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Novel Edible Coating Containing Essential Oil Nanoemulsions to Prolong the Shelf Life of Vegetable Products

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Edible coatings are considered an environmentally friendly technology, able to extend the shelf-life of fruit and vegetables. In the present work, a novel approach to the preservation of vegetable products is proposed, through the development of edible coatings, containing nanoemulsified natural antimicrobial compounds.
Modified chitosan was initially selected as film-forming biopolymer, owing to its inherent antimicrobial activity. Different essential oil nanoemulsions, with variable composition, mean droplet size and surface charge, containing lemon, mandarin, oregano or clove essential oils, were developed to maximize in vitro the interaction with the modified chitosan coating and promote the resulting antimicrobial activity.
The combined use of nanoemulsified lemon essential oil with modified chitosan resulted in the remarkable increase in antimicrobial activity, with respect to other essential oils. Therefore, this formulation, comprising 0.05%wt modified chitosan and 0.1%wt nanoemulsified lemon essential oil, was further tested *in product* for the stabilization of a leaf vegetable, such as rucola, during a shelf-life, consisting of storage at 4°C for 3 d and then at 8°C for a total of 21 d. Microbial viability as well as colour and texture changes were monitored during the shelf-life.
The obtained results showed that the incorporation of nanoencapsulated lemon essential oil into the modified chitosan coating prolonged the shelf life of rucola leaves from 3 to 7 d, in comparison to the untreated sample. Moreover, the modified chitosan containing the nanoemulsified antimicrobial caused a significantly longer shelf-life also in comparison to a coating made of modified chitosan or essential oil alone.
In conclusion, thanks to this novel treatment it is possible to prolong the shelf life of delicate leaf vegetables to about 10 - 14 d, without causing any significant alteration of the organoleptic properties of the product, preventing the loss of firmness and colour changes and preserving palatability during storage.

1. Introduction

In recent years, the market demand of ready-to-eat fruits and vegetables has witnessed a rapid expansion. What consumers perceive as the most appealing attributes of these products include their fresh-like appearance, taste and flavour, in addition to convenience. However, the minimal processing to which fresh-cut fruits and vegetables are submitted renders products highly perishable, requiring chilled storage to ensure a reasonable shelf life. Fresh-cut products are very sensitive to contamination: cutting or slicing operations increase tissue damage, causing the release of intracellular contents (González-Aguilar et al., 2009) that can support and increase the activity of pathogenic microorganisms.
In conventional types of fruit and vegetable processing, these problems are prevented or controlled by heat processing, inactivation of enzymes by the use of protective packaging materials or through the addition of different additives. In the production of fresh-cut products, heat treatments are avoided in order to prevent cooking of the product and the consequent loss of fresh-like appearance and nutritional properties of food.
Therefore, the constantly increasing request for minimally processed foods promoted the investigation of novel approaches to reduce pathogens, simultaneously ensuring both safety and quality of the produce (Birmpa et

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al., 2013). In particular, an increasing interest is arousing by the development of invisible, colourless, odourless, tasteless, edible coatings. These formulations can extend the shelf life without compromising the fresh quality, inhibiting enzymatic browning, maintaining the product's natural texture, preventing sliced product from dehydrating and retaining natural liquids.
In addition to these well-known advantages of coating films, also food deterioration can be retarded by inhibiting the growth of microorganisms, due to the incorporation of antimicrobial compounds (Vu et al., 2011).
The use of essential oils (EOs) as natural antimicrobial agents has recently attracted significant attention for food preservation against foodborne pathogens and spoilage bacteria (Caillet et al., 2006). However, essential oils are more efficiently used in foods when encapsulated in appropriate delivery systems, not only to overcome the dosage limitations related to their low solubility in water (Donsì et al., 2011), but also to increase the physical stability of active substances, protecting them from the interactions with other food ingredients. In addition, the encapsulation of EOs into nanometric delivery systems can enhance their bioactivity, improving the passive mechanisms of cell absorption, enabling the reduction of the doses of essential oils required to ensure antimicrobial activity, thus minimizing the impact on aroma, flavour and taste (Donsì et al., 2011).
Recent studies demonstrated the efficacy of a coating formulation based on modified chitosan and essential oils in extending the shelf life of strawberries (Vu et al., 2011), as well as of chitosan based coating formulations including bergamot essential oil on quality and safety of table grapes (Sànchez-Gonzàlez et al., 2011). A very recent work conducted by our team demonstrated how the antimicrobial efficacy against *L. monocytogenes* of a coating formulation based on modified chitosan and essential oils in broccoli florets is promoted when used in combination with non thermal treatments such as UV-C, ozonated water and γ-irradiation (Severino et al., 2014).The addition of food additives, or 'generally recognized as safe' (GRAS) compounds with antimicrobial properties, to biopolymers in order to form edible coating has been less studied in leaf vegetables.
The aim of this study is the development of novel antimicrobial edible coating, containing nanoemulsified essential oils, in order to extend the shelf life of leaf vegetables.

2. Materials and Methods

Different essential oils (lemon, mandarin, oregano or clove essential oils) were screened as natural antimicrobial agents and used in nanoemulsions preparation. In the emulsion fabrication, a mixture of essential oil and a lipophilic emulsifier, such as glycerol monooleate or soy lecithin, were dispersed in bidistilled water containing a hydrophilic emulsifier (whey protein isolate, pea proteins, Tween 20), using an Ultra Turrax T25 (IKA Labortechnik, Germany) at 24,000 rpm for 3 min, to form a primary emulsion. Subsequently, the primary emulsions were subjected to a high pressure homogenization (HPH) treatment in a custom-made system for 3 min at 200 MPa, to reach a droplet size distribution in the nanometric range. A photon correlation spectrometer (HPPS, Malvern Instruments, Malvern, UK) was used to characterize the particle size distribution of the nanoemulsion droplets.
Modified chitosan (MC) was used as coating matrix for incorporation of antimicrobial emulsions. MC was dissolved in 1 %(v/v) lactic acid solution, where EO nanoemulsions were added at different concentrations. The coating application consisted of immersing rucola samples (10 g) into the coating solution (100 mL) for 3 min. The coated samples were centrifuged and allowed to dry for 30 min in a biological safety cabinet. The samples were then packaged in bags and stored at 4 °C for 3 d and at 8 °C for a total of 21 d. For untreated (control) and coated samples, microbial analysis, colour and texture measurements were conducted at days 0, 3, 7, 10, 14 and 21 during storage.
For the determination of the total microbial load of the samples the UNI EN ISO 4833-2:2003 method was used, which provides the enumeration of colonies growing in Plate Count Agar after aerobic incubation at 30 °C for 72 h. For the determination of yeasts and moulds of the samples the ISO 21527-1:2008 method was used, which specifies a horizontal method for the enumeration of viable yeasts and moulds in products having a water activity greater than 0.95, by means of the colony count technique at 25 °C.
The colour variation of treated and untreated samples was monitored over the entire refrigerated storage, by using a tristimulus colorimeter (CR400 Chroma Meter, Konika Minolta Inc., Japan). The CIE*Lab* color scale was employed: the L* axis represents the lightness from black (L* = 0) to absolute white (L* = 100), the a* axis varies from green (−) to red (+), and the b* axis varies from blue (−) to yellow (+). The global colour difference (ΔE^*ab) was calculated according to Eq (1).

$$\Delta E^* ab = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Texture analysis was carried out on the samples during the storage at refrigerated conditions using a texture analyzer model TA-XT2 (Stable Micro System Ltd., US). Measurements were conducted using force-in-

compression test mode. The values of the force as a function of the distance was measured in each experiment and the maximum force achieved during compression cycle was calculated as the peak force.
Each measurement was replicated three times.

3. Results and discussion

During the screening phase, different formulations for EOs encapsulation were developed using different emulsifiers. The formulations produced were characterized in terms of mean droplet diameter (Z-average) and polydispersity index (PDI), which are reported in Table 1. The combined use of a lipophilic and a hydrophilic emulsifier resulted in a significantly lower mean droplet diameter.
The finer droplet size of the delivery systems, also, corresponds to a higher chemical and physical stability of the encapsulated compounds, reducing the impact on the organoleptic properties of the product in which the antimicrobial compounds are added (Donsì et al., 2011). For these reasons, the Tween 20 - glycerol monooleate-based nanoemulsion was selected for further studies in product, owing to its extremely small mean droplet size and low PDI in comparison with the other formulations.
Moreover, different essential oils were encapsulated in order to evaluate their antimicrobial activity on leaf vegetables. Figure 1 reports the total microbial load of rucola samples treated by dip coating in nanoencapsulated EOs alone or combined with modified chitosan, at fixed EO concentration (0.05 % v/v). As shown in Figure 1, dipping in EOs nanoemulsions alone, caused a decrease in microbial population in a range comprised between 1 and 2 Log-cycles, with higher antimicrobial activity observed for lemon and clove EOs, and lower antimicrobial activity for oregano and mandarin EOs. In addition, modified chitosan alone already exhibited a significant antimicrobial activity, with a reduction of the microbial load of about3 Log compared to the control. Remarkably, the combined use of nanoemulsified lemon essential oil with modified chitosan resulted in a significant synergy with consequent increase in antimicrobial activity, which was not observed for the other essential oils, showing a reduction of the microbial load of approximately 4 Log in comparison to the untreated samples. In particular, this formulation, comprising 0.05 % wt modified chitosan and 0.1 % wt nanoemulsified lemon essential oil, was further tested *in product* for the stabilization of rucola leaves, during a shelf-life that, according to a consolidated industrial procedure, consisted of storage at 4 °C for 3 d and then at 8 °C for a total of 21 d. Microbial viability as well as colour and texture changes were monitored during the shelf-life.

Table 1: Composition, mean droplet diameter (Z-average) and polydispersity index (PDI) of different nanoemulsions produced for the encapsulation of essential oils.

Composition	Z-average (nm)	PDI
5 % EO 3 % soy lecithin 92 % water	254±30	0.97±0.09
5 % EO 3 % pea protein 92 % water	394±11	0.41±0.04
5 % EO 1.5 % Tween 20 1.5 % glycerol monooleate 92 % water	88±10	0.19±0.01
5 % EO 3 % whey protein isolate 92 % water	391±21	0.46±0.07

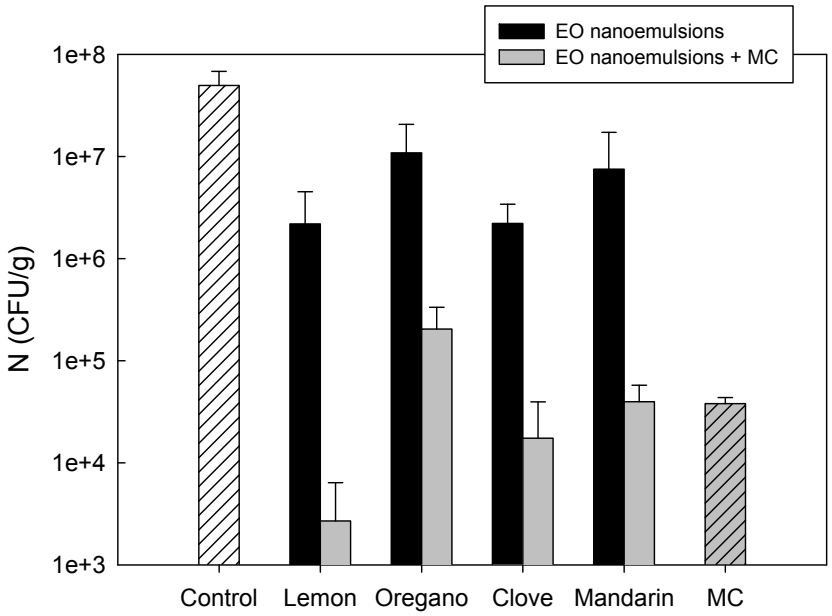

Figure 1: Total microbial load of rucola samples treated by dip coating into different nanoemulsified essential oils alone or with modified chitosan (MC) in comparison with untreated samples (control).

Figure 2 shows the effect of the coating treatment on the shelf life of leaf vegetables. In particular, Figure 2a reports the effect of the bioactive coating on the total microbial load of rucola samples under refrigerated storage conditions. At time zero the rucola leaves have a total microbial load of about 6 Log CFU/g. The formation on the surface of the leaf vegetable of an edible coating, consisting of nanoencapsulated lemon essential oil in a matrix of modified chitosan, determined a reduction of the initial microbial load of approximately 2 Log. Notably, after 3 d, while for the control an increase of the total microbial load of about 1 Log was observed, for the coated samples the microbial load remained almost constant compared to day 0, and about 3 Log lower than untreated samples after 3 d. This result is due to a controlled release of the lemon essential oil from the biopolymeric matrix, in which it is trapped, and which coats the rucola leaves, guaranteeing a prolonged antimicrobial activity over time. After the third day of storage, an increase of the total microbial load was observed also for the treated samples. However, the samples treated with a bioactive coating always exhibited a microbial load lower of about 1 Log than the control until day 14.
Figure 2b reports the evolution over time of the concentration of yeasts and moulds of the samples coated with lemon EO in comparison with the untreated samples. Also in this case, the results show a reduction of the initial microbial load of yeasts and moulds when the rucola leaves were coated with natural antimicrobials.
In the food industry, the commercialization of ready-to-eat leaf vegetables is permissible below a limit value of total microbial load of $5*10^7$ CFU/g. Based on the obtained results, the maximum shelf life of untreated samples is of 7 days ($5.5*10^7$ CFU/g on day 7), whereas the shelf life of samples treated with a natural antimicrobial coating varies between 10 and 14 days ($3.7*10^7$ CFU/g and $8.5*10^7$ CFU/g on day 10 and 14, respectively), as shown in Figure 2.

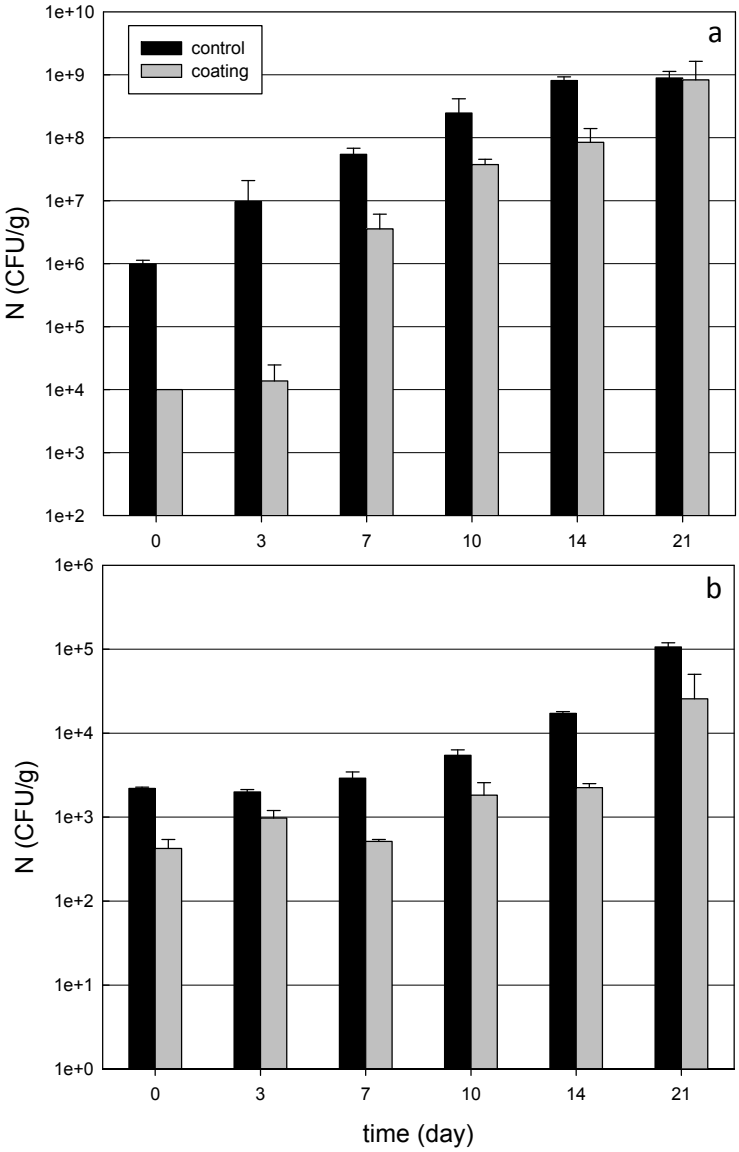

Figure 2: a) Total microbial load and b) yeasts and moulds of rucola samples treated with a bioactive coating, consisting of nanoemulsified lemon EO with modified chitosan, in comparison with the untreated samples (control) as function of the storage time.

*Table 2: Evolution of global colour difference ΔE*ab and of peak force in compression (N) over time of rucola samples treated with a bioactive coating in comparison with the untreated samples (control). The samples are stored at 4 °C for 3 d and then at 8 °C for a total of 21 d.*

	*ΔE*ab*		*Force in compression (N)*	
Storage time (day)	Control	Coating	Control	Coating
0	$1.84\pm0.80_{a}^{A}$	$7.08\pm1.93_{ab}^{B}$	$0.29\pm0.10_{a}^{A}$	$0.24\pm0.04_{a}^{A}$
3	$6.65\pm0.89_{bc}^{A}$	$7.05\pm0.49_{a}^{A}$	$0.43\pm0.09_{a}^{A}$	$0.24\pm0.04_{a}^{B}$
7	$5.18\pm1.12_{b}^{A}$	$5.75\pm0.03_{b}^{A}$	$0.46\pm0.11_{a}^{A}$	$0.38\pm0.06_{ab}^{A}$
14	$8.86\pm1.18_{c}^{A}$	$5.11\pm1.79_{b}^{B}$	$0.62\pm0.22_{ab}^{A}$	$0.62\pm0.27_{b}^{A}$
21	$14.36\pm0.68_{d}^{A}$	$11.38\pm0.80_{c}^{B}$	$1.41\pm0.57_{b}^{A}$	$1.36\pm0.39_{c}^{A}$

Values are means ± standard deviations. Means with different lowercase letters within the same treatment lot (same treatment at different storage times) are significantly different ($P \leq 0.05$), while means with different uppercase letters within each treatment condition (different treatments at fixed storage time) are significantly different ($P \leq 0.05$).

The impact of the bioactive coating on the colour of rucola leaves during storage in terms of total colour variation *ΔE*ab,* calculated with respect to the untreated sample at day 0, is reported in Table 2. Overall, the results show no significant variations in colour between the different treated and untreated samples that follow approximately the same trend during the storage period. In particular, all samples on day 14 are still in good condition, while a deterioration and a yellowing of the samples can be detected at the 21st day of storage. The yellowing of the samples is confirmed by colorimetric measurements with an increase of the parameter b*, a slight increase of the parameter a* and a gradual decrease in brightness (L*).
The firmness of rucola samples treated with modified chitosan and nanoemulsified lemon essential oil based coating during storage was evaluated by texture analysis, with the results being reported in Table 2. At day 0, no significantly changes in the texture of coated samples in comparison with control were observed. For the untreated samples, an increase of the firmness was observed already after 3 d of storage, while for the treated samples an increase of the peak of force in compression was measured only after 7 d. Regarding to these results, the coating treatment with nanoemulsified lemon essential oil and modified chitosan not alters the texture of the leaf vegetable during the first week of storage improving the shelf life of product.

4. Conclusions

This study showed that the use of an edible coating containing nanoencapsulated natural antimicrobial compounds is an efficient way to increase the shelf life of ready-to-eat fruits and vegetables. In particular, the results obtained permit to conclude that the combined use of nanoencapsulated lemon essential oil and modified chitosan on rucola leaves by a dip coating treatment prolongs the shelf life by a minimum of 3 d to a maximum of 7 d compared to the untreated samples. Moreover, the coating treatment does not alter significantly the organoleptic properties of the leaf vegetable.

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