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Supercritical Carbon Dioxide Treatment Combined with Rosemary Essential Oil to Prolong the Shelf-Life of Chicken Breast Meat

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Essential oils are well known for their antimicrobial properties, making them attractive for food preservation. However, when used alone, their effectiveness can be limited. Combining essential oils with other technologies shows promise for enhancing their efficacy.. The present work focuses on a laboratory-scale feasibility analysis investigating the potential synergism between supercritical carbon dioxide ($scCO_2$) and rosemary essential oil (REO) to extend the shelf life of chicken breast meat, a product with a short storage time that contributes significantly to food waste. In this context, a novel method with supercritical carbon dioxide and modified atmosphere packaging ($scCO_2MAP$) was used in combination with 1% REO. This method has a lower impact on product colour change with respect to traditional $scCO_2$ processes while increasing food safety by reducing the number of viable microorganisms. Storage tests were conducted for up to 12 days at 4°C, measuring the load of the total mesophilic and psychrophilic microorganisms, water activity and pH. At day 0, the results showed a significant reduction in both mesophilic and psychrophilic microorganisms, respectively about 2.11 and 2.05 log CFU/g. During storage, the microorganism counts in the treated samples were lower compared to the untreated ones. The pH and water activity were not significantly different during the whole shelf life. This study shows promise for the development of a low-temperature treatment capable of enhancing the safety and of prolonging the shelf life of poultry meat.

1. Introduction

Rosemary *(Rosmarinus officinalis L.)* essential oil (REO) is well recognized for its antimicrobial and antioxidant properties thanks to the presence of active molecules, such as camphor, α-pinene, and β-pinene, making it a valuable candidate for applications in the field of food preservation (Kačániová et al., 2023). Moreover, REO has been approved by the FDA (Food and Drug Administration) as a GRAS (Generally Recognised as Safe) component (Qiu et al., 2024) and it can be therefore freely adopted in the food industry.
Different methods to incorporate REO into food products have been reported in the scientific literature, including, biodegradable food packaging (Göksen et al., 2021), nano-encapsulation (Seyed Hajizadeh et al., 2021), emulsions (Dávila-Rodríguez et al., 2019), and direct use (Saraiva et al., 2021). For instance, a recent study by Brandt et al. (2023) explored the use of a micro-emulsion with REO in tomato paste as an antioxidant and antimicrobial agent.
Thus, essential oils and derived products are often adopted to hinder microbial proliferation in fresh products, but their use does not modify the initial microbial loads. A possible solution is to combine the use of essential oils with decontamination techniques, such as supercritical carbon dioxide ($scCO_2$) processes.
$scCO_2$ processes have been widely studied as effective inactivation methods thanks to the peculiar characteristic of CO_2 in the supercritical state and its effectiveness in decreasing the load of bacteria, yeasts, and molds in both liquid (Silva et al., 2020) and solid (Bae et al., 2011) food products.

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The possibility of combining $scCO_2$ processes with essential oils has been already tested (Santi et al., 2023) for chicken meat and for raw almonds (Chen et al., 2022) .
Chicken breast meat was selected as test product as it is widely consumed (Uzundumlu et al., 2023) and has a relatively short shelf-life of about 5 days (Chouliara et al., 2007) and presents a high risk of contamination by pathogenic bacteria such as *Salmonella* and *Campylobacter* (EFSA report 2021, EFSA story map 2022).
In the previous study (Santi et al. 2023), chicken breast was treated with two different CO_2 processes, with and without the addition of REO, and the results were compared in terms of microbial inactivation and final product aspect. In particular, the traditional $scCO_2$ process was compared with a novel CO_2-based pasteurisation process for solid food products ($scCO_2MAP$) patented by (Spilimbergo et al., 2020), in which the products are treated after the packaging in a CO_2-rich atmosphere, avoiding the risk of post-process contamination (Zulli et al. 2023). It was demonstrated that the addition of REO allowed an enhanced significant inactivation, while maintaining a fresh-like aspect of the product (Santi et al., 2023).
In the present study, chicken breast meat was treated with $scCO_2MAP$, with and without the addition of REO, and compared with the non-treated fresh sample equivalently packaged; furthermore, a shelf life study was carried out at 4°C for 12-days , monitoring the mesophilic and psychrophilic bacteria proliferation, pH alteration and water activity .The aims of this work were to evaluate a possible synergistic effect between $scCO_2$ and REO on food shelf life.

2. Materials and methods

2.1 Sample preparation

Chicken breast meat was purchased from a local market (Padova, Italy) and cut into pieces of 5 ± 0.05 g. Then, 1% (v/w) REO (Erbamea, Perusa, Italy) was added on the surface and left to dry for 15 min under a laminar-flow cabinet (BioAir, Pavia, Italy) for some samples, while a part of chicken breast pieces was not sprinkled with REO. Successively, all the samples were packed in high-barrier multilayer (PA/EVOH/PA/PE) film (Euralpack, Schoten, Belgium) filled with CO_2 (carbon dioxide 4.0, purity > 99.8%, Nippon GasesItalia, Milano, Italy) and closed by using an electrical sealer (FS300, Plastic Film Sealer, China). The volume of each pouch was 100 ± 5 ml. The 4 different theses compared were: modified atmosphere packaging enriched with CO_2 ($MAPCO_2$), $MAPCO_2$ + 1% REO, $scCO_2MAP$ and $scCO_2MAP$ + 1% REO. (Santi et al.,2023) $MAPCO_2$ and $MAPCO_2$ + 1% REO were not subjected to treatment while $scCO_2MAP$ and $scCO_2MAP$ + 1% REO were treated with the process described in 2.2.

2.2 High pressure-system

The high-pressure processes were carried out in a water-driven multi batch reactor system as extensively described by Barberi et al. (2021). The process conditions were the same used bySanti et al., 2023: 14 MPa and 40 °C for 15 min. Process conditions and REO concentration were selected by preliminary optimization tests (Santi et al., 2023) to better verify the synergism between CO_2 and REO.

2.3 Microbial and physicochemical storage test

Treated and non-treated products were monitored for a storage time of 12 days and microbial proliferation, pH and water activity (aw) were assessed at the following time points: 0, 2, 5 7 9 and 12 day.
Microbial count was carried out using the standard plate count for the enumeration of mesophilic and psychrophilic microorganisms, as described by Santi et al. (2023). To perform the analysis, 1000 µL of the selected dilutions were pour-plated on Plate Count Agar (PCA) (Microbiol diagnostici, Cagliari, Italy)). The plates for the enumeration of mesophilic microorganisms were incubated at 30°C for 72 h, while for psychrophilic microorganisms the plates were incubated at 10°C for 120 h. Results are expressed as logarithm of Colony Forming Unit over grams (log CFU/g).
At the same time point, pH was measured using a pH-meter (pH1100, VWR, Leuven, Belgium) with an electrode for solid samples (spear 220, VWR, Leuven, Belgium), while the water activity (a_w) was measured with an aw-meter (HygroPalm HP23-AW-A, Bassersdorf, Switzerland). Each measurement was performed at least in triplicate.

2.4 Statistical analysis

Statistical analyses were performed in Minitab®. Mean values were used to compare different effects of the use of several substances alone and in combination with supercritical carbon dioxide treatment. The existence of significant differences (α = 0.05) between different treatments was studied with an ANOVA and pair comparison within a group with its post hoc analysis (Fisher) were executed.

2. Results

Mesophilic bacteria growth (Table1) highlighted an important effect of $scCO_2$ and $scCO_2$+REO at time 0 of the storage.

Table 1: Loads of Mesophilic bacteria storage test of 12 days at 4°C expressed as log CFU/g. Statistically significant differences within a given day's data are denoted by distinct lowercase superscript letters within the same row, while divergent capital letters within a specific day's column signify significant differences ($p < 0.05$).

Shelf life – mesophilic bacteria				
	$MAPCO_2$	$MAPCO_2$ + 1% REO	$scMAPCO_2$	$scMAPCO_2$ + 1% REO
Day 0	5.38 ± 0.04^{aC}	5.12 ± 0.17^{aCD}	4.10 ± 0.66^{bC}	3.27 ± 0.62^{bC}
Day 2	5.30 ± 0.12^{aC}	4.53 ± 0.64^{abD}	4.75 ± 0.49^{aC}	3.89 ± 0.23^{bBC}
Day 5	5.47 ± 0.20^{aC}	4.23 ± 0.98^{bE}	5.53 ± 0.11^{aB}	4.18 ± 0.10^{bB}
Day 7	6.60 ± 0.23^{aB}	5.67 ± 0.02^{bBC}	6.80 ± 0.12^{aA}	5.76 ± 0.16^{bA}
Day 9	6.94 ± 0.46^{abB}	6.51 ± 0.15^{bAB}	7.05 ± 0.17^{aA}	5.98 ± 0.13^{cA}
Day 12	8.06 ± 0.15^{aA}	7.27 ± 0.44^{abA}	7.06 ± 0.44^{bA}	6.19 ± 0.71^{cA}

The only addition of REO on the fresh sample ($MAPCO_2$ + 1% REO, day 0) did not have an instant effect on the mesophilic microbial load of the fresh sample ($MAPCO_2$, day 0). Similar results were obtained by Alvarez et al. (2019).
The high-pressure treatments with ($scCO_2$MAP + 1% REO) and without ($scCO_2$MAP) the addition of REO led instead to a significant reduction, confirming the data obtained by our previous study (Santi et al., 2023). However, it was not possible to appreciate a significant difference between the microbial loads after the two supercritical carbon dioxide treatments.
After that, the mesophilic microbial load exhibited varying trends across the studied period. The $MAPCO_2$ samples treatment maintained a stable microbial load until day 5, followed by an increase on days 7 and 12. Conversely, the addition of REO leads to a decrease in microbial load on day 5 with an increase in the subsequent days. The same behaviour was also observed by Chouliara et al. (2007) while applying different concentrations of oregano essential oil and different modified atmosphere packaging on chicken breast meat. The same trend was noticed when the MAP was rich in CO_2. The reason for this behaviour could be the substitution of communities, as reported by Hosseini et al. (2021), such as the decrease of *Enterobacteriaceae* coupled with an increase of lactic acid bacteria (LAB). However, this hypothesis needs to be verified with further appropriate studies.
Regarding the high-pressure treated samples, synergistic effect of rosemary essential oil and supercritical carbon dioxide on mesophilic bacteria starting from day 2. $scCO_2$MAP samples showed an increase in mesophilic load on days 5 and 7, while it remained stable during the following days.
Samples treated with $scCO_2$MAP + 1% REO, instead, showed a slower microbial proliferation leading to less contaminated samples compared to all the other theses on every time point
This synergism allowed us to achieve a mesophilic bacteria load of 6.19 log CFU/g after 12 days of storage at 4°C, which is lower than 7 log CFU/g, a value recommended by ICFM (1986) for the consumption of chicken breast meat. In Table 2, the results concerning psychrophilic bacteria loads obtained from the same storage test at 4°C are reported.
Psychrophilic bacteria growth (Table 2) reports significant differences in growth trends comparing the two microbial populations. Considering the samples stored in CO2 ($MAPCO_2$), mesophilic bacteria did not show a significant increase until day 5, while in the same thesis a noticeable increase in psychrophilic bacteria was observed from day 5 onwards. In the samples $MAPCO_2$ + 1% REO, the psychrophilic load decreased from day 0 to day 2, whereas a significant decrease in mesophilic bacteria was only observed on day 5. The $scCO_2$MAP treatment reported a stable microbial population until day 2, with a significant increase observed until day 7 for mesophilic bacteria and day 9 for psychrophilic bacteria. The treatment $scCO_2$MAP + 1% REO showed some differences as well, with the psychrophilic load increasing from day 2 to day 7, in contrast to mesophilic bacteria, which only increased on day 7.

Table 2: Loads of psychrophilic bacteria during the storage test of 12 days at 4°C expressed as log CFU/g. Statistically significant differences within a given day's data are denoted by distinct lowercase superscript letters within the same row, while divergent capital letters within a specific day's column signify significant differences ($p < 0.05$).

Shelf life – psychrophilic bacteria				
	$MAPCO_2$	$MAPCO_2$ + 1% REO	sc$MAPCO_2$	sc$MAPCO_2$ + 1% REO
Day 0	5.24 ± 0.30^{abCD}	5.52 ± 0.47^{aA}	4.56 ± 0.15^{bD}	3.19 ± 0.70^{cD}
Day 2	4.69 ± 0.27^{aD}	4.20 ± 0.59^{abC}	4.56 ± 0.27^{aD}	3.61 ± 0.03^{bD}
Day 5	5.45 ± 0.06^{aCB}	4.05 ± 1.14^{bC}	5.60 ± 0.04^{aC}	4.25 ± 0.11^{bC}
Day 7	6.21 ± 0.50^{aBA}	5.38 ± 0.34^{bB}	6.74 ± 0.17^{aB}	5.40 ± 0.43^{bB}
Day 9	6.62 ± 0.18^{bB}	6.48 ± 0.19^{bA}	7.12 ± 0.08^{aA}	5.98 ± 0.13^{cB}
Day 12	7.78 ± 0.37^{aA}	6.15 ± 0.15^{cAB}	7.10 ± 0.17^{bA}	7.33 ± 0.05^{bA}

Furthermore, it is noteworthy that also for psychrophilic bacteria, a synergistic effect was observed between supercritical carbon dioxide treatment and the addition of rosemary. Importantly, on the 12-day storage test, only sc$MAPCO_2$ + 1% REO exhibited an acceptable bacteria load suitable for chicken meat consumption.
pH and water activity (a_w) results were reported in Table 3 and Table 4.
The only addition of REO did not lead to changes in these two parameters, but high-pressure treatments led to a slight decrease of both pH and a_w. During the storage test, no important variation of pH was observed and the pH was the same for all theses from day 5, while for the water activity a significant decrease of the following theses is appreciable: $MAPCO_2$, $MAPCO_2$ + 1% REO and scCO_2MAP + 1%REO.

Table 3: pH variation during the storage test of 12 days at 4°C.Statistically significant differences within a given day's data are denoted by distinct lowercase superscript letters within the same row, while divergent capital letters within a specific day's column signify significant differences ($p < 0.05$).

pH				
	$MAPCO_2$	$MAPCO_2$ + 1% REO	sc$MAPCO_2$	sc$MAPCO_2$ + 1% REO
Day 0	*5.85 ± 0.05^{aA}*	*5.85 ± 0.10^{aA}*	*5.66 ± 0.09^{bA}*	*5.74 ± 0.07^{abA}*
Day 2	*5.59 ± 0.01^{bB}*	*5.75 ± 0.02^{aAB}*	*5.66 ± 0.09^{abA}*	*5.71 ± 0.05^{aA}*
Day 5	*5.70 ± 0.12^{aB}*	*5.69 ± 0.07^{aB}*	*5.72 ± 0.07^{aA}*	*5.70 ± 0.07^{aA}*
Day 7	*5.67 ± 0.05^{aB}*	*5.82 ± 0.04^{aA}*	*5.72 ± 0.13^{aA}*	*5.82 ± 0.12^{aA}*
Day 9	*5.67 ± 0.07^{aB}*	*5.76 ± 0.08^{aAB}*	*5.63 ± 0.07^{aA}*	*5.69 ± 0.07^{aA}*
Day 12	*5.71 ± 0.07^{aB}*	*5.68 ± 0.06^{aB}*	*5.71 ± 0.13^{aA}*	*5.81 ± 0.06^{aA}*

Table 4: Water activity (aw) variation during the storage test of 12 days at 4°C.Statistically significant differences within a given day's data are denoted by distinct lowercase superscript letters within the same row, while divergent capital letters within a specific day's column signify significant differences ($p < 0.05$).

aw				
	$MAPCO_2$	$MAPCO_2$ + 1% REO	sc$MAPCO_2$	sc$MAPCO_2$ + 1% REO
Day 0	0.971 ± 0.002^{aA}	0.977 ± 0.003^{aA}	0.954 ± 0.007^{bBC}	0.956 ± 0.006^{bB}
Day 2	0.963 ± 0.004^{abAB}	0.967 ± 0.005^{aB}	0.958 ± 0.005^{bAB}	0.960 ± 0.003^{abB}
Day 5	0.970 ± 0.003^{aA}	0.972 ± 0.002^{aAB}	0.967 ± 0.002^{aA}	0.968 ± 0.004^{aA}
Day 7	0.957 ± 0.004^{aBC}	0.954 ± 0.004^{abC}	0.949 ± 0.003^{bBC}	0.958 ± 0.004^{aB}
Day 9	0.950 ± 0.002^{aCD}	0.950 ± 0.002^{aC}	0.945 ± 0.004^{abC}	0.940 ± 0.004^{bC}
Day 12	0.945 ± 0.006^{abD}	0.955 ± 0.006^{aC}	0.947 ± 0.009^{abC}	0.939 ± 0.003^{bC}

3. Conclusions

The $scCO_2MAP$ + 1% REO process resulted in an improved antimicrobial effect compared to $scCO_2MAP$ alone, allowing to prolong the microbial shelf life of chicken breast meat at refrigerated conditions. Future studies will evaluate the microbial inactivation and survival of pathogenic microorganisms such as *Salmonella*, *Listeria monocytogenes*, and *Campylobacter jejuni* strains (Regulation (EC) No 2073/2005), and will also focus on the antioxidant activity of REO, combining different combinations of temperature, pressure, and time. Considerations on different poultry meat are also suggested as possible ways to exploit this technology and to reduce food waste and on its connected environmental impact.

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