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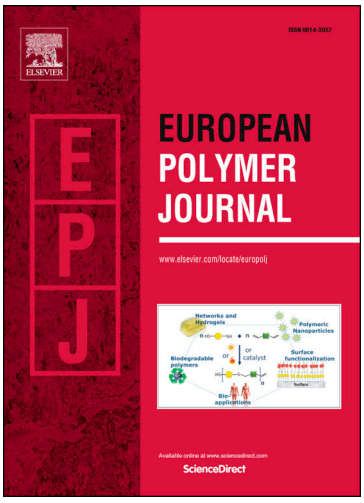

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Antibacterial biopolymeric foams: Structure–property relationship and carvacrol release kinetics

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Abstract

In this work, the feasibility of antibacterial biopolymeric foams containing carvacrol (CRV) for potential food packaging applications was investigated. Sodium bicarbonate (SB) was chosen as foaming agent and a commercial biodegradable polymer, Mater-Bi® (MB), as a matrix. MB/SB and MB/SB/CRV systems were prepared by melt mixing and the foaming process was conducted in a laboratory press. The influence of foaming agent and antibacterial additive content was investigated. The foamed samples were characterized through morphological analysis, mechanical tests and measurements of CRV release kinetics. Moreover, a mathematical model, i.e. power law model, was used to fit the release data in order to investigate the release mechanism. The antibacterial activity of foams containing CRV was tested *in vitro* against pro-technological, spoilage and pathogenic bacteria applying the disc diffusion method.

The results showed that by using 3% of SB it is possible obtain foams with a porosity of about 40%**.** The presence of carvacrol, strongly influenced the properties of the biopolymeric foams but at the higher content ensured antibacterial activity of the samples.

Keywords: Biopolymeric foams; antibacterial properties; food packaging; essential oil.

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1. Introduction

Polymeric foams, due to their low density, find wide range of applications in several industrial fields from insulation to food packaging applications [1–3].

In the recent years, there was an increasing interest of both academic and industrial research on foamed materials paying particular attention in the development of environmentally friendly processes and materials that can be used in these high-volume productions [4–6]. In this context, biodegradable polymers obtained by renewable sources can be considered a possible approach to reduce the overall environmental impact and solve the disposal problem, especially when recycling is difficult or when biodegradation is a functional requirement of the product [7–9]. Biodegradable polymeric foams or mats are already exploited in many biomedical applications, such as wound dressing, bio-resorbable scaffold, and drug delivery systems [10–14]. However, their use in commodity applications, such as packaging or agriculture, is rapidly emerging since economic issues and difficulties related to their processing have been overcoming [15,16]. For instance, there is an increasing interest on thermoplastic polymers based on starch such as the commercial polymer Mater-Bi® (MB). This family of biodegradable and compostable materials is available with different grades based on starch and they are one of the most important commercially available polymer for utility applications including packaging [15,17,18].

Apart the sustainability of food packaging, another main concern in this field is consumer's demand for safe, "healthy" and high-quality foods, ideally with a long shelf-life. It is worth nothing that shelf life of most food products is affected by the biological, chemical, and physical interactions among food, package, and the environment [19,20]. Textural properties such as flavor, odor and color of food can be modified by high levels of bacteria and microorganism that may lead to undesirable deteriorations. Over the last few decades, products for active packaging (AP) have been developed in order to improve the quality and extend the shelf life of food [21]. AP systems can be divided into active scavenging systems (absorbers) and active-releasing systems (emitters). Absorbers are able to remove undesired substances from food or the surrounding environment such as CO_2, O_2 or water vapor while emitters add substances to the packaged food such as antimicrobial or antioxidants molecules or particles [21]. In an effort to prepare all-green packaging, there is an increasing interest on compounds from natural origin that exhibit antimicrobial activity. Among these, essential oils (EOs), secondary metabolites of plant, are gaining more and more scientific attention due to their high inhibitory potential against a wide spectrum of microorganisms [22]. Carvacrol (CRV) is a phenolic essential oil presenting a broad spectrum of biological and pharmacological properties such as antimicrobial, antioxidant and anti-inflammatory activities [10,11,23,24].

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Furthermore, CRV is categorized as safe food additive in the USA and Europe and Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration [10] and it was recently investigated as antimicrobial additive for a wide plethora of polymers [10,22,25,26]**.**

Aim of this study was to investigate the feasibility of Mater-Bi foams containing CRV for food packaging applications. Sodium bicarbonate (SB) was chosen as foaming agent since meet the non-toxicity requirements and because the temperature range of the decomposition reactions is higher than the processing temperature of MB. MB/SB and MB/SB/CRV systems were prepared by melt mixing and the foaming was conducted in hot press. The morphological analyses were carried out with an optical microscope equipped to a scanning electron microscopy while the mechanical behavior and the release profile of Mater-Bi/CRV foams were evaluated by a laboratory dynamometer and by UV-Vis measurements respectively. The antibacterial activity of Mater-Bi foams containing CRV was tested *in vitro* against pro-technological, spoilage and pathogenic bacteria applying the disc diffusion method.

2. Experimental part

2.1 Materials

In this work it was used a commercial biodegradable polymer Mater-Bi® (EF05B) supplied by Novamont. A pharmaceutical grade of sodium bicarbonate, supplied by Solvay (Bicar®Pharma), was chosen as foaming agent. Carvacrol was purchased by Sigma Aldrich.

2.2 Foam preparation

As preliminary study, a series of MB/SB composites (SB wt% equal to 0.5%, 1%, 2% and 3%) were realized in order to find the right amount of SB for an effective foaming. In brief, MB was fed to a batch mixer (Brabender PLE-330 T=130 °C, n=60 rpm) and processed for 4 minutes, then the SB was added and mixed with MB for 1 minute in order to minimize the degradation of the additive. The MB/SB composite was then fed out and rapidly cooled. During the process, the torque was measured as a function of time.

MB/SB systems were then foamed in a Carver laboratory press at 170 °C and 140 bar in 25 mm diameter and 3 mm height cylindrical molds for 3 minutes. The mold was partially filled by MB/SB (60 vol%) in order to give space to the system for blowing.

In order to prepare MB/SB/CRV systems (with CRV wt% equal to 5%, 10%, 20% with respect to MB), MB was fed to a batch mixer and processed for 4 minutes, then the SB and CRV were

added together and processed with MB for another minute in order to minimize the evaporation of CRV and the degradation of SB. The foaming of MB/CRV was conducted as described above. In order to avoid the loss of CRV due to evaporation during the storage stages, all the MB/CRV foams were stored at 2 °C and characterized within 24 h of preparation.

2.3 Spectroscopic analysis

The spectroscopic characterization of the samples was performed by FT-IR/ATR analysis, by using a Perkin-Elmer FT-IR/NIR Spectrum 400 spectrophotometer. The spectra were collected in the range 4000–400 cm^{-1}.

2.4 Differential scanning calorimeter

The calorimetric properties of the composites were studied by using a Differential Scanning Calorimeter (DSC), (Setaram, model DSC131). foamed samples were sealed in aluminum pans with approximately the same weight (~ 10 mg). The analysis was carried out with a cycle of heating from 0 °C to 220 °C at 10 °C/min under nitrogen flow. The crystallization enthalpy of MB/CRV (ΔH_c) systems was found according to equation (1):

$$\Delta H_c = \frac{\Delta H_c^0}{W_p} \quad (1)$$

Where, ΔH_c^0 is the enthalpy value evaluated from the DSC curves and W_p The weight ratio of the polymer phase.

2.5 Morphological analysis

The samples were broken under liquid nitrogen and then attached on an aluminum stub using an adhesive carbon tape. The sections were observed with the optical microscope equipped in a scanning electron microscopy, (Phenom ProX, Phenom-World).

2.6 Porosity

The porosity of the foams was evaluated according to the following equation (2) [27]:

$$Porosity\ (\%) = \left(1 - \frac{\rho_{foam}}{\rho_{MB}}\right) x\ 100 \quad (2)$$

Where ρ_{foam} is the apparent density of the foam while ρ_{MB} is the density of MB evaluated with an helium pycnometer.

2.7 Mechanical properties

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The foams were mechanically tested under compression using an Instron 3365 mechanical testing machine (UK) equipped with BioPuls (Norwood, MA). The cylindrical specimens were tested in compression mode until 80% of deformation with an uniform crosshead speed of 1 mm min^{-1} and with a 1 kN load cell.

2.8 Carvacrol release kinetics

It is well known that the maximum absorbance peak of CRV in water detectable by UV-Vis measurements can be detected at 272 nm [28,29]. A series of CRV/water solutions containing from 1 to 50 mg/L of CRV were prepared and analyzed via UV-Vis (model UVPC 2401, Shimadzu Italia s.r.l., Milan, Italy) in order to obtain a calibration curve correlating the absorbance peak intensity and the CRV concentration (mg/L). In this range of concentration, the calibration curve was found to be linear (ABS_{272nm} = 0.0142 [CRV]; R^2 = 0.99998). The release of the essential oils from the films was investigated by immersing a pre-weighed sample (a section of 3 × 20 × 3 mm, approximately 120 mg) in 10 mL of distilled water at 37 °C. The absorbance of the medium at 272 nm was measured at specific time intervals and converted CRV concentration by using the calibration line. After each measurement, the samples were immersed in 10 mL of fresh distilled water at 37 °C, and the cumulative release of CRV here reported was calculated by sequentially adding the CRV released after each step.

2.9 Microbial strains

In order to test the inhibitory properties of MB foams containing 5%, 10% and 20% of CRV, three bacterial strains of food origin and belonging to the culture collection of the Agricultural Microbiology Unit of the Department of Agricultural, Food and Forest Science – University of Palermo (Italy) – were used as indicators (sensitive strains). In particular, *Lactococcus lactis* PON153, *Pseudomonas poae* 4G558 and *Listeria monocytogenes* 13BO were used as representative of the pro-technological, spoilage and pathogenic bacterial groups, respectively.
Listeria monocytogenes was subcultured in Brain Heart Infusion (BHI) broth incubated at 37°C for 24 h, *Lc. lactis* in Medium 17 (M17) incubated at 30°C for 24 h, and *P. poae* in Nutrient Broth (NB) incubated at 30°C for 24 h. All media were purchased from Oxoid (Milan, Italy).

2.9 Antibacterial activity determination

Mater-Bi foams containing CRV at different percentages were tested for antibacterial activity applying the paper disc diffusion method [30] with a few modifications. Briefly, a water agar (2% w/v) base support [31] was overlaid with 7 mL of the optimal soft agar (0.7% w/v) medium for

each indicator strain inoculated at approximately 10^7 CFU/mL. MB foams discs (6-mm diameter) containing CRV were placed onto the surface of the double agar layer. Sterile filter paper discs (Whatman no. 1) of the same diameter were soaked with streptomycin (10% w/v) and used as positive controls, while discs of MB foams without CRV represented the negative controls.

The inhibitory activity was evaluated after incubation at the optimal temperature (reported above) for each strain. The inhibitory activity was scored positive when a definite clear area was detected around the discs. The diameters of the inhibitory halos around the paper discs were measured. The experiments were performed in triplicate.

3. Results and discussion

3.1 Effect of sodium bicarbonate content on the properties of Mater-Bi foams

As preliminary study, a series of MB/SB composites (SB wt% equal to 0.5%, 1%, 2% and 3%) were realized in order to find the right amount of SB for an effective foaming. Since SB decomposes over a broad temperature range (100–180 °C), it was mixed within the polymer melt for only 1 minute in order to preserve it from decomposition [32].

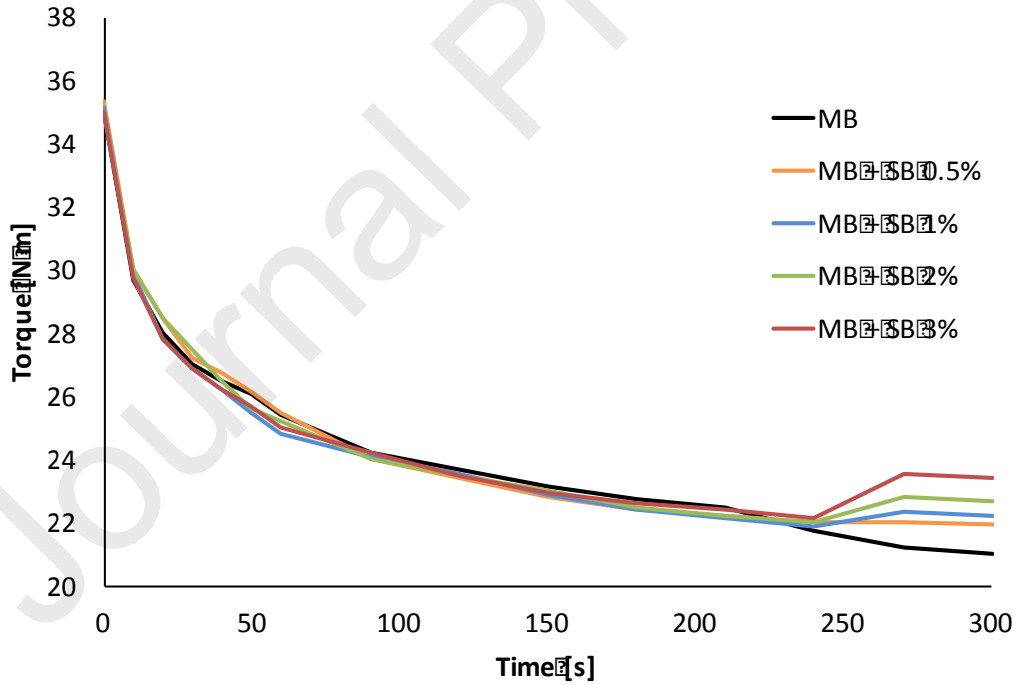

Figure 1 – *MB torque measured during melt mixing as a function of time and amount of SB*

During melt mixing at 130 °C at 60 rpm, it was recorded the torque offered by the melt during mixing (Figure 1). As known the torque is a measure of the viscosity of the melt and then of the processability of the polymer system. As expected the MB curve is characterized by a monotonic decreasing behavior which tends to reach a steady state, starting around 50 seconds of processing,

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indicating the attainment of a thermo-rheological steady state. The addition of SB in the last 60 seconds induced an increment of the torque value. This result was expected since it is well known that solid particles tend to increase the viscosity of polymer melts [33,34]. Furthermore, it is possible to observe that the torque increases upon increasing the SB amount.

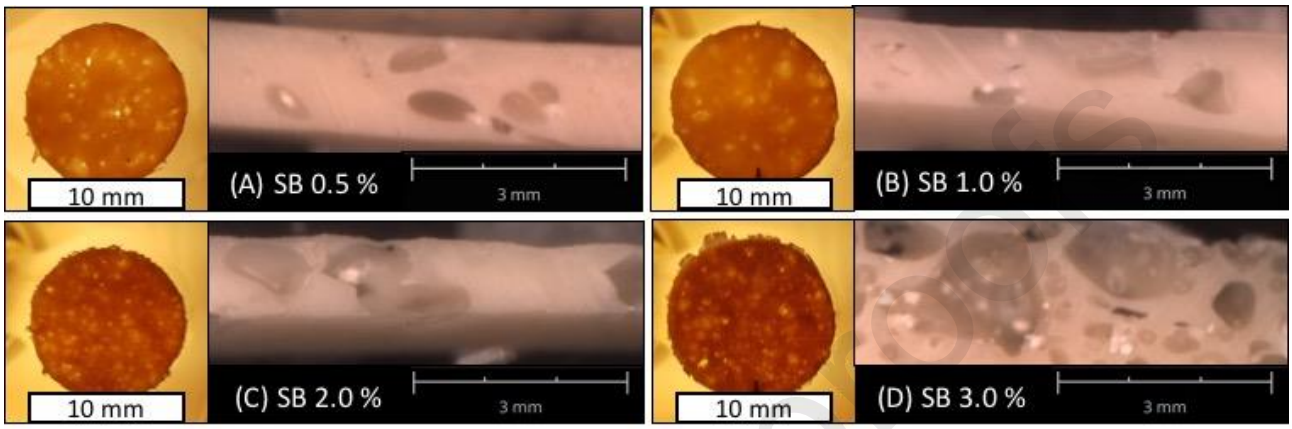

Figure 2 - Pictures and micrographs of MB based foams as a function of SB wt% filled in the polymer matrix. A) 0.5 wt% of SB; B) 1 wt% of SB; C) 2 wt% of SB and D) 3 wt% of SB.

In Figure 2 (A-D) it is possible to observe the morphology of the foams as a function of SB content incorporated in the polymer matrix while in Table 1 are showed the porosity values of the same systems.

Table 1 – Porosity evaluated as a function of SB incorporated in MB

Material	Porosity [%]
MB/SB 0.5%	24.7 ± 1.6
MB/SB 1%	27.1 ± 1.8
MB/SB 2%	33.2 ± 1.3
MB/SB 3%	38.2 ± 1.7

The morphological analysis revealed that the pores are in general in the macroscale (hundreds of µm) and that the morphology of the foams depends on the SB concentration in the MB/SB composites. More in detail, the number of pores increased upon increasing the SB amount. For all the systems, the pores morphology is not spherical, probably because of a partial collapse of the melted structure in the hot press during the polymer expansion.

Porosity measurements shown in Table 1 confirmed that the porosity increased upon increasing the SB amount in the polymer matrix reaching the maximum value of 38.2 % for the system filled with 3 wt% of SB. This porosity value is comparable with those reported in the scientific literature

for other biopolymeric systems [35,36]. For this reason, the foamed samples containing the antibacterial agent were prepared by adding 3% of SB.

3.2 Effect of carvacrol addition on the propertied of the Mater-Bi based foams

In Figure 3 it is shown the behavior of torque as a function of time during melt mixing of MB/SB/CRV systems. As described in experimental section, CRV was added to the polymer melt together with SB, in the last 60 seconds of processing, in order to avoid its evaporation.

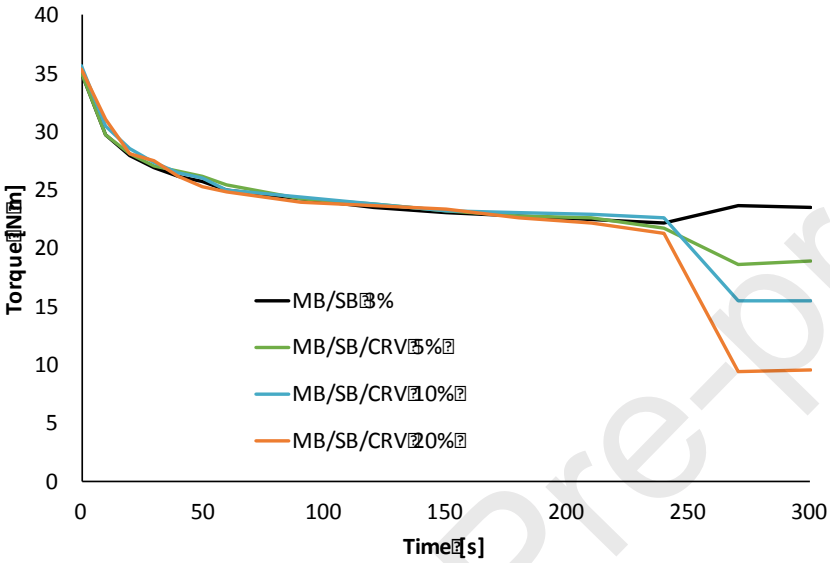

Figure 3 - Torque of MB/SB 3% and MB/SB/CRV as a function of time

It is well known that essential oils can act as plasticizer for thermoplastic polymers [11,37]. As visible from Figure 3, the reduction of viscosity due to the essential oil is stronger than the increment due to addition of SB particles.

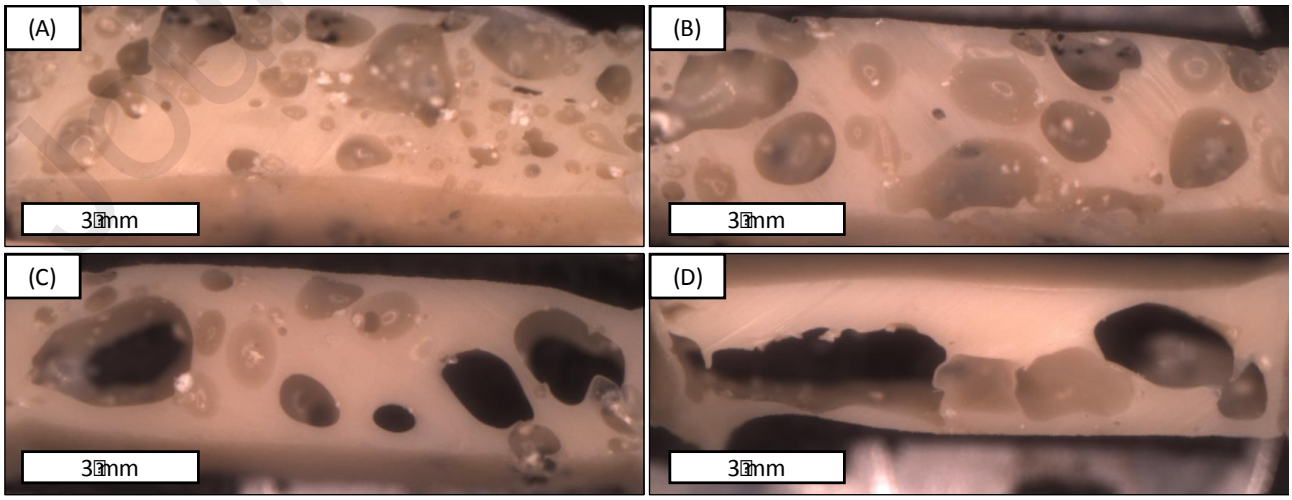

Figure 4 Micrographs of foamed MB as a function of CRV wt% incorporated in the polymer matrix. A) 0 wt% of CRV; B) 5 wt% of CRV; C) 10 wt% of CRV and D) 20 wt% of CRV.

Micrographs of MB and MB/CRV foams cross section are shown in Figure 4A-D. In general, the pores of MB/CRV foams exhibited higher diameters if compared with foamed MB. More in detail, the pores number decrease and the pores diameter increases upon increasing the CRV amount in the polymer matrix. Comparing foamed MB (Figure 4A) and foamed MB/CRV 5% (Figure 4B) it is possible to observe an increase of the number of pores with diameter higher than 1 mm and a neat decrease of pores with dimeter lower than 1 mm. MB/CRV 10% (Figure 4C) showed pores with higher diameter than MB and MB/CRV 5%. Finally, MB/CRV 20% morphology is characterized by a very low number of pores exhibiting high diameter that in some case exceeded 3 mm (Figure 4D).

These phenomena can be likely ascribed to the lower viscosity of the polymer matrix filled with the essential oil as observed with torque measurements. In fact, as a result of the decreased matrix viscosity, the polymer is unable to overcome the biaxial strain that occurs during cell growth, thus promoting coalescence or collapse of cells [36,38]. This feature can explain the formation of a low number of macropores that characterize the morphology of the foams containing CRV.

Table 2 – Porosity evaluated as a function of CRV incorporated in MB/SB 3%

Material	Porosity [%]
MB/SB 3%	38 .2 ± 1.7
MB/SB/CRV 5%	41.8 ± 2.1
MB/SB/CRV 10%	41.1 ± 1.9
MB/SB/CRV 20%	38.8 ± 2.5

Porosity of the foams containing CRV are shown in Table 2. Results revealed that CRV induced a slight increase of the porosity of the foams despite the different morphology of the pores. Also this results can be likely ascribed to the reduced viscosity of MB due to CRV.

3.3 ATR and DSC analysis

In order to verify the effective inclusion of CRV in MB polymer matrix, FTIR-ATR measurements were performed on MB, CRV and MB/CRV foams (Figure 5). MB formula is not available since it is proprietary. The pure MB spectrum (Figure 5) shows the characteristic peaks of poly-caprolactone (PCL) corresponding to the C=O lactone stretching vibrations (1720 cm^{-1}) and C-O lactones stretching (1180 cm^{-1}) were found, as already observed in other works [39]. As visible in Figure 5, carvacrol shows characteristic peaks at 3378 cm^{-1} (OH), 2960 cm^{-1} (CH stretching), 1459 cm^{-1}, 1382 cm^{-1} and 1346 cm^{-1} (CH deformation) and 866 cm^{-1} and 812 cm^{-1} (aromatic ring) [11]. FTIR-

ATR of MB/CRV systems showed that part of the CRV bands were overlapped by the more intense MB peaks. Nevertheless, CH stretching at 2960 cm^{-1} and the CH deformation at 1459 cm^{-1} of CRV can be easily depicted also in MB/CRV systems (Figure 5). Furthermore, it can be noticed that the peak intensity related with CRV included in MB increased upon increasing the essential oil concentration in the foam.

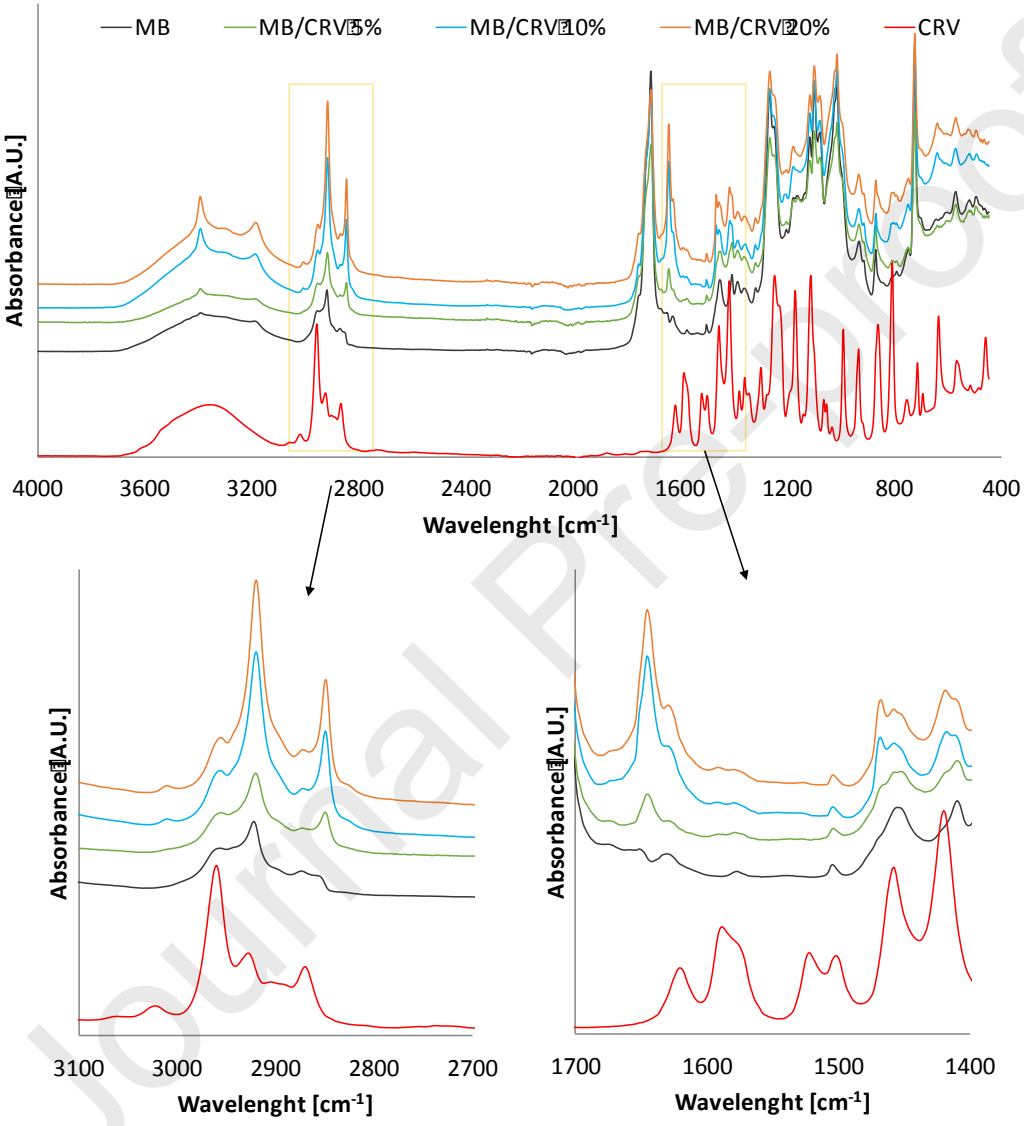

Figure 5 - ATR measurements of CRV and MB or MB/CRV foams

In order to evaluate the miscibility of polymer-essential oil system, DSC analysis was carried out (Figure 6). Thermodynamically, due to both entropic and enthalpic effects, when a miscible drug is added to the polymer matrix, the chemical potential of the amorphous phase decreases while the chemical potential of the crystalline part does not vary. As a consequence, the equilibrium between the crystalline and the amorphous phase of the polymer is established at lower temperatures [40,41]. This equilibrium can be monitored trough both the depression of the melting point (Tm) or the

depression of the crystallization temperature (Tc). In fact, the decrease of Tc is considered to be directly related to the motion ability of the polymer chains [42]. In Figure 6 the cooling thermograms of MB and MB/CRV foams are reported.

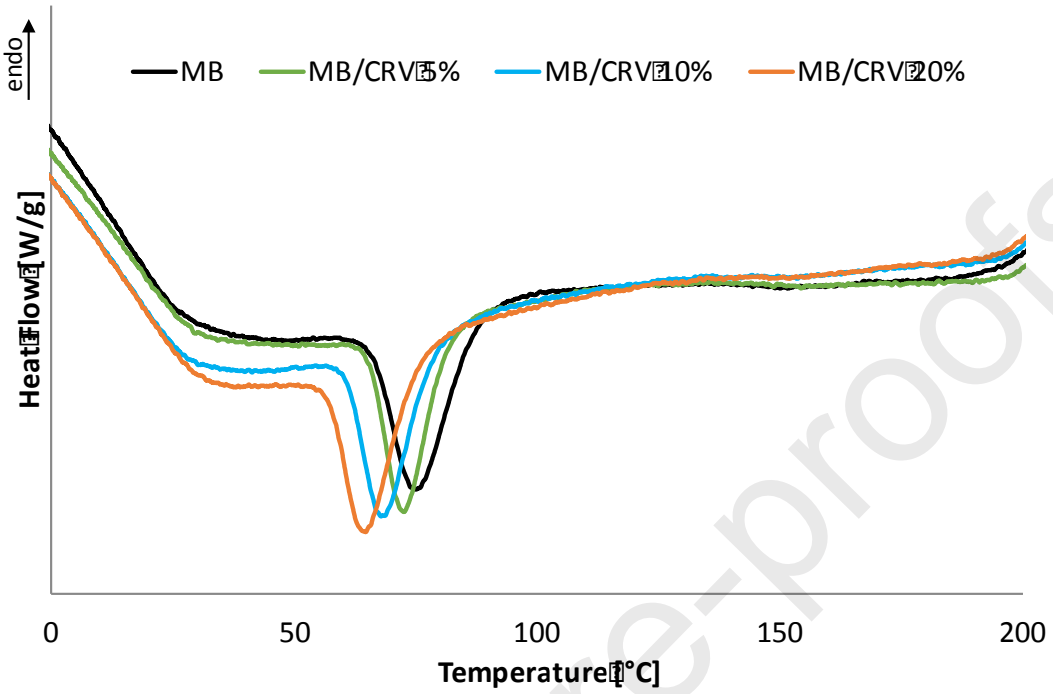

Figure 6 -Cooling curves of MB and MB/CRV foams

As clearly visible from Figure 6, upon increasing the CRV content, MB crystallization temperature (Tc) strongly decreased (Table 3). In particular, Tc decreased from 74.7 °C of MB to 64.6 °C of MB/CRV 20%. The modified crystallization kinetic of this polymer indicates a higher mobility of MB macromolecules thus confirming the molecular scale miscibility between MB and CRV. At the same time, the crystallization enthalpy was found to remain almost constant thus demonstrating that CRV amount did not affect the crystallization capacity of MB.

Table 3 - Thermal properties of MB and MB/CRV foams

Material	T_c [°C]	ΔH_c [J/g]
MB	74.7	17.74
MB/CRV 5%	72.6	17.25
MB/CRV 10%	68.0	17.32
MB/CRV 20%	64.6	17.22

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3.4 Mechanical test

Mechanical behavior of foamed MB and MB/CRV samples with different amount of CRV are shown in Figure 7. All the samples showed an initial section, up to 20 % of strain, characterized by a linear-elastic region (see the inset), likely associated with the bending of the walls of the pores, followed by a transition region that can be associated to the beginning of permanent plastic deformation. The last region, starting from 70% of strain, is characterized by a steep increase of the stress due to the pore's walls collapse that fill the void of the porous structure.

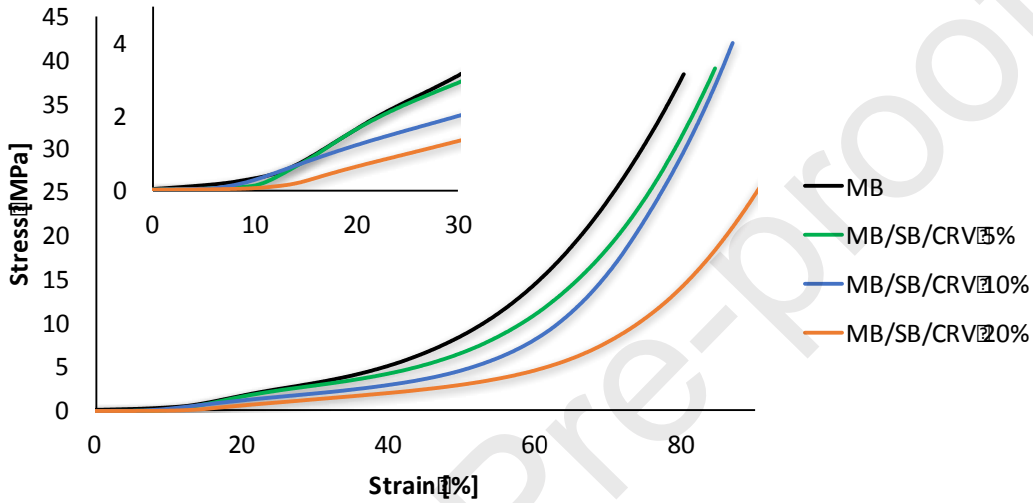

Figure 7 - Stress strain curves of foamed MB and MB/CRV samples

The elastic modulus of the samples is summarized in Table 4. Foamed MB has an *E* value nearly to 19 MPa. The addition of 5% of CRV induced a reduction of this value to almost 16 MPa. Upon increasing the CRV amount up to 10% and 20%, the elastic modulus is ~ 9 MPa, so approximately one half of the neat MB foam. These results can be explained considering the interaction between CRV and the polymer matrix. In fact, it is well known that CRV can act as plasticizer of thermoplastic polymers and it is due to the phase slipping induced by the low molecular weight of the oil included into the polymeric matrix [10,11,26,28].

Other crucial parameters affecting mechanical properties in foams are porosity and pore size distribution [43,44]. In fact, usually, the higher the porosity the lower the elastic modulus and the higher the pore size the higher the elastic modulus [12]. In this work the addition of CRV induced an increment of the pore size, still maintaining almost constant the porosity but the plasticizer effect of CRV seemed to overcome the potential reinforcing effect due to the pores structure.

Table 4 -Elastic modulus of MB and MB/CRV foams

Material	*E* [MPa]
MB/SB	18.83 ± 0.66
MB/SB/CRV 5%	15.94 ± 1.18
MB/SB/CRV 10%	9.33 ± 0.96
MB/SB/CRV 20%	9.10 ± 0.77

The plasticizer effect of CRV in MB also affected the transition and densification region of the stress-strain curves. In particular, the stress offered at the early stage of the plastic deformation (around 20% of strain) is approximately 1.7 MPa for MB and MB/CRV 5%, 1.2 MPa for MB/CRV 10% and 0.65 MPa for MB/CRV 20%.

3.5 Carvacrol release kinetics

In Figure 8 the release of CRV is expressed as the ratio M_t/M_∞ where M_∞ is the theoretical weight of oil components incorporated in the polymer matrix and M_t is the amount of oil components released at time t. For all the systems, the release of CRV was characterized by two phases: (i) a burst phase in the first 5 hours of release (highlighted in the insets of Figure 8) and (ii) a second phase characterized by slower release rate.

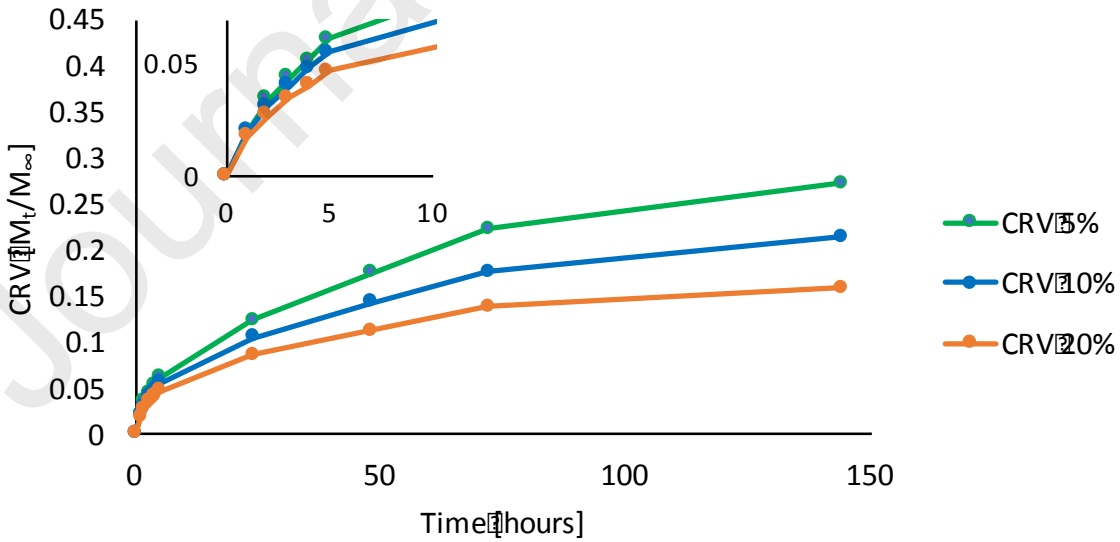

Figure 8 - Release kinetics of carvacrol from MB/CRV foams in distilled water at 37 °C expressed as M_t/M_∞.

Figure 8 highlights that the fraction of incorporated oil released in the medium (M_t/M_∞) at the end of the test decreased upon increasing CRV concentration in the polymer matrix. More in detail,

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during the first 5 hours the fraction released is similar for all the systems (approximately around 5%) and it seems to be poorly affected by CRV concentration in MB but after 144 hours of immersion the foam containing 5%, 10% and 20% of CRV released 27.2%, 21.4% and 15.9% of essential oil respectively. However, it is worth noting that the amount of released CRV (mg of CRV for 1 g of MB/CRV foam) increased upon increasing the CRV incorporated in the polymer matrix.

In order to better understand the release mechanism of CRV in water, the experimental data were fitted using the power law model:

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

where *t* is the release time, *k* is a kinetics constant and *n* is the diffusion exponent related with the release mechanism. In particular, the release is diffusion-controlled when *n* is lower than 0.5 (Fickian) and it is swelling-controlled when *n* is equal to 1.0. If *n* is in the range 0.5-1.0 it can be assumed a release due to the superposition of both phenomena that is defined as anomalous transport by Peppas et al. [45].

In Table 5 there are summarized *n*, *k* and the R^2 values for each system here investigated.

Table 5 - Power law parameters obtained from the release kinetics of CRV.

CRV	k x 10^{-3} [h^{-1}]	*n*	R^2
5%	24.1	0.5110	0.9903
10%	23.4	0.4663	0.9895
20%	20.8	0.4345	0.9866

According to Peppas all the systems here investigated showed a diffusion-controlled mechanism of release. The value of *n* for the foam containing 5% of CRV is slight above 0.5 thus indicating a very small contribute of swelling-controlled release mechanism. Usually, the value of *k* was related to the release kinetics, i.e., the faster the release, the higher the *k* value [11]. The highest value of *k* was observed for the foams containing 5% of CRV and it decreased gradually upon increasing the CRV amount. This result is rather unusual but it can be likely ascribed to two phenomenon: i) the pores morphology of the foams [11] and ii) the evaporation of CRV during melt processing. More in detail, SEM images revealed that upon increasing the CRV concentration in the polymer matrix the pores diameter increases, the number of pores decrease as well as their distribution in the structure. So, the specific surface offered by the foams to the medium decreased upon increasing the CRV filled in MB and this can affect release kinetics of the essential oil in water. At the same time, CRV partially evaporates during the melt mixing process because of the high temperatures. It can be assumed that the ratio of evaporated CRV

increased upon increasing the CRV concentration thus reducing the actual content of essential oil in the polymer matrix.

3.6 Antibacterial activity of MB foams

The antibacterial efficacy of MB foams containing different percentages of CRV were tested after direct contact with three bacterial strains chosen as representative of the three groups generally associated with foods, pro-technological, spoilage and pathogenic [46] by means of disc diffusion method. In particular, *Lc. lactis* represents the typical mesophilic starter culture for dairy use [47], *P. poae* is responsible for the alteration of several foods of animal and plant origin [48,49], while *L. monocytogenes*, able to grow in a wide range of pH and temperatures of vegetables, meat and fish products as well as dairy foods, is cause of human listeriosis [50–53].

Table 6 - Inhibition halos [mm] of MB/CRV foams for different bacterial strains. Values are given as mean ± SD.

Bacterial strain	MB/CRV 5%	MB/CRV 10%	MB/CRV 20%
Lc. lactis PON153	-	-	-
P. poae 4G558	-	-	12.2 ± 0.2
L. monocytogenes 13BO	-	-	12.4 ± 0.1

Results indicate mean ± SD of three independent experiments.
Abbreviations: *Lc.*, *Lactococcus*; *P.*, *Pseudomonas*; *L.*, *Listeria*; -, no inhibition found.

Pseudomonas poae 4G558 and *L. monocytogenes* 13BO showed a clear sensitivity to MB foams containing 20% of CRV with an average diameter of the inhibition area around the discs of 12.2 and 12.4 mm, respectively (see Table 6). Interestingly, *Lc. lactis* PON153 was not inhibited by any of the MB foams tested, indicating their harmless activity against pro-technological bacteria. This is a relevant finding since several lactic acid bacteria used as starter or protective cultures are able to inhibit undesired bacteria through their primary (energetic) and secondary (biosynthetic) metabolisms, due to the production of organic acids and bacteriocins, respectively [54,55]. For this reason, MB foams have been proven to be safe system for food application since directly inhibit spoilage and pathogenic bacteria while do not compromise the viability of the useful strains.

In general, spoilage and pathogenic bacteria contaminate foods and their presence is attributable to environmental contaminations during manufacturing [56,57]. Thus, MB foams containing 20% of CRV represent suitable systems to be applied during storage in order to keep the growth of the above species under control.

4. Conclusions

Antibacterial biopolymeric foams incorporating carvacrol as biocide, were prepared by via melt-compounding in a batch mixer followed by foaming process in a laboratory press by using sodium bicarbonate as foaming agent. The influence of the foaming agent content on the morphology of the foamed samples and furthermore, of the antibacterial additive content on properties of the samples was investigated.

A preliminary study on the content of foaming agent revealed that the porosity increased upon increasing the SB amount in the polymer matrix reaching the maximum value of 38.2 % for the system filled with 3 wt% of SB.

The presence of CRV led to foams that exhibited pores with higher diameters if compared with neat foamed MB. More in detail, the pores diameter increased upon increasing the CRV amount in the polymer matrix. At the same time, the number of pores decrease as well as their homogeneity in the foam although the porosity remained almost unchanged. The CRV acted as a plasticizer thus leading to a reduction of compressive modulus.

The amount of released CRV from foams increased upon increasing the CRV incorporated in the polymer matrix although the fraction of incorporated oil released in the medium at the end of the test decreased upon increasing its concentration in the polymer matrix.

The foamed samples incorporating the higher CRV content exhibited antibacterial activity against spoilage and pathogenic bacteria but the pro-technological one was not inhibited by any of the MB foams tested.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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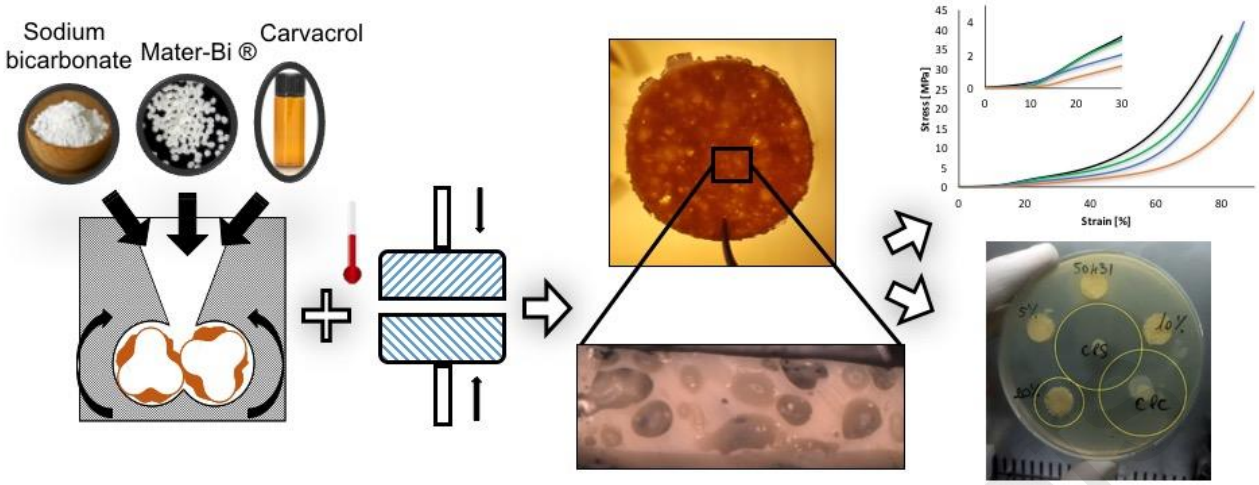

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HIGHLIGHTS

- Antibacterial biopolymeric foams incorporating carvacrol were prepared by using sodium bicarbonate as foaming agent.
- The porosity increased upon increasing the foaming agent amount in the polymer matrix
- The presence of carvacrol strongly influenced the properties of the biopolymeric foams
- The carvacrol acted as a plasticizer thus leading to a reduction of compressive modulus
- The foamed samples incorporating 20% of carvacrol exhibited antibacterial activity against spoilage and pathogenic bacteria

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The authors declare no conflict of interest.

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