, during which they</del>  $\frac{8}{9}$   $197$   $\;\;$  were periodically tested for appearance and nutritional quality. Films having a content of 198 active phase of 5 wt% or 10 wt% were not effective to delay the microbial growth of the  $13\,$   $199$  selected foods (data not shown). This is in accordance with literature (Shen et al., 2010), in  $^{15}_{16}$  200 which a minimum content of 15 wt% PS in starch films was required to exhibit *in vitro*  $\frac{17}{18}$  201  $^-$  antimicrobial function, due to the hydrogen bonding interaction between the hydroxyl group 202 of the polymer matrix and the carboxyl group of potassium sorbate. Therefore, only the <sup>22</sup> 203 Fresults corresponding to films with an active phase content of 15wt% are presented. 1 3  $5 \quad \cdots \quad \cdots$  $7^{120}$  allocal compositions and store <sub>9</sub> 197 <del>were periodically tested for appe</del> 10  $11\,$  198 active phase of 5 wt% or 10 wt% 12 14  $16^{200}$  morrism minimum content of 10  $18\,$  201 and an interviewed to the set of the distribution of  $18\,$ 19 and the set of the  $20\,202$  of the polymer matrix and the ca 21 23

 $\frac{24}{25}$  204 Effect of antimicrobial films on shredded carrot quality  $25$   $20$ <sup> $\pm$ </sup>  $20$   $\pm$   $20$ 

 $^{\rm 26}_{\rm 27}$   $\rm 205$   $\;$  The physicochemical and color parameters and the microbiological quality of shredded 29 206 earrots packed in the control and in the antimicrobial films were monitored during refrigerated  $31\,207$  storage at  $4^\circ$ C. 27 28 30

 $_{2.4}^{33}$  208  $\;\;\;$  The pH values and titratable acidity of shredded carrots during 10 days of storage are  $\frac{35}{36}$  209  $^-$  reported in **Table 1**. Since the antimicrobial activity of both PS and SB is pH-dependent 210 (Davidson et al., 2005; Musyoka et al., 2018), lemon juice was added during processing of  $40\,211$  carrots in order to reduce their initial pH. The lemon juice addition led to a decrease in the  $^{42}_{42}$  212 initial pH value of shredded carrots from 6.2 ± 0.5 to 4.1 ± 0.1, which is consistent with the  $^{4\,4}_{4\,5}\,$  213  $^-$  antimicrobial activity of both PS and SB. 34  $36\,209$  reported in **Table 1.** Since the a 37 38 210 (Davidson et al., 2005; Musyoka 39 41  $43$   $212$   $\ldots$   $212$   $\ldots$   $212$   $\ldots$   $212$   $\ldots$  $45\,213$  antimicropial activity of both FS

 $\frac{46}{47}$   $214$   $\;\;\;\;$  During the storage of shredded carrots, a pH increase, though not significant for all 49 215 conditions, was observed during the first three days, followed by a slight decrease until day  $^{51}_{-2}$  216  $-$  10. As expected, TA followed the opposite trend. The changes of pH and TA during the first  $^{53}_{5.4}$  217  $\;\;$  days can be explained by spatial repartition at the bottom of the pack of the acidic liquid  $\frac{55}{56}$   $218$   $\;$  (lemon juice), whereas the further decrease could result from microbial acidification, as 58 219 previously reported (Alegria et al., 2010; Piscopo et al., 2019; Pushkala et al., 2012). 47 214 During the storage of shredded 48 50 52<sup>-1</sup>  $_{54}$  217 aays can be explained by spalla  $_{56}$  218 (lemon juice), whereas the furth 57

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220 Color measurement of shredded carrots were also performed, since color variation of food 2 221 during the time is generally perceived by the consumers as a loss of freshness (Piscopo et  $^{4}$  222 al., 2019). For shredded carrots, surface dehydration and production of lignin could result in  $\frac{6}{7}$  223 the discoloration of the vegetable during the storage (Alegria et al., 2010; Pushkala et al.,  $\frac{8}{9}$  224  $-$  2012). At the initial day, shredded carrots exhibited a low brightness (L\*) and an intense red 225 (a\*) and yellow (b\*) color (**Online Resource 1**). Fai et al. (Fai et al., 2016) reported  $13\,226$  comparable values for shredded carrots of a\* and b\*, but also a higher value of brightness.  $^{15}_{16}$  227 Poorly significant color differences to day 0 were observed during storage for the control  $\frac{17}{18}$  228  $^-$  packaging film (Table 1). This could be owed by the presence of the film that acted as a 229 barrier to water, reducing dehydration and thus discoloration of the packed food. Also  $22\,230$  Piscopo et al. (Piscopo et al., 2019) did not observe any relevant discoloration of shredded  $^{24}_{2}$  231 carrots packaged in polypropylene (PP) pouches up to 10 days of storage at 4°C, while  $\frac{26}{27}$  232  $^-$  Alegria et al. (Alegria et al., 2010) found a fading/whitening of the characteristic color of 233 shredded carrots packed in PP bags, significant after 7 days. A large variability of L\*, a\* and 28 31 234 b\*, and thus color difference was observed (Table 1 and Online Resource 1), which can  $33, 235$  explain the observation. Moreover, as reported in Table 1, no relevant differences in color of  $\frac{35}{36}$  236 carrots packed in the films compared to day 0 were observed, except for the 15 wt% SB + 15  $_{38}^{37}$  237  $\;\;$  wt% PS film after 10 days, sign that the active phase did not modified the global color of the 40 238 packed food. To support that data, the visual appearance of shredded carrots stored in the  $42, 239$  different films for 7 days is reported in Figure 1. No relevant browning or widespread change  $^{44}_{45}$  240 was observed during the time, but localized white and black tiny spots appeared on carrot  $_{\rm 47}^{\rm 46}$  24 $1$  surface whatever the packaging film. 1 3 5  $7 \, \text{22}$  are discolated of the vegetable  $\frac{1}{9}$   $224$  2012). At the initial day, shifted 10  $11\,225$  (a\*) and yellow (b\*) color (Onlin 12 14  $16$   $22$ , conf eighteen color amore no  $18\,220$  packaging ilini (**rabie** 1). This co 19 20 229 barrier to water, reducing dehyd 21 23  $25$  $27$   $232$   $\phantom{1}$  Alegria et al. (Alegria et al., 2010  $29\;233$  shredded carrots packed in PP  $\mu$ 30 32 34  $36\,230\,$  carrots packed in the films comp  $38\,237$  Wr% PS film after TU days, sign  $3$ 39 41 43  $45$  2.10  $\mu$  map obtained during the time, b  $_{47}$   $_{241}$  surface whatever the packaging 48

242 Microbial counts of shredded carrots are reported in **Figure 2a** and **Figure 2b**. The TPC of  $51,243$  shredded carrots packed in the control film significantly increased during the storage, being  $^{53}_{-2}$  244 significantly different to day 0 from 3 days of storage, and reaching 11.3 ± 0.1 log CFU/g  $^{55}_{56}$  245  $^{\circ}$  after 10 days. During the same time, the increase of YMC was significant only after 10 days  $_{58}\,$   $246$   $\quad$  of storage compared to the initial count. YMC of control conditions reached 4.7  $\pm$  0.1 log 247 CFU/g. 60 49 242 Microbial counts of shredded ca 50 52 54  $_{56}$   $^{24}$  and TV days. During the same to 57 and 200 million and 200 mil 59 61

248 The European Regulation (EUR-Lex - 32005R2073 - EN - EUR-Lex, 2005) sets a threshold 2 249 for the total microbial load of minimally processed vegetables equal to 7 log CFU/g.  $^{4}$  250 According to this limit, the shelf life of the packed carrots was comprised between 3 and 7  $\frac{6}{7}$  251 days, because of bacterial development. Different results are reported in literature with a  $\frac{8}{9}$  252  $-$  range of shelf-life between 6 and 10 days. Corbo *et al.* (Corbo et al., 2004) found a shelf life 253 of shredded carrots treated with chlorinated water equal to 6 days considering a threshold of <sup>13</sup> 254 7.7 log CFU/g. Alegria et al. (Alegria et al., 2010) found that shredded carrots treated with  $^{15}_{2.2}$  255  $\phantom{1}$  chlorinated water reached the European threshold value in 7 days, whereas Piscopo *et al.*  $\frac{17}{18}$  256  $\;\;$  (Piscopo et al., 2019) reported that shredded carrots stored in PP bags reached the limit 257 after 10 days of storage. 1 3  $5$  $7\frac{231}{2}$  adjs, because of bacterial development  $9\,$   $232$  ange of shell-life between 6 and 10  $11\,253$  of shredded carrots treated with 12 14  $16$   $255$  conomitation match redefined the  $\Gamma$  $18\,230\,$  (Fiscopo et al., 2019) reported to 19  $20\,25\%$  after 10 days of storage.

 $22\,258$  The comparison of the microbial counts of shredded carrots according to the different  $^{24}_{2.5}$  259  $^{\circ}$  packaging films showed that the presence of the active phases slightly reduced the TPC  $\frac{26}{27}$  260  $\;\;$  (Figure 2a). The strongest inhibitory effect was observed for the film 15 wt% PS after 7 and <sup>28</sup> 261 10 days. The TPC counts exceeded 7 log CFU/g between 3 and 7 days, as for the control, 31 262 but a difference of 0.8 log CFU/g was observed between the control and the carrots packed  $^{33}_{2}$  263 in 15 wt% PS after 7 days. A similar tendency was observed for YMC of carrots packed  $\frac{35}{36}$  264 antimicrobial films, though with lower populations (Figure 2b). Only the film 15 wt% SB was  $\frac{37}{38}$   $265$   $\quad$  able to maintain YMC at a level not different from the control at day 0, whatever the storage 40 266 duration, and the fungal population after 7 days of storage was 3.4 log CFU/g, i.e. 1.3 log  $42\,267$  CFU/g less than the control. 23  $25$  $27^{200}$  (rigult  $2a$ ). The subligest little  $29\,201$   $-10$  days. The TPC counts excee 30 32 34  $36\,204\,$  and the obtaining, along that is  $38\,203$  able to maintain YMU at a level 39 41

 $^{44}_{45}$  268  $\phantom{1}$  The results of carrot shelf-life tests suggested that the developed films were able to release  $\frac{46}{47}$  269  $\;\;$  the antimicrobial agents, as they exerted their antimicrobial function on TPC reduction for the 270 film containing PS as active agent and on YMC for the film containing SB. No synergistic 49 51 271 effect between PS and SB was observed. 45  $47\,209$  and the animicropial agents, as they 48 50

 $^{53}_{54}$  272 Effect of antimicrobial films on pineapple juice quality  $_{54}$   $_{212}$  Lifect of antimicropial limits on p

 ${\frac{55}{56}}$   ${\frac{273}{273}}$  . The active films were assessed in order to evaluate their impact on the quality of fresh 58 274 pineapple juice during storage up to 7 days at 4°C. Fresh pineapple juice is naturally acidic,  $^{60}_{2}$  275 with a pH value comprised between 3.1 and 4.2 and its main organic acids are citric and  $56\,213$  The active films were assessed 57 59 61

276 malic (Leneveu-Jenvrin et al., 2020). Hence, juice pH was suitable for PS and SB 2 277 antimicrobial effect. Until now, beverage active packaging has been mostly investigated for  $^{4}$  278 shelf-stable beverages which require high barrier properties regarding oxygen (Palomero et  $\frac{6}{7}$  279 al., 2016; Ramos et al., 2015). 1 3  $5$ 

 $\frac{8}{9}$  280  $\;\;\;\;$  Table 2 shows that the pH value of pineapple juice in the control film did not significantly 281 change during storage, whereas a slight decrease of TA was noticed after 7 days. After 7  $13\,282\quad$  days of juice storage, pH values were not different according to the packaging film, but  $^{15}_{16}$  283  $\phantom{10}$  differences in TA were observed. In fresh pineapple juice, TA variability is in the range 0.66- $\frac{17}{18}$   $284$   $-$  1.35 g/100 mL (Leneveu-Jenvrin et al., 2020) and the values obtained in this study were 285 consistent with these data, except for juice stored for 7 days in 15 wt% SB film. <sup>22</sup> 286 The color of the juice stored in the control film changed during storage: a\* and b\* significantly  $^{24}_{2}$  287  $\phantom{1}$  decreased (**Online Resource 2**), resulting in a color difference of 23.8  $\pm$  1.2 after 7 days  $\frac{26}{27}$  288  $^-$  compared to the initial color (**Table 2**). This difference is high, as a difference above 5 can be  $^{\rm 28}_{\rm 29}$   $^{\rm 28}$  assigned to two different colors (Mokrzycki & Tatol, 2011). It is also consistent with previous 31 290 reports indicating color modification and browning during storage of pineapple juice  $33\overline{)291}$  (Leneveu-Jenvrin et al., 2020). For the juice stored in the antimicrobial films, the decrease in  $\frac{35}{36}$  292 a\* and b\* was less pronounced than for the control film, leading to a color variation compared  $\frac{37}{38}$  293  $^-$  to the initial juice in the range 4.4 ± 0.1 - 7.4 ± 0.1. The color change was even less marked 294 for the juice stored in films with 15 wt% SB or 15wt% PS as active phase, compared to the 40  $^{42}$  295 one in films with both the antimicrobial compounds, which behave differently with an increase 296 in L\*.  $\frac{9}{9}$  280 **Table 2** shows that the pH value 10 11 281 Change during storage, whereas 12 14  $16\,$   $205\,$  amoronous in Transfer specified  $18\,204$  1.33 g/TOO THE (Lefteved-Jerry III 19 and the contract of the con 20 285 consistent with these data, exce 21 23 25  $27\,200$  compared to the linual color (Tai 29 289 assigned to two different colors 30 32 34  $36 \frac{2}{2}$  a and b was loss pronounced  $38\,293$  to the initial juice in the range 4. 39 41 43  $^{44}$  206 in  $\mathsf{I}^*$ 

 $_{\rm 47}^{\rm 46}$  297  $\;$  Figure 2c and Figure 2d shows the development of enterobacteria and yeasts and molds 298 respectively in pineapple juice stored in control or antimicrobial films. For the control film, a 49 51 299 significant increase is observed after two days for EC and YMC. Both EC and YMC of the  $^{53}_{-3}$  300  $^{-}$  control condition increased by 2 log CFU/g within the 7 days of storage. Initial counts and  $^{55}_{56}$  301  $^{\circ}$  population increases were consistent with previous observations (Leneveu-Jenvrin et al., <sup>57</sup> 302 2020). The comparison of microbial counts <mark>after two days of storage of</mark> juice whatever the 60 303 antimicrobial packaging film showed lower counts for the two microbial groups compared to  $_{47}$   $_{29}$   $\prime$  Figure 2C and Figure 2d shows 48 50 52  $54$   $500$  complete condition increased by  $\pm$  $56\,$   $301$  population increases were constructed  $58\,302\,$  2020). The comparison of micro 59

304 the control one. After 7 days of storage, the difference of counts between the control 2 305 condition and juice packed into antimicrobial films represented 1.4-1.5 log CFU/g and 1.0-1.6  $\frac{4}{5}$  306 log CFU/g, respectively for EC and YMC. Each antimicrobial proved to be of the same  $\frac{6}{7}$  307 efficacy than their combination for the inhibition of yeast and molds and of enterobacteria.  $\frac{8}{9}$  308  $\;\;$  All together, these results showed that the use of biodegradable films containing 309 antimicrobial compounds at a concentration of 15 wt% in the coating layer is strongly  $13,310$  effective to limit microbial development in pineapple juice, but also to limit color modification,  $\frac{15}{16}$  311 extending the juice's shelf life. 1 3  $5$   $\phantom{0}$   $7^{307}$  choddy than their combination is  $9\,$   $300$  All together, these results show 10  $11\,309$  antimicrobial compounds at a com 12 14  $16^{311}$  chronomy the jailor condition.

 $\frac{17}{18}$  312  $\;\;\;\;$  The impact of the active films is much more pronounced on pineapple juice than on shredded 313 carrots, likely because of the lower pH of pineapple compared to carrots that allowed to the <sup>22</sup> 314 active phases to better exert their antimicrobial action. Moreover, the diffusion of the  $^{24}_{2}$  315 antimicrobials, which are non-volatile but water soluble, was probably favored by the liquid  $\frac{26}{27}$  316  $^{-}$  nature of the pineapple juice (Wu et al., 2018). In addition, the ratio food quantity / film  $^{\rm 28}_{\rm 29}$   $\,317$   $\,$  surface was more favorable for pineapple juice than for carrots. Among the tested 31 318 antimicrobial compounds, films containing PS were more effective than SB or their  $33\overline{3}$  319 combination to delay the microbial growth in both the analyzed food, in accordance with data  $\frac{35}{36}$  320 literature reported for fresh noodles (Wangprasertkul et al., 2021).  $18\,$   $312\,$  The impact of the active films is 19 and the contract of the con  $20\,313$  carrots, likely because of the lov 21 23 25 and the contract of the con 27  $29\,31$  / surface was more favorable for  $\vert$ 30 32 34

## 38 321 Film properties

 $\frac{40}{4}$  322 The main functional properties of the films and their heat-sealing ability were evaluated in  $^{42}_{43}$  323  $^{\circ}$  order to analyze the effect of the incorporation of the active phases on the film's performance  $\frac{44}{45}$  324  $\;\;$  and thus on their suitability as food packaging materials. **Table 3** reports the mechanical, 47 325 barrier and optical properties of the films with the highest levels of active phase. All the  $^{49}_{-2}$  326 coated films showed values of the main mechanical parameters in the same range of  $^{51}_{52}$  327 PLA/PBAT based blends (Pietrosanto, Scarfato, Di Maio, & Incarnato, 2020; Pietrosanto,  $_{54}^{53}$   $328$   $\,$  Scarfato, Di Maio, Nobile, et al., 2020) and comparable to those of polyethylene (PE)  $\,$ 329 (Mangaraj et al., 2009), one of the most used conventional polymers for flexible packaging 56  $58,330$  applications. The presence of the active phases did not significantly affect the stiffness of the  $^{60}_{61}$  331 films, while it led to a slight increase of the yield stress. Moreover, it caused a slight reduction 41  $43\,$   $323\,$  order to analyze the effect of the  $45\,324$  and thus on their suitability as for 46 48 50  $\frac{52}{2}$  $54\,328$  Scarrato, DI Maio, Nobile, et al., 55 57 59  $61$   $551$  mms, while it lod to a slight more 62

332 in the ductility of the films, more pronounced for the SB phase. It may be hypothesized that 2 333 SB, having aromatic nature, and thus lower chemical affinity than the aliphatic PS towards  $4\,$  334 the PLA matrix of the coating layer, could tend to aggregate into the polymer, creating small  $\frac{6}{7}$  335 particles that act as stress concentration points and reduce the toughness. However, in all  $^8_{\rm o}$  336  $^8$  cases the change was not large enough to compromise the ductile failure mode of the films 11 337 (Table 4). 1 3  $5$  $7^{333}$  particles that act as stress correct  $9\,$   $330$   $\,$  cases the change was not large 10

 $13\,$  338  $\,\,\,\,\,$  Since the oxygen is one of the main factors that lead to food spoilage, the developed films  $^{15}_{33}$  339  $^{\circ}$  were also tested for their oxygen permeability. All coated films showed comparable values of  $\frac{17}{18}$  340  $^-$  permeability coefficients (approx. 40 (cm $^3$ ×mm)/(m $^2$ ×d×bar)), independently on the 341 formulation of the active layer. These values are in the typical range for food packaging films,  $22\,$   $342\,$  far above those of polyolefins, but lower than polyethylene terephthalate (PET) (Piergiovanni  $^{24}_{2}$  343 and Limbo, 2010). Compared to the uncoated substrate film, which permeability coefficient is  $^{26}_{27}$  344  $^{\circ}$  equal to 51.5 (cm $^3$ ×mm)/(m $^2$ ×d×bar), a significant improvement of the oxygen barrier  $^{\rm 28}_{\rm 29}$  345  $^-$  performance of the coated films was observed. This finding can be explained by the lower 31 346 molecular mobility of the polymer chains of the coating layer (PLA, at glassy state at 23°C)  $33\overline{3}$  347 with respect to those of the substrate (PLA/PBAT blend, where the PBAT constituent is in the  $\frac{35}{36}$  348  $^-$  rubbery state at 23°C) (Apicella et al., 2019; Pietrosanto, Scarfato, Di Maio, Nobile, et al.,  $\frac{37}{38}$  349  $-$  2020). On the opposite, the addition of both the preservatives did not lead to significant 40 350 changes of oxygen permeability of coated films. Different results were reported in literature  $^{42}$  351 on other polymer substrates (Wangprasertkul et al. 2021), where the incorporation of PS and  $^{44}_{45}$  352 SB, which are polar molecules, prevented the permeability to oxygen molecules.  $\frac{46}{47}$  353  $^-$  Films optical properties in terms of "see through" possibility were investigated by measuring  $\rm _{49}$   $\rm 354$   $\,$  the transparency, *i.e.* the transmission of visible light at 560 nm (TR $\rm_{560}$ ). The transparency of  $51$  355 the developed films was lower than the conventional used polymers (e.g. PE and PET)  $^{53}_{-3}$  356  $\phantom{1}$  (Moreno-Vásquez et al., 2017; Nogi et al., 2013), due to the presence of PBAT, which is  $^{55}_{56}$   $357$  white and opaque, in the substrate (Wang et al., 2016). Moreover, the addition of the 358 preservatives led to a further reduction of the transparency value, which was more significant 57 60 359 for SB than for PS. The transparency of a polymeric system increases with the increase of 14  $16 \frac{333}{16}$  More also tooted for them exygen  $18\,$   $340$  permeability coefficients (approx 19  $20\,341$  formulation of the active layer. I 21 23  $25$  $27 \text{ } 27$ 29 345 performance of the coated films 30 32 34  $36^{3}$  $38\,349$  2020). On the opposite, the addi 39 41 43  $45^{332}$   $\omega$ , which are polar molecules,  $47$   $333$  Films optical properties in terms 48 50 52  $54$   $50$   $($   $\ldots$   $\ld$  $56 \frac{337}{200}$  write and opaque, in the substra  $58\,338$  preservatives led to a further red 59 61

360 the dispersed composite size (Schulz et al., 2007). Thus, the lower transparency of 15SB film  $2\,361$  can be attributable to the lower compatibility of SB active agent with PLA compared to PS,  $^{4}$  362 that caused a worse dispersion in the polymer matrix. Since the antimicrobial activity is  $\frac{6}{7}$  363 affected by the dispersion of the active phase in the polymer matrix (Kuplennik et al., 2015),  $\frac{8}{9}$  364  $\;\;$  this result can also explain the lower antimicrobial effect on both the shredded carrots and 11 365 pineapple juice that was observed for films containing SB comparatively to those containing 13 366 PS. 1 3  $5 \qquad \qquad \blacksquare$  $7^{300}$  anched by the dispersion of the  $9\,$   $304$   $\,$  uns result can also explain the K 10 12

 $^{15}_{3.67}$  Finally, to investigate the capability of the coating layer to provide the heat-sealing ability of  $\frac{17}{18}$   $368$   $\quad$  the films, hot-tack tests were carried out. Packaging systems able to self-adhere at a 369 convenient processing condition and provide a flawless hermetic seal are of fundamental  $22\,$   $370$  importance for food packaging applications, because it ensures the tightness of the package  $^{24}_{2}$  371 and thus the protection of the food. Proper heat-sealing ability also makes the packaging  $\frac{26}{27}$  372  $\;\;$  suitable for applications in aseptic processing and packaging where special technologies as <sup>28</sup> hot-filling or <mark>steam</mark> sterilization are used. In the case of the <mark>films</mark> developed in this work, <mark>heat</mark>-31 374 sealing ability is made possible by the use of an amorphous PLA coating layer able to self- $33\overline{3}$  375 adhere. **Table 4** reports the results of the hot tack tests. As it can be seen, the addition of the  $\frac{35}{36}$  376 PLA coating layers to the substrate, which is not sealable, allowed to impart to all of them  $\frac{37}{38}$   $377$   $\;\;$  heat-sealing ability. In the case of the control film sample, the hot seal strength is 383 g/15  $\;\;$ 40 378 mm and the failure happens mainly through a delamination mechanism, which means that  $^{42}$  379 the adhesion of the PLA coating layer to the substrate was lower than its seal strength.  $^{44}_{4}$  380  $\phantom{1}$  The incorporation of the preservatives in the coating layer affected both the seal strength and  $\frac{46}{47}$   $381$   $\quad$  the failure type. The seal strength of the active films was lower than that of the control one: 49 382 the presence of active agent salts in the polymer matrix hindered the interactions between  $51\,$  383 in the two melted surfaces of the PLA layer during the heat-sealing process. The seal strength  $\frac{53}{2}$  384 was so much reduced that it became lower than the adhesion of the PLA coating to the  $^{55}_{56}$  385  $\phantom{0}$  substrate, resulting in an adhesive failure mechanism of the active films. In addition, films  $_{58}\,$   $386$   $\quad$  containing PS had a lower seal strength than those containing SB, because the better  $16^{30}$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$  $18\,$   $300\,$  and mins, not-tack tests were can 19 and the contract of the con 20 369 convenient processing condition 21 23 25<sup>2</sup>  $27 \frac{3}{2}$  suitable for applications in asept 29 30 32 34  $36^{370}$  TEN country tayors to the substitution  $38\,377$  neat-sealing ability. In the case ( 39 41 43  $45\,$   $\frac{300}{100}$   $\frac{100}{100}$  potation of the probably  $47.381$  and railure type. The sear strength 48 50 52  $54$  $56\,$   $30\,$  substrate, resulting in an additional 57 and the second contract of the seco

- compatibility and dispersion of PS into the polymer matrix might have exerted a greater
- hindering effect on the interaction between the PLA layers (Voon et al., 2012). 2 388 hindering effect on the interactio

## 389 Conclusion

2 390 Multifunctional, eco-friendly active packaging films were developed by coating a  $4\overline{3}$  391 biodegradable PLA/PBAT substrate with a heat-sealable layer loaded with antimicrobial  $\frac{6}{7}$  392 agents. The incorporation of the active phases did not relevantly affect the mechanical and  $\frac{8}{9}$  393  $-$  barrier properties of the films, while it led to a slight reduction of transparency and hot-tack 394 strength. Only the films at highest level of active agents showed effective antimicrobial <sup>13</sup> 395 activity in shelf-life test performed on shredded carrots and fresh pineapple juice.  $^{15}_{16}$  396  $\phantom{10}$  The film with PS was very efficient to reduce microbial development and color modification of  $\frac{17}{18}$  397  $\;\;$  pineapple juice. A better dispersion of PS in the polymer matrix probably explains its better 398 performance compared to films containing SB or a combination of both antimicrobials. 22 399 The better impact on shelf-life of pineapple juice compared to shredded carrots, probably  $^{24}_{24}$  400 sowed to the liquid state and the lower pH of pineapple juice, that may have favored both the  $^\circ$  $\frac{26}{27}$  401 diffusion of the antimicrobial into the food and the antimicrobial activity of the active  $\frac{28}{29}$  402  $^-$  compounds. In conclusion, these results open new possibilities for the development of 31 403 sustainable active packaging for beverages with a short shelf-life. 1 3  $5$  $7^{32}$  agains. The moorporation of the  $9\,$   $393$  barner properties of the limits, with 10  $11\,$   $394$  strength. Only the films at highe 12 14  $16^{390}$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$   $\ldots$  $18\,$   $327$  place proper junct. A better dispers 19 and the contract of the con 20 398 performance compared to films 21 23 25 and 2012 and 2012 and 2012 and 2013  $27$  401 annually of the antimological line 29 30

#### $33\overline{)}$  404 Acknowledgements 34

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Table 1. pH value, titratable acidity (TA, g/100g) and color difference of shredded carrots packed in the different films during refrigerated storage up to 10 days. Control: no active phase, 15SB: sodium benzoate at 15 wt% in the coating layer, 15PS: potassium sorbate at 15 wt% in the coating layer, 15SB+15PS: sodium benzoate and potassium sorbate in a 50/50 mass ratio at 15 wt% in the coating layer. Means and standard deviations of triplicates are indicated. Values in the same column with different letters show significant differences at p<0.05.



Table 2. pH value, titratable acidity (TA, g/100g) and color difference of fresh pineapple juice packed in control film or in antifungal films and stored up to 7 days at 4°C. Means and standard deviations of triplicates are shown. Different letters in the same column indicate significant differences (p-value < 0.0001). Control: no active phase, 15SB: sodium benzoate at 15 wt% in the coating layer, 15PS: potassium sorbate at 15 wt% in the coating layer, 15SB+15PS: sodium benzoate and potassium sorbate in a 50/50 mass ratio at 15 wt% in the coating layer.





<b>Film</b>	<b>Hot tack strength</b> $(g/15$ mm)	<b>Failure mode</b>
Uncoated substrate	Not sealable	۰
Control	$383 \pm 14$	<b>Delamination</b>
15SB	$283 \pm 29$	Adhesive
15 <sub>PS</sub>	$245 \pm 25$	Adhesive
$15PS+SB$	$267 \pm 29$	Adhesive

Table 4. Hot tack strength (according to ASTM F 1921 – 98) of the films.

# Figure 1









 $(c)$  (d)



## Figure captions

Fig. 1 Visual appearance of shredded carrots packed into the films containing antimicrobials after 7 days of storage. Arrows indicate black or white spots on the carrot surface. Film names: Control, no active phase; 15SB, sodium benzoate at 15 wt% in the coating layer; 15PS, potassium sorbate at 15 wt% in the coating layer; 15SB+15PS, sodium benzoate and potassium sorbate in a 50/50 mass ratio at 15 wt% in the coating layer.

Fig. 2 Total aerobic plate counts of shredded carrots (a), yeast and mold counts of shredded carrots (b), enterobacterium counts of pineapple juice (c) and yeast and mold counts of pineapple juice (d), packed in the control film (white bars), in 15SB (grey bars), in 15PS (black bars), in 15SB+15PS (spotted bars) during refrigerated storage. Film names: Control, no active phase; 15SB, sodium benzoate at 15 wt% in the coating layer; 15PS, potassium sorbate at 15 wt% in the coating layer; 15SB+15PS, sodium benzoate and potassium sorbate in a 50/50 mass ratio at 15 wt% in the coating layer. Means and standard errors are indicated. Same letter in a graph indicates no significant difference at p<0.05.