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Antilisterial activity of olive-derived polyphenols: an experimental study on meat preparations

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Abstract
Pork meat and processed pork products have been linked to multiple listeriosis outbreaks worldwide during the past years. Specifically, it has been highlighted that minced pork meat is easily perishable and may increase the growth of *Listeria monocytogenes*, which could be harmful to the general public's health. This study aimed to investigate the potential application of olive oil mill wastewater polyphenolic and red beet extracts as natural antimicrobial agents for *L. monocytogenes* growth control in burgers. The minced pork meat was mixed with the extracts and experimentally inoculated with *L. monocytogenes*, then molded into vacuum-packaged and cold-stored (4±1°C) burgers kept under alternating exposure to fluorescent light. The *L. monocytogenes* enumeration was performed on burgers at 0, 2, 5, and 10 days of shelf life. In uninoculated burgers, physicochemical (pH, water activity, color) and sensory determination (descriptive sensory analysis) were also conducted. At the end of storage, the samples treated with olive-derived extract showed the lowest value of *L. monocytogenes* (approximately 1.3 Log CFU/g). The physicochemical and sensory traits of burgers have benefited from the addition of both olive-derived and red beet extracts. Results suggest that olive mill wastewater polyphenolic extracts could be added to minced pork meat products to act as a natural antimicrobial agent.

Introduction
Listeria monocytogenes is the microorganism responsible for listeriosis, a severe illness primarily transmitted through contaminated food, including pork products (Branciari *et al*., 2020; Zamuz *et al.*, 2021; EFSA and ECDC, 2023). Pork is the world's second-most-consumed meat, following poultry (FAO, 2021), and in 2021, 19.9 and 104.8 million tons of pork meat were consumed in Europe and globally, respectively (Lagarde *et al.*, 2023). Over the past two decades, pork meat and processed pork products, including ready-to-eat food products, contaminated with the pathogenic bacterium *L. monocytogenes* have been incriminated in several listeriosis outbreaks around the world (Painset *et al*., 2019; EFSA and ECDC, 2023).
Studies have shown that *L. monocytogenes* is a major hazard along the value chain from pig farming to the pork industry (Lagarde *et al*., 2023). Contamination of pork products can occur at various steps, from primary production up to retail, and different sources such as the living pigs and the food processing environment (Lagarde *et al*., 2023).
Furthermore, it is known that the mincing process of raw meat causes the disruption of muscle structure and impairs the physicochemical stability of the food matrix thus favoring microbial growth (Mancini *et al.*, 2017). In particular, it has been observed that minced pork has the potential to support the growth of *L. monocytogenes*, posing a risk to public health (Meloni, 2015).
Additionally, the prevalence of *L. monocytogenes* in meat products, including pork, has been increasing over the years and European food safety authorities nowadays consider “mixed meat and products thereof” (including pork meat) as one of the three most represented categories in listeriosis foodborne outbreaks (EFSA Panel on Biological Hazards *et al.,* 2018). In this context, it is crucial to implement measures to prevent and control *L. monocytogenes* contamination in minced pork and other pork products to ensure food safety.
Antimicrobial additives can be effective in controlling *Listeria* in pork minced meat, and several studies have explored the use of natural antimicrobials in meat products to inhibit the growth of *L. monocytogenes*.
Plant powders, such as apple, onion, black currant berries, garlic, tomato, and rhubarb petioles, have shown inhibitory effects on microbial growth in raw minced pork (Ranucci *et al*., 2019; Zamuz *et al*., 2021).
However, natural preservatives with antilisterial effects in meat products, such as essential oils and plant extracts, when applied *in vivo*, often result in reduced antimicrobial activity compared to *in vitro* studies (Zamuz *et al*., 2021).
Among different natural antimicrobials used in food, treated olive oil by-products have been considered for their high content of phenolic substances (Roila *et al.,* 2022). For instance, olive mill

wastewater (OMWW), a liquid by-product in the olive industry, is characterized by a high content of phenolic compounds (Servili *et al*., 2009). The use of these bioactive molecules in food resulted in the growth inhibition of different bacterial strains (Roila *et al.,* 2019; Roila *et al*., 2022). The red beetroot (*Beta vulgaris*) is known to represent a source of bioactive phytochemicals, such as polyphenols and flavonoids and to contain water-soluble pigments known as betalains (Marrone *et al*., 2021, Stagnari *et al.*, 2014).
The aim of this work was to investigate the potential application of olive oil mill wastewater polyphenolic extract and red beet extract as a natural antimicrobial agent for *L. monocytogenes* growth control in minced pork meat products.

Materials and Methods

Natural extracts

Red beet (*Beta vulgaris*) natural extract is obtained from fresh raw material (100% beetroot) subjected to technological processes including the freeze-dried method to ensure its durability and stability and it is represented by a fine powder (<0.5 mm). The other tested extract, Stymon 50W(Stymon Natural Products P.C., Patras, Greece), derives from OMWW produced during the olive oil production from Koroneiki cultivar olive (*Olea Europaea L.*) and it is formulated in encapsulated fine powder(<0.5 mm). It is produced based on a patented process (GR1010150, EP4049543A1) based on green technologies. This latter by-product extract was chosen by the authors because it has already been attributed to the food grade certification (certificate released by General Chemical State Laboratory of Greece n. 30/003/000/3810) and the extraction treatment was carried out using only water without the addition of any chemicals.

Bacterial cultures and inoculum preparation

A multi-strain mix of *L. monocytogenes* was used in the study to carry out a challenge test. The mix consisted of an authenticated reference strain (WDCM 00021) and two strains isolated from pork meat and meat products. The inoculum to be used in the study was prepared according to the guidelines of the European Union Reference Laboratory for *L. monocytogenes* (2021).
The bacterial cultures used in the study were regenerated into Brain Heart Infusion (BHI) (Oxoid, Basingstoke, UK) by incubation at 37°C for 24 hours. Aliquots of each activated culture (0.1 mL) were transferred into tubes containing BHI and incubated for 72 hours at 10±1°C, a temperature close to the storage condition of the product. At the end of the incubation time, the cultures were equally combined to form the multi-strain cocktail, which was subsequently centrifuged at 2178 g at 10°C for 5 minutes, the supernatant was discarded and the pellet was resuspended in 10 mL of sterile physiological solution. Counts were performed by serial decimal dilution and inoculation in Agar Listeria Ottaviani Agosti (ALOA Selective Supplement, ALOA Enrichment Supplement; Biolife, Italy) incubated at 37°C for 24-48 hours and the multi-strain suspension was diluted with a sterile physiological solution in order a final concentration in meat batter of approximately 2 Log CFU/g. The contamination of the pork hamburger was performed on ground meat before the shaping.

Burger formulation

The meat for the preparation of pork burger was obtained from cuts (shoulder muscle and loin) of pig reared and slaughtered in Italy in accordance with European Union Regulation (European Parliament and Council of the European Union, 2004 - Regulation (EC) No. 853/2004). Meat preparations were produced in a pilot plant of the Department of Veterinary Medicine, University of Perugia. Meat cuts were ground twice in a professional trimmer equipped with a 4 mm hole plate. The ground meat was divided into four different formulations destined for the evaluation of physicochemical characteristics and sensory analysis: i) control hamburger (CTR-H) - the minced meat was mixed with a sterile saline solution and 1% of salt; ii) control hamburger + Stymon 50 W (CTR-S1) produced by adding and hand mixing 0.0156 g/g of Stymon 50W to control hamburger dough; iii) control hamburger + Stymon 50 W (CTR-S2) produced by adding and hand mixing

0.03125 g/g of Stymon 50W to control hamburger dough; iv) control hamburger + beetroot extract (CTR-B) produced by adding and hand mixing 0.15% of beetroot extract;
Moreover, to evaluate the effect of the tested extracts on the behavior of *L. monocytogenes* during hamburger shelf-life, the following experimental groups were formulated: i) *Lm*-control burger (Lm-C); the minced meat was mixed with a suspension of the multi-strain mix of *L. monocytogenes* at a final concentration of approximately 2 Log CFU/g; ii) *Lm* hamburger + S (Lm-S1); produced by adding and hand mixing 0.0156 g/g of Stymon 50W to *Lm*-control burger dough; iii)*Lm* hamburger + S (Lm-S2); produced by adding and hand mixing 0.03125 g/g of Stymon 50W to *Lm*-control burger dough; iv) *Lm* hamburger + beetroot(Lm-B); produced by adding and hand mixing 0.15 % of beetroot extract to *Lm*-control burger dough.

Storage conditions and sampling
Each hamburger was molded (about 100g each), vacuum packaged and stored at 4±1°C for 10 days under alternating exposure to fluorescent light (12 hours light/12 hours darkness) to simulate retail storage conditions.

Detection and enumeration of Listeria monocytogenes
The detection of *L. monocytogenes* was conducted according to the EN ISO 11290-1 reference method (ISO, 2017a) before burger preparation in the untreated meat dough to assess the natural presence of the pathogen.
The enumeration of *L. monocytogenes* in meat preparation was conducted in triplicate in Lm-C, Lm-S2, Lm-S1, Lm-B samples on the day of burger preparation (day 0) and after 2, 5, and 10 days following the EN ISO 11290-2 (ISO, 2017b) reference method for bacterial enumeration. Counting results were reported in terms of Log CFU g^{-1}.

Physicochemical determinations
The physicochemical determinations were carried out at 0 (day of production), 5 and 10 days of storage time on uncontaminated samples (CTR-H, CTR-S1, CTR-S2, CTR-B).
All analyses were carried out on three replicates. The pH measurements were performed through a puncture electrode probe connected to a portable pH meter (Mettler Toledo Inc., Columbus, OH, USA). Water activity (a_w) was measured at 25°C with the a_w recorder AquaLab, series 3, Model TE (Decagon Devices Inc., Pullman, WA, USA).
The color measurements were performed on raw pork burger surface by measuring Color coordinates (CIE, 1976) using a Minolta Chromameter CR400 (Minolta, Osaka, Japan — light source of D65 calibrated against a standard white tile). The results were expressed as lightness (L*), redness (a*), and yellowness (b*). To examine and relate differences in color values to visually perceived differences, the calculation of ΔE was made with Equation 1:

$$\Delta E=[(L^*-L^*_0)^2 + (a^*-a^*_0)^2+(b^*-b^*_0)^2]^{0.5} \qquad [Eq. 1]$$

where ΔE is equal to the square root of the sum of squares of the differences between L*, a*, and b* coordinates of the treated sample and control.

Sensory analysis
A descriptive sensory analysis was performed using an 8-member panel, which was trained following the criteria of ISO 85861:1993 (ISO, 1993). The attributes were generated in three pre-testing sessions as described by Branciari *et al.*(2015). For quantification of the intensity of the attribute's discoloration, off-odor, and off-flavor, the panelist used a five-point descriptive scale according to ISO 13299:2003 (ISO, 2003). The assessors evaluated the off-odor and discoloration on raw hamburgers while for off-flavor and overall liking tasted samples of burgers, which were placed on steel trays covered with aluminum foil and oven-cooked at 180°C (10% relative humidity) for approximately 25 minutes to an internal temperature of 71.1°C, which was measured

using a thermometer with a handheld probe (TES-1300, TES Electrical Electronic Co., Taipei, Taiwan). Hamburgers were cooked without salt and spices. The cooked burger was cut into 2×2×2 cm pieces and kept warm until they were served. In the session, one sample/group was monadically served on white plastic plates identified by 3 random digit codes.

Results and Discussion

The microbial analysis on pork meat dough performed before the formulation of experimental groups, attested that *L. monocytogenes* was undetectable, thus excluding the natural contamination of the product.

The analytical results of *L. monocytogenes* growth during storage of refrigerated and vacuum-packed pork burgers are reported in Table 1. As shown, a significant ($p<0.05$) increase for the entire duration of the shelf-life was observed for Lm-C as well as for Lm-B, corroborating the results reported elsewhere referring to a variable or negligible antimicrobial effect for similar extracts (Marrone et al., 2021, Roila et al., *in press*). However, it is important to consider that the effect of these extracts is strongly influenced by the differences in microbial species, food matrices and extract composition.

The results of the Lm-S1 group show that during the shelf-life the loads of *L. monocytogenes* were equivalent, suggesting that this specific concentration of polyphenolic extracts is able to inhibit the growth of the tested pathogen without exerting strong bactericidal effects. The behavior of *L. monocytogenes* in the Lm-S2 group shows an initial phase of no significant fluctuation of the microbial concentration, while from day 5 a decrease of the initial inoculum was registered ($p<0.05$).

Considering the end of the shelf-life (day 10), significant ($p<0.05$) differences between experimental groups were observed and the lowest value was registered in those burgers formulated with the highest amount of Stymon 50 W (Lm-S2). This outcome suggests that at this concentration the bioactive molecules contained in the extract are able to affect the pathogen behavior by limiting its growth, as preliminary suggested by an *in vitro* assay.

Previous results suggest an existing antibacterial activity of polyphenol extracts against the *L. monocytogenes*; the antimicrobial activity of polyphenol extracts seems to be exerted more efficaciously towards Gram-positive bacteria (Altissimi *et al.*, 2024).

L. monocytogenes growth curves for Lm-C, Lm-S1, Lm-S2 and Lm-B were drawn using DMFit version 2021 (ComBase online freeware) by fitting the experimental data to the model of Baranyi and Roberts (1994). From this analysis, the growth parameters, namely the initial values (Log CFU/mL), the duration of the lag phase (λ, hours), the maximum growth rate (μmax) (Log CFU/mL/h) and final values (Log CFU/mL), were defined.

The estimated growth curves of the four different pork burger groups formulation are reported in Figure 1. The trend described by the modeled data confirms, graphically, the behavior of *L. monocytogenes* outlined by experimental enumeration data collected during pork burger shelf-life.

The kinetic parameters characterizing the growth curves of *L. monocytogenes* in the four experimental groups were obtained by modeling growth data by means of the Baranyi equation (Table 2).

These data reveal that the addition of polyphenols in pork burgers resulted in an extended lag phase for Lm-S2 ($p<0.05$). The values of μmax for all the experimental groups are equivalent to Lm-C ($p>0.05$) albeit Lm-S2 even reached negative values, likely determining the bactericidal effect of this concentration of polyphenolic extract.

Analogous conclusions have previously been reported for the same compounds against other microbial populations (Roila *et al.,* 2019; Roila *et al.,* 2022). The mechanisms by which olive-derived polyphenols exert antimicrobial activity have been discussed in the literature. In particular, their interaction with amino acids, proteins and membrane molecules, which leads to membrane permeabilization and bacterial cell lysis, is likely responsible for their antimicrobial properties (Medina *et al.*, 2009).

Furthermore, it has been reported that tyrosol hinders cyclooxygenase enzyme activity, while hydroxytyrosol has been attributed to the capacity of denaturing proteins (Medina *et al.*, 2009). Additionally, polyphenolic compounds have been shown to be effective iron scavengers and a lack of iron can inhibit bacteria growth by affecting nucleic acid synthesis (Lim *et al.*, 2013).

The results regarding the pH and a_w were not influenced by the treatment either at the beginning or at the end of storage, the sample exhibits a similar value.

Colorimetric parameters (L*, a*,b* coordinates, mean±standard deviation) of the four types of burgers packed under anaerobic conditions at 1 and 10 days of storage are shown in Figure 2. CTR-B exhibited different color characteristics in comparison with the other burgers depending on the addition of beetroot both at 0 and 10 days of storage (Table 3). The color characteristics of pork burgers changed significantly during refrigerated storage in the control group (Table 3). Lightness (L* values) and redness (a* values) decreased during refrigerated storage, and the yellowness (b* values) tended to increase over time as reported in the literature (Estévez *et al*., 2005). On the contrary in the case of L* and a* both values were similar to initial values at the end of the storage in all groups, except for CTR-H. Usually, a* values have been related to meat red components (hemopigments) and their interconversions, due to oxidation processes that can affect both the hemopigments and fat. The discoloration of pork burger during refrigeration was affected by the addition of polyphenolic and beetroot extracts because the total color difference (ΔE) values in CTR-S1, CTR-S2, and CTR-B were significantly smaller than in CTR-H. According to Clydesdale (1991), the color modifications instrumentally measured can be considered as slightly noticeable when the total color difference (ΔE) values are higher than 2 and noticeable for consumers when are higher than 5 (Dos Santos *et al.,* 2023). In this sense, the addition of polyphenols successfully inhibited, albeit partially, the discoloration of the burger after 10 days, since the changes instrumentally measured were almost imperceptible.

The results of sensory analysis of the four formulations of pork meat preparations are reported in Table 4.

The panel confirmed color stability in treated groups in comparison with the CTR, indicating that polyphenols and beetroot can prevent color modification. The color stability in the sample with the addition of olive polyphenols could be attributed to the action of these compounds, which act as potent radical quenchers and metal chelators, most likely interfering with the oxidation cycle at the propagation stage, preventing the formation of additional lipid radicals (Choe and Min, 2009; Allen and Cornforth, 2010). Polyphenol most likely inhibits lipid oxidation by binding metals, iron and copper, and stabilizing them in an inactive or insoluble form. Meat color can thus be maintained due to the antioxidant ability to limit lipid oxidation, preventing the formation of reactive aldehydes and iron-catalyzed lipid oxidation (Allen and Cornforth, 2010; Dal Bosco *et al*., 2012).

On the other hand, burgers with the addition of beetroot show color stability due to betalains, the most important compounds present in red beetroot. Betalains are hydrophilic nitrogenous pigments, and these compounds have an extreme potential as natural colorants. Studies in the literature demonstrate that refrigeration storage increases the stability of the pigment (Domínguez *et al.*, 2020). The time for the formation of off-odors and off-flavors (Table 4) was also influenced by phenolic compounds, confirming the findings of other studies that showed that off-flavors and off-odors of meat preparation were caused by aldehydes produced during lipid oxidation. The increased presence of polyphenols in ground meat most likely affects lipid stability during product storage, resulting in delayed development of off-odor and off-flavor (Descalzo *et al.*, 2008). Such a delay in off-flavor development was not found in the sample with beetroot addition. This could be explained by the fact that betalains do not inhibit lipid oxidation, as shown by Jin *et al.* (2014). Indeed, the authors reported a significant increase in redness and sensory acceptability of color in pork sausages produced with beetroot powder extract, but no significant effect in terms of lipid oxidation. In terms of overall palatability, panelists perceived a slightly bitter taste in the CTR-S2 sample, whereas the bitter taste was almost imperceptible in the CTR-S1 sample, resulting in the most preferred sample at the end of storage. The bitter taste did not negatively affect the CTR-S2 sample at the end of

storage compared to CTR-H and CTR-B, probably due to the inhibition of lipid oxidation by the added polyphenols (Roila *et al.,* 2022).

Conclusions

The adoption of polyphenolic extract in pork meat burgers is able to hinder the growth of *L. monocytogenes* during refrigerated and under vacuum shelf-life. Furthermore, both olive-derived and red beetroot extracts prevented physicochemical and sensory characteristics depletion. It can be concluded that the use of natural compounds in meat products, to act as natural preservatives, could represent a feasible and valuable strategy to improve their quality and safety traits. Specific studies are needed to define the most suitable application of each natural compound in relation to its chemical composition and the targeted food product.

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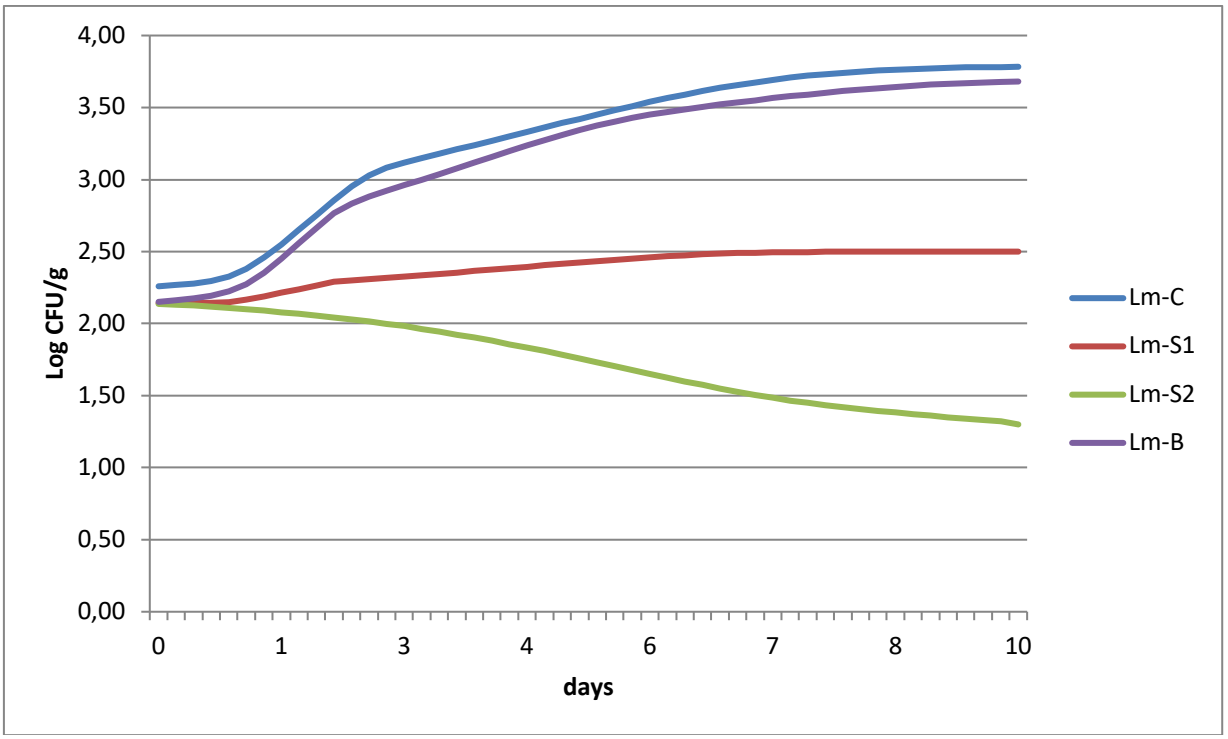

Figure 1. Estimated growth curves of *Listeria monocytogenes* applying the Baranyi and Roberts model to growth data of experimental groups. Lm-C, basic pork burger recipe with the addition of the *L. monocytogenes* inoculum; Lm-S1, basic pork burger recipe with the addition of 0.0156 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-S2, basic pork burger recipe with the addition of 0.03125 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-B, basic recipe with the addition of 0.15% of beetroot extract and the *L. monocytogenes* inoculums.

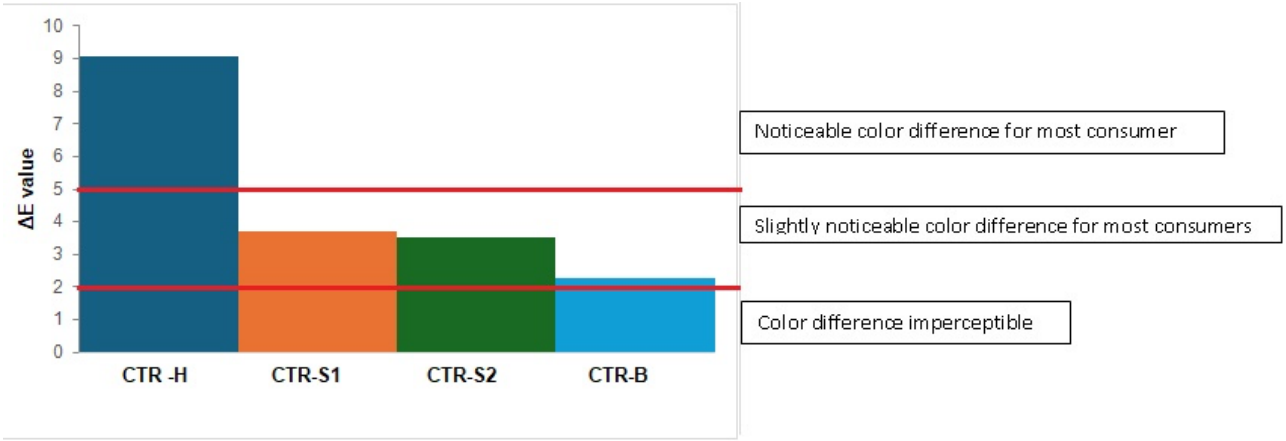

Figure 2. Global color difference within each treatment comparing 10 days of storage to 0 days of storage. CTR-H, burger with no addition; CTR-S1, burger with an addition of 0.0156 g/g of Stymon 50 W; CTR-S2, burger with an addition of 0.03125 g/g of Stymon 50 W; CTR-B, burger with an addition of 0.15 % of beetroot extract; ΔE, color difference - $\Delta E=[(L^*-L^*_0)^2+(a^*-a^*_0)^2+(b^*-b^*_0)^2]^{0.5}$

Table 1. Microbial counts (Log CFU/g) of *Listeria monocytogenes* in pork burger stored at 4°C under vacuum for 10 days.

	Day 0	Day 2	Day 5	Day 10
Lm-C	2.27 ± 0.32a	2.70 ± 0.56 Bb	3.37 ± 0.15 Cc	3.87 ± 0.35 Cd
Lm-S1	2.15 ± 0.10	2.30 ± 0.10 A	2.43 ± 0.03 B	2.5 ± 0.10 B
Lm-S2	2.11 ± 0.11 b	2.02 ± 0.04 Ab	1.73 ± 0.23 Aab	1.30 ± 0.14 Aa
Lm-B	2.14 ± 0.20 a	2.59 ± 0.08 Ba	3.44 ± 0.28 Cb	3.62 ± 0.59 Cb

Lm-C, basic pork burger recipe with addition of the *L. monocytogenes* inoculum; Lm-S1, basic pork burger recipe with the addition of 0.0156 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-S2, basic pork burger recipe with the addition of 0.03125 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-B, basic recipe with the addition of 0.15 % of beetroot extract and the *L. monocytogenes* inoculum. Different letters in the same row (a, b, c, d) indicate differences between mean values during sampling times ($p \leq 0.05$); different letters in the same column (A, B, C,) indicate differences between mean values for different experimental groups ($p \leq 0.05$).

Table 2. Growth dynamic parameters estimated by the Baranyi and Roberts model for *Listeria monocytogenes* growth in pork burgers treated with Stymon 50W at different levels (Lm-S1 and Lm-S2) and beetroot extract (0.1%) compared to control samples (Lm-C).

	Lm-C	Lm-S1	Lm-S2	Lm-B
Initial values (Log CFU/g)	2.26 ± 0.32	2.14 ± 0.10	2.10 ± 0.15	2.15 ± 0.14
λ (h)	24.41 ± 3.94 a	30.03 ± 10.96 a	61.76 ± 54.45 b	23.54 ± 5.53 a
μmax (Log CFU/g/h)	0.04 ± 0.02	0.01 ± 0.01	-0.01 ± 0.00	0.03 ± 0.02
Final value (Log CFU/g)	3.79 ± 0.37	2.55 ± 0.07		3.69 ± 0.36

λ, lag phase; μmax, maximum growth rate; Lm-C, basic pork burger recipe with the addition of the *L. monocytogenes* inoculum; Lm-S1, basic pork burger recipe with the addition of 0.0156 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-S2, basic pork burger recipe with the addition of 0.03125 g/g of Stymon 50 W and the *L. monocytogenes* inoculums; Lm-B, basic recipe with the addition of 0.15 % of beetroot extract and the *L. monocytogenes* inoculum. Different letters in the same row (a, b, c, d) indicate differences between mean values during sampling times ($p \leq 0.05$).

Table 3. Lightness, redness, and yellowness values of burger samples immediately after production (day 1 of storage) and at the end of storage (days 10 of storage).

	1 day of storage				P-value	10 days of storage				P-value
sample	**CTR - H**	**CTR-S1**	**CTR-S2**	**CTR-B**		**CTR - H**	**CTR-S1**	**CTR-S2**	**CTR-B**	
L	51.48± 0.83a	51.66± 1.46a	51.80± 1.32a	55.57± 0.71b	0.02	46.18± 0.11a	50.11± 1.92b	50.71± 1.75b	55.04± 1.52c	<0.001
a*	12.63± 1.20	11.30± 0.75	11.41± 0.32	17.62± 1.35	<0.001	8.94±0.08a	10.93± 0.54b	11.03± 0.58b	16.99± 0.31c	<0.001
b*	4.97±1.66a	6.28±0.19a	5.77±0.35a	8.60±1.07b	0.005	11.06± 0.4c1	9.43±0.25b	9.03±0,26b	7.90±0.29a	<0.001

CTR-H, burger with no addition; CTR-S1, burger with an addition of 0.0156 g/g of Stymon 50 W; CTR-S2, burger with an addition of 0.03125 g/g of Stymon 50 W; CTR-B, burger with an addition of 0.15 % of beetroot extract; L, lightness; a*, redness; b*, yellowness.

Table 4. Mean sensory values of pork burger.

Attributes	**Sample**	**Days of Storage**			**S**	**T**	**SXT**	**SEM**
		0	**5**	**10**				
Off odour[1]	CTR-H	1.0A	2.63bB	4.5bC	<0.001	<0.001	<0.001	0.191
	CTR-S1	1.13A	1.25aA	2.88aB				
	CTR-S2	1.38A	1.25bA	3.13aC				
	CTR-B	1.00A	2.38bB	4.38bC				
Discolouration[1]	CTR-H	1.00A	4.0bB	4.88bC	<0.001	<0.001	<0.001	0.092
	CTR-S1	1.13A	1.38aA	2.00aB				
	CTR-S2	1.13A	1.25aA	1.88aB				
	CTR-B	1.00A	1.00aA	1.88aB				
Off flavour[1]	CTR-H	1.00aA	3.00cB	5.00bC	<0.001	<0.001	<0.001	0.161
	CTR-S1	1,25bA	1.38aB	2.88 aC				
	CTR-S2	1.5cA	1.87bA	3.125 aB				
	CTR-B	1.00aA	2.75cB	4.63bC				
Overall liking[2]	CTR-H	5.00bC	2.88aB	1.13aA	<0.001	<0.001	<0.001	0.152
	CTR-S1	4.88bB	4.75cB	4.13cA				
	CTR-S2	4.00aA	3.63bA	3.63dA				
	CTR-B	5.00bC	4.5cB	2.88bA				

CTR-H, burger with no addition; CTR-S1, burger with an addition of 0.0156 g/g of Stymon 50 W; CTR-S2, burger with an addition of 0.03125 g/g of Stymon 50 W; CTR-B, burger with an addition of 0.15 % of beetroot extract; [1]scale: 1, none; 2, slight; 3, small; 4, moderate 5, extreme; [2]scale: 1 (1=dislike very much, 2=dislike slightly, 3=neither like nor dislike, 4=like slightly, 5=like very much); abc values in the same column with different letters means significant differences ($p<0,05$); ABC values in the same row with different letters means significant difference ($p<0,05$); S, sample; T, time.