

## Phytochemical contents, antioxidant activity, and functional properties of encapsulated girgir (*Eruca sativa*), figl (*Raphanus sativus*), and cabbage (*Brassica oleracea var. capitata*) extracts

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### Abstract

The leaves of *Eruca sativa* (commonly known as girgir), the roots of *Raphanus sativus* (figl), and *Brassica oleracea var. capitata* (cabbage) are rich in bioactive compounds such as carotenoids, alkaloids, phenolic acids, flavonoids, tannins, and essential vitamins (B3, B6, B9, and C), all of which contribute to their potent antioxidant and antimicrobial effects. This study evaluates the microbiological activity, quality attributes, and sensory properties—specifically color and texture—of fish burgers enriched with encapsulated extracts of *E. sativa* (Encaps-ES), *R. sativus* (Encaps-RS), and *B. oleracea var. capitata* (Encaps-BO), which were encapsulated in alginate beads. The fish burgers were refrigerated ( $4 \pm 2^\circ\text{C}$ ) for 15 days. To assess the quality and stability, various physicochemical parameters, including pH, total volatile nitrogen (TVN), thiobarbituric acid (TBA), trimethylamine (TMA), water-holding capacity (WHC), and plasticity, were analyzed. The highest pH value of 5.75 was recorded for Encaps-BO, followed by Encaps-RS and Encaps-ES, with no significant differences ( $p > 0.05$ ). In the control sample, TMA levels increased significantly from  $1.35 \pm 0.39$  to  $5.55 \pm 0.20$  mg/100g over the 15-day period. However, the encapsulated extracts, including Encaps-ES, Encaps-RS, and Encaps-BO, exhibited initial TMA values of 1.35, 1.40, and 1.55 mg/100g, respectively, which increased to 5.83, 5.55, and 4.15 mg/100g by day 15. Among the encapsulated extracts, Encaps-RS showed superior pH stability and WHC, while Encaps-BO exhibited better plasticity. Microbiological analysis revealed a reduction in the total aerobic plate count (APC) for Encaps-RS, Encaps-BO, and Encaps-ES, from initial levels of  $3.55 \times 10^3$ ,  $3.80 \times 10^3$ , and  $3.15 \times 10^3$  cfu/g to  $2.12 \times 10^3$ ,  $2.22 \times 10^3$ , and  $2.03 \times 10^3$  cfu/g, respectively, after 15 days. Encaps-BO samples maintained superior texture and color stability, followed by Encaps-RS and Encaps-ES. These results emphasize the potential of encapsulated plant extracts as effective natural antioxidants and antimicrobial agents, enhancing the preservation, quality, and sensory appeal of fish-based products. The findings support the application of these encapsulated extracts as a means of extending the shelf life and improving the safety of perishable meat products.

**Keywords:** Encapsulation, antioxidant activity, functional properties, Phytochemical contents

### Introduction

Plant-derived bioactive compounds are crucial for various biological functions and can significantly influence

physiological changes that enhance human health (Niaz *et al.*, 2020; Tran *et al.*, 2020). These compounds include a wide range of phytochemicals and nutrients such as proteins, carbohydrates, lipids, vitamins, and minerals,

which are abundant in plant-based products (Deledda *et al.*, 2021; Narzary *et al.*, 2016). Phytochemicals serve as important antioxidants, safeguarding plants against reactive oxygen species while also providing numerous health benefits to humans (Allam *et al.*, 2021a; Narzary *et al.*, 2016). Notably, a single plant species can harbor over a thousand unique phytochemicals (Kumar and Khanum, 2012).

The commercialization of underutilized plants presents an opportunity to enhance food supply, nutritional diversity, and economic revenue. In developing countries, lesser-known edible plants could play a vital role in alleviating concerns over food and nutrition (Al Jumayi *et al.*, 2022; Ebert, 2014; Hughes, 2008). The interest in exploring these underutilized species for their bioactive compounds has increased within the food and pharmaceutical industries because of their potential health benefits and ability to promote sustainable practices.

This study focuses on the phytochemical composition, antioxidant activity, and functional properties of encapsulated extracts from *Eruca sativa* (girgir), *Raphanus sativus* (figl), and *B. var. capitata* (cabbage). These plants have rich nutritional profiles and medicinal attributes, including antioxidant and antibacterial effects, that prolong the shelf life of food and improve product quality. Bioactive compounds from plants are essential for human health as they facilitate physiological processes and help mitigate the risk of noncommunicable diseases (Niaz *et al.*, 2020; Tran *et al.*, 2020). Phytochemicals like polyphenols, alkaloids, flavonoids, and vitamins are present in plants, providing substantial antioxidant protection against reactive oxygen species along with various health benefits (Allam *et al.*, 2021a; Narzary *et al.*, 2016). Remarkably, individual plant species may contain hundreds of distinct phytochemicals (Kumar and Khanum, 2012).

The commercialization of these underutilized plants can significantly improve dietary diversity and nutrition while generating an income, particularly in underdeveloped areas where they can help address food insecurity (Ebert, 2014; Al Jumayi *et al.*, 2022). For instance, the leaves of girgir and figl are excellent sources of dietary fiber, essential minerals, and vitamins (Allam, 2023; Keyata *et al.*, 2021). Traditionally, leaves and roots of figl have been used to treat various ailments such as gastrointestinal disorders, liver inflammation, urinary infections, cardiovascular diseases, and ulcers due to their secondary metabolites like glucosinolates, polyphenols, and isothiocyanates (Singh *et al.*, 2003; Teklić *et al.*, 2021).

Cabbage (*B. oleracea* var. *capitata*), one of the most widely cultivated vegetables worldwide, belongs to the Cruciferae family along with kale, broccoli, and

cauliflower (Tsao and Lo, 2004). Originally from Western Europe, cabbage cultivars exhibit considerable diversity in leaf and head characteristics (Capinera, 2020). Cabbage is a significant source of antioxidants and other bioactive compounds that contribute to its health benefits. Girgir (*E. sativa*) in particular contains phenolic acids, flavonoids, carotenoids, glycosides, and alkaloids. These compounds have antidiabetic, antibacterial, and antihypertensive effects and promote hair growth (Keyata *et al.*, 2021; Marwat *et al.*, 2016). Both figl and girgir leaves are recognized for their high content of antioxidants and flavonoids (Mazzucotelli *et al.*, 2018; Sarikurkcü *et al.*, 2017). However, there is only limited research on the phytochemical profiles and functional attributes of these indigenous plants in regions like Taif, Saudi Arabia (Allam *et al.*, 2021b). It is crucial to investigate and validate their potential as functional ingredients in food formulations catering to diverse populations.

This study aims to characterize the phytochemical content and antimicrobial and antioxidant activities of *E. sativa*, *R. sativus*, and *B. oleracea* var. *capitata*, using high-performance liquid chromatography (HPLC). By evaluating their functional properties, the study seeks to explore opportunities for the development and commercialization of these plants, contributing to food security and health initiatives (Figure 1).

## Materials and Methods

### Extraction technique

The roots of *E. sativa* (girgir), *R. sativus* (Figl), and *B. oleracea* var. *capitata* (cabbage) were sourced from a local market in Taif, Saudi Arabia, and quickly transported to the Meat Products Laboratory at Taif University's Department of Food Science and Technology. The transportation was conducted in plastic containers, ensuring that the samples arrived within 1 to 2 h at room temperature. Upon reaching the laboratory, the plant materials were thoroughly rinsed under running tap water to eliminate any contaminants, followed by manual peeling of the roots. The cleaned samples were freeze-dried and then ground into a fine powder using an electric blender to create raw extracts. To ensure complete dehydration, these crude extracts were subjected to further drying in an oven set to 30–35°C for a duration of 24 to 30 h. For the preparation of the extracts, 500 mg of the powdered crude extract was mixed with 100 mL of either ethanol or distilled water. This mixture was continuously agitated for 24 h using a mechanical shaker to enhance the extraction process. Afterward, the solution was filtered to remove solid residues and stored at 4°C until further analysis, following the protocols outlined by Allam *et al.* (2021).



**Figure 1.** Visual representation of the integration of natural, plant-based extracts, specifically from *B. oleracea var. capitata* (cabbage), *E. sativa* (girgir), and *R. sativus* (figl), in fish burger formulations. It highlights their antioxidant and antimicrobial properties, shelf-life extension benefits, and potential to replace synthetic additives for safer and more sustainable food systems.

### Total polyphenol

The total flavonoid content (TFC) of the extract was determined by the method established by Sakanaka *et al.* (2005). The reaction mixture was prepared in a 10 mL volumetric flask. Initially, 0.5 mL of the extract was mixed with 5 mL of distilled water and 0.3 mL of 1:20 sodium nitrite ( $\text{NaNO}_2$ ) solution. This mixture was allowed to react for 5 min. Subsequently, 3 mL of 1:10 aluminum chloride ( $\text{AlCl}_3$ ) solution was added, and after an additional 6 min, 2 mL of a 4% sodium hydroxide ( $\text{NaOH}$ ) solution was introduced. The final volume was adjusted to 10 mL with distilled water. The resulting solution was thoroughly mixed, and its absorbance was measured at 510 nm using a spectrophotometer (Schoot Instrument, UV Line 9400, EU), with a blank sample serving as the reference. The flavonoid content was calculated and expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g). To ensure precision and reproducibility, all measurements were performed in triplicate, in accordance with the protocol outlined by Sakanaka *et al.* (2005).

### Total flavonoids

The TFC of the extract was assessed using the method described by Sakanaka *et al.* (2005). A 10 mL volumetric flask was used to create the reaction mixture. To begin

with, 0.5 mL of the extract was mixed with 5 mL of distilled water and 0.3 mL of 1:20  $\text{NaNO}_2$  solution. This mixture was allowed to react for 5 min. Next, 3 mL of 1:10  $\text{AlCl}_3$  solution was added, and after another 6 min, 2 mL of 4%  $\text{NaOH}$  solution was incorporated. The final volume was then adjusted to 10 mL with distilled water. The resulting solution was thoroughly mixed, and its absorbance was measured at 510 nm using a spectrophotometer (Schoot Instrument, UV Line 9400, EU), with a blank sample serving as a reference. The flavonoid content was calculated and reported as milligrams of quercetin equivalents per gram of sample (mg QE/g). To ensure precision and reproducibility, all measurements were performed in triplicate, in accordance with the procedures outlined by Sakanaka *et al.* (2005).

### Encapsulation of BE

Ethanolic extracts of *E. sativa* (ES), *R. sativus* (RS), and *B. oleracea var. capitata* (BO) were encapsulated in sodium alginate according to the protocol established by Ribeiro and Veloso (2020). Initially, the extracts were diluted and then combined with sodium alginate (% w/v) to create a homogeneous solution. The mixtures of ES, RS, and BO with sodium alginate were weighed and injected into a 1.5% (w/v) calcium chloride solution using a syringe fitted with a 0.80 mm × 25 mm needle. The resulting beads were thoroughly washed, filtered through

sterile Whatman® Grade I filter paper, and air-dried for 20 min. After drying, the beads were weighed to confirm that no unencapsulated extract remained from the pelleting process. Digital images of the encapsulated beads were captured using a Brother MFC-7360N scanner, and their sizes were analyzed with the open-source software ImageJ1, as outlined by Aguirre and Santagapita (2016). The average diameter of the beads was calculated based on digital measurements, evaluating the total distance (mm) between various points along the bead boundaries. Standard deviation (SD) was employed to assess variability in bead size, ensuring accuracy and consistency throughout the encapsulation process.

### Fish burger patty preparation

#### Marine fish

The carp fish (*Cyprinus carpio* L.) were thoroughly rinsed under running tap water to remove any blood and the dark lining from the gut cavity. Following this cleaning process, the head, skin, and bones were carefully removed. The cleaned fish flesh was then processed using a meat mincer (Moulinex 65, Taif, Saudi Arabia) to produce minced fish meat, which was prepared for further processing and analysis.

#### Preparation of carp

The carp fish were washed meticulously under running tap water to eliminate blood and the dark lining within the gut cavity. Following this, the head, skin, and bones were removed. The cleaned fish flesh was then processed using a meat mincer (Moulinex 65, Taif, Saudi Arabia) to obtain minced fish meat, which was prepared for subsequent processing and analysis.

#### Defatted textured soybean

Defatted textured soy protein, containing 46% protein and 5.86% fat, was sourced from the Food Technology Research Institute at the Agricultural Research Center in Giza, Egypt.

#### Preparation of texturized soybean and bulgur

Defatted texturized soy protein was hydrated in water at a 1:2 (w/v) ratio for 30 min and then processed using a meat mincer (Moulinex 65, Taif, Saudi Arabia). Meanwhile, bulgur was cleaned to remove any impurities, rinsed thoroughly with water multiple times, and soaked in water at a ratio of 1:2 (w/v) for 2 h. After soaking, the bulgur was also minced using the same meat mincer (Moulinex 65, Taif, Saudi Arabia).

#### Preparation of spices mixture

Dried natural herbs were finely ground into a powder using a laboratory mill (Moulinex 65, France). A spice blend was then created by combining the following

ingredients in specific proportions: 32% black pepper, 22.5% coriander, 15% cumin, 10% cardamom, 9% red pepper, 7.5% cubeb, and 4% clove.

#### Fish burger

A total of 1 kg of minced fish was mixed with 0.8% (w/w) NaCl in a mixing bowl for 3 min to create a uniform blend. This mixture was then divided into four experimental groups, each weighing 250 g: (1) Control group, which included sterile distilled water; (2) encapsulated with *E. sativa* extract (Encaps-ES 5%, v/w); (3) encapsulated with *R. sativus* extract (Encaps-RS 5%, v/w); and (4) encapsulated with *B. oleracea* extract (Encaps-BO 5%, v/w). The 5% (v/w) extract concentration was selected based on previous studies involving fish slices (Allam *et al.*, 2021; Aminzare *et al.*, 2015). Burger patties were then formed using a patty maker to ensure uniform weight and size, with each patty weighing approximately 50 g and having a diameter ranging from 5 cm to 7 cm, as outlined in Table 1. The burger samples were organized into four distinct groups.

All burger samples were placed in an aerobic environment on food trays, covered with polyethylene plastic film, and refrigerated at a temperature of  $4 \pm 2^\circ\text{C}$ . The samples were arranged on foam plates, wrapped in polyethylene film, and kept in the refrigerator for up to 15 days. Evaluations were conducted at 5-day intervals, that is, on days 0, 5, 10, and 15 of storage, with each sample from every batch being analyzed for pH, physicochemical properties, cooking characteristics, microbiological parameters, and sensory attributes.

### Physicochemical properties

#### Analytical method

The total volatile nitrogen (TVN), pH, trimethylamine (TMA), and thiobarbituric acid (TBA) levels in the samples were analyzed using the methods outlined by Baldi *et al.* (2018). Water-holding capacity (WHC) and

**Table 1. Beef burger formulation used in the study.**

Treatment	Ingredients in burger patties
Control	Minced fish + 0.8% (w/w) NaCl + 5% (v/w) sterile distilled water
Encaps-es	Minced fish + 0.8% (w/w) NaCl + 5% (v/w) of <i>Eruca sativa</i> (ES) encapsulated in alginate beads
Encaps-rs	Minced fish + 0.8% (w/w) NaCl + 5% (v/w) <i>Raphanus</i> ( <i>Raphanus sativus</i> ) extract encapsulated in alginate beads
Encaps-bo	Minced fish + 0.8% (w/w) NaCl + 5% (v/w) <i>Brassica</i> [ <i>Brassica oleracea</i> var. <i>capitata</i> ]- (BO)] extract encapsulated in alginate beads

plasticity were evaluated using the filter press technique as described by Honikel and Hamm (1994). The pH value was measured following the procedure suggested by Chemists (2010).

#### Cooking characteristics

Cooking loss, fat retention, and moisture retention were determined using the methods described by (Sánchez-Zapata *et al.*, 2010) as follows:

**Cooking loss:** The weight difference before and after cooking was measured to calculate the cooking loss, which is expressed as a percentage of the initial weight.

$$\% \text{Cooking loss} = \frac{\text{Uncooked burger weight} - \text{Cooked burger weight}}{\text{Uncooked burger weight}}$$

Burger samples were thawed at 5°C then fried in deep corn oil for 2–3 min until they turned light yellow, according to (Hachmeister and Fung, 1993). Cooking loss was calculated as the difference in the mass according to Marius *et al.* (2012).

**Fat retention:** The fat content of the cooked samples was determined, and fat retention was calculated as the ratio of the fat content in the cooked sample to the initial fat content before cooking, expressed as a percentage.

$$\% \text{Fat retention} = \frac{\text{Cooked burger weight} \times \% \text{Fat in cooked burger sample}}{\text{Uncooked burger weight} \times \% \text{Fat in uncooked burger sample}} \times 100$$

**Moisture retention:** Moisture content was determined before and after cooking, and moisture retention was calculated as the ratio of the moisture content in the cooked sample to the initial moisture content, expressed as a percentage.

$$\% \text{Moisture retention} = \frac{\text{Cooked burger weight} \times \% \text{Moisture in cooked burger sample}}{\text{Uncooked burger weight} \times \% \text{Moisture in uncooked burger sample}} \times 100$$

#### Microbiological examination

Fish burger samples were prepared according to the guidelines provided by the American Public Health Association (Young and Nestle, 2021). Microbiological analysis was conducted to evaluate the following parameters:

- **Total viable bacterial count (TVBC):** This was determined by preparing appropriate dilutions of the samples, plating them on suitable agar media, and incubating under standardized conditions to quantify the total number of viable bacteria.

- **Psychrophilic bacteria:** These microorganisms were quantified by incubating the samples at low temperatures (typically around 4°C), which promote the growth of psychrophilic species.
- **Coliform group bacteria:** Coliforms were identified using selective media, followed by the most probable number (MPN) method or plating techniques for bacterial enumeration (Korzeniewska *et al.*, 2009).
- **Salmonella spp. count:** The presence of Salmonella species was evaluated through selective enrichment and plating methods, as described by Nam *et al.* (2005), utilizing specific media to isolate and identify these bacteria.

#### Sensory evaluation

The sensory evaluation of the fish burgers was conducted by trained panelists who assessed various sensory attributes, including taste, odor, texture, color, and overall acceptability. These characteristics were rated on a hedonic scale of 1 to 10, where 1 indicated “extremely disliked” and 10 represented “extremely liked,” as outlined by Huang *et al.* (2021). The data were analyzed using Costas version 6.311 (Copyright 1998-2005, CoHort Software). If a significant main effect was detected, means were separated using the completely randomized design (CRD) test. A significance level of 5% ( $P \leq 0.05$ ) was applied to all comparisons, in accordance with the guidelines provided by Curtis *et al.* (2015).

#### Statistical analysis

The experimental results are presented as mean values  $\pm$  SDs. A one-way analysis of variance (ANOVA) was conducted to evaluate and compare data from multiple tests. Fisher’s test ( $p \leq 0.05$ ) was used to identify significant differences between means, following the methodologies described by Artimage and Berry (1987) and Kowalczewski and Andreani (2015), with minor modifications. The results are reported as mean  $\pm$  SD. Significant differences between means within the same storage periods (0, 5, 10, and 15 days) are indicated by different letters in each row, based on Fisher’s least significant difference test ( $p \leq 0.05$ ).

## Results and Discussion

### Chemical components of beetroots

Proximate composition analysis is an essential technique in food manufacturing, utilized to assess the nutritional profile and potential applications of raw materials in food products. This analysis provides critical information on the concentrations of key macromolecules, including moisture, protein, fat, carbohydrates, fiber, and ash,

which are crucial for determining the nutritional value of food ingredients. Such data are vital for accurate nutritional labeling and for evaluating the suitability of raw materials in food processing. In this study, we evaluated the proximate composition and energy content of ES leaves, RS roots, and BO on a dry matter basis to explore their nutritional potential. The moisture content of the fresh samples was measured as follows:  $11.1 \pm 0.22$  g/100 g for *E. sativa* leaves,  $13.90 \pm 0.32$  g/100 g for *R. sativus* roots, and  $12.1 \pm 0.1$  g/100 g for *B. oleracea* var. *capitata*. These values illustrate the differing water retention capacities of these plant parts, which can impact their shelf life, processing stability, and nutritional density. The results indicate that moisture content is relatively comparable among the three plant materials, with *R. sativus* roots showing the highest moisture level. This variation could influence the texture and potential applications of these plants in various food formulations. Understanding these differences is important for predicting how these plant materials will behave during processing and storage, as well as their suitability for specific applications such as dehydration, freezing, or incorporation into processed food products.

The chemical composition of *Eruca sativa* (girgir), *Raphanus sativus* (figl), and *Brassica oleracea* var. *capitata* (cabbage) extracts, including moisture content (MC), protein, fat, fiber, ash, uncalculated carbohydrates (UCC), and energy values, aligns with findings from previous studies (Kadous, 2008; Ribeiro and Veloso, 2020). The variations in macronutrient composition can be attributed to genetic differences and environmental factors affecting the crops (Carbone and Pasiakos, 2019; Ahmed *et al.*, 2023) (Table 2).

The moisture content of fresh radish (*R. sativus*) leaves (89.5 g/100 g) and roots (95.24 g/100 g) sourced from Argentina closely aligns with findings by Goyeneche Ortégón and Jiménez Sánchez (2015), who reported similar moisture levels in dried radish leaves (88.5 g/100 g).

Moisture content is a critical factor influencing food quality, preservation, and susceptibility to spoilage. Fresh vegetables, especially leaves and roots, are highly vulnerable to microbial decay because of their elevated moisture levels, highlighting the necessity for preservation methods such as drying to enhance shelf life and storage stability. Drying effectively reduces available water, thereby mitigating microbial growth and extending the longevity of these products (Gao *et al.*, 2017). The importance of the intake of adequate protein for maintaining health is well-documented, particularly during periods of growth and aging (Carbone and Pasiakos, 2019). The dried roots of *R. sativus* and *B. oleracea* var. *capitata* contain significant levels of crude protein, measuring  $32.01 \pm 0.98$  g/100 g and  $34.55 \pm 0.48$  g/100 g, respectively. No significant differences ( $P > 0.05$ ) were observed between the two plant roots, indicating that both serve as excellent sources of protein. This protein content supports the findings of Carbone and Pasiakos (2019), who emphasize the role of plant-based proteins in promoting health, particularly in populations with limited access to animal-derived proteins. The dried roots of these plants can be particularly beneficial in addressing protein–energy malnutrition (PEM), especially among children. In addition to protein, fats and oils play vital roles in various food products such as confectionery, baked goods, emulsions, and sauces (Rios *et al.*, 2014). Notably, *B. oleracea* var. *capitata* roots exhibit a higher fat content ( $1.6 \pm 0.12$  g/100 g) compared to other plant parts, making them a valuable ingredient for food formulations designed to provide essential fatty acids and enhance energy content. The fat present in these roots may also aid in the absorption of fat-soluble vitamins, contributing to overall health. Furthermore, if these fats are predominantly unsaturated, they could offer cardiovascular benefits by helping to lower cholesterol levels (Rios *et al.*, 2014). Overall, the moisture content in the leaves and roots of *E. sativa*, *R. sativus*, and *B. oleracea* var. *capitata* is crucial for food processing, storage, and preservation. The high moisture levels in fresh radish leaves (89.5 g/100 g)

**Table 2.** Chemical composition of leaves of girgir (ES), roots of figl (RS), and *Brassica oleracea* var. *capitata* (BO) extracts.

Raw Material	MC (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	UCC (%)	Energy (kcal)
ES*	$11.1 \pm 0.22^b$	$6.71 \pm 0.42^b$	$0.65 \pm 0.09^c$	$18.5 \pm 0.8^c$	$16.7 \pm 0.45^b$	$25.1 \pm 2.11^c$	$237 \pm 1.06$
RS**	$13.90 \pm 0.32^{ab}$	$32.01 \pm 0.98^a$	$0.84 \pm 0.3^{bc}$	$21.5 \pm 0.8^b$	$16.5 \pm 0.32^b$	$46.11 \pm 2.8^b$	$254.6 \pm 1.3$
BO***	$12.1 \pm 0.13^{ab}$	$34.55 \pm 0.48^a$	$1.6 \pm 0.12^a$	$25.4 \pm 0.22^{ab}$	$18.1 \pm 0.41^{ab}$	$64.9 \pm 2.15^a$	$286.4 \pm 1.08$

\*ES = *Eruca sativa* (girgir)

\*\*RS = *Raphanus sativus* (figl)

\*\*\*BO = *Brassica oleracea* var. *capitata* (cabbage)

Data are expressed as mean  $\pm$  SD.

Means with different lowercase letters within a row are significantly different ( $p \leq 0.05$ ).

This means that different uppercase letters across a column are significantly different ( $p \leq 0.05$ ).

and roots (95.24 g/100 g) highlight the need for effective preservation strategies to extend the shelf life while maintaining nutritional quality. The findings regarding protein content further highlight the potential of dried root powders from these plants as rich sources of essential nutrients that can fortify diets and address PEM among vulnerable populations, particularly children.

Fats play a crucial role in various food products, including confectionery, ice cream, baked goods, and emulsions (Rios *et al.*, 2014). The study found that the fat content in the roots of *B. oleracea var. capitata* was significantly higher ( $1.6 \pm 0.12$  g/100 g) compared to other plant parts studied ( $P \leq 0.05$ ). These findings highlight the nutritional potential of *E. sativa*, *R. sativus*, and *B. oleracea*, emphasizing the importance of understanding their composition. This knowledge not only informs their incorporation into food systems but also presents opportunities for enhancing food security, particularly in regions facing widespread PEM. High moisture content, particularly in fresh leaves and roots (e.g., 89.5% in *E. sativa* leaves), encourages microbial growth and enzymatic activity, leading to rapid spoilage during storage. This highlights the need for effective preservation methods such as drying or refrigeration. Dehydration reduces microbial activity by limiting available water, thus extending the shelf life (Gao *et al.*, 2017). Therefore, drying the leaves and roots of *E. sativa*, *R. sativus*, and *B. oleracea var. capitata* could enhance their stability for use in food products with longer shelf lives. Research into optimal drying conditions—such as temperature and air circulation—could provide valuable insights for improving the preservation of these plant parts. Protein is a vital macronutrient essential for growth, immune function, and tissue repair (Carbone and Pasiakos, 2019). The protein content in the dried roots of *R. sativus* and *B. oleracea* was found to be  $32.01 \pm 0.98$  g/100 g and  $34.55 \pm 0.48$  g/100 g, respectively, indicating that these roots are rich in protein and valuable in combating malnutrition, especially in areas where animal-based proteins are scarce or unaffordable. These findings reinforce the significance of plant-based proteins for vulnerable populations like children. The inclusion of these plant roots in protein-fortified foods could help address PEM, a common issue in developing countries. In addition, comparing the amino acid profiles of these plants with other protein sources—such as legumes and grains—would help to assess their nutritional completeness and bioavailability. Incorporating these roots into food formulations can diversify diets with locally available, sustainable ingredients. Nayak *et al.* (2020) emphasized the role of plant-based proteins in functional foods aimed at improving nutritional status, which is particularly relevant for *E. sativa*, *R. sativus*, and *B. oleracea*.

The significant fat content found in *B. oleracea* roots *var. capitata* ( $1.6 \pm 0.12$  g/100 g) is also important for

food formulations, as fats are essential for the absorption of fat-soluble vitamins and provision of necessary fatty acids. Rios *et al.* (2014) noted that fats are integral to many processed foods, including emulsions and spreads; thus, the high-fat content in *B. oleracea var. capitata* roots could serve as a valuable ingredient for nutrient-dense, high-energy food products. If the fats present are primarily unsaturated, they may offer cardiovascular benefits by lowering cholesterol levels and reducing the risk of heart disease (Rios *et al.*, 2014).

From a sustainability perspective, utilizing plant parts like the roots and leaves of *E. sativa*, *R. sativus*, and *B. oleracea*, which are often discarded during agricultural practices, can help reduce food waste and promote a circular economy. By incorporating these plants into diets, we can enhance nutritional quality while minimizing the environmental impact associated with agricultural by-products. The findings suggest that these plants could be used to develop functional foods and fortification strategies across a range of products such as breads, snacks, or beverages, providing a cost-effective means to enhance nutritional profiles. For instance, adding protein-rich dried roots or fat-rich extracts into bakery items could improve their nutritional value for health-conscious consumers or those with specific dietary requirements. Furthermore, the antioxidants, vitamins, and bioactive compounds present in these plants—such as vitamin C and polyphenols—may contribute to overall health by reducing the risk of diseases associated with oxidative stress (Brahmachari *et al.*, 2020). Further research into developing functional foods from these plants is warranted. The proximate composition analysis of *E. sativa*, *R. sativus*, and *B. oleracea var. capitata* highlights their significant nutritional value, positioning them as promising candidates for food fortification and functional food development. Their high moisture content necessitates proper storage and preservation methods like drying to extend the shelf life effectively. The protein-rich roots of *R. sativus* and *B. oleracea var. capitata* have potential applications in combating malnutrition, while the fat content in *B. oleracea var. capitata* could provide health benefits when incorporated into suitable formulations. In addition, these plants offer opportunities to improve sustainability and food security by utilizing underused plant parts, thereby reducing food waste and environmental impact. Future studies should focus on exploring the amino acid profiles, digestibility, and bioavailability of proteins within these plants as well as their integration into various food products. Examining the environmental and economic benefits associated with large-scale production and processing will further elucidate their broader applications within food systems. The bioactive compounds found in the leaves of *E. sativa* and the roots of *R. sativus* and *B. oleracea var. capitata* demonstrate their potential as functional foods because

of their diverse phytochemical profiles that contribute to nutritional and therapeutic applications. The total phenolic content measured in *E. sativa* ( $26.33 \pm 1.25$  mg/g DW), *R. sativus* ( $25.89 \pm 2.31$  mg/g DW), and *B. oleracea* var. *capitata* ( $22.56 \pm 1.25$  mg/g DW) supports their antioxidant properties (Rasouli *et al.*, 2017). Phenolic compounds act as free radical scavengers that inhibit lipid peroxidation and protect against oxidative damage linked to aging and chronic diseases such as cardiovascular disease, diabetes, and cancer (Liu *et al.*, 2018). Incorporating these plant extracts into diets could help mitigate oxidative stress while improving metabolic health outcomes. The higher flavonoid content observed in *R. sativus* roots ( $2.36 \pm 0.54$  mg/g DW) compared to both *B. oleracea* var. *capitata* ( $1.99 \pm 0.24$  mg/g DW) and *E. sativa* ( $1.11 \pm 0.25$  mg/g DW) emphasizes their potential role in reducing inflammation and enhancing cardiovascular health (Bondonno *et al.*, 2019). Flavonoids also interact with cell signaling pathways involved in oxidative stress responses, suggesting their applicability in functional foods targeting inflammatory diseases. The tannin content found in *E. sativa* leaves ( $5.14 \pm 0.18$  mg/g DW) indicates potential antimicrobial properties since tannins can bind to bacterial membranes disrupting their functions while inhibiting microbial growth (Borges *et al.*, 2013). This characteristic makes them ideal candidates for food preservation or natural remedies against bacterial infections. Carotenoid levels present in *E. sativa*, *R. sativus*, and *B. oleracea* (approximately 1.3 mg/100 g FW) highlight their potential benefits for vision health while boosting immune responses due to carotenoids like lutein and beta-carotene serving as precursors to vitamin A, which helps protect against age-related macular degeneration (Tang *et al.*, 2019). The identified phenolic acids—including catechol, gallic acid, ferulic acid, p-coumaric acid, and o-cinnamic acid—are recognized for their anti-inflammatory properties along with anti-cancer effects (Cheyner *et al.*, 2018). The higher catechol content found in BO roots ( $6.18 \pm 0.71$  mg/100 g DW) compared to both ES and RS emphasizes its potential utility within functional foods aimed at treating inflammation-related disorders. The diverse bioactive compounds present within these plants position them favorably as candidates for fortifying foods while increasing antioxidant intake; they may also serve as natural preservatives thus reducing reliance on synthetic additives within food systems. Overall, the ethanolic extracts from *E. sativa* leaves along with the roots from both *R. sativus* and *B. oleracea* var. *capitata* reveal a wide array of flavonoids alongside phenolic compounds, vitamins, and minerals, showcasing significant nutritional and medicinal potentials inherent within these botanical resources.

The substantial fat content in BO roots, measured at  $1.6 \pm 0.12$  g per 100 g, is significant for food formulation because of the essential role of fats in the absorption of

fat-soluble vitamins and the provision of vital fatty acids. Rios *et al.* (2014) highlighted that fats are a fundamental component in numerous processed foods, such as emulsions and spreads. Therefore, the elevated fat levels in BO roots could be used effectively as an ingredient in nutrient-rich, high-energy food products. Moreover, if these fats are predominantly unsaturated, they may offer cardiovascular advantages since unsaturated fats are linked to lower cholesterol levels and a decreased risk of heart diseases (Rios *et al.*, 2014). From a sustainability standpoint, incorporating plant parts like the roots and leaves of ES, RS, and BO—which are often discarded during agricultural practices—can significantly mitigate food waste and foster a circular economy. This practice not only enhances nutritional value but also lessens the environmental impact associated with agricultural by-products. Such an approach is particularly pertinent given the global challenges of climate change, food security, and resource scarcity. The FAO (2021) highlights the importance of promoting plant-based diets and leveraging underutilized plant species to achieve food security and sustainable agricultural practices.

The findings of the study indicate that plants such as ES, RS, and BO have significant potential in the development of functional foods and food fortification strategies. These plants can be integrated into various products, including breads, snacks, and beverages, offering a cost-efficient method to enhance their nutritional profiles. For instance, adding protein-rich dried roots or fat-rich plant extracts to baked goods could elevate their nutritional content, appealing to health-conscious consumers and individuals with specific dietary requirements. Moreover, the presence of antioxidants, vitamins, and bioactive compounds—such as vitamin C and polyphenols—found in these plants may contribute to improved health outcomes and a reduction in diseases linked to oxidative stress (Brahmachari *et al.*, 2020). Further investigation into the development of functional foods from these plants is warranted. The proximate composition analysis reveals that ES, RS, and BO possess substantial nutritional value, making them promising candidates in initiatives for food fortification. Their high moisture content requires effective storage and preservation methods, such as drying, to prolong the shelf life. Specifically, the protein-rich roots of RS and BO could play a crucial role in addressing malnutrition, while the fat content in BO may provide health benefits when incorporated into suitable formulations. In addition, these plants present an opportunity to enhance sustainability and food security by utilizing underutilized parts of plants, thereby reducing food waste and minimizing environmental impact. Future research should focus on examining the amino acid profiles, digestibility, and bioavailability of proteins from these plants, along with their incorporation into food products.

In addition, examining the environmental and economic benefits of large-scale production and processing of these plants will offer insights into their broader applications in food systems. The bioactive compounds found in ES leaves and the roots of RS and BO demonstrate their potential as functional foods. These plants exhibit a variety of phytochemicals, contributing to their nutritional and therapeutic applications. The total phenolic content in ES ( $26.33 \pm 1.25$  mg/g DW), RS ( $25.89 \pm 2.31$  mg/g DW), and BO ( $22.56 \pm 1.25$  mg/g DW) supports their antioxidant properties (Rasouli *et al.*, 2017). Phenolic compounds act as free radical scavengers, inhibit lipid peroxidation, and protect against oxidative damage, which is linked to aging and chronic diseases like cardiovascular disease, diabetes, and cancer (Liu *et al.*, 2018). Incorporating these plant extracts into diets could help reduce oxidative stress and improve metabolic health. The higher flavonoid content in RS roots ( $2.36 \pm 0.54$  mg/g DW) compared to BO ( $1.99 \pm 0.24$  mg/g DW) and ES ( $1.11 \pm 0.25$  mg/g DW) highlights their potential in reducing inflammation and improving cardiovascular health (Bondonno *et al.*, 2019). Flavonoids also interact with cell signaling pathways involved in oxidative stress and inflammation, indicating their potential for functional foods aimed at inflammatory diseases. The tannin content in ES leaves ( $5.14 \pm 0.18$  mg/g DW) suggests antimicrobial applications. Tannins bind to bacterial membranes, disrupting their function and inhibiting microbial growth (Borges *et al.*, 2013). This property makes them suitable for food preservation or as natural remedies against bacterial infections. The carotenoid levels in ES, RS, and BO (around 1.3 mg/100 g FW) highlight their potential to support vision health and boost immune responses. Carotenoids like lutein and beta-carotene are precursors to vitamin A and help protect against age-related macular degeneration (Tang *et al.*, 2019). The phenolic acids identified, including catechol, gallic acid, ferulic acid, p-coumaric acid, and o-cinnamic acid, are known for their anti-inflammatory and anti-cancer properties (Cheyner *et al.*, 2018). The higher catechol content in BO roots ( $6.18 \pm 0.71$  mg/100 g DW) compared to ES and RS highlights its potential for developing functional foods targeting inflammation-related disorders. The bioactive compounds present in these plants make them ideal candidates for fortifying foods to boost antioxidant intake. These compounds could also serve as natural preservatives, reducing the need for synthetic additives in food systems. With their diverse bioactive profiles, these plants are suitable for the development of nutraceutical products aimed at alleviating oxidative stress and inflammation, key factors in chronic diseases. The ethanolic extracts from ES leaves, RS roots, and BO roots revealed a broad range of flavonoids, phenolic compounds, vitamins, and minerals, showcasing the nutritional and medicinal potential of these plants.

The extracts analyzed were found to be abundant in bioactive compounds, with myricetin identified as the predominant flavonoid across all three samples. The concentrations of myricetin were measured at  $21.17 \pm 0.24$  mg/100g DW in ES,  $18.47 \pm 0.56$  mg/100g DW in RS, and  $19.54 \pm 0.35$  mg/100g DW in BO. Another significant flavonoid, naringenin, was also present in notable quantities, ranging from  $17.55 \pm 0.52$  mg/100g DW in BO to  $18.63 \pm 0.33$  mg/100g DW in ES. Although detected in lower concentrations, kaempferol and apigenin contributed to the overall flavonoid profile, with statistically significant differences noted among the extracts ( $p \leq 0.05$ ). In terms of vitamins, the extracts contained several water-soluble vitamins, including vitamin C, B3 (niacin), B6 (pyridoxine), and B9 (folic acid), with vitamin C being the most abundant. Its concentrations were recorded as  $23.87 \pm 0.77$  mg/100g DW in BO,  $22.54 \pm 0.42$  mg/100g DW in ES, and  $21.87 \pm 0.17$  mg/100g DW in RS, indicating the potential of extracts as natural antioxidants and immune boosters. The phenolic content was also noteworthy, with gallic acid being the most prevalent compound, found in concentrations between 9.45 mg/100g DW and 11.55 mg/100g DW across the samples. Other phenolic compounds such as catechol, p-coumaric acid, and cinnamic acid were present in smaller amounts but still contributed to the overall antioxidant activity that can mitigate oxidative stress and inflammation. Mineral analysis revealed significant levels of essential minerals including sodium (Na), potassium (K), calcium (Ca), phosphorus (P), and iron (Fe). Notably, potassium levels exceeded 40 mg/100g DW across all extracts, while iron was consistently measured at  $15.11 \pm 0.12$  mg/100g DW, indicating the potential role of extracts in addressing mineral deficiencies. The total phenolic content was highest in ES at  $26.33 \pm 1.25$  mg gallic acid/g DW, followed by RS at  $25.89 \pm 2.31$  mg/g DW and BO at  $22.56 \pm 1.25$  mg/g DW. In contrast, TFC peaked in RS at  $2.36 \pm 0.54$  mg quercetin/g DW, compared to ES with  $1.11 \pm 0.25$  mg/g DW and BO at  $1.99 \pm 0.24$  mg/g DW. Overall, these findings highlight the rich bioactive profile of the extracts, indicating their potential applications in health and nutrition because of their antioxidant properties and mineral content.

The variations in phytochemical compositions among different plant species are significantly influenced by their unique metabolic pathways and environmental conditions. Recent studies have shown that ethanolic extracts from ES, RS, and BO are rich in bioactive compounds that contribute to their noteworthy antioxidant, anti-inflammatory, and therapeutic properties. The high levels of myricetin and naringenin, along with substantial phenolic content, indicate a strong antioxidant potential in these extracts, corroborating previous research that highlights the benefits of flavonoid-rich plant extracts.

Furthermore, the presence of essential vitamins such as C, B6, and B9 enhances the nutritional value of these

extracts, suggesting their potential as functional food ingredients. These vitamins are crucial for immune function, metabolic processes, and reducing the risk of chronic diseases. The mineral content, particularly iron and potassium, also suggests that these extracts could be beneficial in addressing anemia and maintaining the electrolyte balance. The higher concentrations of flavonoids and phenolic compounds found in ES and RS suggest that these extracts may possess greater therapeutic potential. This finding can guide future research aimed at exploring their applications in nutraceutical formulations. Further investigations should focus on the bioavailability and synergistic effects of these compounds *in vivo*, as well as their clinical efficacy in managing oxidative stress-related disorders. These findings have significant implications for the development of functional foods, dietary supplements, and cosmetics. The antioxidant properties of these compounds could enhance the shelf life of food and stability. In summary, the ethanolic extracts of *E. sativa*, *R. sativus*, and *B. oleracea var. capitata* present a rich source of bioactive compounds with considerable antioxidant and nutritional benefits. Their potential applications span various industries, including food, pharmaceuticals, and cosmetics. Future research should aim to optimize extraction methods to maximize yield and explore broader therapeutic benefits for human health.

The quantitative analysis of phenolic and flavonoid compounds, including total phenolics, flavonoids, tannins, alkaloids, and specific compounds like gallic acid and kaempferol, corroborates prior findings on the nutritional profile of these species (Aguirre and Santagapita, 2016; Kowalczewski and Andreani, 2015). The vitamin content (e.g., ascorbic acid, niacin, pyridoxine) and mineral composition, such as sodium and potassium levels, are consistent with reported data in the literature (FAO, 2021; Nayak *et al.*, 2020; Brahmachari *et al.*, 2020). The statistical analysis ( $p \leq 0.05$ ) highlights significant differences among the samples for most parameters, reflecting the diversity of bioactive compounds among these plant extracts (Gao *et al.*, 2017; Rios *et al.*, 2014) (Table 3).

The assessment of the quality of fish burger and the stability of storage involves evaluating several key physicochemical parameters, including TVN, pH, TBA, TMA, WHC, and plasticity. These parameters are crucial for understanding the chemical changes and spoilage processes that occur in fish products during refrigerated storage, which ultimately affect their shelf life and consumer acceptability.

### Initial pH values and storage impact

At the outset, the pH values of the fish burger samples ranged from 5.22 to 5.35, with encapsulated treatments

exhibiting slightly elevated initial pH levels. Specifically, the encapsulated treatment with bioactive extracts (Encaps-BO) recorded the highest initial pH at 5.75, followed closely by Encaps-RS at 5.51 and Encaps-ES at 5.49. Statistical analysis revealed no significant differences ( $p > 0.05$ ) in pH among these treatments at the beginning of the storage period. Throughout a 15-day storage period at  $4 \pm 2^\circ\text{C}$ , the average pH for all fish burger samples increased significantly from 5.34 to 5.88. This rise in pH is indicative of microbial activity, particularly the production of volatile basic compounds like ammonia and TVN, which are by-products of bacterial spoilage (Lawrie and Ledward, 2006). The encapsulation of bioactive extracts in alginate beads likely contributed to moderating pH changes by inhibiting microbial growth to some extent. Nevertheless, the gradual increase in pH suggests that microbial and enzymatic spoilage persisted, albeit at a reduced rate.

### Microbial spoilage indicators

An increase in pH during storage is a recognized marker of microbial spoilage in fish products, primarily driven by bacteria such as *Shewanella spp.* and *Pseudomonas spp.*, which degrade proteins and release amines along with nitrogenous volatile compounds (Gram and Huss, 1996). The use of plant extracts such as ES, RS, and BO appears to slow down this increase in pH compared to untreated controls, indicating that these extracts possess antimicrobial and antioxidative properties that could extend the shelf life. Among the treatments, Encaps-BO demonstrated slightly better stability over time, suggesting variability in effectiveness based on the type of extract used. These findings hold significant implications for the fish processing industry; encapsulating plant extracts presents a promising strategy for enhancing the quality and shelf life of fish burgers during refrigerated storage. By optimizing the concentration of extracts like BO, RS, or ES, it may be possible to further control microbial growth and oxidative deterioration. In summary, encapsulating bioactive plant extracts in alginate beads effectively moderates pH changes in fish burgers and demonstrates potential for slowing spoilage processes. The gradual rise in pH highlights the necessity for additional preservation techniques to maintain product quality during storage. This encapsulation strategy aligns with increasing consumer demand for natural preservatives and clean-label products while providing a sustainable solution for extending the shelf life of fish products (Lawrie and Ledward, 2006). Similar findings on pH stability influenced by plant-based encapsulated extracts were reported by Das *et al.* (2021) and Rezaeifar *et al.* (2022) (Figure 2).

The analysis of physicochemical changes in fish burgers during refrigerated storage ( $4 \pm 2^\circ\text{C}$ ) over a 15-day period

**Table 3.** Quantitative chemical composition, flavonoid and phenolic compounds, and water-soluble vitamins of *Eruca sativa* (ES) leaves, *Raphanus sativus* (RS) roots, and *Brassica oleracea* var. *capitata* (BO) extracts analyzed using HPLC.

Component	ES (mg/g DW or µg/100 g FW)	RS (mg/g DW or µg/100 g FW)	BO (mg/g DW or µg/100 g FW)	Statistical analysis	Large variation	Small variation
Total Phenolic (mg Gallic acid/g DW)	26.33±1.25	25.89±2.31	22.56±1.25	p ≤ 0.05	ES > RS > BO	Minimal
Total Flavonoid (mg Quercetin/g DW)	1.11±0.25	2.36±0.54	1.99±0.24	p ≤ 0.05	RS > BO > ES	Moderate
Total Tannin (mg Tannic acid/g DW)	5.14±0.18	3.54±0.84	4.54±0.54	p ≤ 0.05	ES > BO > RS	Large
Total Alkaloid (g/100 g DW)	2.54±0.08	3.04±0.46	1.89±0.74	p ≤ 0.05	RS > ES > BO	Large
Total Anthocyanin (µg/100 g FW)	55.14±0.45	57.87±0.84	56.48±0.12	p ≤ 0.05	RS > BO > ES	Small
Carotenoids (mg/100 g FW)	1.33±0.21	1.33±0.14	1.25±0.54	p ≤ 0.05	RS = ES > BO	Small
Phenolic Compounds (mg/100 g DW)						
Gallic Acid	9.45±0.23	11.55±0.41	10.17±0.45	p ≤ 0.05	RS > BO > ES	Small
Catechol	4.41±0.52	5.64±0.11	6.18±0.71	p ≤ 0.05	BO > RS > ES	Moderate
p-Coumaric Acid	0.58±0.14	0.22±0.18	0.44±0.26	p ≤ 0.05	ES > BO > RS	Small
Ferulic Acid	0.78±0.41	0.72±0.59	0.42±0.13	p ≤ 0.05	ES > RS > BO	Small
o-Coumaric Acid	1.02±0.18	1.86±0.47	1.26±0.23	p ≤ 0.05	RS > BO > ES	Large
Cinnamic Acid	0.88±0.11	0.42±0.14	0.68±0.34	p ≤ 0.05	ES > BO > RS	Moderate
Flavonoid Compounds (mg/100 g DW)						
Myricetin	21.17±0.24	18.47±0.56	19.54±0.35	p ≤ 0.05	ES > BO > RS	Moderate
Naringenin	18.63±0.33	18.02±0.87	17.55±0.52	p ≤ 0.05	ES > RS > BO	Small
Kaempferol	1.54±0.23	2.08±0.27	2.42±0.22	p ≤ 0.05	BO > RS > ES	Moderate
Apigenin	2.78±0.42	2.18±0.22	2.44±0.32	p ≤ 0.05	ES > BO > RS	Moderate
Vitamins Contents (mg/100 g DW)						
Ascorbic Acid (Vit. C)	22.54±0.42	21.87±0.17	23.87±0.77	p ≤ 0.05	BO > ES > RS	Small
Niacin (Vit. B3)	1.77±0.27	1.47±0.45	1.67±0.16	p ≤ 0.05	ES > BO > RS	Small
Pyridoxine (Vit. B6)	4.89±0.48	5.52±0.45	5.89±0.45	p ≤ 0.05	BO > RS > ES	Small
Folic Acid (Vit. B9)	3.09±0.11	2.89±0.24	2.56±0.25	p ≤ 0.05	ES > RS > BO	Small
Mineral Contents (mg/100 g DW)						
Sodium (Na)	51.44±0.31	55.0±0.31	52.0±0.31	p ≤ 0.05	RS > BO > ES	Small
Potassium (K × 102)	44.45±0.22	42.0±0.22	42.0±0.22	p ≤ 0.05	ES > RS = BO	Small
Calcium (Ca × 102)	7.2±0.25	8.45±0.25	7.2±0.25	p ≤ 0.05	RS > ES = BO	Small
Phosphorus (P × 10)	40.02±1.02	41.82±1.02	40.02±1.02	p ≤ 0.05	RS > ES = BO	Small
Iron (Fe)	15.11±0.12	15.11±0.12	15.11±0.12	No Significant Diff.	-	No Variation

**Explanation of Statistical Analysis:**

Statistical Significance (p ≤ 0.05): For all components, if the p-value is less than or equal to 0.05, it indicates significant differences between the groups (ES, RS, and BO). This is reflected in the table, where each comparison has been marked with "p ≤ 0.05."

Large Variation: A component with large variation shows a higher SD, indicating more fluctuation across replicates.

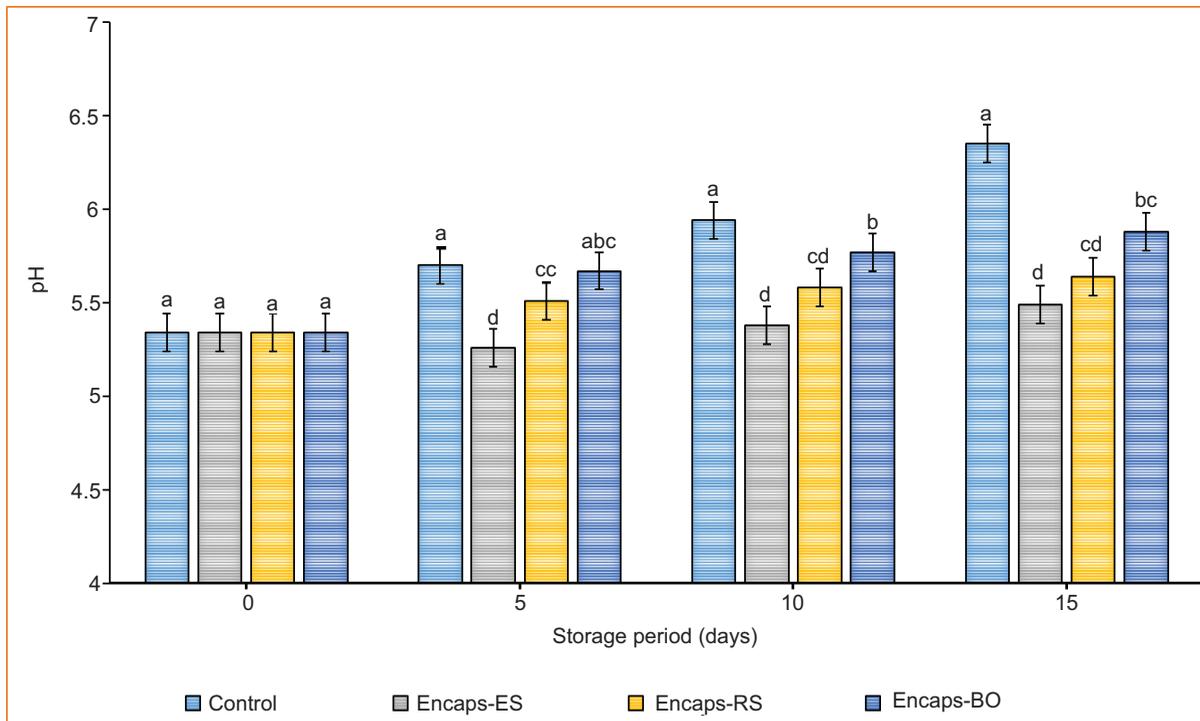
Small Variation: Small variation corresponds to a lower SD, suggesting consistent measurements with minimal fluctuation.

Comparative Analysis: The "large variation" column identifies which components show the most fluctuation between the plant samples.

"Small variation" highlights components with the least fluctuation, indicating consistency in the data across the samples.

reveals significant insights into spoilage indicators, particularly TVN, TBA, and pH levels. The study also examines the stability of WHC, plasticity, and TMA under these conditions. TVN serves as an indicator of protein degradation and microbial activity. Throughout the storage period, TVN levels varied from 10.99 to 18.19 mg/100 g. Notably, fish burgers made with Encaps-ES extract exhibited lower TVN values (11.22 mg/100 g) compared to those containing Encaps-RS extract, although

no significant difference was noted between Encaps-ES and Encaps-BO treatments (p > 0.05). These findings align with previous research by Darwesh (2017), which reported TVN levels of 10.33 mg/100 g for carp and 13.22 mg/100 g for little tuna, indicating that encapsulated bio-active extracts can mitigate protein breakdown during storage and reduce microbial spoilage. TBA values, indicative of lipid oxidation, are crucial for assessing the sensory and nutritional quality of fish burgers. The type



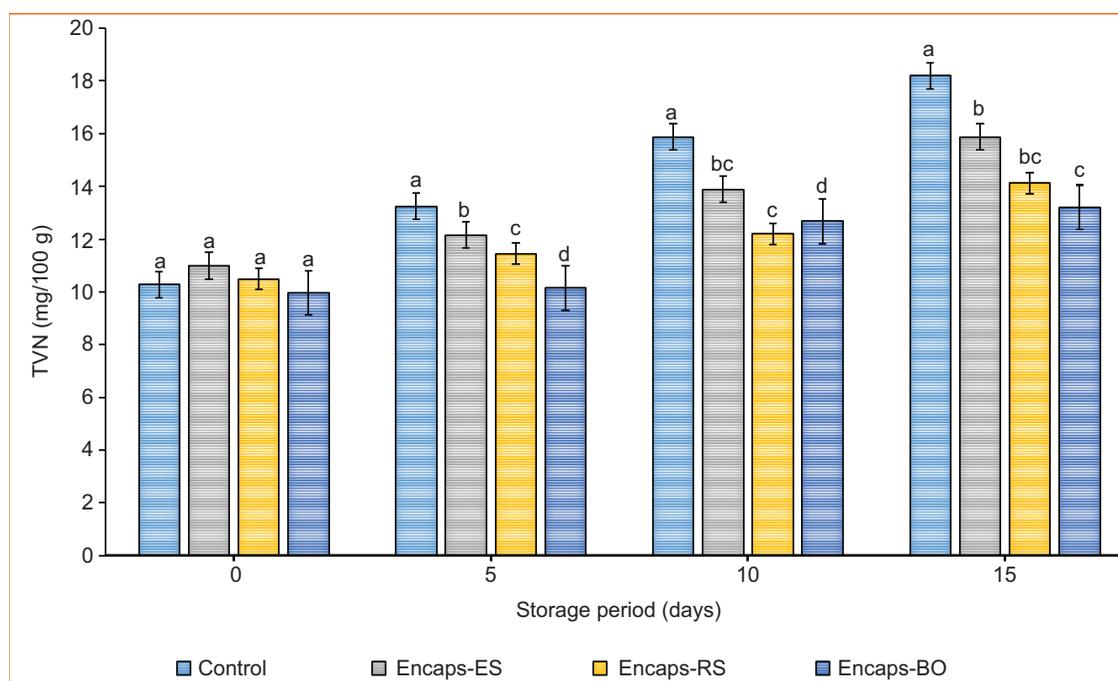
**Figure 2.** Change in pH values of fish burger samples treated with sterile distilled water (Control), encapsulated in alginate beads with *Eruca sativa* (ES) extract (Encaps-ES 5%, v/w), *Raphanus sativus* (RS) extract (Encaps-RS 5%, v/w), and *Brassica oleracea* var. *capitata* (BO) extract (Encaps-BO 5%, v/w) during storage at refrigerated temperatures (up to 15 days at  $4 \pm 2^\circ\text{C}$ ).

of fish meat significantly influenced TBA levels ( $p \leq 0.05$ ), with encapsulated treatments generally showing reduced lipid oxidation. This suggests that encapsulation may create a protective barrier that limits lipid exposure to pro-oxidants during storage.

The pH of the fish burgers was significantly affected by the duration of storage ( $p \leq 0.05$ ), although variations across different fish meat types were minimal. Encapsulation treatments resulted in smaller increases in pH compared to control samples, demonstrating their effectiveness in slowing microbial spoilage. In contrast to TVN and TBA, WHC, plasticity, and TMA levels did not show significant changes related to the type of fish meat used ( $p > 0.05$ ). However, these parameters were influenced by refrigerated storage due to alterations in protein structure and water-binding capacity over time. TMA levels increased gradually but remained within acceptable limits throughout the storage period. The results highlight the importance of bioactive encapsulation in enhancing the quality of fish burgers during refrigerated storage. Encapsulated extracts, particularly ES and BO, effectively lower spoilage indicators such as TVN and TBA, suggesting their potential as natural preservatives. The study highlights how different types of extract and techniques of encapsulation can affect spoilage dynamics, emphasizing that while all encapsulated treatments provide benefits, their efficacy varies based on the bioactive

profiles of the extracts utilized. The role of encapsulated plant extracts in reducing nitrogen degradation in meat and fish products during refrigerated storage has been demonstrated by Ganesan *et al.* (2019) and El-Dieb *et al.* (2023) (Figure 3).

The storage duration significantly influenced the average TVN values across all fish burger treatments. After 15 days of refrigeration at  $4 \pm 2^\circ\text{C}$ , the TVN levels were recorded as follows: 18.19 mg/100 g for the control group, 13.12 mg/100 g for the Encaps-ES treatment, 15.88 mg/100 g for Encaps-RS, and 11.22 mg/100 g for Encaps-BO. Initially, at time zero, the mean TVN value across all treatments was 10.33 mg/100 g, which exhibited a significant increase ( $p \leq 0.05$ ) by the end of the storage period, with the Encaps-BO treatment reaching 13.22 mg/100 g. This increase in TVN levels during storage can be attributed to microbial activity that degrades proteins into volatile nitrogenous compounds, as noted in studies by El-Nashi *et al.* (2015), Fallah *et al.* (2016), and Mahdavi-Roshan *et al.* (2022). Importantly, all measured TVN values remained within the acceptable range of 35–40 mg N/100 g for fish muscle, as recommended by Immaculate and Jamila (2018) and Lim (2022). In addition, TMA, which is produced from the reduction of trimethylamine oxide (TMAO) because of bacterial spoilage and enzymatic reactions (Bekhit *et al.*, 2021; Odeyemi *et al.*, 2018), was also analyzed. The mean



**Figure 3.** Changes in total nitrogen value (TVN) (mg/100 g) of fish burger samples treated with sterile distilled water (Control) or encapsulated in alginate beads containing *Eruca sativa* (ES) extract (Encaps-ES 5%, v/w), *Raphanus sativus* (RS) extract (Encaps-RS 5%, v/w), and *Brassica oleracea var. capitata* (BO) extract (Encaps-BO 5%, v/w) during storage at refrigerated conditions (up to 15 days at  $4 \pm 2^\circ\text{C}$ ).

TMA values across all treatments ranged from 2.67 to 5.55 mg/100 g during the storage period, with no significant differences observed between treatments ( $p > 0.05$ ). In the control group, TMA levels rose from  $1.35 \pm 0.39$  mg/100 g to  $5.55 \pm 0.20$  mg/100 g over the course of storage. Initial TMA levels for Encaps-ES, Encaps-RS, and Encaps-BO were 1.35, 1.40, and 1.55 mg/100 g, respectively. By the end of the 15-day period, these values had significantly increased to 5.83, 5.55, and 4.15 mg/100 g, respectively. Notably, there were no significant changes in TMA levels between days 0 and 5 or between days 10 and 15; however, significant differences were observed between days 5 and 15. This analysis highlights the impact of storage duration on spoilage indicators in fish burgers and highlights the importance of monitoring these parameters to ensure product quality during refrigerated storage. The mitigation of TMA formation in seafood through natural plant-based treatments was highlighted by Tavakoli *et al.* (2020) and Sharma *et al.* (2021) (Figure 4).

The TBA value serves as a recognized indicator of lipid oxidation in food items (Aheto *et al.*, 2019; Sørensen and Jørgensen, 1996). Among the different treatments applied to fish burgers, those incorporating Encaps-RS exhibited the highest TBA value at 0.513 mg malonaldehyde/kg, significantly surpassing the values of other treatments

(Figure 5). Conversely, the Encaps-ES treatment recorded the lowest TBA value of 0.328 mg malonaldehyde/kg, while Encaps-BO presented similar results, although no significant differences were noted between these two. Throughout the duration of storage, average TBA values for all fish burger treatments rose markedly from an initial 0.279 mg malonaldehyde/kg to a final value of 0.577 mg malonaldehyde/kg. This increase is linked to oxidative rancidity, a common occurrence in fish products during storage. These findings are consistent with those reported by Kadous (2008), who documented TBA values of 0.350 and 0.340 mg malonaldehyde/kg for carp and little tuna burgers, respectively (Aheto *et al.*, 2019; Sørensen and Jørgensen, 1996). The antioxidative impact of encapsulated bioactive compounds in reducing lipid oxidation during storage was discussed in studies by Siripatrawan and Vitthayakitti (2016) and Zhao *et al.* (2022) (Figure 5).

WHC quantifies the ability of meat and meat products to retain moisture (Pearce *et al.*, 2011), while plasticity indicates the tenderness of the meat (Jankowiak *et al.*, 2021). Throughout the storage period, no significant differences ( $p > 0.05$ ) were detected in the average WHC values, which varied between 2.44 and 2.78 cm<sup>2</sup>/0.3 g. Similarly, plasticity values remained consistent across all fish burger treatments, ranging from 3.81 to 4.12 cm<sup>2</sup>/0.3 g (Figure 6).

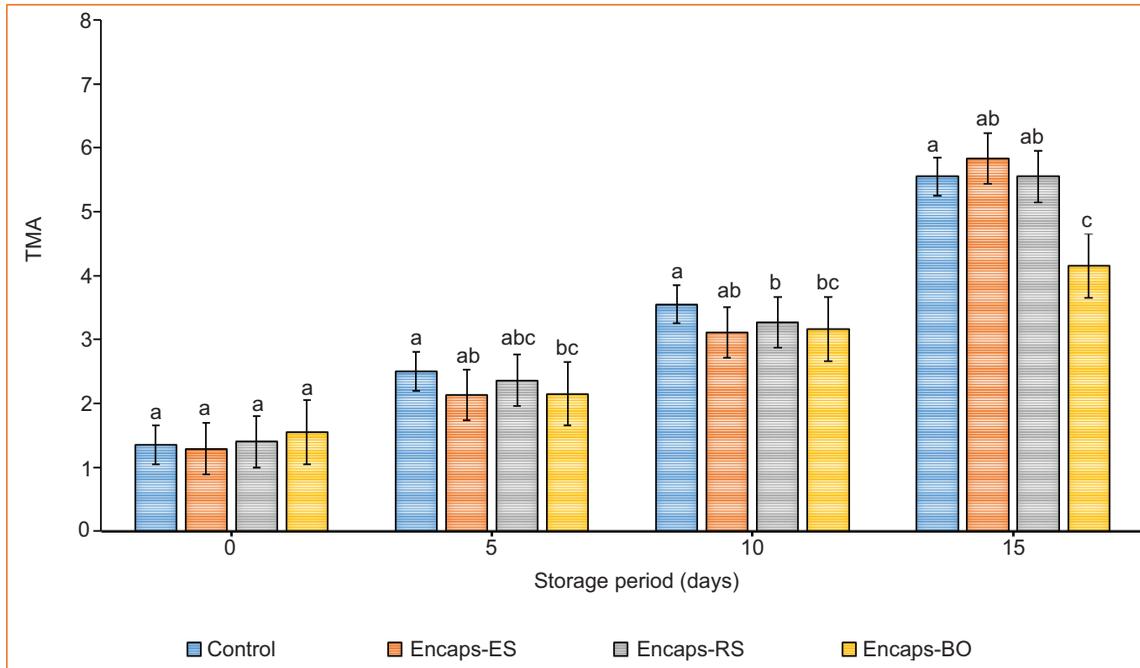


Figure 4. Changes in trimethylamine (TMA) levels of fish burger samples during refrigerated storage (up to 15 days at  $4 \pm 2^\circ\text{C}$ ). The treatments include a control sample treated with sterile distilled water (Control) and samples encapsulated in alginate beads containing *Eruca sativa* (ES) extract (Encaps-ES, 5%, v/w), *Raphanus sativus* (RS) extract (Encaps-RS, 5%, v/w), and *Brassica oleracea* var. *capitata* (BO) extract (Encaps-BO, 5%, v/w).

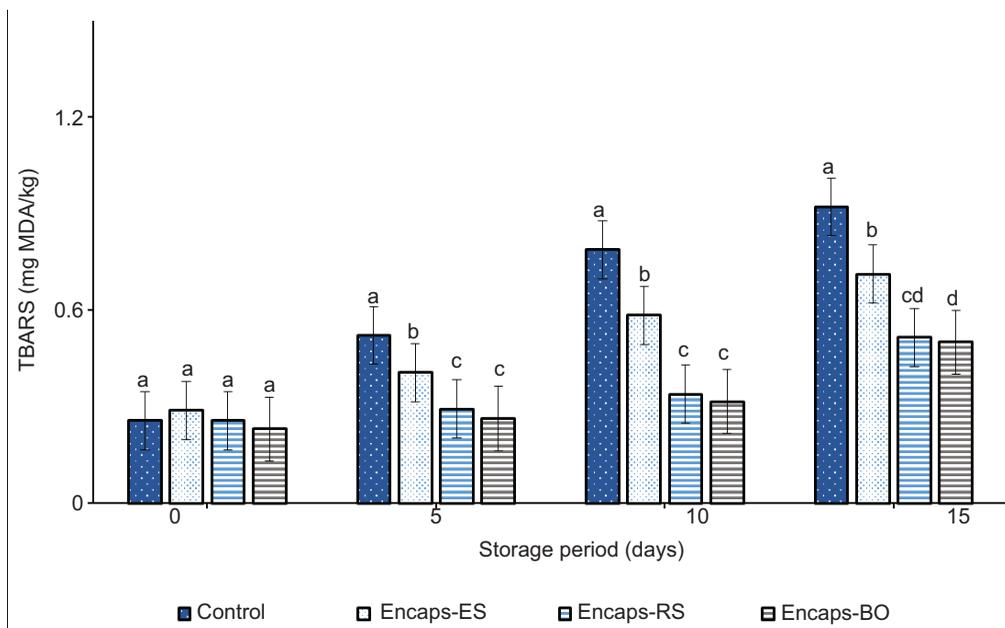
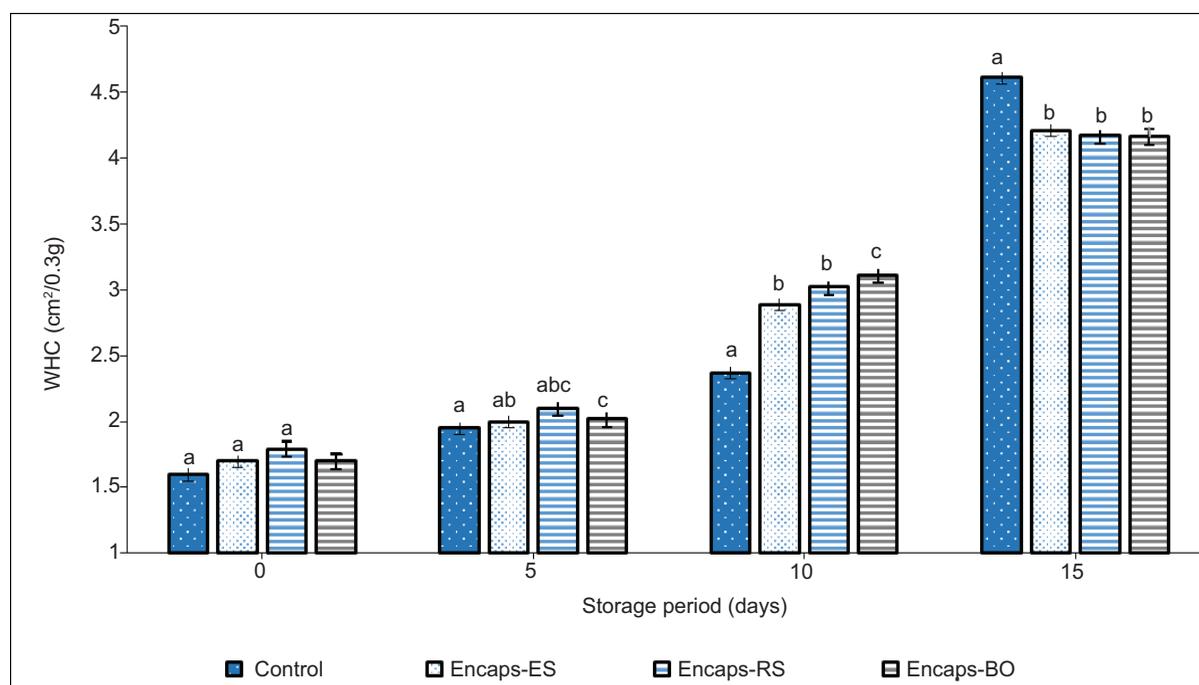


Figure 5. Changes in TBARS values (mg malondialdehyde/kg) of fish burger samples during refrigerated storage (up to 15 days at  $4 \pm 2^\circ\text{C}$ ). Treatments include the control (treated with sterile distilled water) and fish burgers encapsulated in alginate beads with ES extract (Encaps-ES, 5% v/w), RS extract (Encaps-RS, 5% v/w), and BO extract (Encaps-BO, 5% v/w).



**Figure 6.** Changes in water-holding capacity (WHC, cm<sup>2</sup>/0.3 g) of fish burger samples treated with sterile distilled water (Control) and samples encapsulated in alginate beads with ES extract (Encaps-ES 5%, v/w), RS extract (Encaps-RS 5%, v/w), and BO extract (Encaps-BO 5%, v/w) during refrigerated storage (up to 15 days at 4 ± 2°C).

The Encaps-RS treatment demonstrated the highest WHC with a minimal value of 2.31 cm<sup>2</sup>/0.3 g, while the Encaps-ES treatment exhibited the greatest plasticity at 4.12 cm<sup>2</sup>/0.3 g. Throughout the refrigerated storage period, both WHC and plasticity experienced significant declines. Specifically, the separated free water for WHC increased from 1.70 to 4.16 cm<sup>2</sup>/0.3 g, and plasticity decreased from 4.51 to 3.26 cm<sup>2</sup>/0.3 g. This reduction in WHC and plasticity during storage is attributed to protein denaturation and decreased protein solubility, as highlighted by Kramer (2013).

### Cooking characteristics of fish burgers during storage

Table 4 illustrates that cooking loss, cooking yield, fat retention, and water retention of fish burger treatments were significantly affected ( $p \leq 0.05$ ) by both the type of fish meat used and the duration of refrigerated storage at 4 ± 2°C for up to 15 days. Cooking loss, which indicates the volume of water lost during cooking, is closely related to the WHC of the meat (Alakhrash *et al.*, 2016; Allam *et al.*, 2021). Among the treatments, Encaps-ES recorded the highest cooking loss at 24.28%, markedly exceeding that of other treatments. In contrast, Encaps-RS and Encaps-BO had lower cooking losses of 19.98% and 21.39%, respectively. The burgers made with Encaps-RS achieved the highest mean values for cooking yield (79.22%), fat retention (136.38%), and water retention

(77.81%), followed by Encaps-BO and Encaps-ES, with significant differences ( $p \leq 0.05$ ) observed among the treatments.

The study of fish burgers stored at 4 ± 2°C for 15 days reveals significant changes in cooking loss, yield, fat retention, and water retention. At the start of the storage period, cooking loss was recorded at 20.59%, which increased to 22.71% after 15 days. Cooking yield decreased from 79.41% to 77.29%, while fat retention rose from 131.97% to 135.14%, and water retention increased from 75.33% to 77.66%. The increase in cooking loss is attributed to protein denaturation and decreased protein solubility, as noted by Carroll *et al.* (2007). This observation aligns with the rise in WHC values, indicating that the ability of the protein matrix to retain water and bind fat contributed to the increases in the retention of fat and water, supported by findings from Jairath *et al.* (2018) and Shen *et al.* (2022). Alexandre *et al.* (2022) also reported similar trends in fish burgers, noting increases in water retention from 74.11% to 74.55% and fat retention from 129.44% to 129.49% over a 5-month refrigerated storage period.

### Microbiological examination

The microbiological analysis conducted on the fish burger samples indicated a decline in total aerobic plate

**Table 4.** Physicochemical properties of fish burger samples treated with sterile distilled water (control) and samples encapsulated in alginate beads with ES extract (Encaps-ES 5%, v/w); encapsulated with RS extract (Encaps-RS 5%, v/w); and encapsulated with BO extract (Encaps-BO 5%, v/w) during storage at refrigerated temperature (up to 15 days at  $4 \pm 2$  °C).

Properties	Storage period (Day)	Encaps-RS	Encaps-BO	Encaps-ES	Mean
Cooking Loss (%)	0	18.91±0.21 <sup>Cd</sup>	20.51±0.22 <sup>Bb</sup>	23.82±0.25 <sup>Ac</sup>	21.08
	5	18.71±0.22 <sup>Cc</sup>	20.45±0.22 <sup>Bb</sup>	23.99±0.28 <sup>Ac</sup>	21.05
	10	20.77±0.23 <sup>Cb</sup>	22.44±0.36 <sup>Ba</sup>	24.13±0.31 <sup>Ab</sup>	22.44
	15	21.54±0.24 <sup>Ca</sup>	22.18±0.22 <sup>Ba</sup>	25.19±0.28 <sup>Aa</sup>	22.97
Mean		19.98	21.39	24.28	
Cooking Yield (%)	0	80.88±0.25 <sup>Aa</sup>	78.84±0.22 <sup>Bb</sup>	77.15±0.44 <sup>Ba</sup>	78.95
	5	80.23±0.33 <sup>Aa</sup>	79.04±0.24 <sup>Aab</sup>	76.94±0.28 <sup>Ba</sup>	78.73
	10	78.58±0.25 <sup>Ab</sup>	77.55±0.36 <sup>Abc</sup>	75.49±0.29 <sup>Bb</sup>	77.20
	15	77.21±0.25 <sup>Ac</sup>	76.16±0.24 <sup>Bc</sup>	74.59±0.36 <sup>Cc</sup>	75.98
Mean		79.22	77.89	76.04	
Fat Retention (%)	0	133.95±0.12 <sup>Ad</sup>	132.79±0.33 <sup>Ad</sup>	129.57±0.33 <sup>Bc</sup>	13210
	5	135.26±0.22 <sup>ABc</sup>	134.3±0.34 <sup>Bc</sup>	130.95±0.33 <sup>Cbc</sup>	133.50
	10	137.74±0.23 <sup>ABb</sup>	135.08±0.36 <sup>Bbc</sup>	131.35±0.41 <sup>cab</sup>	134.72
	15	138.58±0.33 <sup>Aa</sup>	136.14±0.36 <sup>Aab</sup>	132.45±0.42 <sup>Ba</sup>	135.72
Mean		136.38	134.57	131.08	
Moisture Retention (%)	0	76.97±0.22 <sup>Ab</sup>	75.45±0.11 <sup>Ac</sup>	74.9±0.22 <sup>Ab</sup>	75.77
	5	77.08±0.32 <sup>Aa</sup>	77.72±0.23 <sup>ABb</sup>	75.2±0.32 <sup>Bab</sup>	76.66
	10	78.05±0.22 <sup>Aa</sup>	78.25±0.14 <sup>Aab</sup>	76.5±0.33 <sup>Ba</sup>	77.6
	15	79.17±0.34 <sup>Aa</sup>	79.06±0.12 <sup>Aa</sup>	76.3±0.34 <sup>Ba</sup>	78.17
Mean		77.81	77.62	75.72	
Overall Acceptability	0	8.2±0.24 <sup>Aa</sup>	7.4±0.36 <sup>Ba</sup>	8.3±0.36 <sup>Aa</sup>	7.96
	5	7.2±0.33 <sup>Ab</sup>	6.9±0.33 <sup>Aa</sup>	7.5±0.34 <sup>Aa</sup>	7.20
	10	5.9±0.22 <sup>Ac</sup>	5.2±0.34 <sup>Ab</sup>	5.6±0.24 <sup>Ab</sup>	5.56
	15	5.7±0.36 <sup>Ac</sup>	5.2±0.24 <sup>Ab</sup>	5.4±0.33 <sup>Ab</sup>	5.43
Mean		6.75	6.17	6.71	

Means with different small letters across a row are significantly different ( $p \leq 0.05$ ). Means with different capital letters across a column are significantly different ( $p \leq 0.05$ ).

counts (APC) over the 15-day storage period. Initial counts were recorded as  $3.55 \times 10^3$ ,  $3.80 \times 10^3$ , and  $3.15 \times 10^3$  cfu/g for the Encaps-RS, Encaps-BO, and Encaps-ES samples, respectively. By Day 15, these counts decreased to  $2.12 \times 10^3$ ,  $2.22 \times 10^3$ , and  $2.03 \times 10^3$  cfu/g, demonstrating that the encapsulated extracts effectively inhibited bacterial growth. Psychrophilic bacteria counts also showed a decrease from initial levels of  $7.55 \times 10^2$ ,  $7.80 \times 10^2$ , and  $6.96 \times 10^2$  cfu/g for Encaps-RS, Encaps-BO, and Encaps-ES, respectively, down to  $3.45 \times 10^2$ ,  $3.19 \times 10^2$ , and  $2.45 \times 10^2$  cfu/g by Day 15, indicating significant suppression of these bacteria because of the encapsulated extracts. Notably, coliform bacteria were absent throughout the duration of storage in all samples, suggesting a high level of hygiene in the products tested, while *Salmonella spp.* was not detected at any point, implying potential antimicrobial properties of the extracts used. Mold and yeast counts were initially higher in Encaps-ES

( $9 \times 10$  cfu/g) compared to Encaps-BO ( $8 \times 10$  cfu/g) and Encaps-RS ( $7 \times 10$  cfu/g), but by Day 15, these counts had decreased across all samples: Encaps-RS ( $2 \times 10$  cfu/g), Encaps-BO ( $3 \times 10$  cfu/g), and Encaps-ES ( $4 \times 10$  cfu/g). This reduction highlights an effective control of mold and yeast proliferation during storage, particularly in refrigerated conditions. Encapsulated extracts, as used in the fish burgers, appear to play a significant role in curtailing microbial growth, especially psychrophilic bacteria, as well as molds and yeasts, during storage. This aligns with previous studies indicating that such extracts possess antimicrobial properties, likely due to the bioactive compounds that disrupt microbial cell walls. The encapsulation of these extracts can enhance their stability and prolong their activity, ensuring they remain effective throughout storage. Additionally, previous research suggests that the formation of ice crystals during refrigeration can cause structural damage to microbial cells, which

may contribute to the antimicrobial effects observed in the current study (Jayasena *et al.*, 2013; Shirin *et al.*, 2020).

For example, Jayasena *et al.* (2013) reported that encapsulated essential oils exhibited significant antimicrobial activity against foodborne pathogens and spoilage microorganisms, including molds and yeasts, in meat products stored under refrigeration. Similarly, Shirin *et al.* (2020) demonstrated that encapsulation of plant extracts not only protected the active compounds from degradation but also enhanced their antimicrobial efficacy by promoting ice crystal formation during refrigeration, which contributed to microbial cell wall disruption.

These findings are consistent with earlier studies on the application of encapsulated antimicrobial agents in various food matrices, suggesting that encapsulation can be an effective strategy for improving the shelf life and microbial safety of refrigerated products, such as fish burgers (Donsi *et al.*, 2010; Cai *et al.*, 2014; Su *et al.*, 2019; Abid *et al.*, 2021).

The microbiological evaluation of fish burger samples treated with encapsulated plant extracts—namely, rosemary (RS), basil oil (BO), and essential safflower (ES)—during refrigerated storage provides significant insights into their preservation efficacy and microbial inhibition capabilities. These findings indicate that encapsulating these plant extracts effectively reduces the microbial load in fish burgers, which is crucial for ensuring food safety and maintaining quality throughout storage. Initially, the total APC for the fish burgers were recorded at  $3.55 \times 10^3$ ,  $3.80 \times 10^3$ , and  $3.15 \times 10^3$  cfu/g for Encaps-RS, Encaps-BO, and Encaps-ES, respectively. Over a 15-day storage period, these counts decreased to  $2.12 \times 10^3$ ,  $2.22 \times 10^3$ , and  $2.03 \times 10^3$  cfu/g. This reduction in APC indicates that the encapsulated plant extracts not only act as preservatives but also possess antimicrobial properties that inhibit the growth of aerobic bacteria during storage. Such findings align with previous research by Ramezani *et al.* (2015), which demonstrated reduced bacterial counts in refrigerated fish burgers treated with antimicrobial agents. Their study showed that antimicrobial treatments effectively inhibited the growth of spoilage microorganisms, including psychrophilic bacteria, in fish products stored under refrigeration conditions. This supports the potential of using natural antimicrobial agents to enhance food safety and extend shelf life in perishable products like fish burgers (Ramezani *et al.*, 2015). Initial counts of psychrophilic bacteria were recorded at  $7.55 \times 10^2$ ,  $7.80 \times 10^2$ , and  $6.96 \times 10^2$  cfu/g for Encaps-RS, Encaps-BO, and Encaps-ES, respectively. By the end of the storage period, these counts decreased to  $3.45 \times 10^2$ ,  $3.19 \times 10^2$ , and  $2.45 \times 10^2$  cfu/g. This decline highlights the effectiveness of the encapsulated extracts in curtailing

the growth of psychrophilic bacteria—an essential factor for preserving the quality and safety of refrigerated foods. Similar results were noted by Vanitha *et al.* (2015), where treated fish burgers exhibited lower psychrophilic bacterial counts during storage. Initially, mold and yeast counts were highest in Encaps-ES ( $9 \times 10$  cfu/g) compared to Encaps-BO ( $8 \times 10$  cfu/g) and Encaps-RS ( $7 \times 10$  cfu/g). However, by Day 15, these counts dropped to  $2 \times 10$  for Encaps-RS,  $3 \times 10$  for Encaps-BO, and  $4 \times 10$  for Encaps-ES. This trend supports the notion that encapsulated extracts can effectively inhibit fungal growth—a finding corroborated by Cai *et al.* (2014), who indicated that encapsulated antimicrobial agents could significantly reduce fungal contamination in refrigerated products.

Throughout the entire duration of storage, no coliform bacteria or *Salmonella spp.* were detected in any of the samples, indicating a high level of hygiene in the fish burgers. The absence of these pathogens—commonly associated with fecal contamination—suggests that encapsulated extracts may contribute to preventing harmful bacterial proliferation. In summary, the results indicate that encapsulated plant extracts (RS, BO, and ES) are effective at reducing microbial growth—particularly psychrophilic bacteria as well as molds and yeasts—during refrigerated storage. These findings are consistent with existing literature and suggest that such extracts could serve as natural alternatives to synthetic preservatives in food products while enhancing food safety and extending the shelf life. Further research is warranted to explore their broader applications in food preservation strategies and safety practices. The implications of this study are significant for the food industry as they highlight the potential of using natural plant extracts as effective preservation methods that not only maintain food quality but also promote consumer health by minimizing reliance on synthetic additives.

The absence of coliforms and *Salmonella spp.* in fish burger products highlights their safety, suggesting that the encapsulated extracts may possess either bactericidal or bacteriostatic properties that inhibit the growth of pathogenic bacteria. This is particularly significant for refrigerated food items, where the presence of such pathogens can lead to foodborne illnesses. The use of natural antimicrobial agents, like plant extracts, could effectively mitigate the risk of contamination in food supply. Encapsulated plant extracts have shown promising results in reducing microbial contamination during refrigerated storage. Notable reductions in APCs, psychrophilic bacteria, and mold/yeast counts indicate that specific extracts—such as rosemary (RS), basil (BO), and eucalyptus (ES)—can act as natural preservatives, enhancing both the safety and shelf life of fish-based products. The complete absence of coliforms and *Salmonella spp.* further highlights the effectiveness

**Table 5.** Microbiological examination of fish burger samples treated with sterile distilled water (control) and samples encapsulated in alginate beads with *Eruca sativa* (ES) extract (Encaps-ES 5%, v/w), *Raphanus sativus* (RS) extract (Encaps-RS 5%, v/w), and *Brassica oleracea* var. *capitata* (BO) extract (Encaps-BO 5%, v/w) during storage at  $4 \pm 2^\circ\text{C}$  for 15 days.

Properties	Storage Period (Day)	ENCAPS-RS	ENCAPS-BO	ENCAPS-ES
Aerobic Plate Count	0	$3.55 \times 10^3$	$3.80 \times 10^3$	$3.15 \times 10^3$
	5	$3.21 \times 10^3$	$3.55 \times 10^3$	$3.17 \times 10^3$
	10	$2.18 \times 10^3$	$2.42 \times 10^3$	$2.16 \times 10^3$
	15	$2.12 \times 10^3$	$2.22 \times 10^3$	$2.03 \times 10^3$
Psychrophilic Bacteria	0	$7.55 \times 10^2$	$7.80 \times 10^2$	$6.96 \times 10^2$
	5	$6.30 \times 10^2$	$6.78 \times 10^2$	$5.22 \times 10^2$
	10	$5.10 \times 10^2$	$5.26 \times 10^2$	$4.13 \times 10^2$
	15	$3.45 \times 10^2$	$3.19 \times 10^2$	$2.45 \times 10^2$
Total Coliform Count	0	ND	ND	ND
	5	ND	ND	ND
	10	ND	ND	ND
	15	ND	ND	ND
Salmonella spp.	0	ND	ND	ND
	5	ND	ND	ND
	10	ND	ND	ND
	15	ND	ND	ND
Mold and Yeast	0	$7 \times 10^2$	$8 \times 10^2$	$9 \times 10^2$
	5	$5 \times 10^2$	$6 \times 10^2$	$7 \times 10^2$
	10	$4 \times 10^2$	$5 \times 10^2$	$6 \times 10^2$
	15	$2 \times 10^2$	$3 \times 10^2$	$4 \times 10^2$

ND = Not detected; Cfu/g = Colony-forming unit/gram.

of these extracts in preventing pathogen proliferation. The encapsulation process not only protects these bioactive compounds from degradation but also enhances their stability and bioavailability, allowing for controlled release during storage. This approach maximizes their antimicrobial potential while minimizing any adverse effects on food quality. Studies have demonstrated that encapsulated plant extracts can significantly inhibit the growth of spoilage bacteria and reduce the risk of food-borne diseases, making them a valuable tool in food preservation strategies.

### Sensory evaluation of fish burgers during refrigerated storage

Sensory evaluation plays a crucial role in determining food quality from the consumer's perspective, focusing on attributes such as taste, odor, color, texture, and overall acceptability. This study investigates the sensory characteristics of fish burgers stored under refrigerated conditions ( $4 \pm 2^\circ\text{C}$ ) over various intervals, assessing how the duration of storage influences the quality of the product.

According to the statistical analysis presented in Table 6, sensory attributes (taste, odor, color, texture, and overall acceptability) were not significantly influenced ( $p > 0.05$ ) by the type of fish meat used in the formulation. The mean sensory scores for all fish burger samples (Encaps-RS, Encaps-BO, and Encaps-ES) ranged from 7.23 to 7.93 for taste, 7.46 to 7.93 for odor, 7.38 to 8.73 for color, 7.73 to 7.96 for texture, and 7.74 to 7.91 for overall acceptability, with no significant differences ( $p > 0.05$ ) observed among different treatments. However, sensory attributes were significantly affected ( $p \leq 0.05$ ) by the duration of refrigerated storage at  $4 \pm 2^\circ\text{C}$ . Initial mean scores for taste (8.68), odor (8.53), color (8.55), texture (8.63), and overall acceptability (8.52) declined significantly over time, reaching values of 6.59 for taste, 7.08 for odor, 7.96 for color, and 7.24 for overall acceptability after 15 days of refrigeration.

The decline in sensory properties of the fish burgers can indeed be attributed to the formation of volatile low-molecular-weight compounds, which arise from protein degradation and lipid oxidation during storage. This is well-documented in previous studies (Ganhão *et al.*, 2013; Mariutti and Bragagnolo, 2017; Tokur *et al.*, 2004),

**Table 6.** Sensory evaluation of fish burger treatments during storage period at  $4 \pm 2$  °C for 15 days.

Properties	Storage Period (months)	ENCAPS-RS	ENCAPS-BO	ENCAPS-ES	Mean
Color	0	8.98±0.21	8.85±0.22	7.84±0.25	8,55±0.44 <sup>A</sup>
	5	8.74±0.22	8.55±0.22	7.13±0.28	8,14±0.44 <sup>AB</sup>
	10	8.64±0.23	8.25±0.36	7.34±0.31	8,07±0.41 <sup>AB</sup>
	15	8.56±0.24	8.11±0.22	7.22±0.28	7,96±0.15 <sup>B</sup>
Means		8,73±0.44 <sup>a</sup>	8,44±0.55 <sup>a</sup>	7,38±0.41 <sup>a</sup>	
Taste	0	8.34±0.25	8.84±0.22	8.87±0.44	8,68±0.41 <sup>A</sup>
	5	7.84±0.33	8.01±0.24	7.94±0.28	7,93±0.25 <sup>B</sup>
	10	6.55±0.25	7.76±0.36	7.99±0.29	7,43±0.44 <sup>B</sup>
	15	6.21±0.25	6.63±0.24	6.94±0.36	6,59±0.64 <sup>C</sup>
Means		7,23±0.84 <sup>a</sup>	7,81±0.41 <sup>a</sup>	7,93±0.35 <sup>a</sup>	
Odor	0	8.75±0.12	8.70±0.33	8.14±0.33	8,53±0.44 <sup>A</sup>
	5	8.21±0.22	8.15±0.34	7.29±0.33	7,88±0.65 <sup>B</sup>
	10	7.65±0.23	7.82±0.36	7.11±0.41	7,52±0.78 <sup>BC</sup>
	15	7.12±0.33	6.84±0.36	7.30±0.42	7,08±0.42 <sup>C</sup>
Means		7,93±0.94 <sup>a</sup>	7,87±0.88 <sup>a</sup>	7,46±0.54 <sup>a</sup>	
Texture	0	8.92±0.22	8.44±0.11	8.54±0.22	8,63±0.35 <sup>A</sup>
	5	8.17±0.32	8.09±0.23	7.87±0.32	8,04±0.42 <sup>A</sup>
	10	7.58±0.22	7.30±0.14	7.55±0.33	7,47±0.41 <sup>AB</sup>
	15	7.20±0.34	7.11±0.12	7.38±0.34	7,23±0.65 <sup>AB</sup>
Means		7,96±0.61 <sup>a</sup>	7,73±0.34 <sup>a</sup>	7,83±0.43 <sup>a</sup>	
Overall Acceptability	0	8.72±0.24	8.49±0.36	8.37±0.36	8,52±0.46 <sup>A</sup>
	5	7.95±0.33	8.11±0.33	8.10±0.34	8,05±0.48 <sup>AB</sup>
	10	7.64±0.22	7.54±0.34	7.34±0.24	7,50±0.42 <sup>B</sup>
	15	7.35±0.36	7.21±0.24	7.17±0.33	7,24±0.14 <sup>B</sup>
Means		7,91±0.44 <sup>a</sup>	7,83±0.63 <sup>a</sup>	7,74±0.48 <sup>a</sup>	

**Statistical Analysis:**

Analysis of Variance (ANOVA): The sensory evaluation data for each property (color, taste, odor, texture, and overall acceptability) were analyzed using ANOVA to assess significant differences between different treatments (ENCAPS-RS, ENCAPS-BO, and ENCAPS-ES) over the storage period.

**Significant Differences:**

Small letters (e.g., a, b, c) across rows indicate significant differences between different storage periods for each treatment at the 95% confidence level ( $p \leq 0.05$ ).

Capital letters (e.g., A, B) across columns indicate significant differences between the treatments for each storage period at the 95% confidence level ( $p \leq 0.05$ ).

which highlight the role of these processes in affecting the taste, aroma, and overall sensory quality of refrigerated food products. Texture deterioration is often associated with protein denaturation during freezing (de Oliveira Ferreira *et al.*, 2019; Yang *et al.*, 2020). Despite these declines, all fish burger treatments remained acceptable throughout the entire refrigeration period, with overall acceptability scores exceeding 7.0 even after full duration of storage.

These findings indicate that while storage time negatively impacts sensory properties, the type of fish used does not significantly affect the sensory quality of fish burgers,

which is vital for maintaining product consistency and consumer satisfaction. Furthermore, the incorporation of encapsulated plant extracts did not lessen the sensory appeal of the burgers, suggesting that this method is a viable strategy for enhancing food preservation without compromising taste or texture—a conclusion supported by Liao *et al.* (2020).

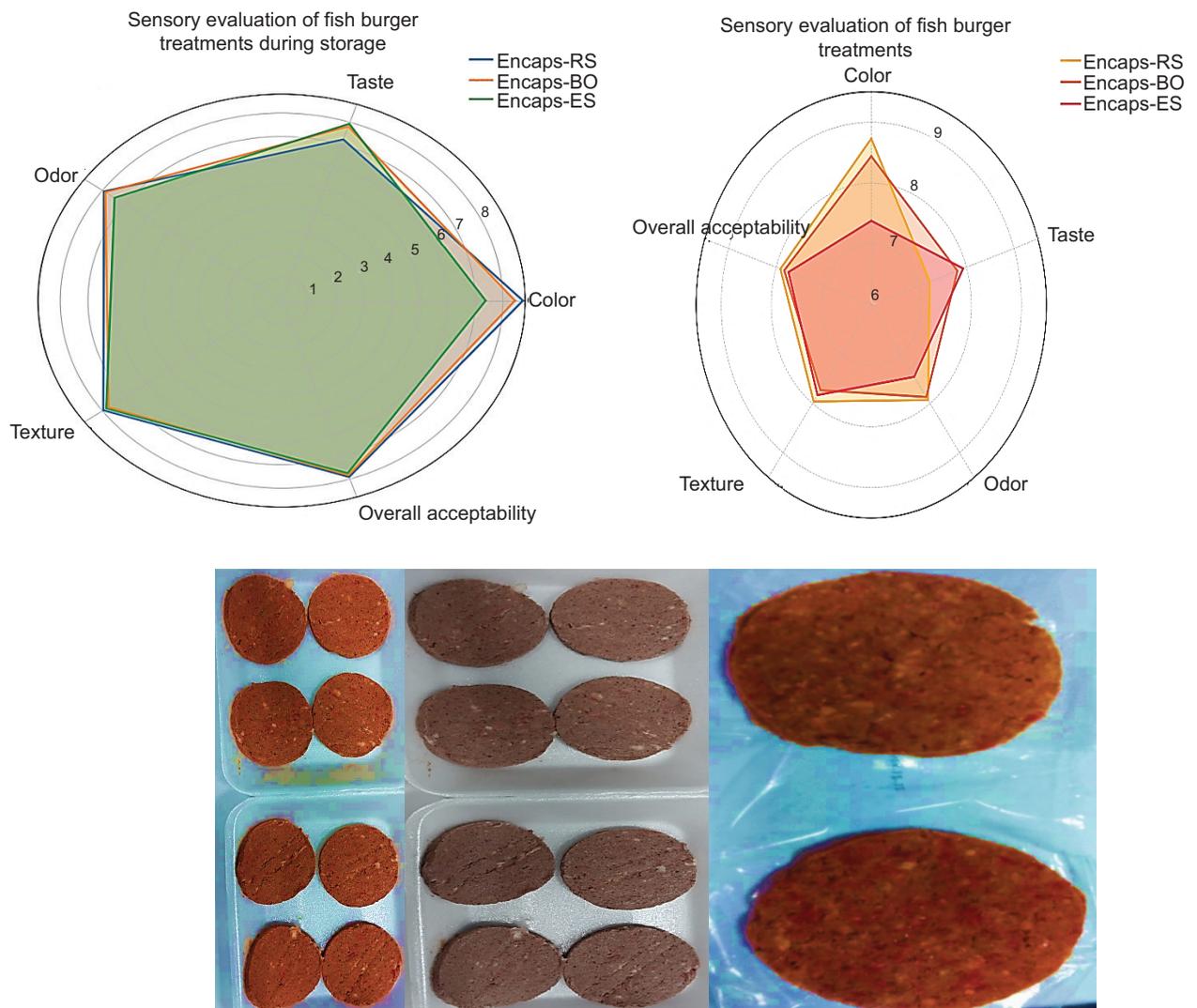
The production of volatile compounds related to lipid oxidation and protein degradation adversely affects odor and taste (Ganhão *et al.*, 2013; Mariutti and Bragagnolo, 2017). Fish proteins are particularly susceptible to degradation under refrigeration conditions, leading to

texture changes such as softening and loss of firmness (de Oliveira Ferreira *et al.*, 2019; Yang *et al.*, 2020). This deterioration is reflected in the significant reduction in texture scores over time.

Despite these declines in sensory properties over time, all fish burger treatments remained acceptable according to consumer standards, with overall acceptability scores exceeding 7.0 even after 15 days of storage. This indicates that while storage induces some sensory deterioration, products remain within an acceptable quality range from a sensory perspective. The results from this sensory evaluation align with previous research on storage effects on fish products; Ganhão *et al.* (2013) and Mariutti and Bragagnolo (2017) have shown that microbial spoilage along with lipid oxidation and protein breakdown during storage contribute to diminished sensory

properties—especially concerning odor and taste. In summary, while there is a notable decrease in sensory properties associated with increased storage time, all fish burger treatments maintained acceptable quality levels until the end of the refrigeration period at  $4 \pm 2^\circ\text{C}$ . This study highlights the potential of using encapsulated plant extracts as natural preservatives that can extend the shelf life without significant compromises to consumer acceptance or product quality—offering a sustainable alternative to conventional synthetic preservatives while also contributing to reduced food waste and improved food safety.

The radar graph (Figure 7) depicts sensory attributes (color, taste, odor, texture, and overall acceptability) for fish burgers enriched with encapsulated extracts of girgir (*E. sativa*), figl (*R. sativus*), and cabbage (*B. oleracea*



**Figure 7.** Radar graph of the sensory evaluation results for fish burgers prepared with encapsulated extracts of girgir (*E. sativa*), figl (*R. sativus*), and cabbage (*B. oleracea var. capitata*). Sensory attributes of each treatment, including color, taste, odor, texture, and overall acceptability, are represented over a 15-day period of storage at  $4 \pm 2^\circ\text{C}$ .

*var. capitata*), evaluated over 15 days of refrigerated storage ( $4 \pm 2^\circ\text{C}$ ). ENCAPS-BO exhibited superior color stability (highest scores on average) across the storage period, retaining an attractive appearance. This can be attributed to the pigments in cabbage extracts (e.g., anthocyanins) which are known for their stability under varying conditions (Xie *et al.*, 2020). ENCAPS-RS and ENCAPS-ES showed slight declines in color over time, with ENCAPS-ES having the lowest score by day 15. The degradation of color in ENCAPS-ES might be linked to a loss of carotenoids due to oxidation (Ahmed *et al.*, 2022). ENCAPS-ES achieved higher initial taste scores, potentially because of glucosinolates and volatile compounds in *E. sativa*, which can enhance flavor profiles (Fahey *et al.*, 2020). ENCAPS-RS and ENCAPS-BO had moderate but stable taste scores throughout the storage period. The phenolic compounds in Figl and Brassica extracts contribute to taste stability (Javed *et al.*, 2021). Over time, a decline in taste was observed across all samples, with ENCAPS-ES maintaining better taste acceptability. This aligns with findings that encapsulated bioactive compounds can help preserve taste-related qualities (Yadav *et al.*, 2023). ENCAPS-RS and ENCAPS-BO showed higher odor scores initially, indicating the positive impact of encapsulated phenolic acids in mitigating rancidity and preserving freshness (Opara *et al.*, 2020). Although the odor stability of ENCAPS-ES was slightly lower than that of the other treatments, it was still well-maintained, indicating that encapsulated *E. sativa* extracts help preserve microbial suppression and oxidative stability. Across treatments, the odor declined over time, which may be related to volatile compound degradation, but encapsulation significantly delayed this process.

ENCAPS-BO achieved the best texture scores on average, likely due to the high plasticity and WHC of cabbage-derived bioactive compounds (Lutz *et al.*, 2022). ENCAPS-ES and ENCAPS-RS also maintained good textural integrity, though slightly less consistent than ENCAPS-BO. The encapsulation matrix (alginate) likely contributed to reduced water migration and texture stability in all samples (Ahmed *et al.*, 2023). ENCAPS-BO consistently was rated the highest for overall acceptability, demonstrating superior balance across all sensory attributes. ENCAPS-ES and ENCAPS-RS showed competitive performance, with ENCAPS-ES slightly trailing due to lower color scores. The overall acceptability of all treatments declined slightly over time, with ENCAPS-BO showing the least decline, affirming its strong preservation capabilities. The antioxidant and antimicrobial properties of bioactive compounds (e.g., phenolics, glucosinolates) in encapsulated extracts are critical for sensory quality. Studies by Opara *et al.* (2020) highlighted that such extracts extend the shelf life and preserve sensory characteristics. Encapsulation technology in alginate beads minimizes oxidation and

microbial activity, as shown in research by Yadav *et al.* (2023). This technology is particularly effective in maintaining color, taste, and odor. Similar studies on encapsulated natural extracts have demonstrated their ability to outperform synthetic preservatives in sensory quality (Ahmed *et al.*, 2022). For instance, encapsulated rosemary and thyme extracts showed comparable benefits in meat products. Cabbage extracts (*B. oleracea*) are rich in anthocyanins and glucosinolates, offering superior antioxidant protection (Lutz *et al.*, 2022). Figl (*R. sativus*) contains isothiocyanates, which contribute to its antimicrobial properties and odor stability (Javed *et al.*, 2021). Girgir (*E. sativa*) enhances flavor profiles with its unique glucosinolate content (Fahey *et al.*, 2020).

The radar graph confirms that encapsulated extracts improve sensory quality, extend the shelf life, and maintain the freshness of fish burgers. ENCAPS-BO emerges as the most effective treatment, showcasing the highest stability in sensory attributes. However, ENCAPS-ES and ENCAPS-RS also demonstrate significant potential, making them valuable natural additives for enhancing food quality. These findings align with global trends in using plant-based, encapsulated extracts for functional food applications.

## Conclusions

This study highlights the value of utilizing underexploited plants for their nutritional and medicinal properties, contributing to food security and the development of sustainable food systems. Through a detailed analysis of the phytochemical, antioxidant, antibacterial, and functional properties of *E. sativa* (girgir), *R. sativus* (radish roots), and *B. oleracea var. capitata* (cabbage), it was evident that cabbage extract exhibited superior antioxidant and antibacterial activity. These findings position cabbage as a natural and effective alternative to synthetic antioxidants in enhancing the quality and safety of fish-based food products. The incorporation of cabbage extract (BVE) and its encapsulated form (Encaps-BVE) into fish burger formulations led to notable improvements in microbial quality compared to control samples. Microbiological assessments demonstrated the ability of these extracts to significantly inhibit the growth of harmful microorganisms—including APCs, psychrophilic bacteria, total coliforms, *Salmonella spp.*, and molds and yeasts—during 15 days of refrigerated storage at  $4 \pm 2^\circ\text{C}$ . These results highlight the strong antibacterial properties of cabbage extract, which can effectively extend the shelf life and enhance the safety of fish burgers.

Furthermore, the encapsulated extracts from *B. oleracea* (Encaps-BO), *R. sativus* (Encaps-RS), and Encaps-ES were effective in maintaining stable pH levels (5.2–5.5) in

fish burgers throughout storage. This stability contrasts with the significant pH increases observed in control samples ( $p < 0.05$ ), attributed to bacterial activity and protein degradation. By mitigating pH fluctuations, encapsulated plant extracts preserved meat quality and delayed spoilage, emphasizing their functional role as natural preservatives. In terms of sensory quality, the encapsulated extracts maintained superior texture and color in fish burgers compared to other treatments. Among these, Encaps-BO consistently outperformed, followed by Encaps-RS and Encaps-ES. These results reaffirm the potential of plant-based extracts—particularly in their encapsulated forms—as natural antioxidants and antimicrobial agents in meat product formulations. Their ability to preserve physical attributes and extend the shelf life highlights their practical application as viable alternatives to synthetic additives. In conclusion, integrating natural plant-based extracts, especially from *B. oleracea var. capitata*, into fish burger formulations offers a promising strategy for improving product quality and safety during storage. This approach not only provides a healthier alternative to synthetic preservatives but also supports the broader goals of promoting sustainable food systems and public health. The use of underutilized plants in functional food formulations aligns with global objectives of reducing environmental impact and enhancing resource efficiency. Future research should explore the optimization of extract concentrations, encapsulation techniques, and formulations to facilitate commercial adoption within the food industry. Such advancements could unlock the full potential of natural plant-based additives, paving the way for innovative and sustainable food product development.

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## Ethical Statement

This study does not need ethical approval.

## Conflict of Interest

There is no conflict of interest.

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