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Research Article

Increasing the shelf life of cauliflower (*Brassica oleracea* var. *botrytis*) by using pre-formed chitosan films enriched with basil (*Ocimum basilicum* L.) essential oil

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Abstract

This study investigates the efficacy of chitosan films enriched with *Ocimum basilicum* L. (basil) essential oil in extending the shelf life of cauliflower (*Brassica oleracea* var. *botrytis*). Incorporating natural preservatives into packaging materials could provide an eco-friendly alternative to synthetic chemicals. Chitosan films, recognized for their biodegradable and antimicrobial properties, were augmented with 100 µl basil essential oil/100 ml chitosan film and 200 µl basil essential oil/100 ml chitosan. The control group consisted of cauliflower florets wrapped in polyethylene perforated bags. The impact of these treatments on cauliflower quality was assessed over a storage period, evaluating parameters such as color retention, phenolic content, antioxidant activity, and bacterial contamination. Results indicated that both concentrations of basil essential oil significantly enhanced the antioxidant properties and phenolic content of the cauliflower, compared to the control. Color analysis showed better retention in treated groups, suggesting a delay in the browning process. Bacteriological assays demonstrated a reduction in microbial growth on cauliflower wrapped in films with basil oil, with 200 µl basil essential oil/100 ml chitosan showing the most substantial antimicrobial effect. These findings suggest that chitosan films enriched with basil essential oil could be an effective solution for extending the shelf life of cauliflower by maintaining quality and reducing spoilage.

Keywords: cauliflower curd; chitosan film; essential oil; microorganisms; storage

Introduction

Vegetables are very important for human nutrition because of their content in bioactive principles that ensure good health. However, due to the seasonal nature of production and the short marketing period, there is a significant loss of production (Pusik *et al*., 2019). Extending their marketing and consumption period is a major concern for producers and traders. This involves maintaining the quality of vegetables by using methods to delay ripening, spoilage (particularly microbiological spoilage) and physiological collapse (Kaynaş, 2020).

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The combination of biological, chemical and physical methods to maintain the quality of vegetables has great potential to extend the shelf life of fresh vegetables. Maintaining the firmness and freshness of fresh vegetables over a longer period of time is a consumer acceptability criterion (Volden *et al*., 2009)

Microbiological spoilage of fresh plant products and foods is a worldwide problem and the main cause of shortening their shelf life as well as a food safety issue. That's why a lot of research is currently being carried out to find various methods to control micro-organisms on the surface of fruit, vegetables and in food. These methods must be safe and must not affect the quality of the products or the health of the people who consuming them. Several researches have been conducted on the mechanism of microbiological spoilage as well as on methods to slow down this process (Alegbeleye *et al*., 2022; Fan *et al*., 2021; Wong *et al*., 2021; Miceli *et al.,* 2019). Coating fresh fruit and vegetables with biocompatible polymers is a solution to extend their shelf life and that is why many scientists focus on creating coatings using natural preservatives (Duan *et al*., 2019). Chitosan is a readily soluble substance in aqueous solutions of many inorganic and organic acids with antimicrobial activity, being one of the most abundant natural biopolymers (Fei Liu *et al*., 2001). Chitosan is obtained from crab shells, but other species such as lobster are also used, each with different chitin contents (Kou *et al.,* 2021).

Chitosan consists of a group of de-acetylated chitin polymers. Water-soluble chitosan is used to de-acidify products such as cosmetics, medicines, and foods. This is why chitosan is frequently used in biotechnology, food, and pharmaceutical industries (Edo *et al*., 2025; Azmana *et al.,* 2021). Chitosan is reported to be a bioactive compound with antifungal, antimicrobial and antioxidant properties (Shariatinia *et al.,* 2019). However, the antimicrobial action of chitosan is different depending on the type of microorganisms (Ke *et al.,* 2021).

Chitosan films function as a barrier on the surface of fruits and vegetables, keeping the water in them, thus delaying ripening. Currently, the most research is focused on improving the efficiency of these coatings using different substances with antimicrobial potential such as plant essential oils (EO), however the main problem is being the way of their incorporation (Nair *et al.,* 2020).

Essential oils are volatile compounds produced by plants with various protective functions (Franz *et al.,* 2020). It has been proven that EOs have an antimicrobial potential, but weaker than that of synthetic compounds, Gram-positive bacteria being much more sensitive to the essential oil compared to Gram-negative ones. Moreover, due to their volatility, the time of action is limited, hence the need to find methods of encapsulation and gradual release of them. However, the fact that they are of natural origin make them helpful for use as antimicrobial agents in food or in coatings of fresh fruits and vegetables (Wińska *et al.,* 2019).

One of the EOs used in the food industry described in the scientific literature is that of basil (*Ocimum basilicum* L.), one of the most cultivated aromatic plants. There are several varieties of *O. basilicum* species, which have different EO contents. Basil essential oil (BEO) has biological activity: antioxidant, antifungal and antibacterial, with linalool, eugenol, 1,8-cineole, epi-α-cadinol and α-trans-bergamotene in its composition (Ionica *et al*., 2023; Amor *et al*., 2021; Milenković *et al*., 2019).

White cauliflower (*Brassica oleracea* var. *botrytis*) is the second most popular species from the *Brassica* genus, the *Brassica oleracea* species cultivated in Romania. From cauliflower, the inflorescence primordia are used for consumption, which makes it very perishable with a short shelf life. At the same time, cauliflower is sensitive to fungal and bacterial attacks. Increasing the shelf life while maintaining the nutritional value of cauliflower florets during refrigerated storage is one of the most important issues for technologists (Palumbo *et al.,* 2022). The use of edible coatings is seen as one of the possibilities to fulfil this requirement (Lin *et al.,* 2007).

Previous research claims that edible films consisting of various biologically active substances have contributed to extending the shelf life and quality of cauliflower (Jourabian *et al.,* 2023). The application of

these films consists of immersing fresh fruit and vegetables in various solutions (films) prepared in advance followed by dripping.

A common problem with edible coatings is their non-acceptability by consumers, who often consider them to contain toxic substances. In addition, in the case of cauliflower florets, dipping in such coatings creates problems of adherence of the film to the substrate. This study aimed to extend the cold storage life of cauliflower florets wrapped in a pre-formed BEO-enriched chitosan film. Although several investigations have been carried out on the use of preformed chitosan films, so far this type of film has not been used to extend the shelf life of cauliflower. It was thus revealed which concentration of BEO used had the maximum effect on maintaining the quality of cauliflower florets and increasing their shelf life.

Materials and Methods

Reagents used

n-hexane, water – LiChrosolv® grade Merck (Burlington, MA, USA). Sodium carbonate and methanol have been acquired from Merck Darmstadt, Germany; food grade chitosan, high purity Folin–Ciocalteu reagent, gallic acid, sodium acetate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (TROLOX) were provided by Sigma-Aldrich (Taufkirchen, Germany). The other chemicals were of analytical grade.

Plant material

Cauliflower curds belonging to the variety Casper RZ F1 were used as plant material. It is a hybrid for summer-autumn crops with a growing period of 95 days in autumn crops and excellent adaptability to dry autumns. The curd is white, compact, and smooth, reaching a weight of 2-3 kg. The flowers were procured from a field crop in Grădinari (44°33′33″N 24°16′36″E), Olt County, Romania.

Cauliflower harvested early in the morning in the compact curd phase, sorted with covering leaves were transported with pre-cooling (6 °C) to the Faculty of Horticulture in Craiova, Romania.

Here they were cut into bunches (separated into experimental variants) and pre-packaged with chitosan films enriched with different concentrations of BEO prepared in advance. The prepared florets were then packed in cardboard crates for the transport of fruit and vegetables equalized to 15 kg and stored by refrigeration at 2 °C. Two cardboard boxes containing 15 kilograms of cauliflower florets were used for each experimental variant.

Ocimum basilicum essential oil

The BEO was obtained by hydro distillation using an installation consisting of a heating mantle (NAHITA, Barcelona, Spain) connected directly to a Neo Clevenger continuous distiller (Glasco, Seaside, OR USA) type. The BEO characteristics were described previously (Ionica *et al.,* 2022).

Preparation of chitosan film

A chitosan film consisting of 3% chitosan with the addition of 2.5% glycerol and 5% lactic acid was prepared (Cîrstea *et al*., 2023) by first introducing water and glycerol into a flask, then placing it on a magnetic stirrer and heating it to 90 °C. After adding the chitosan and ensuring its complete dissolution, lactic acid was added. Following thorough homogenization, the flask was placed in an ultrasound bath for 60 minutes to remove air bubbles. The cooled film was then spread on a flat ceramic surface and left for a few days to dry. After drying, it was peeled off and kept in an incubator at 60 °C for 4 hours. The prepared film was used to wrap cauliflower florets, which were then refrigerated at 2 °C (Figure 1).

For the BEO-enriched film variants after cooling the film to room temperature, BEO was incorporated into the film in different concentrations (experimental design).

Figure 1. Preparation and application of chitosan film. A) Formation of chitosan film. B) Dried chitosan film ready for use. C) Cauliflower florets wrapped in chitosan film for bio-preservation studies

Experimental design

To determine the effect of BEO-enriched chitosan films on the storage of cauliflower florets, 4 experimental variants were proposed (Table 1).

Table 1. Experimental variants and their description

Variant	Description
C (Control)	Pre-wrapped florets of cauliflower in polyethylene perforated bags and packed in cardboard boxes and stored at a temperature of 2 °C
CHI	Cauliflower florets wrapped in chitosan film, packed in cardboard boxes and stored at 2 °C
BEO1	Cauliflower florets wrapped in a chitosan film enriched with 100 µl basil essential oil/100 ml chitosan film, packed in cardboard boxes and stored at 2 °C
BEO2	Cauliflower florets wrapped in a chitosan film enriched with 200 µl basil essential oil/100 ml chitosan film, packed in cardboard boxes and stored at 2 °C

During storage, a series of chemical parameters were periodically determined to evolve the storage response of cauliflower. Every 7 days samples (film-wrapped cauliflower florets) were taken from the cardboard boxes from which, after removal of the film, chemical analysis and determinations were made. Weight loss, dry matter content, total soluble solids content, color, total polyphenol content and antioxidant activity were analyzed. The microbiological examination of cauliflower florets consisted in the determination of aerobic mesophilic bacteria present on the surface of the florets. The shelf life of the cauliflower was 4 weeks.

The properties of chitosan film

The thickness of the film was measured using a digital micrometer (Mitutoyo Corporation, 20-1, Sakado 1-Chome, Takatsu-ku, Kawasaki-shi, Kanagawa 213-8533, Japan) with a measurement range of 0 - 25 mm, a resolution of 0.001 mm, and a flatness of measuring faces of 0.6 µm. Measurements were taken at three points on the film, with the result representing the average of the replicates.

The moisture content of the films was determined by maintaining the pre-weighed films in an oven at 102 °C until a constant weight was achieved (Ahmed *et al.,* 2017). The moisture content was expressed as a percentage using the formula:

$$\text{Moisture} = (Wi - Wf/Wi) \times 100$$

where Wi represents the initial water content and Wf represents the final water content of the films.

Tensile strength (TS) was determined using a texture analyzer (SHIMADZU CORPORATION, Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) with a grip separation fixed at 30 mm and a test speed range of 50 mm/min. Strips cut from the prepared films with dimensions of 50 mm x 10 mm were used for this test.

Analytical methods

Weight loss was evaluated by weighing cauliflower florets with a digital scale (Kern, PFB 6000-1, KERN-SOHN, Balingen, Germany with an accuracy = 0.01 g) both at the beginning of the experiment and every 7 days of storage, and the results were reported as percentage of weight loss from the initial weight (Ionica *et al.,* 2022).

The dry matter (DM%) was determined as a percentage of the cauliflower dry weight in relation to its fresh weight. A total of 5 g of finely divided cauliflower was dried in a laboratory oven (Memmert, Germany) at 105°C to a constant weight. A digital refractometer (Hanna Instruments, Woonsocket, RI, USA) was used to determine Total Soluble Solids (TSS) and the results were expressed as percentages (Paul *et al.,* 2010).

Determining color

The surface color of cauliflower florets was measured using a colorimeter (CHROMA METER CR400, Colorimeter, Osaka, Japan). Color was measured in three florets of each experimental variety at the same location. CIELAB (L*, a*, b*) parameters were determined which were then converted by the CIE system to Chroma $[(C=a^{*2} + b^{*2})^{0.5}]$ and Hue angle ($H= {}^{tan\text{-}}1\ b^*/a^*$). The colorimeter was calibrated using the manufacturer's standard white plate (Gu *et al*., 2015).

Determining total phenolic content

The total phenolic content (TP) of the cauliflower methanolic extracts was measured using a Varian Cary 50 178 UV spectrophotometer (Varian Co., Palo Alto, CA, USA), coupled to a VISION Pro-software computer (Thermo Scientific), by measuring the absorbance at 765 nm.

Extraction

Extraction was carried out using methanol from three grams of cauliflower homogenate for 60 minutes (on ultrasonic bath) at room temperature. The procedure was followed by centrifugation at 6000 RPM for 15 minutes, the resulting supernatants were collected and stored at -40 °C.

Evaluation of TP content

Subsequently, 100 µL of extract was combined with 5 mL of distilled water and 500 µL of Folin-Ciocalteu reagent was added, followed by the addition of 1.5 mL of sodium carbonate solution (20% w/v). This mixture brought to a total volume of 10 mL with distilled water was shaken vigorously and incubated in the dark at 40 °C for 30 min., then the absorbance was measured at 765 nm. The results were quantified in milligrams of gallic acid equivalent (GAE) per 100 fresh weight (f.w.), determined from a pre-constructed calibration curve using standard gallic acid solutions (Ionica *et al.,* 2023).

Determining antioxidant activity (AOA Mmol Trolox/100 g f.w.)

The scavenging activities of the methanolic extracts from cauliflower samples against DPPH radicals were measured according to Cosmulescu *et al*. (2017) from the methanolic extracts obtained as in the determination of TP. Methanolic extracts (50 µl) of cauliflower diluted 1:10 were mixed with 0.004% (v/v) DPPH in methanol (3 ml) and incubated in the dark at room temperature.

The absorbance was measured at 517 nm. To establish a baseline, a blank sample was created by 0.004% (v/v) DPPH in methanol solution. The results (mMol TROLOX/100 g f.w.) were calculated using Trolox as

standard of different concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.5 mM). For each analysis or determination performed there were three replicates with the results representing the arithmetic mean of the three replicates.

Bacteriological analysis

The determination of the total aerobic mesophilic germ number was determined as follows the protocol presented by Aycicek *et al.* (2006). Ten grams cauliflower was mixed with 90 ml of dispersion solution (0.1% peptone water) and homogenized for 120 s. A 0.1 ml inoculum of dilutions of 10^{-3} and 10^{-4} was applied to the surface of standard culture medium (Granucult Merck, Burlington, MA, USA) distributed in Petri dishes. For incubation, the temperature was adjusted to 30 °C for 48 hours. Quantitative assessment was carried out by counting colonies. The results, expressed as log CFU/g, were derived from the mean of three replicates and subjected to statistical validation. Standard methods (morphology, tinctorial affinities, cultural characteristics) were used for identification.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.1. The relationships between the groups were examined using one-way ANOVA. This approach was specifically applied to identify significant differences between each experimental group and the control group (C).

Results

This study demonstrated the chemical and microbiological changes that occur in cauliflower during storage as well as the differences that occur as a result of the effect of packaging the florets with chitosan films enriched with basil essential oil. Changes in weight loss during storage, total dry matter content (DM%), total soluble solids (TSS%), polyphenols (TP), antioxidant activity (AOA), color parameters (lightness, chroma, hue angle) as well as the total number of aerobic mesophilic bacteria were thus highlighted.

Chitosan film characterization

All the obtained films were transparent with a slight yellowish tint, allowing visualization of the wrapped product. Additionally, the films had a uniform thickness and did not exhibit any bubbles within their structure (Figure 2).

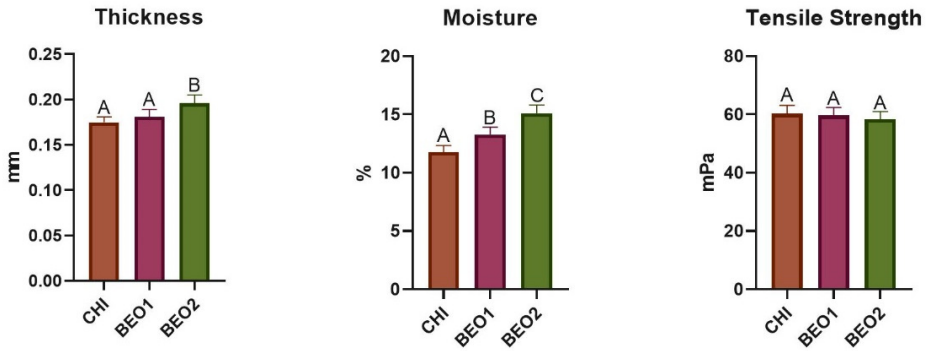

Figure 2. Comparison of film thickness, moisture content, and tensile strength for chitosan films (CHI) and chitosan films incorporated with essential oils (BEO1 and BEO2)

Means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Capital letters indicate the effect of BEO on the characteristics of the chitosan film

The thickness of the chitosan films varied depending on the amount of essential oil added. The thickness ranged from 0.175 mm (CHI) to 0.196 mm (BEO 2). Thickness of the chitosan film was not affected by BEO addition in 100 µl dose. The amount of BEO added in doses of 200 ul affected the thickness of the chitosan film with significant differences compared to the other two variants (CHI and BEO1). The film moisture was between 11.77% (CHI) and 15.09% (BEO 2). There are significant differences between variants, with the addition of BEO increasing the moisture of the film. The enhancement was directly proportional to the dose of BEO added. Tensile strength measurements showed variations between 58.37 (BEO 2) and 60.21 (CHI), without significant differences between variants, which correspond to values reported in the literature (Peh *et al.,* 2000; Cazón *et al.,* 2020).

Overall, the inclusion of essential oils influences the physical properties of the chitosan films, particularly thickness and moisture content, while tensile strength remains almost unaffected. Still the fortification of chitosan films with BEO may affect their properties, which will be investigated in further studies.

Weight loss

Weight loss during storage is shown in Figure 3. Weight loss during storage is influenced by exogenous and endogenous factors. Endogenous factors are represented by cauliflower chemical composition, metabolism, and curd structure. Exogenous factors include storage conditions and packaging methods (Pusik *et al.,* 2019).

After seven days of storage the weight losses ranged from 0.6 to 0.9% with the highest values recorded in the control (0.9%). The coated cauliflower florets of CHI and BEO1 did not differ significantly from the control (0.8%). The lowest weight loss after 7 days was observed in the BEO2 variant (0.6%) with significant differences from the other variants. After 14 days of storage, weight losses increased to values between 0.8 and 1.3% with significant differences between BEO1, BEO2 and C, CHI variants. This increased rate of weight loss was maintained throughout the shelf life. Thus, at the end of the retention period, weight loss was between 1.2 and 2.2%. The highest value was observed in the control (2.2%) while the BEO2 variant recorded the lowest weight loss value (1.2%).

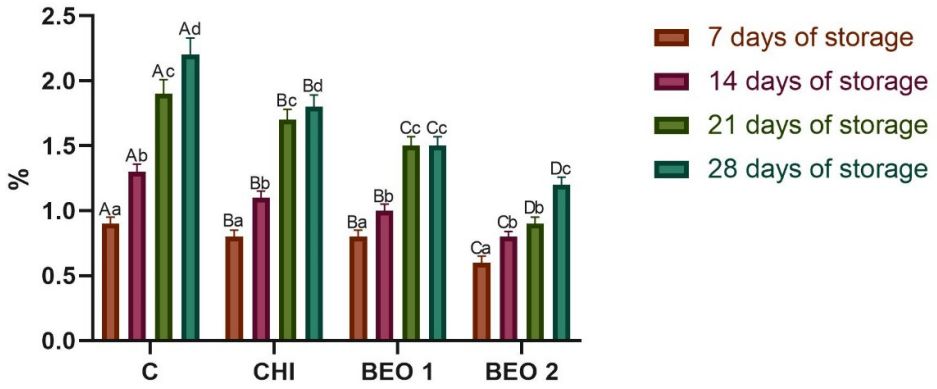

Figure 3. Graphical representation of the cauliflower weight loss during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

Dry matter

The results for DM evolution during storage are shown in Figure 4.

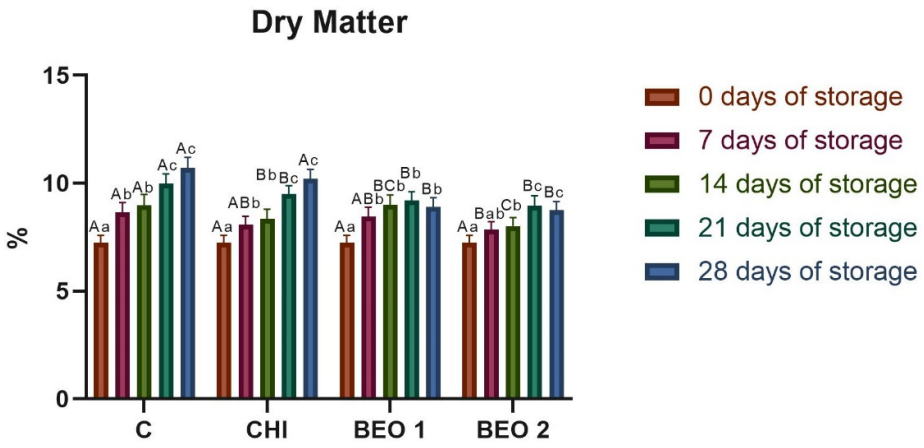

Figure 4. Graphical representation of the cauliflower dry matter during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

The initial DM content (%) of cauliflower was 7.24%, which is lower than the data presented by EL-Bauome *et al*. (2022). It increased during storage to between 8.74% at BEO2 and 10.7% at Control. Significant differences are thus found for the experimental variants ($p \leq 0.05$). At the end of storage, the lowest DM value (%) was found for BEO2, i.e. cauliflower florets wrapped in chitosan yarn enriched 200 µl basil essential oil. The BEO-enriched chitosan film-coated variants showed a lower rate of increase in DM content (%) than the control and CHI variants.

Total soluble solids

The initial TSS content (%) was 4.9% and increased steadily to values between 6.1% (BEO2) and 7.4% (Control) with significant differences between the variants (Figure 5). The data found are consistent with those shown by Nasrin *et al*. (2022).

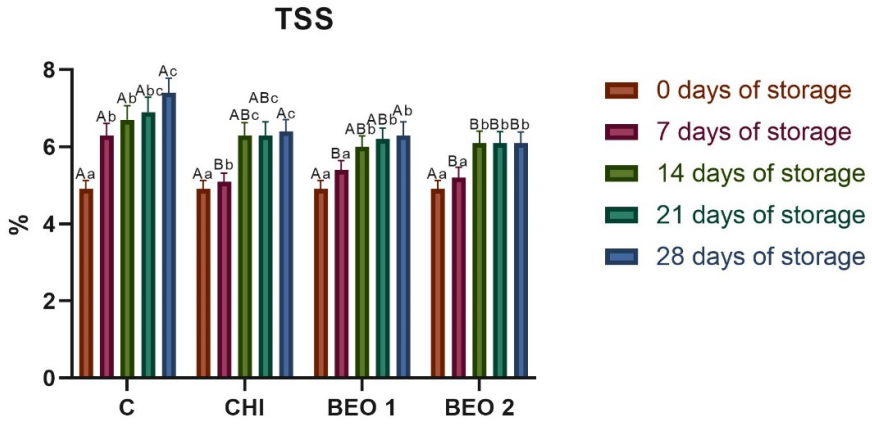

Figure 5. Graphical representation of the cauliflower total soluble solids during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

At the end of storage, the lowest TSS was found in BEO2 (6.1%) and BEO1 (6.3%) with significant differences from the control. There is a strong effect of coating cauliflower florets with BEO-enriched chitosan film on TSS (BEO1 and BEO2) compared to the control and CHI. Also, the cumulative effect of the storage period as well as the wrapping of the bunches with film is significant.

Color

The color variation of cauliflower florets was expressed using the CIELAB parameters: Lightness (L), Chroma (C) and Hue angle (H), these most accurately capture the variation of cauliflower florets during the storage period and are shown in Figures 5, 6 and 7. Changes in the color of the external areas of cauliflower vary enormously depending on both the storage period and the type of film wrapping (Figure 6). Initially L had the value of 81.3. For all variants L decreased during the first 7 days of storage, then increased contact up to 21 days of storage and then decreased sharply to values between 85.03 (BEO2) and 86.7 (Control). The decrease in L was sharper in the CHI and Control variants.

However, there were no significant differences between the variants in terms of lightness.

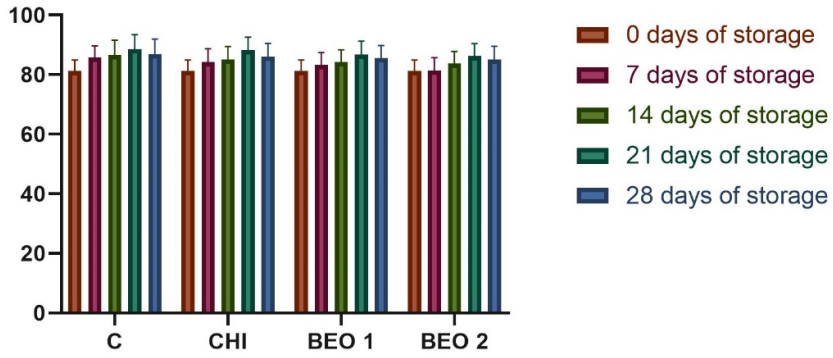

Figure 6. Graphical representation of the cauliflower lightness during storage
It was found that Chroma (C) increased during the first 7 days of storage (from 23.11 to 24.81) and then decreased continuously until day 21 of storage with significant differences from the beginning of storage. Very significant differences were found between the experimental variants especially towards the end of the storage period, expressed by a pronounced color change in the Control and CHI

It was found that Chroma (C) increased during the first 7 days of storage (from 23.11 to 24.81) and then decreased continuously until day 21 of storage with significant differences from the beginning of storage (Figure 7). Very significant differences were found between the experimental variants especially towards the end of the storage period

Similar values and evolution were found by Gu *et al*. (2015) in four varieties of cauliflower stored by refrigeration at 4 °C and Sanz Cervera *et al*. (2007) in cauliflower packed in 4 types of films (a microperforated PVC and propylene).

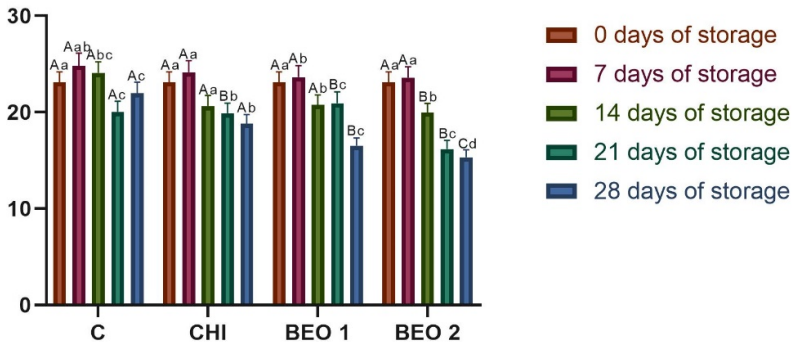

Figure 7. Graphical representation of the cauliflower chroma during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

The initial hue angle (Figure 8) was 84.69 and remained almost constant during the first 7 days of storage for all variants except BEO2 (83.54). Starting from day 14 of storage H decreased dramatically in the control and CHI while in the BEO variants it remained almost constant with a slight decrease in the last 7 days of storage. There were no significant differences between variants at the end of storage.

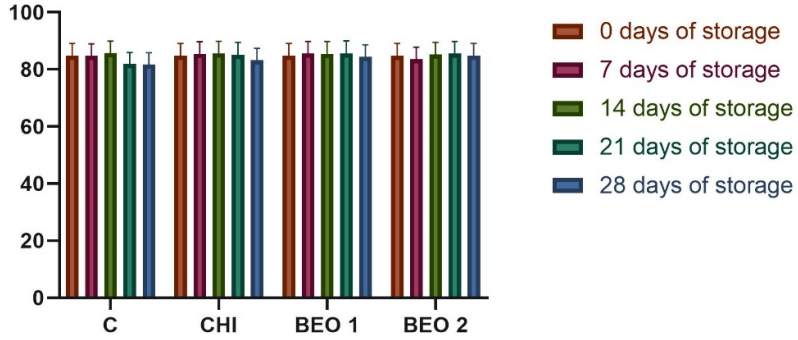

Figure 8. Graphical representation of the cauliflower hue angle during storage

The cumulative effect of the storage period and the films used was significant and was mainly determined by the presence of BEO in the films used for wrapping. Moreover, the noticeable color change in the control and CHI is also due to the appearance of fungi on the surface of the florets (Figure 9).

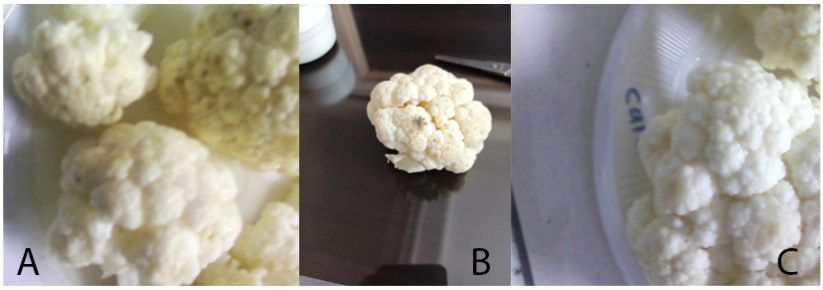

Figure 9. Change in color of cauliflower during storage (A = Control; B = CHI; C = BEO2)

Total phenolic content

At the beginning of the experiment TP was 48.12 mg/100g f.w. It showed increases during storage except during 14-21 days of storage. Between 21 and 28 days of storage TP increased sharply less in the control where growth was slower. At the end of storage TP had values between 58.42 mg/100g f.w. (CHI) and 74.06 mg/100g f.w. (BEO1). Significant differences ($p \leq 0.05$) were found between variants. The results found are similar to those reported by Raja *et al*. (2011) (Figure 10).

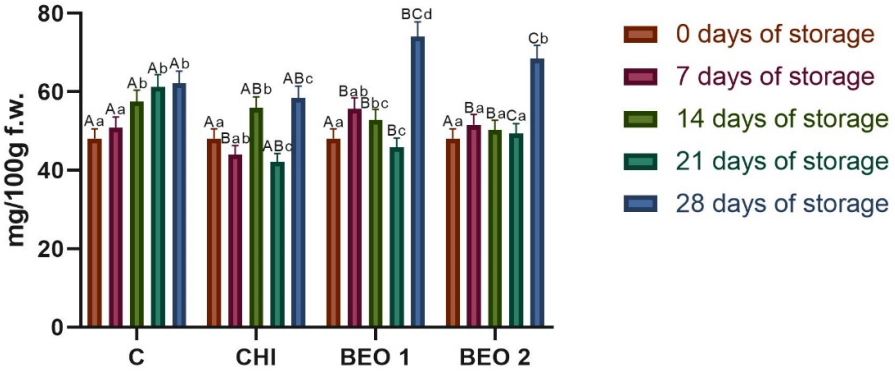

Figure 10. Graphical representation of the cauliflower total phenolic content during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

Antioxidant activity

The variation of the antioxidant activity of cauliflower florets during storage in relation to the type of film used is shown in Figure 11. The initial antioxidant activity was 1.6 mmol Trolox/100g f.w., a value which was similar to that found by Volden, Bengtsson & Wicklund (2009). In the first 7 days of storage AOA decreased. The smoothest decrease was observed in the BEO2 variant (1.57 mMol TROLOX/100 g.f.w.), while the sharpest decrease was found in the CHI variant (0.98 mMol TROLOX/100g f.w.). At the end of the storage period, it was found that AOA increased from the initial value with significant differences between variants ($p \leq 0.05$).

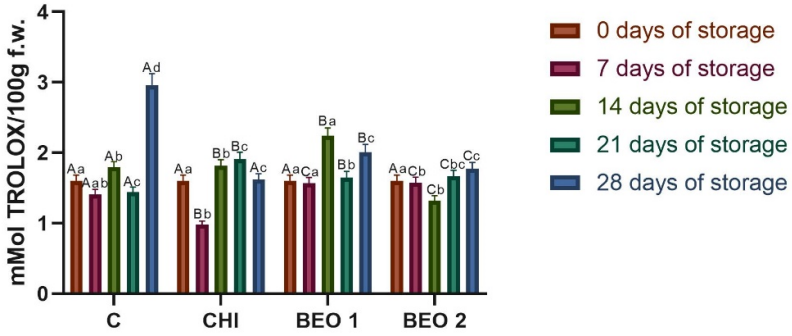

Figure 11. Graphical representation of the cauliflower antioxidant activity during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

Bacteriological analysis

The bacteriological analysis consisting in the determination of the total number of aerobic mesophilic germs (TAMG) is shown in Figure 12 The TAMG on raw material has been 0.47 log CFU/g. Bacterial load decreased during the first 7 days of storage in BEO1 and BEO2 (chitosan film enriched with BEO) while it increased in control and CHI. Moreover, in the BEO2 variant TAMG showed a value of 0 with significant differences ($p \leq 0.05$) from the C (1.04 log CFU/g) and CHI (1.00 log CFU/g). Over the next 21 days of storage the TAMG increased steadily with very significant differences ($p \leq 0.05$) observed at the end of storage between the variants (Figure 13). However, at the end of storage it was found that the BEO1 (0.6 log CFU/g) and BEO 2 (1.47 log CFU/g) variants had much lower TAMG loading compared to the Control (2.96 log CFU/g) and CHI (2.01 log CFU/g). The effect of BEO-enriched chitosan films had a strong effect on the TAMG keeping the TAMG at low values throughout storage.

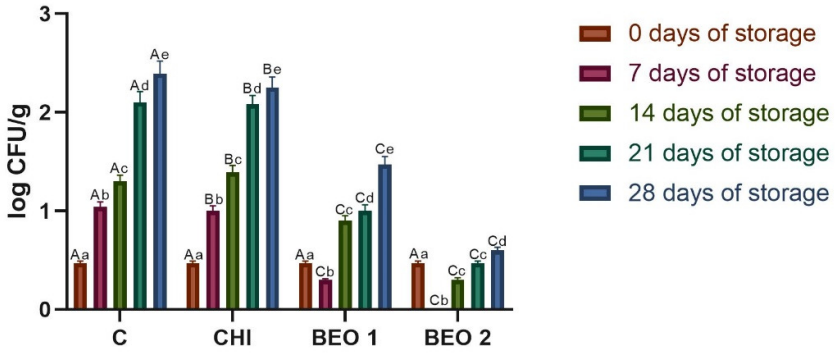

Figure 12. Graphical representation of the cauliflower bacteriological analysis during storage
Large letters indicate the influence of essential oil addition, while small letters indicate the influence of storage duration. The means of the replicates were tested by the Least Significant Difference (LSD) test ($p < 0.05$). Error bars represent standard deviations. Different letters above the bars denote statistically significant differences

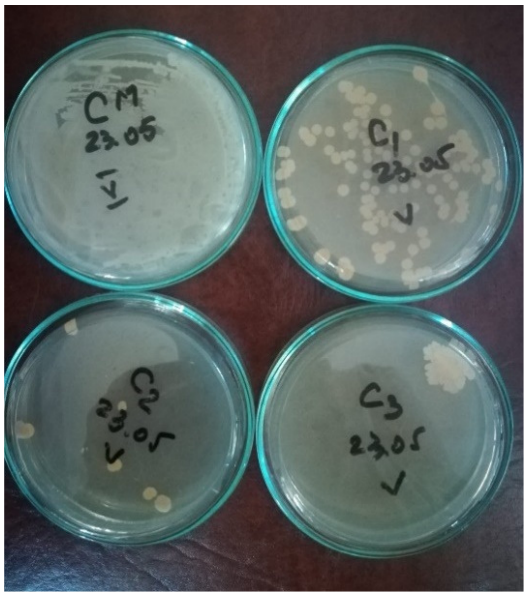

Figure 13. Microbiological analysis of cauliflower florets at the end of storage

Discussion

Due to its perishability, the shelf life of cauliflower is extremely short. Moreover, because of this, the marketing life of cauliflower is short. Nevertheless, cauliflower is an attractive vegetable for consumers. Finding ways to extend the shelf life of cauliflower while maintaining its quality and consumer acceptability is a major challenge for researchers. Since from the cauliflower plant the inflorescences are consumed, delaying their ripening and prolonging their shelf life is of particular importance. Ripening is an irreversible process that eventually leads to physiological collapse. It undergoes a series of chemical and physiological changes that result in loss of quality and unacceptability for consumption. At the same time these changes lead to loss of firmness of the inflorescences which creates a favourable environment for the development of pathogens.

Therefore, delaying the ripening of the inflorescences allows their quality to be maintained over a longer period of time and their acceptability for consumption to be maintained. Over time a number of methods such as controlled atmosphere or refrigerated storage at temperatures between 0 °C and 4 °C have been previously studied (Fasake *et al*., 2022; Mu *et al.,* 2022; Kaynaş *et al*., 2020). Chitosan is a substance with antimicrobial effect that is why in recent years it has been used as a coating agent for fruits and vegetables (Confederat *et al.,* 2021; Dewi *et al.,* 2017; Goy *et al.,* 2009). The enriched chitosan films with BEO (with high antimicrobial activity) in different concentrations resulted in the extension of the shelf life of cauliflower florets by 7 days (storage length 28 days compared to 21 days mentioned by Raja *et al*. (2011). By using these films, weight loss during storage was reduced by about 50%, with the BEO2 version recording the lowest values followed by BEO1The TSS amount also differed according to the films used, with BEO2 and BEO1 variants again standing out. Cauliflower color during storage evolved adversely in the control and CHI variants while it was maintained in BEO2. The change in color may be due to mechanical shocks and pressure points that have subsequently been affected by fungal attack (Mohamed Mahroop Raja *et al*., 2011*)*. At the end of storing, an increase in the number of brownish-brown spots was observed in the control variant. Cauliflower is a source of antioxidant substances with a high content of phenolic substances (Hashem *et al.,* 2021). BEO-enriched chitosan films significantly influenced the evolution of TP and AOA where they were found to increase at the end of storage compared to the beginning of storage in contrast to the data found by Raja *et al*., (2011) for cauliflower packed in polyethylene bags. Cauliflower can be infested and carry large numbers of microorganisms after harvest and during storage (Zhang *et al.,* 2022). These microorganisms are mostly responsible for the spoilage of cauliflower during storage. Chitosan films enriched with BEO had a dramatic effect on aerobic mesophilic bacteria present on curds. After 7 days of storage TAMG reached 0 in BEO2. Moreover, the effect of BEO on TAMG can be observed throughout the storage period as its concentration significantly influences the results.

The aim of this study was not only to investigate the characteristics of the film itself but also its effect on the preservation of cauliflower. However, we performed some basic determinations and some results that without significant differences was omitted from the paper. It is true that the literature is abundant with studies on packaging films, but there are few studies on the effect of such films on extending the shelf life of cauliflower. The purpose of this work was not to develop the film and its characteristics but to examine the effect of basil essential oil-enriched film on the preservation of cauliflower. The specialized literature does not refer to this aspect. This paper focuses strictly on a packaging technology that leads to an extended shelf life of cauliflower.

Conclusions

Packaging cauliflower in preformed chitosan films enriched with basil essential oil can be a way to extend its shelf life. The concentration of basil essential oil influences the load of micro-organisms, thus prolonging its durability and shelf life. The combined effect of chitosan and basil essential oil was major on the microbiological load on the cauliflower curds. This effect was also manifested on the variation of the chemical components

during the storage of the cauliflower. The use of basil essential oil in concentrations of 200 µl per 100 ml of chitosan film for packaging found as the most effective option for extending the shelf life of cauliflower.

Authors' Contributions

Conceptualization, M.E.I.; methodology, M.E.I., F.T., A.R.C. and A.B.; software, A.B.; validation, M.E.I., F.T., A.R.C. and A.B.; formal analysis, M.E.I., F.T., A.R.C. and A.B.; investigation, M.E.I., F.T., A.R.C. and A.B.; resources, M.E.I., F.T., A.R.C. and A.B.; data curation, M.E.I., F.T., A.R.C. and A.B.; writing—original draft preparation, M.E.I. and A.B.; writing—review and editing, M.E.I. and A.B.; visualization, M.E.I. and A.B.; supervision, M.E.I. and A.B.; project administration, M.E.I. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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