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ORIGINAL ARTICLE

Application of edible coating with starch and carvacrol in minimally processed pumpkin

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Abstract The present study evaluated the effect of an edible coating of cassava starch and carvacrol in minimally processed pumpkin (MPP). The minimal inhibitory concentration (MIC) of carvacrol against *Escherichia coli*, *Salmonella enterica* serotype Typhimurium, *Aeromonas hydrophila*, and *Staphylococcus aureus* was determined. The edible coating that contained carvacrol at the MIC and 2 × MIC was applied to MPP, and effects were evaluated with regard to the survival of experimentally inoculated bacteria and autochthonous microflora in MPP. Total titratable acidity, pH, weight loss, and soluble solids over 7 days of storage under refrigeration was also analyzed. MIC of carvacrol was 312 μg/ml. Carvacrol at the MIC reduced the counts of *E. coli* and *S.* Typhimurium by approximately 5 log CFU/g. *A. hydrophila* was reduced by approximately 8 log CFU/g, and *S. aureus* was reduced by approximately 2 log CFU/g on the seventh day of storage. Carvacrol at the 2 × MIC completely inhibited all isolates on the first day of Storage. *coli*forms at 35 °C and 45 °C were not detected (< 3 MPN/g) with either treatment on all days of shelf life. The treatment groups exhibited a reduction of approximately 2 log CFU/g in psychrotrophic counts compared with controls on the last day of storage. Yeast and mold were not detected with either treatment over the same period. The addition of carvacrol did not affect total titratable acidity, pH, or soluble solids and improved weight loss. The edible coating of cassava starch with carvacrol may be an interesting approach to improve the safety and microbiological quality of MPP.

Keywords Minimally processed vegetables · Pumpkin · Antimicrobial activity · Phenolic compounds · Microbiological quality · Natural antimicrobials

Highlights
- Carvacrol was used to inhibit microbiota in minimally processed pumpkin (MPP)
- Carvacrol effectively reduced pathogenic bacteria in MPP
- Carvacrol reduced autochthonous bacteria in MPP
- Carvacrol influenced physicochemical parameters in MPP

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Introduction

Minimally processed vegetables (MPV) are products that go through a process that usually involves trimming, peeling, cutting, washing, and disinfecting while retaining the characteristics of fresh food (Sousa et al. 2012). Minimally processed vegetables have gained great acceptance by consumers who want the quality of fresh products and convenience of a ready-to-eat product (Azeredo et al. 2011).

In Brazil, the cultivation of pumpkin has high economic and social value, with substantial consumption of approximately 27.08 kg per capita (IBGE 2010). Because of their large size and weight, harvesting, handling, packing, transporting, storing, and marketing can become difficult, leading to enormous waste and laborious preparation (Habibunnisa et al. 2001). Thus, pumpkin should be preserved

in a minimally processed form that is more convenient to store and use, thereby reducing waste.

However, in the last two decades, the association of MPV with foodborne diseases outbreaks has been observed (Maistro et al. 2012). Studies have shown the occurrence of pathogenic bacteria, such as *Salmonella* spp., *Escherichia coli* (Maistro et al. 2012), and *Staphylococcus aureus* (Seo et al. 2010) in different MPV. These foods can also contain psychrotrophic microorganisms, such as *Aeromonas hydrophila* and *Listeria monocytogenes* (Maistro et al. 2012; Sousa et al. 2012), in addition to hygiene-associated microorganism, such as coliform, mold, and yeast (Maistro et al. 2012).

Considering the possibility of microbial contamination, the use of plant essential oils and their constituents may be an alternative because they have antimicrobial properties, do not present toxicity to humans, and sometimes improve the sensorial quality of vegetables (Molinos et al. 2009). Additionally, some essential oils are Generally Recognized as Safe (GRAS) by the Food and Drug Administration (Guarda et al. 2011), thus raising interest in their application in MPV (Sousa et al. 2012).

Carvacrol is a component of the essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. It has been shown to be active against several microorganisms, including *E. coli* (Guarda et al. 2011), *Salmonella* spp. (Yun et al. 2013), *S. aureus* (Guarda et al. 2011), and *A. hydrophila* (Sousa et al. 2012).

The promising use of essential oils and their constituents in food consists of their incorporation into an edible coating in an attempt to control pathogens and prolong the shelf life of perishable products (Iturriaga et al. 2012). Interest has been seen in the use of starch-based edible coatings because they are tasteless, colorless, and odorless and exhibit selective permeability to oxygen and carbon dioxide. They are also relatively inexpensive, making then an excellent alternative as antimicrobial agents (Ehivet et al. 2011; Kuorwel et al. 2011).

To our knowledge, no studies have evaluated carvacrol in edible coatings for minimally processed vegetables (MPV). Thus, the present study investigated the antimicrobial effects of carvacrol in an edible coating that was applied to minimally processed pumpkin (MPP). We also evaluated the effects of this coating on total titratable acidity, pH, weight loss, and soluble solids of these products.

Material and methods

Bacterial isolates

Escherichia coli (ATCC 25922), *Salmonella enterica* serotype Typhimurium (ATCC 14028), *Aeromonas hydrophila* (ATCC 7966), and *Staphylococcus aureus* (ATCC 25923) stored in Brain and Heart Infusion Broth (Difco, Becton Dickinson, Sparks, MD, USA) that contained 20 % glycerol at −20 °C in the Laboratory of Food Microbiology, State University of Maringá, Paraná, Brazil were used in this study.

Determination of minimal inhibitory concentration

MIC was determined using the broth microdilution method according to M100-S22/2012 methods of the Clinical and Laboratory Standards Institute (CLSI 2012). Carvacrol (Sigma-Aldrich, St. Louis, MO, USA) was serially diluted in 100 μl of Mueller Hinton Broth (Difco, Becton Dickinson, Sparks, MD, USA) at concentrations of 19–5000 μg/ml in 96-well microplates. Standardized bacterial suspensions were made in 0.85 % saline using a McFarland scale at an interval of 0.5 and diluted 1:20, and 10 μl was inoculated in each well of the microplate. The microplates were incubated at 35 °C for 24 h, and the MIC was visually determined as the lowest concentration of carvacrol that inhibited bacterial growth.

Preparation of edible coating and carvacrol incorporation

The edible coating of native cassava starch was prepared according to Chiumarelli et al. (2010). Briefly, the starch that is marketed regionally was weighed, diluted in distilled water to a concentration of 3:97 (*w*/*v*), and homogenized at 70 °C to complete gelatinization. After cooling, carvacrol was added at concentrations of 312 μg/ml (MIC) and 625 μg/ml (2 × MIC), based on antimicrobial activity in vitro.

Preparation of minimally processed pumpkin

Mature pumpkins (*Cucurbita moschata* Duch.) were purchased in local markets and processed according to Cortez-Vega et al. (2014), with modifications. After selection, the pumpkins were washed and immersed in cold water that contained 200 ppm chloride for 2 min. Processing occurred manually to obtain cubes with 3 cm sides that were free of peels, seeds, and spongy parts. The pumpkins were sanitized with 200 ppm chloride solution for 10 min, and they were then rinsed with 2 ppm chloride solution, drained for approximately 2 min, and bleached in boiling water for 2 min. The pieces were then immersed in cold water to stop the cooking process and then drained for approximately 2 min.

Minimally processed pumpkin was subjected to analysis as required by Brazilian law (BRASIL 2001). We performed counts of bacteria that were experimentally inoculated to ensure that no sample was previously contaminated.

Minimally processed pumpkin pieces were immersed in the coating solution for 1 min and placed in trays. To pack the product, we used expanded polystyrene trays (3 mm thickness, 4 cm height, 15 cm width, 21 cm length) that were covered with a film of stretchable polyvinyl chloride (PVC; 0.02 mm thick). Each tray had an average weight of 300 g of product. The trays were stored in a refrigerator at 4 °C (± 2 °C)

for 7 days according to general storage conditions in Brazil. A flowchart of the controls, treatments, storage, and analyses is presented in Fig. 1.

The experiment had two control groups: MPP (C1 group) and MPP coated with cassava starch without carvacrol (C2 group), and two treatment groups: MPP coated with cassava starch that contained carvacrol at the MIC (312 μg/ml; T1 group) and 2 × MIC (625 μg/ml; T2 group).

Effect of carvacrol on the survival of experimentally inoculated bacteria in minimally processed pumpkin with an edible coating of cassava starch

To evaluate the survival of experimentally inoculated bacteria, the control and treatment groups were contaminated with *E. coli* (ATCC 25922), *S.* Typhimurium (ATCC 14028), *A. hydrophila* (ATCC 7966), and *S. aureus* (ATCC 25923). The bacterial suspensions were prepared as described in the "Determination of minimal inhibitory concentration" section. The pumpkin samples were immersed in each of the bacterial suspensions for 10 min. The control and treatment groups were stored in a refrigerator and analyzed on days 0, 3, and 7 of shelf life.

Inoculated bacteria were counted by diluting 25 g of the MPP in 225 ml of sterile peptone water (1 g/L). Serial dilutions were performed and plated on Agar Enteric Hektoen (Difco, Becton Dickinson, Sparks, MD, USA) for *Salmonella* spp. and *A. hydrophila*, Agar Eosin Methylene Blue (Difco, Becton Dickinson, Sparks, MD, USA) for *E. coli*, and Agar Baird Parker (Difco, Becton Dickinson, Sparks, MD, USA) for *S. aureus*. Incubation was performed under specific conditions (time and temperature) for each bacterium. The results are expressed in log colony-forming units (CFU) per gram (Downes and Ito 2001; Sant'Ana et al. 2012).

Effect of carvacrol on the survival of autochthonous microbiota in minimally processed pumpkin

To evaluate the survival of autochthonous microbiota in MPP, the control and treatment groups were stored in a refrigerator and analyzed on days 0, 3, and 7 of shelf Life. *coli*forms at 35 °C and 45 °C, mold, yeast, and psychrotrophic bacteria were evaluated according to the methodology described by Downes and Ito (2001).

Coliforms were determined by the Most Probable Number (MPN) technique using a three-tube series of Lauryl Sulfate Tryptose broth (Difco, Becton Dickinson, Sparks, MD, USA) that were incubated at 35 °C for 48 h. The tubes that presented gas production were transferred to Green Bile Lactose broth (Difco, Becton Dickinson, Sparks, MD, USA) and incubated at 35 °C for 48 h and EC broth (Difco, Becton Dickinson,

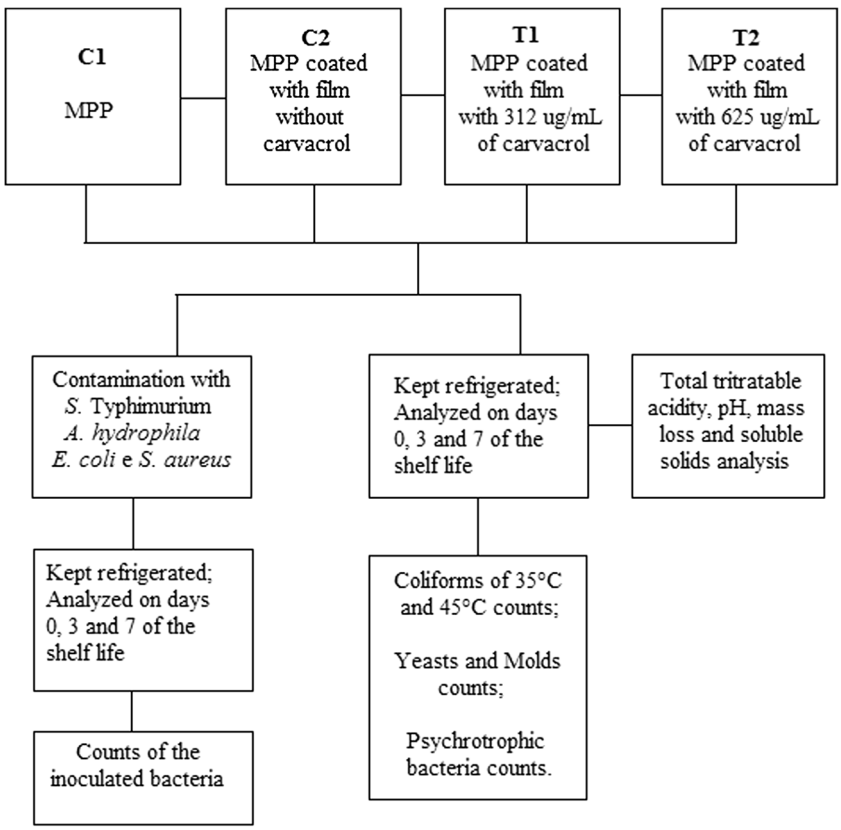

Fig. 1 Flowchart of the controls, treatments, storage, and analyses

Sparks, MD, USA) and incubated at 45 °C for 48 h. The results are expressed as MPN/g.

Psychrotrophic bacteria were counted using the pour-plating technique in Plate Count Agar (Difco, Becton Dickinson, Sparks, MD, USA), and the plates were incubated at 6 °C for 7 days. The results are expressed as log CFU/g.

To count mold and yeast, we used the spread-plating technique in Agar Dicloran Glycerol (Difco, Becton Dickinson, Sparks, MD, USA), and the plates were incubated at 25 °C for 5 days. The results are expressed as log CFU/g.

Physicochemical analyses

The control and treatment groups of MPP, without contamination, were analyzed with regard to weight loss, soluble solids, total titratable acidity, and pH according to the methodology recommended by AOAC (1992) during 7 days of storage. Weight loss was determined by weighing MPP on an analytical scale and considering the initial weight of each sample. The results are expressed as a percentage. The amount of soluble solids was determined by refractrometric analysis of the juice from the crushed samples. The results are expressed as Brix degrees (°Bx). Total titratable acidity was determined in three samples from the titration of 10 g of homogenate pulp diluted in 100 ml of distilled water and a standard solution of 0.1 N sodium hydroxide. The endpoint was indicated by phenolphthalein. The results are expressed as a percentage. Hydrogenionic potential (pH) was determined in ground pumpkin pulp using a digital potentiometer.

Statistical analysis

The analyses were performed in duplicate with two repetitions. The results are expressed as mean and standard deviation. The data were analyzed using analysis of variance (ANOVA). The level of significance was set to 5 %. *Post hoc* comparisons were performed using Tukey's test. GraphPad Prism 5.01 software was used for the statistical analyses.

Results and discussion

Minimal inhibitory concentration

MIC was 312 μg/ml for *S.* Typhimurium (ATCC 14028), *E. coli* (ATCC 25922), *A. hydrophila* (ATCC 7966), and *S. aureus* (ATCC 25923). Previous studies reported the effectiveness of carvacrol at different concentrations in inhibiting the growth of the same bacteria (Guarda et al. 2011; Sousa et al. 2012; Yun et al. 2013).

Effect of applying an edible coating of cassava starch with the addition of carvacrol on the survival of bacteria inoculated in minimally processed pumpkin

The effects of the edible coating with carvacrol that was applied to MPP that was previously contaminated with *E. coli* (ATCC 25922), *S.* Typhimurium (ATCC 14028), *A. hydrophila* (ATCC 7966), and *S. aureus* (ATCC 25923) on different days of shelf life are shown in Table 1.

The C1 and C2 groups generally exhibited elevations in bacterial multiplication during the 7 days of storage, even under refrigeration. *E. coli* and *S.* Typhimurium reached values of approximately 7 log CFU/g on day 7. These results are important because these values are higher than the infectious dose of *E. coli*, which varies from 10 to 10^{12} CFU/g, depending on the pathogenic group (Feng 2012), and the infectious dose of *Salmonella* spp., which can be a single cell, depending on serotype, age, and the immune state of the host (Hammack 2012). According to Lampel (2012), no consensus has been reached about the infectious dose of *A. hydrophila*. Nonetheless, outbreaks have been reported with 10^7 CFU/g contamination of food, a count that was found in the C1 and C2 groups already on the third day of storage. The counts of *S. aureus* increased approximately 1 log CFU/g during the storage period and reached levels that were less than those necessary to produce toxins (i.e., 10^6 CFU/g; Hait 2012).

The results for the four bacterial isolates indicated that the T1 and T2 groups were significantly different ($p < 0.05$) from the C1 and C2 groups on all days of shelf life. The application of carvacrol at 625 μg/ml in MPP (T2 group) completely inhibited bacterial growth on all days of storage. The counts of *E. coli* and *S.* Typhimurium in the T1 group (312 μg/ml carvacrol) were reduced by 5 log CFU/g on day 7. For *A. hydrophila*, a reduction of approximately 8 log CFU/g was observed in the same period. A reduction of approximately 2 log CFU/g was observed for *S. aureus*. These results demonstrate the efficiency of carvacrol at the MIC of 312 μg/ml, even when bacterial counts in the control groups were elevated.

Baskaran et al. (2013) tested carvacrol at concentrations of 0.15 % and 0.3 % in water that was used to wash apples and found counts of *E. coli* (O157:H7) that were <1 log CFU per apple. Muriel-Galet et al. (2012) also reported reductions of 0.32 and 0.5 log CFU/g of *S. enterica* (ATCC 13076) in salads that were packaged with a film that contained oregano essential oil that contained 2.8 % and 6.7 % carvacrol, respectively. Sousa et al. (2012) detected <2 log CFU/g of *A. hydrophila* in fresh vegetables that were treated with carvacrol at 0.6 μl/ml. The present findings were better than those in these previous studies, in which carvacrol at a lower concentration more effectively reduced bacterial counts.

When evaluating carvacrol at 312 and 625 μl/ml, *S. aureus* and *S.* Typhimurium counts were significantly different

Table 1 Counts of *E. coli* (ATCC 25922), S. Typhimurium (ATCC 14028), *A. hydrophila* (ATCC 7966) and *S. aureus* (ATCC 25923) in MPP with an edible coating of cassava starch and carvacrol

Group	*E. coli* (ATCC 25922) (log CFU/g)			*S. typhimurium* (ATCC 14028) (log CFU/g)			*A. hydrophila* (ATCC 7966) (log CFU/g)			*S. aureus* (ATCC 25923) (log CFU/g)		
	Day			Day			Day			Day		
	0	3	7	0	3	7	0	3	7	0	3	7
C1	3.335 ± 0.104^{A}	4.904 ± 0.0559^{A}	7.525 ± 0.445^{A}	3.738 ± 0.151^{A}	4.310 ± 0.097^{A}	7.793 ± 0.031^{A}	3.656 ± 0.175^{A}	7.349 ± 0.102^{A}	9.480 ± 0.082^{A}	3.072 ± 0.106^{A}	3.901 ± 0.092^{A}	4.825 ± 0.110^{A}
C2	3.299 ± 0.153^{A}	4.920 ± 0.0240^{A}	7.901 ± 0.093^{B}	3.429 ± 0.222^{B}	4.444 ± 0.104^{A}	7.710 ± 0.324^{A}	3.575 ± 0.197^{A}	7.260 ± 0.087^{A}	9.186 ± 0.092^{A}	3.330 ± 0.158^{B}	3.515 ± 0.107^{A}	4.871 ± 0.113^{A}
T1	1 ± 1.069^{B}	1.044 ± 1.118^{B}	2.516 ± 0.279^{C}	$<2 \pm 0^{C}$	0.849 ± 0.908^{B}	2.373 ± 0.113^{B}	0.678 ± 0.768^{B}	0.425 ± 0.786^{B}	0.425 ± 0.786^{B}	$<2 \pm 0^{C}$	1.044 ± 1.118^{B}	2.294 ± 0.084^{B}
T2	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$	$<2 \pm 0^{D}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$	$<2 \pm 0^{B}$	$<2 \pm 0^{B}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$

Values are mean log CFU/g followed by standard deviation. Means in the same column with different capital letters are significantly different ($p < 0.05$; Tukey's test). C1, MPP inoculated; C2, MPP inoculated + coating of cassava starch; T1, MPP inoculated + coating with MIC of carvacrol; T2, MPP inoculated + coating with 2MIC of carvacrol

between the T1 and T2 groups on the third and seventh days of storage, and *E. coli* counts were significantly different on all days of storage. These findings are consistent with previous reports that indicated that a higher carvacrol concentration was associated with a higher antimicrobial effect in food (Baskaran et al. 2013; Muriel-Galet et al. 2012; Kuorwel et al. 2011). For *A. hydrophila*, no significant difference ($p > 0.05$) was found between the T1 and T2 groups. Azeredo et al. (2011) reported that the MIC of oregano essential oil completely inhibited *A. hydrophila* in fresh vegetables (e.g., lettuce, beet root, and arugula). During the storage period, *A. hydrophila* was the bacteria with the highest counts, which may be explained by its psychrotrophic characteristics. However, it was also the bacterium that presented the highest reduction of counts after carvacrol treatment. According to Burt (2004), *A. hydrophila* is considered one of the most sensitive bacteria to essential oils.

The significant reductions of bacterial counts that were observed in the present study may also be attributable to the method of carvacrol application in the food. The incorporation of antimicrobial compounds in edible coatings can cause gradual release from the polymer matrix to the food surface (Hyldgaard et al. 2012; Sangsuwan et al. 2009). Thus, the concentration that is necessary for microbial growth inhibition can be maintained for a longer period of time.

Effect of application of the edible coating of cassava starch with the addition of carvacrol on the development of bacteria that are naturally present in minimally processed pumpkin

The effects of application of the edible coating with carvacrol to MPP on the development of coliforms at 35 °C and 45 °C, psychrotrophic bacteria, mold, and yeast on various days of shelf life are shown in Table 2.

The counts of coliforms at 35 °C and 45 °C in the T1 and T2 groups were <3 MPN/g on all days of shelf life. The C1 and C2 groups presented counts of coliform at 45 °C < 3 MPN/g on all days of shelf life and coliforms at 35 °C ≤ 1100 MPN/g on the seventh day of shelf life. These coliform at 35 °C counts may be attributable to the multiplication of bacteria that were initially present in the MPP or contamination during storage. These results indicate the effectiveness of carvacrol with regard to coliform inhibition. In Brazil, the analysis of coliforms at 35 °C in MPV is not required by law (BRASIL 2001). However, this result becomes relevant because the presence of this group of bacteria reflects inadequate hygiene and sanitization conditions (Moore and Griffith 2002). Emiroğlu et al. (2010) reported a reduction of coliform counts of 1.6 log CFU/g in edible soybean coatings with *Oreganum heracleoticum* L. essential oil.

The T1 and T2 groups were treated with 312 and 625 μg/ml carvacrol, respectively, and both presented significant

Table 2 Psychrotrophic bacteria, mold and yeast and coliforms in MPP with an edible coating of cassava starch and carvacrol

Group	Coliforms at 35 °C (MPN/g)			Coliforms at 45 °C (MPN/g)			Psychrotrophic bacteria (log CFU/g)			MoldandYeast (log CFU/g)		
	Day											
	0	3	7	0	3	7	0	3	7	0	3	7
C1	$3.075 \pm 0{,}212^{A}$	998.08 ± 286.04^{A}	1100 ± 0^{A}	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$2.157 \pm 0{,}139^{A}$	$4.393 \pm 0{,}035^{A}$	$7.344 \pm 0{,}310^{A}$	2.476 ± 0.279^{A}	$3.466 \pm 0{,}128^{A}$	$4.835 \pm 0{,}222^{A}$
C2	$<3 \pm 0^{A}$	1100 ± 0^{A}	1100 ± 00^{A}	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$1.979 \pm 0{,}186^{A,B}$	4.227 ± 0.106^{B}	$6.957 \pm 0{,}024^{B}$	$2.024 \pm 0{,}265^{A,B}$	$2.882 \pm 0{,}230^{A}$	$2.581 \pm 0{,}266^{B}$
T1	$<3 \pm 0^{A}$	$<3 \pm 0^{B}$	$<3 \pm 0^{B}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$1.894 \pm 0{,}218^{B}$	$3.929 \pm 0{,}030^{C}$	$5.710 \pm 0{,}067^{C}$	$1.387 \pm 0{,}867^{B}$	$0.849 \pm 0{,}908^{B}$	$<2 \pm 0^{C}$
T2	$<3 \pm 0^{A}$	$<3 \pm {}^{B}$	$<3 \pm 0^{B}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$<3 \pm 0^{A}$	$1.894 \pm 0{,}160^{B}$	$3.960 \pm 0{,}024^{C}$	$5.467 \pm 0{,}219^{C}$	$2.048 \pm 0{,}373^{A,B}$	$<2 \pm 0^{C}$	$<2 \pm 0^{C}$

Values are mean followed by standard deviation. Means in the same column with different capital letters are significantly different ($p < 0.05$; Tukey's test). C1, MPP inoculated; C2, MPP inoculated + coating of cassava starch; T1, MPP inoculated + coating with MIC of carvacrol; T2, MPP inoculated + coating with 2MIC of carvacrol

reductions ($p < 0.05$) of psychrotrophic bacteria counts compared with the C1 and C2 groups on the third and seventh days of storage. A reduction of 1.9 log CFU/g was observed in the T2 group compared with the C1 group on the seventh day of shelf life. No significant difference ($p > 0.05$) was found between the T1 and T2 groups. Sousa et al. (2012) also observed a significant reduction of psychrotrophic bacteria counts in minimally processed leaf vegetables that were exposed to a solution of carvacrol at 600 μg/ml. Azeredo et al. (2011) studied *O. vulgare* essential oil and observed a reduction of psychrotrophic bacteria counts in MPV, confirming the results of the present study. The psychrotrophic bacteria counts reached 7 log CFU/g on the seventh day of storage in the C1 group, thus compromising the shelf life of the product, even when maintained under refrigeration.

Mold and yeast counts also significantly decreased ($p < 0.05$) in the T1 and T2 groups compared with the control groups. On the third day of shelf life, the T1 and T2 groups presented counts of 0.849 log CFU/g and <2 log CFU/g, respectively. On the seventh day, the T1 and T2 groups presented counts <2 log CFU/g. At this same time point, the C1 group presented counts of 3.466 and 4.835 log CFU/g, indicating the effectiveness of carvacrol in reducing mold and yeast. Sousa et al. (2012) evaluated the effect of carvacrol in MPV and observed a reduction of 2.9 log CFU/g in mold and yeast counts. These microorganisms are important in the deterioration of MPV because they reduce their shelf life and cause economic losses. In such cases, the application of an edible coating with carvacrol may be a viable option.

Effect of application of an edible coating of cassava starch with carvacrol on physicochemical parameters in minimally processed pumpkin

The effects of the edible coating with carvacrol that was applied to MPP on total titratable acidity, pH, weight loss, and soluble solids at various time points of shelf life are shown in Table 3. All groups (T1, T2, C1, and C2) presented an increase in total titratable acidity on the third day of shelf life, followed by a decline on the seventh day. This oscillation in total titratable acidity may be related to biochemical processes that are associated with respiratory metabolism, in which organic acids are utilized and converted to sugars (Martínez-Ferrer et al. 2002). A significant reduction ($p < 0.05$) of total titratable acidity was found between the treatment groups (T1 and T2) and control groups (C1 and C2) on the third and seventh days of storage. However, Habibunnisa et al. (2001) observed a slight increase in total titratable acidity (0.01 %) in MPP during 8 days of storage. The reduction of total titratable acidity is relevant because elevated acidity in MPV influences the sensorial characteristics of the product and decreases its shelf life.

A significant difference ($p < 0.05$) in pH was found between the treatment groups (T1 and T2) and C1 group on the first and

Table 3 Total titratable acidity, pH, soluble solids and mass loss in MPP with an edible coating of cassava starch and carvacrol

Group	Total titratable acidity (%)			pH			Soluble solids(%)			Mass loss (%)		
	Day											
	0	3	7	0	3	7	0	3	7	0	3	7
C1	0.196 ± 0.006^{A}	0.466 ± 0.003^{A}	0.195 ± 0.003^{A}	6.510 ± 0.033^{A}	6.506 ± 0.032^{A}	6.169 ± 0.042^{A}	4.450 ± 0.378^{A}	5.088 ± 0.516^{A}	5.263 ± 0.575^{A}	0 ± 0^{A}	2.650 ± 0.058^{A}	4.080 ± 0.044^{A}
C2	0.194 ± 0.003^{A}	0.391 ± 0.002^{B}	0.276 ± 0.002^{B}	$6.349 \pm 0.049^{B,C}$	6.409 ± 0.035^{A}	6.393 ± 0.0423^{B}	$4.125 \pm 0.212^{A,B}$	4.938 ± 0.350^{A}	5.150 ± 0.537^{A}	0 ± 0^{A}	0.660 ± 0.023^{B}	0.950 ± 0.035^{B}
T1	0.096 ± 0.302^{A}	0.285 ± 0.003^{C}	0.294 ± 0.002^{C}	6.303 ± 0.034^{B}	6.434 ± 0.048^{A}	6.401 ± 0.042^{B}	3.925 ± 0.225^{B}	$4.725 \pm 0.423^{A,B}$	5.188 ± 0.644^{A}	0 ± 0^{A}	0.200 ± 0.012^{C}	0.300 ± 0.014^{C}
T2	0.096 ± 0.002^{A}	0.267 ± 0.002^{C}	0.185 ± 0.002^{D}	6.361 ± 0.036^{C}	6.475 ± 0.030^{A}	6.365 ± 0.049^{B}	4.400 ± 0.424^{A}	4.238 ± 0.342^{B}	4.900 ± 0.453^{A}	0 ± 0^{A}	0.240 ± 0.013^{D}	0.350 ± 0.020^{D}

Means in the same column with different capital letters are significantly different ($p < 0.05$; Tukey's test). C1, MPP inoculated; C2, MPP inoculated + coating of cassava starch; T1, MPP inoculated + coating with MIC of carvacrol; T2, MPP inoculated + coating with 2MIC of carvacrol

seventh days of storage. The C2 group also differed from the C1 group on the same days. Similar results were reported by Oliveira and Cereda (2003), who observed lower pH values in controls compared with fruits that had an edible coating of cassava starch. In both the control and treatment groups, the pH was elevated on the third day of shelf life, with a slight decline on the seventh day. These varying pH values have been previous observed in MPV (Martínez-Ferrer et al. 2002; Lucera et al. 2010) and may be a consequence of metabolism during respiration, an increase in CO_2 headspace concentrations, and compounds that are produced by microorganisms (Martínez-Ferrer et al. 2002; Lucera et al. 2010; Rico et al. 2007).

The amounts of soluble solids slightly increased over the days of storage in both the control and treatment groups, with values of 4.9 to 5.2°Bx on the last day of shelf life. Sasaki et al. (2006) found that MPP presented a slight increase in the amount of soluble solids during the storage period (5.3°Bx). According to these authors, this increase in soluble solids was attributable to the stress that is caused by minimal processing. According to Silva et al. (2009), this observation is also attributable to the loss of water from the product, which may occur immediately after processing. Cortez-Vega et al. (2014) evaluated different edible coatings in pumpkin and observed reductions of soluble solids in all of the samples during the storage period.

Overall, no significant difference ($p < 0.05$) in soluble solids was found between the treatment and control groups. Souza et al. (2009) studied eggplant with an edible coating of cassava starch and did not find significant differences in the amount of soluble solids between the treatment and control groups.

A significant difference ($p < 0.05$) in weight loss was observed between the treatment and control groups and between the C1 and C2 groups on the third and seventh days of shelf life. Carvacrol treatment significantly reduced weight loss in the T2 group compared with the C1 group (3.73 %) and in the T1 group compared with the C1 group (3.78 %) on the last day of storage. Chiumarelli et al. (2010) reported that fresh-cut mango with an edible coating of cassava starch presented a significant reduction of weight loss compared with the uncoated control group. According to Chiumarelli et al. (2010), the use of hydrophilic coatings, such as starch, has limitations that are related to water vapor barrier properties. The presence of carvacrol in the edible coating, because of its hydrophilic characteristics (Burt 2004), may help lessen water loss from vegetables. Controlling weight loss in MPV is important because it directly affects the appearance of the product, a factor that influences the purchase decision of these products.

Conclusion

The results of the present study showed that carvacrol, when added to an edible coating of cassava starch, effectively

inhibited both pathogenic bacteria and autochthonous microbiota in MPP. The addition of carvacrol in the edible coatings also did not affect the physicochemical parameters analyzed and was protective against weight loss in MPP. These results strengthen the possibility that carvacrol can be added to edible coatings to improve the microbiological safety of MPP.

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References

Association of oficial analytical chemists – AOAC (1992) Official methods of analysis, 16th edn. AOAC, Washington

Azeredo GA, Stamford TLM, Nunes PC, Gomes Neto NJ, Oliveira MEG, Souza EL (2011) Combined application of essential oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. Food Res Int 44:1541–1548

Baskaran SA, Upadhyay A, Kollanoor-Johny A, Upadhyaya I, Mooyottu S, Amalaradjou MAR, Schreiber D, Venkitanarayanan K (2013) Efficacy of plant-derived antimicrobials as antimicrobial wash treatments for reducing enterohemorrhagic *Escherichia coli* O157:H7 on apples. J Food Sci 78:1399–1404

BRASIL (2001) Ministério da saúde, Agência Nacional de Vigilância Sanitária - ANVISA. Resolução RDC nº 12 de 02 de janeiro de 2001. Regulamento Técnico Sobre Os Padrões Microbiológicos para Alimentos. Diário Oficial, Brasília

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Chiumarelli M, Pereira LM, Ferrari CC, Sarantópoulos CIGL, Hubinger MD (2010) Cassava starch coating and citric acid to preserve quality parameters of fresh-cut "tommy Atkins" mango. J Food Sci 75: E297–E304

Clinical Laboratory Standards Institute (2012) (2012). performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. CLSI document M100-S22. Wayne, PA, Clinical and Laboratory Standards Institute

Cortez-Vega WR, Piotrowicz IBB, Prentice C, Borges CD (2014) Influence of different edible coatings in minimally processed pumpkin (*Cucurbita moschata* duch). Int Food Res J 21:2017–2023

Downes FP, Ito K (2001) Compendium of methods for the microbiological examination of foods, 4a edn. American Public Health Association, Washington, DC

Ehivet FE, Min B, Park MK, Oh JH (2011) Characterization and antimicrobial activity of sweet potato starch-based edible film containing origanum (*Thymus capitatus*) oil. J Food Sci 76:178–184

Emiroğlu ZK, Yemiş GP, Coşkun BK, Candoğan K (2010) Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci 86:283–288

Feng P (2012) *Escherichia coli* (ETEC, EPEC, EHEC, EIEC). In: Lampel KA (ed) Bad Bug Book: foodborne, pathogenic microorganisms and natural toxins, 2nd edn. FDA, New Hampshire, pp 68–81

Guarda A, Rubilar JF, Miltz J, Galotto MJ (2011) The antimicrobial activity of microencapsulated thymol and carvacrol. Int J Food Microbiol 146:144–150

Habibunnisa B,R, Prasad R, Shivaiah KM (2001) Storage behaviour of minimally processed pumpkin (*Cucurbita maxima*) under modified atmosphere packaging conditions. Eur Food Res Technol 212:165–169

Hait J (2012) *Staphylococcus aureus*. In: Lampel KA (ed) Bad Bug Book: foodborne, pathogenic microorganisms and natural toxins, 2nd edn. FDA, New Hampshire, pp 85–91

Hammack T (2012) *Salmonella* species. In: Lampel KA (ed) Bad Bug Book: foodborne, pathogenic microorganisms and natural toxins, 2nd edn. FDA, New Hampshire, pp 9–13

Hyldgaard M, Myaind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:1–24

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (2010) *Pesquisa de orçamento familiares*: *2008–2009*. IBGE, Rio de Janeiro, 282p

Iturriaga L, Olabarrieta I, Marañón M (2012) Antimicrobial assays of natural extracts and their inhibitory effect against *listeria innocua* and fish spoilage bacteria, after incorporation into biopolymer edible films. Int J Food Microbiol 158:58–64

Kuorwel KK, Cran MJ, Sonneveld K, Miltz J, Bigger SW (2011) Antimicrobial activity of natural agents coated on starch-based films against *Staphylococcus aureus*. J Food Sci 76:531–537

Lampel KA (2012) *Aeromonas* species. In: Lampel KA (ed) Bad Bug Book: foodborne, pathogenic microorganisms and natural toxins, 2nd edn. FDA, New Hampshire, pp 54–56

Lucera A, Costa C, Mastromatteo M, Conte A, Nobile MA (2010) Influence of different packaging systems on fresh-cut zucchini (*Cucurbita pepo*). Innov Food Sci Emerg Technol 11:361–368

Maistro LC, Miya NT, Sant'Ana AS, Pereira JL (2012) Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP e Brazil, as assessed by traditional and alternative methods. Food Control 28:258–264

Martínez-Ferrer M, Harper C, Pérez-Muñoz F, Chaparro M (2002) Modified atmosphere packaging of minimally processed mango and pineapple fruits. J Food Sci 67:3365–3371

Molinos AC, Abriouel H, López RL, Omar NB, Valdivia E, Gálvez A (2009) Enhanced bactericidal acitivity of enterocin AS-48 in combination with essential oils, natural bioactive compounds and chemical preservatives against *Listeria monocytogenes* in ready-to-eat salad. Food Chem Toxicol 47:2216–2223

Moore G, Griffith C (2002) A comparison of surface sampling methods for detecting coliforms on food contact surfaces. Food Microbiol 19: 65–73

Muriel-Galet V, Cerisuelo JP, López-Carballo G, Lara M, Gavara R, Hernández-Muñoz P (2012) Development of antimicrobial films for microbiological control of packaged salad. Int J Food Microbiol 157:195–201

Oliveira MA, Cereda MP (2003) Postharvest of peaches (*Prunus persica* L. Bastsch) covered with film-forming of cassava starch as alternative to the commercial wax. Ciênc Tecnol Aliment 23:28–33

Rico D, Martín-Diana AB, Barat JM, Barry-Ryan C (2007) Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci Technol 18:373–386

Sangsuwan J, Rattanapanone N, Auras RA, Harte BR, Rachtanapun P (2009) Factors affecting migration of vanillin from chitosan/methyl cellulose films. J Food Sci 74:549–555

Sant'Ana AS, Barbosa MS, Destro MT, Landgraf M, Franco BDGM (2012) Growth potential of *salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. Int J Food Microbiol 157:52–58

Sasaki FF, Del Aguila JS, Gallo CR, Ortega EMM, Jacomino AP, Kluge RA (2006) Alterações fisiológicas, qualitativas e microbiológicas Durante o armazenamento de abóbora minimamente processada em diferentes tipos de Corte. Hortic Bras 24:170–174

Seo YH, Jang JH, Moon KD (2010) Occurrence and characterization of enterotoxigenic *Staphylococcus aureus* isolated from minimally processed vegetables and sprouts in Korea. Food Sci Biotechnol 19:313–319

Silva AVC, Oliveira DSN, Yaguiu P, Carnelossi MAG, Muniz EN and Narain N (2009) Temperature and packaging of minimally processed pumpkin (Curcubita moschata). Food Sci Technol 29:391–394

Sousa JP, Azerêdo GA, Torres RA, Vasconcelos MAS, Conceição ML, Souza EL (2012) Synergies of carvacrol and 1,8-cineole to inhibit bacteria associated with minimally processed vegetables. Int J Food Microbiol 154:145–151

Souza PA, Aroucha EMM, Souza AED, Costa ARFC, Ferreira GS, Bezerra Neto F (2009) Conservação pós-colheita de berinjela com revestimentos de fécula de mandioca ou filme de PVC. Hortic Bras 27:235–239

Yun J, Fan X, Li X (2013) Inactivation of *Salmonella enterica* serovar typhimurium and quality maintenance of cherry tomatoes treated with gaseous essential oils. J Food Sci 78:458–464