REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE MOULOUD MAMMERI DE TIZI-OUZOU
FACULTE DES SCIENCES BIOLOGIQUES ET DES SCIENCES AGRONOMIQUES

THÈSE

Présentée

Par **M^me^ Lidia AIT OUAHIOUNE Epse BENREJDAL**

En Vue de l'Obtention du Diplôme : Docteur en Sciences Alimentaires

Spécialité : Certification et Assurance Qualité dans les Industries Agro-Alimentaires (I.A.A.)

Thème

Valorisation des co-produits de caroube (*Ceratonia siliqua* L.) : Application dans un emballage actif pour la conservation du saumon *(Salmo salar)*

Présentée devant le jury :

Mr HOUALI Karim	Professeur (UMMTO)	Président
Mr DJENANE Djamel	Professeur (UMMTO)	Directeur de thèse
Mme NERIN Cristina	Professeur des universités (Université de Zaragoza : Espagne)	Co-Directrice de thèse
Mme YAHIAOUI Karima	Professeur (Université de Boumerdes)	Examinatrice
Mr NEBTI El Hafid	Professeur (Université de Béjaia)	Examinateur
Mr SADOUDI Rabah	MCA (UMMTO)	Examinateur

Année Universitaire : 2021-2022

REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE MOULOUD MAMMERI DE TIZI-OUZOU
FACULTE DES SCIENCES BIOLOGIQUES ET DES SCIENCES AGRONOMIQUES

THÈSE

Présentée

Par **M^me^ Lidia AIT OUAHIOUNE Epse BENREJDAL**

En Vue de l'Obtention du Diplôme : Docteur en Sciences Alimentaires

Spécialité : Certification et Assurance Qualité dans les Industries Agro-Alimentaires (I.A.A.)

Thème

Valorisation des co-produits de caroube (*Ceratonia siliqua* L.) : Application dans un emballage actif pour la conservation du saumon *(Salmo salar)*

Présentée devant le jury :

Mr HOUALI Karim	Professeur (UMMTO)	Président
Mr DJENANE Djamel	Professeur (UMMTO)	Directeur de thèse
Mme NERIN Cristina	Professeur des universités (Université de Zaragoza : Espagne)	Co-Directrice de thèse
Mme YAHIAOUI Karima	Professeur (Université de Boumerdes)	Examinatrice
Mr NEBTI El Hafid	Professeur (Université de Béjaia)	Examinateur
Mr SADOUDI Rabah	MCA (UMMTO)	Examinateur

Année Universitaire : 2021-2022

Acknowledgements

The realization of a thesis work is far from being an individual exercise. Indeed, without the support and the contribution of many people, this work could not have been completed. I would like to ask them to welcome here all my feelings of gratitude, which come from the bottom of my heart.

- *In the first place, I would like to thank Pr. DJENANE Djamel and Pr. NERIN Cristina, my thesis supervisors, for entrusting me with this thesis work, for encouraging my research and for allowing me to grow as a research scientist. Also, I would like to thank them for their kindness and availability, which allowed me to carry out this work in a very good conditions. That they find here the expression of my respect and my most sincere thanks for all the time they devoted to me from the beginning of this thesis work to the writing and the corrections of articles and for all the knowledge they brought me in the field of bioactive packaging.*
- *My sincere thanks go to Dr. WRONA Magdalena, for her help in the conceptualization of my research work. Her valuable suggestions and her enlightened advices enabled me to carry out my thesis work. Thank you also for Pr. SALAFRANCA Jesús and Dr. BECERRIL Raquel for their technical assistance and their help in the realization of some parts of my work.*
- *I would also like to address my thanks to the members of the Analytical Chemistry laboratory of the University of Zaragoza, especially Janira JAEN, Xuechao SONG, Qi-Zhi SU, Sara ÚBEDA-JASANADA and Jazmin OSORIO for their help in some situations where I really needed them.*
- *My thanks are addressed to the members of the Analytical Biochemistry and Biotechnology laboratory, especially for Pr. HOUALI Karim.*
- *I thank the doctoral students: CORDOSO Léticia that I met during my stay in Zaragoza and BELASLI Azem, RAMDINI Ramdane, BENALI Thalsa, MER Sarah and BOUKSIL Djaffar from the university of Tizi-Ouzou, for their encouragement and friendship.*

- *My thanks are addressed also to my jury members: Pr. HOUALI Karim, Pr. YAHIAOUI Karima, Pr. NEBTI El Hafid and Dr. SADOUDI Rabah. Thank you for all of you. It will be a real pleasure and a privilege to discuss with you my thesis work and to benefit from your expertise on this thematic of research.*
- *I am very grateful to my dear father Mohammed Said and to my dear mother Fatima who have always supported and encouraged me. All my gratitude to my sister Katia and to my brothers: Meziane, Djamel and to my angel Mickael. For my dear cousin Ghilas, our artist, without you, none of this would be possible.*
- *I want particularly to thank my husband BENREJDAL Ghilas and his family, who were always supportive and encouraging.*
- *Finally, I would like to thank the Ministry of Higher Education and Scientific Research that awarded me a one-year "PNE" scholarship, which was essential for me to carry out my thesis work under a very good conditions.*

Forever grateful to all of you.

AIT OUAHIOUNE Lidia

Dedications

To my beloved parents

To my dear brothers: Meziane, Djamel,

Mickael, and to my dear twin sister Katia

To my husband Ghilas

To my friends

I LOVE YOU SO MUCH

To all those who will use this thesis, take care as it is the fruit of a lot of efforts.

Without forgetting

My supervisors Professor DJENANE Djamel and Professor NERIN Cristina, this work is the result of your advices.

AIT OUAHIOUNE Lidia

“Success is not final, failure is not fatal: it is the courage to continue that counts”

Winston Churchill

Valorization of scientific works resulting from this thesis

The results obtained during this thesis were the subject of:

International publications

(1) Ait Ouahioune, L., Wrona, M., Becerril, R., Salafranca, J., Nerin, C., & Djenane, D. (2021). *Ceratonia siliqua* L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications. *Food Packaging and Shelf Life*. 31, 100764 https://doi.org/10.1016/j.fpsl.2021.100764. Impact Factor = 6.429

(2) Ait Ouahioune, L., Wrona, M., Nerín, C., Djenane, D. (2022). Novel active biopackaging incorporated with macerate of carob (*Ceratonia siliqua* L.) to extend shelf-life of stored Atlantic salmon fillets (*Salmo salar* L.). *LWT - Food Science and Technology*. 156, 113015. https://doi.org/10.1016/j.lwt.2021.113015. Impact Factor=4.952

Scientific communications

(1) Ait Ouahioune L., Djenane D. Teneur en composés phénoliques et l'étude de l'activité antioxydante d'extrait ethanolique des feuilles de *Ceratonia siliqua* L.1^{ére} Journée Nationale sur « Cancer, Environneemnt et Alimentation », 4 Février 2019, Boumerdes, Algérie.

(2) Ait Ouahioune L., Wrona M., Nerin C., Djenane D. Teneur en composès phénoliques et l'étude de l'activité antioxydante et antimicrobienne des extraits actifs des feuilles de *Ceratonia siliqua* L. et *Pistacia lentiscus* L. Congrès National sur la Valorisation des Substances et Matériaux naturels. 05 au 06 juin 2021, Tlemcen, Algérie.

(3) Ait Ouahioune L., Wrona M., Jesus S., Nerin C., Djenane D. Superaliment Algérien : coproduits de caroube (*Ceratonia siliqua* L.) comme source de composés volatiles bioactifs pour les emballages bio-antioxidants. Congrès National sur la Valorisation des Substances et Matériaux naturels. 05 au 06 juin 2021, Tlemcen, Algérie.

(4) Ait Ouahioune L., Wrona M., Jesus S., Nerin C., Djenane D. Evaluation de la capacité antioxydante des emballages bioactifs par génération *in situ* de radicaux hydroxyles en phase

gazeuse et detection par chromatographie liquide haute performance-fluorescence. Webinaire National sur la Valorisation des Matières Résiduelles du Secteur Agro-alimentaire ». 16 juin 2021, Tlemcen, Algérie.

(5) Ait Ouahioune L., Wrona M., Becerril R., Salafranca J., Nerin C., Djenane D. Identification of volatile compounds from *Ceratonia siliqua* L. by HS-SPME-GC-MS. 1er Séminaire National en Biologie Végétale et Environnement (SNBVE). 24 au 25 novembre 2021, Souk-Ahras, Algérie.

(6) Ait Ouahioune L., Wrona M., Becerril R., Salafranca J., Nerin C., Djenane D. Etude de migration et évaluation des risques de nouveaux films de bioemballages actifs multicouches à base de cellulose. 1er Webinaire International sur les Applications Innovantes des Biotechnologies en Industrie Alimentaire : du Laboratoire à l'Entreprise (WAIBIA), 1 au 2 décembre 2021, Constantine, Algérie.

(7) Ait Ouahioune L., Wrona M., Nerin C., Djenane D., Utilisation des sous-produits de la caroube pour la fabrication d'un emballage actif pour la conservation d'un produit alimentaire. 1er Séminaire International, Pollution, Health et bio-surveillance, 27 au 28 décembre 2021, Skikda, Algeria.

Table of contents

3. Food active packaging as a new technique

III. MATERIAL AND METHODS

1. Development of a new multilayer active packaging

2. ***The effectiveness study on the stored fresh salmon fillet***

IV. RESULTS AND DISCUSSION

1. Ceratonia siliqua L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications (Article 1)

2. Novel active biopackaging incorporated with macerate of carob (Ceratonia siliqua L.) to extend shelf-life of stored Atlantic salmon fillets (Salmo salar L.) (Article 2)

a*: Redness

AA: Adhesive

$\mathbf{A_{control}}$: Absorbance Control

$\mathbf{A_{sample}}$: Absorbance Sample

ATTC: American Type Culture Collection

a_w: Water Activity

b*: Yellowness

BHA: Butylated Hydroxyanisole

BHT: Butylated Hydroxytoluene

CAS: Chemical Abstracts Service

CFU: Colony-Forming Unit

CIE: Internationale Commission on Illumination

CKA: Carob Kibbles Acetone

CKE: Carob kibbles Ethanol

CL: Cellulose

CLA: Carob Leaves Acetone

CLE: Carob Leaves Ethanol

CS: Carob Seeds

CSA: Carob Seeds Acetone

CSE: Carob Seeds Ethanol

$\mathbf{D_0}$: Dry Matter of the Muscle.

DHB: 2,5-Dihydroxybenzoic acid

DI-GC-MS: Direct Injection Gas Chromatography-Mass Spectroscopy

DL: Drip Loss

DPPH: 2,2-Diphényl 1-picrylhydrazyl

FAS: Fresh Atlantic Salmon

EC: European Commission

EDTA: Ethylenediaminetetraacetic acid

EFSA: European Food Safety Authority

EU: European Union

FAO: Food Agricultural Organization

FSF: Fresh Salmon Fillet

G6PDH: Glucose-6-phosphate déshydrogénase

$\mathbf{H_2SO_4}$: Sulfuric Acid

HPLC: High-Performance Liquid Chromatography.

HS-SPME-GC-MS: Headspace Solid Phase Microextraction and Gas Chromatography–Mass Spectrometry.

$\mathbf{IC_{50}}$: Median Inhibitory Concentration

ISO: International Standard Organization

L*: Lightness

LBG: Locust Bean Gum

m/z: mass/load

$\mathbf{m_0}$: Sample weight at t_0

MDA: Malondialdehyde

$\mathbf{m_x}$: Sample weight at t_x

N: Normality

NaOH: Sodium Hydroxide

NIAS: Non-Intentionally Added Substances

NIST: National Institute Standard and Technology.

NLS: Not Listed Substances

Ø: diameter

O-R: Oxidation Reduction

PDMS: Polydimethylsiloxane

PEG: Poly-Ethylene Glycol

PLA: Polylactic acid

pH: Potentiel Hydrogène

PM: Sample Weight

ROS: Radical Oxygen Species

SML: Specific Migration Limits

TAC: Total Antioxidant Capacity

TBA: Thiobarbituric acid

TBA-RS: Thiobarbituric Acid Reactive Substances

TCA: Trichloroacetic acid

TTC: Threshold of Toxicological Concern

TVB-N: Total Volatile Basic Nitrogen

UPLC-ESI-Q-TOF-MS^{E}: Ultra-high Performance Liquid Chromatography - Quadrupole Time of Flight-Mass Spectrometry

USA: United States of America

UV: Ultra-Violet

V_0: Water content of the muscle.

Vac: Volume Acid

Vba: Volume Base

Vm: Sample Volume

W/V: Percent Weight/ volume

w/w: Percent Weight/ Weight

WHC: Water Holding Capacity

WHO: World Health Organization

ΔE: Total Colour Difference

ΔV_0: Weight of the liquid separated from the sample during centrifugation.

Abstract

The objective of this thesis project is to design an active bio-packaging from natural resources as an alternative to conventional packaging of fossil origin. Carob (*Ceratonia siliqua* L.) is undoubtedly the characteristic vegetation of the Mediterranean basin. In addition to its commercial importance, it has medicinal properties. It is widely used by several agro-food industries, which generate several industrial wastes that cause serious environmental problems, however, their valorization should be encouraged.

Through this study, we tried on the one hand to characterize and identify the different volatile compounds of *Ceratonia siliqua* L. leaves, seeds and kibbles and to assess their antioxidant and antimicrobial potential. Then, the different extracts were prepared and analyzed for their application in the manufacture of a new multilayer active packaging based on cellulose polymer. On the other hand, a bio-preservation test was carried out to evaluate its effectiveness on fresh salmon fillets. The results obtained in this work showed a strong antioxidant activity for carob seeds macerates with two different methods, where IC 50 values of 112.75 ± 0.32 and 153.61 ± 3.65 µg/mL, respectively were obtained using the DPPH test. Whereas, CSE and CSA showed the best antioxidant activity by the phosphomolybdenum assay with values of 72.81 ± 0.09 and 67.22 ± 3.91 µg/mL, respectively. Chromatographic analysis (HS-SPME-GC-MS) of the different macerates demonstrated the presence of forty three volatile compounds. The antimicrobial activity of the different extracts showed low antimicrobial activity limiting our study to evaluate only the antioxidant performance of the new biofilm. The results of food safety assessment did not show any migration of volatile compounds including non-volatile ones in the food simulants used

.Fresh salmon fillet covered with the active bio-packaging stored at refrigeration temperatures 4 ± 1 °C presented satisfactory off-odor and overall acceptability results than the control samples until the 5^{th} storage day. For color analysis, active samples preserved better the characteristics of fresh salmon than the controls during the first storage days. Samples covered with active packaging presented on the 5^{th} storage day a lower values of pH (6.54 ± 0.05 and 6.60 ± 0.11), drip loss (3.17 ± 0.76 and 2.83 ± 0.29%), thiobarbituric acid reactive substances (0.056 ± 0.033 and 0.088 ± 0.054 µg MDA/g of salmon.) and total volatile basic nitrogen (30.04 ± 3.54 and 32.67 ± 4.81 mg N/100 g of salmon), whereas, the highest water holding capacity values (92.23 ± 1.09 and 92.91 ± 3.07%) for CSE and CSA respectively, as compared to those of blank bio-packaging.

Therefore, this new active bio-packaging extended the shelf-life of fresh salmon fillet, which is promising for future production of developed active biofilms at industrial scale.

Keywords: *Ceratonia siliqua* L., bio-packaging, cellulose, active packaging, carob seeds, food safety, fresh salmon fillets, shelf-life.

I. Introduction

Food quality deteriorate during transport, processing and storage, and the oxidative reactions being those having the greatest impact in limiting the shelf-life of perishable foods **(Tovar *et al*., 2005; Nerin *et al*., 2008),** the destruction of valuable nutrients, the generation of toxic compounds **(Moudache *et al*., 2017),** and colour changes (degradation of pigments), which are important in the consumers' decision to purchase the food **(Djenane *et al*., 2001; Nerín *et al*., 2008).** A wide variety of organic molecules are susceptible to chemical attack by oxygen (O_2) and among them more attention has been paid to lipids due to the importance of their oxidative damage **(Mcclements & Decker, 2000; Shahidi & Zhong, 2005; Waraho *et al*., 2011).**

Due to the great economic impact of this phenomenon, the food industry is constantly looking for new methods to reduce the effects of oxidation. Although the reduction of O_2 content (by vacuum or modified atmosphere packaging) has an effect on the control of oxidation reactions, this is only partial because the O_2 dissolved in the food at the time of packaging, which is sometimes very difficult to or cannot be completely eliminated **(López-de-Dicastillo *et al*., 2010)**.Therefore, the food industry has long recognized the importance of using synthetic antioxidants for this purpose **(Sanchez-Silva *et al*., 2014).** Recently, consumer's health concerns in relation to food ingredients have led to an increase in the request of foods processed without the addition of synthetic chemical preservatives **(Djenane, 2015),** resulting in a growing trend in the use of natural antioxidants as substitutes of artificial ones **(Barbosa-Pereira *et al*., 2014; Sanches-Silva *et al*., 2014; Fang *et al*., 2017; Djenane *et al*., 2020).** Therefore, the use of plant extracts and essential oils became the main substitutes for synthetic additives **(Djenane *et al*., 2016; Granato *et al*., 2017; Poojary *et al*., 2017; Vinceković *et al*., 2017; Lorenzo *et al*., 2018).** This has another positive effect, since part of the agro-industrial by-products become an economical and practical source of potent antioxidants **(Balasundram *et al*., 2006; Lorenzo *et al*., 2018; Ait Ouahioune *et al*., 2020).**

Massive research efforts have been made to limit oxidation **(Djenane *et al*., 2016, Moudache *et al*., 2017)**, but currently, the most widely applied solution is still direct addition of antioxidants to foods. This is due to cost effectiveness and convenience **(Borzi *et al*., 2019)**. However, there are two limitations with this approach: antioxidants may have reduced or inhibited activity due to their interactions with food components during processing and the food quality will decline rapidly after the consumption of the active compounds in the food **(Mastromatteo *et al*., 2010).** To overcome these defects, researchers have examined the

addition of natural antioxidants directly into the polymer packaging **(López *et al*., 2005; Camo *et al*., 2011; Akrami *et al*., 2015; Djenane *et al*., 2016).**

Active packaging, an emerging technology compared with traditional "inert packaging", incorporates inside the polymer active components such as oxygen scavengers, antioxidants, and antimicrobial agents. They release or absorb substances into or from the packaged food or the environment surrounding the food **(Yildirim *et al*., 2017)**, which can slow down the oxidation and extend the shelf-life of packaged food **(Sanches-Silva *et al*., 2014; Borzi *et al*., 2019).** Several studies have proven the effectiveness of these news antioxidant active packaging **(López *et al*., 2005; Camo *et al*., 2011).** Moreover, a large number of research efforts have laid increased emphasis on increasing the bio-based polymer-based film which are drawn considerable attention as substitute candidate for synthetic materials **(Ku *et al*., 2011; Koronis *et al*., 2013),** as a response for environmental concerns, increasing burden of disposing off the plastic waste and using the industrial food waste **(Al-Tayyar *et al*., 2019).**

The carob tree (*Ceratonia siliqua* L.) is mostly found in the Mediterranean basin and it is considered as an underutilized crop **(Goulas & Georgiou, 2019)**. It is an evergreen tree belonging to the *Fabaceae* family **(Ben Ayache *et al*., 2020)**. The carob fruit contains two major parts: the pulp (90%) and the seeds (10%) **(Goulas *et al*., 2016)**. It holds potentially significant importance for the food industry due to its chemical constituents, flavoring properties, and nutrition benefits **(Fidan *et al*., 2020)**. In terms of its health benefits, *Ceratonia siliqua* L. exhibits a myriad of biological effects including antibacterial, antidiarrheal, antidiabetic, anti-hypercholestrolemic and hepatoprotective **(Farag & El-Kersh, 2017)**.

In this context, the objective of this thesis is to explore the antioxidant potential of carob by-products, namely the leaves, the seeds and the kibbles for the development of an antioxidant bio-packaging, with a view to an application in the field of food packaging. For this, our goal was-on a practical level-to recover the three parts of the carob, as they are abandoned in nature and according to an ecological approach. The scientific challenge of this work was to study the antioxidant activity of carob by-products in order to incorporate them into a cellulose-based packaging matrix. In order to answer the problem of this thesis project, this manuscript is divided into three parts:

The **first part** is devoted to a bibliographical review in which we present an overview on the carob tree waste, food preservation and food active packaging as a new technique.

The **second part** will present the different raw materials used, the different analysis carried out on the *Ceratonia siliqua* L., leaves, seeds and kibbles macerates, the different analysis on the active biopackaging based on cellulose incorporated with carob macerates, and the study of the effectiveness of the new active biopackaging designed by a salmon-type fish preservation test.

Then the results of the work carried out and their discussions are presented in the form of scientific articles in the **third part** of the thesis. The first article treated the identification and the quantification of the profile of the volatile bioactive compounds, the measurement of the antioxidant activity of the macerates, the evaluation of the active packaging's antioxidant capacity using in-situ hydroxyl radical generation method developed by **Pezo *et al*. (2006, 2008)** and 2,2-diphenyl-1-picrylhydrazyle (DPPH) assay and the assessment of the safety of the new antioxidant films for biopackaging applications by migration tests. The second article interests on the determination of the antioxidant efficiency of the new antioxidant active biopackaging material containing carob seeds macerates in the inhibition of the lipid oxidation of the stored fresh Atlantic salmon (FAS), and therefore extend its shelf life at 4 ± 1 °C. Therefore, the organoleptic assay, colour, drip loss and water holding capacity (WHC), pH, *Thiobarbituric acid reactive substances* (TBA-RS) and the total volatile basic nitrogen (TVB-N) parameters were evaluated.

II. Bibliographic review

1. Carob tree wastes

Carob tree wastes

1. Introduction

According to the Food and Agriculture Organization (FAO), one-third of the produced food is wasted **(FAO, 2011)**. The large amounts of agro-food wastes represent a challenge for the food processors, but also an important issue for both environment and international economy, since they are one of the causes for landfilling to be no longer sustainable **(Campos *et al*., 2020; Cattaneo *et al*., 2020)**. Nowadays, there has been considerable attention toward the recovery of the waste plant matrices as possible sources of functional compounds with health properties **(Hsouna *et al*., 2015)**, and recent studies showed that agro-food wastes must be considered renewable source of added value bioactive compounds **(Faustino *et al*., 2019; Panzella *et al*., 2020)**, such as the carob fruit (*Ceratonia siliqua* L.) that has been used for centuries in the food field **(Santonocito *et al*., 2020).**

Figure 1: Photograph of carob tree **(Kaderi *et al*., 2014)**.

2. Botanical classification

Ceratonia siliqua L. is an evergreen tree cultivated or naturally grown in the Mediterranean area such as the Maghreb countries in Nord-Africa including: Tunisia, Algeria and Morocco **(Rejeb, 1995).** The botanical classification of the carob tree according to **Cronquist (1981)**:

- Reign: *Plantae*
- Sub-reign: *Tracheobionta*
- Division: *Magnoliophyta*

- Class: *Magnoliopsida*
- Sub-class: *Rosidae*
- Order: *Fabales*
- Family: *Fabaceae*
- Subfamily: *Caesalpinioideae*
- Genus: *Ceratonia*
- Species: *Ceratonia siliqua* L.

3. Common terminology

The scientific name of the carob tree "*Ceratonia siliqua"* is derived from the Greek word ''keras" meaning small horn and the species name ''*siliqua*'' designates in Latin a "silique" or "pod", alluding to the hardness and shape of the pod. The naming of the species "*Ceratonia siliqua"* in different countries has followed a general form of the arabic name "*al kharroub*", as in the case of "*algarrobo*" or "*garrofero*" in spanish **(Albanell *et al*., 1991).**

4. Traditional uses and toxicological study

The fruit of carob tree has been widely used classically for the treatment of gastrointestinal affections such as diarrhea. In this context, the pods, bark and leaves are used in folk medicine as an antidiarrheal and diuretic **(Baytop, 1984)**.The carob juice is also used for the treatment of diarrhea, because of its richness in electrolytes **(Gulay *et al*., 2012)**. On another hand, these fruits are traditionally used as an antitussive and against warts **(Amico & Sorce, 1997; Merzouki *et al*., 1997)**. **Gulay *et al*. (2012)** have investigated the toxicological properties of carob on male New Zealand white rabbits. In this context, in the treatment group, rabbits received 10 cc of carob cures by boiling the fruit of carob. No toxicological signs or death related to carob extract were observed throughout the 7 weeks period of experiment. Regarding the hematological and physiological parameters, and histological aspects of the organs such as liver, kidney, lung, brain and heart in this study, the use of carob to animals did not cause any toxicological effects and can be used for human consumption **(Rtibi *et al*., 2017)**.

5. Carob as food wastes

Food processing processes generally produce a large amount of wastes. These food processing agro-industrial waste consist of high amounts of proteins, sugars, and lipids along with specific organic compounds as well. Therefore, this could be used as a cheap and abundant source of fine chemicals and secondary metabolites **(Liang *et al*., 2021).**

Carob tree (*Ceratonia siliqua* L.) (*Fabaceae*) is one of the most useful trees in the Mediterranean basin and other Mediterranean-like regions, because of its economic and environmental implications **(Rodríguez-Solana *et al.*, 2021)**. Among the different carob products (husk, endosperm, germ and pulp), the economic importance of carob pod comes from the utilization of seeds by the agro-food industry, which are considered the most valuable part of the fruits, and the main marketed product carob seed based is the locust bean gum (LBG), a natural hydrocolloid, widely used in food industry as stabilizer and thickener **(Dakia *et al.*, 2007)**, whereas carob kibbles, germ and seed peels could be considered as by-products susceptible of valorization (**Battle & Tous, 1997; Albertos *et al.*, 2015; Rico *et al.*, 2019)**. However, in the pharmaceutical field the seeds remain as a food waste **(Santonocito *et al.*, 2020).**

6. The uses of carob

The carob tree is a tree of indisputable ecological, industrial and ornamental importance. The tree and all its parts (especially the fruits) are used in a wide variety of sectors. Carob tree is considered currently one of the most successful fruit and forest trees in the Mediterranean basin since all its parts (leaves, flowers, fruits, wood, bark and roots) can be used. Whether the wood can be used directly as fuel, it is also widely used for making charcoal **(Batlle & Tous, 1997)**.

The carob fruit is very rich in nutrients and has long been used as a food for the livestock and human consumption. The pod consists of a pulp surrounding the seeds. Carob product research and development has recently received attention due to the nutritional potential of carob pods. **Figure 2** represent a scheme of the different parts of carob fruit, their products and composition.

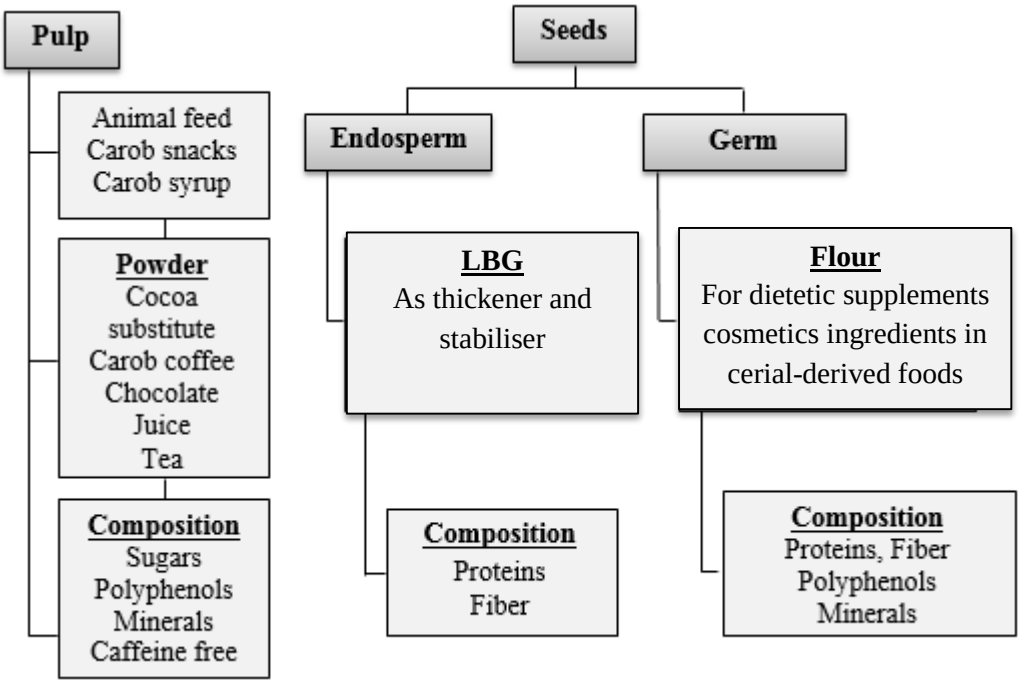

Figure 2: Parts of carob fruit, their products and composition **(Stavrou *et al*., 2018)**.

6.1. Pulp

The pulp, which is very rich in sugar, is often used to prepare concentrated sweet juices or syrups and molasses **(Marakis, 1996, Diaz, 1997).**

6.2. Germ

The carob germ is used to make germ flour, which contains proteins, unsaturated oil at high levels, and it is proposed as a dietetic human food **(Dakia *et al*., 2007)**. It is used in the food industry for the preparation of biscuits, juices, and as substituting cocoa and this can be attributed to the fact that carob powder contains no caffeine, thiobromine or oxalic acid, and has low fat content **(Yousif & Alghzawi, 2000; Biner *et al*., 2007; Srour *et al*., 2016; Aydın & Özdemir, 2017).**

Caroubin, a protein of the carob germ, has similar properties to gluten, so gluten-free products have been developed for celiac people by using carob germ flour **(Smith *et al*., 2010; Tsatsaragkou *et al*., 2013).**

6.3. Seeds

The seeds consist of three different parts: an envelope (brown cuticle, 30-33%), an endosperm (white and translucent, 42-46%) and an embryo or germ (23-25%). The seeds are mainly composed of galactomannans, which are found in the endosperm.

Carob seeds are primarily used for production of LBG, which is widely used as a stabilizer in various commercial applications in the food, pharmaceutical, cosmetic, and biotechnology industries **(Fidan *et al*., 2020)**. Commercial production and processing of carob pod was presented in **figure 3**.

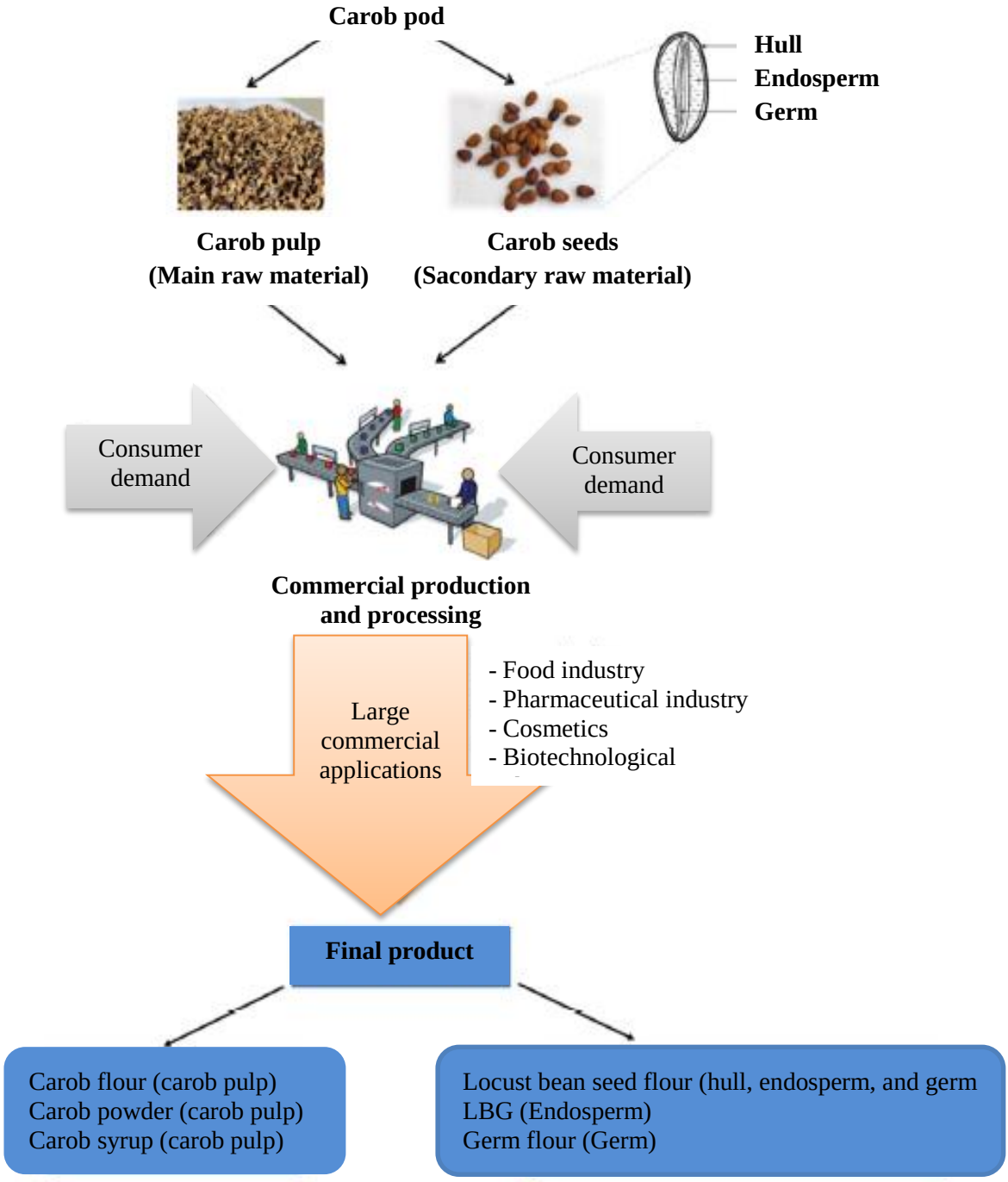

Figure 3: Commercial production and processing of carob pod **(Fidan *et al*., 2020)**.

This source of LBG is used in the food industry as a thickener and as a food stabilizer (E410) **(Calixto & Cañellas, 1982; Battle & Tous, 1997; Barak & Mudgil, 2014)**, and for the manufacture of a large number of foodstuffs: ice cream, soup, sauce, biscuit, pie,

confectionery, bakery products and in animal feed, but also in the cosmetics sector and pharmacy **(Ndir *et al*., 2000)**.

6.4. Leaves

The leaves are rich in tannins and the nutritional quality of the tannins is improved by the use of the leaves with polyethylene glycol (PEG). The bark of the carob tree has long been used in tanning, particularly in finishing and enameling skins **(Batlle & Tous, 1997)**. In Turkey, it was also used by the traditional medicine as an anti-diarrhea remedy. There is, however, no known use for the flowers and roots **(Batlle & Tous, 1997).**

7. Chemical composition of carob

Carob fruit is a complex mixture of primary and secondary metabolites, with the presence of sugars and fibers being characteristic of these fruits, followed by a great diversity of polyphenols. Numerous minerals and amino acids are also present in carob fruits **(Goulas *et al*., 2016). Figure 4** summarizes the major constituents in carob pulp and seed.

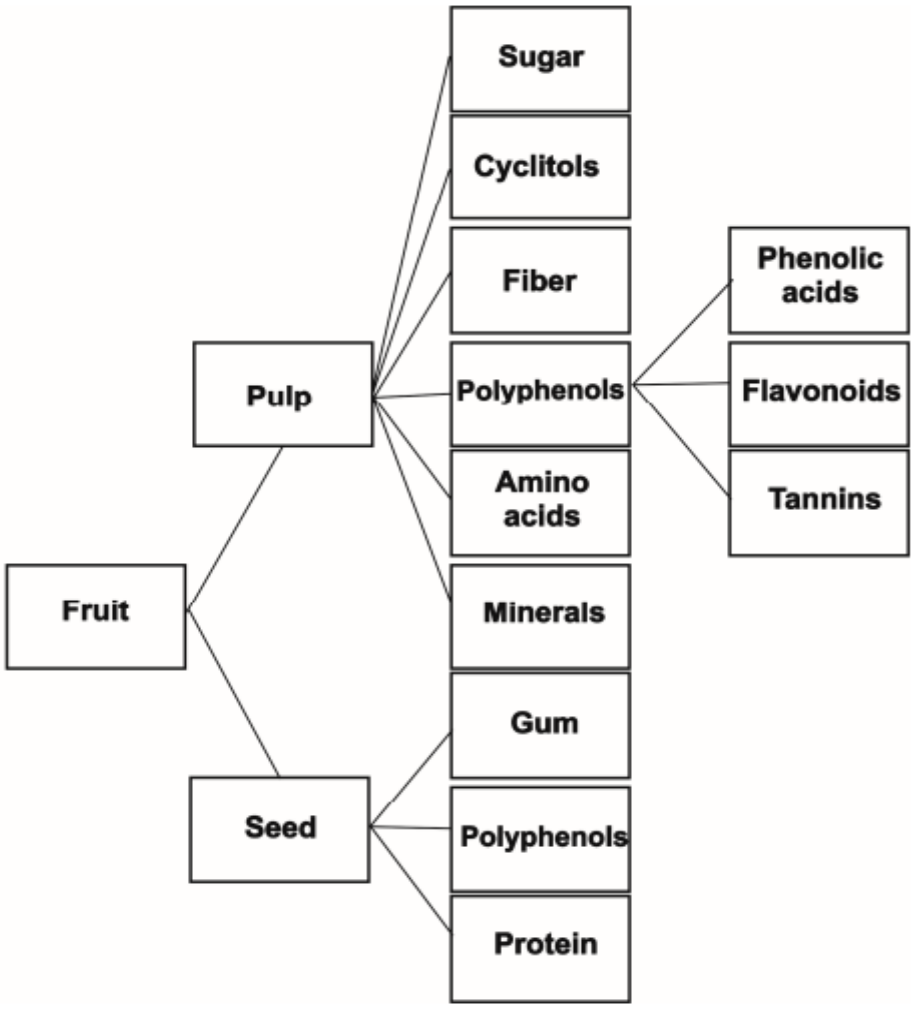

Figure 4: Main chemical constituents in carob pulp and seed with nutritional and health-promoting properties (**Goulas *et al*., 2016)**.

7.1. Sugar

Carob fruit is known for its high sugar content that is responsible for the nutritional value of beans **(Battle & Tous, 1997)**. Regarding the sugar composition, sucrose, fructose and glucose are the major carbohydrate in carob bean. Carob sugars are usually extracted for the production of natural carob syrup. Furthermore, an innovative process has been patented for their recovery in order to produce carob syrup **(Sigge *et al*., 2011).**

7.2. Fiber

Dietary fiber is a heterogeneous group of substances commonly divided into soluble and insoluble fibers. The total dietary fiber content of carob pulp usually ranged 30-40%, and it is produced by water extraction of the carob pulp to remove the majority of soluble carbohydrate **(Owen *et al*., 2003)**. The insoluble dietary fiber fraction consists of cellulose, hemicellulose, lignin and insoluble polyphenols and represented 70% of carob fiber. On the other hand, the amount of soluble dietary fiber is significantly lower and contains simple carbohydrate **(Nasar-Abbas *et al*., 2015)**.

7.3. Gum

LBG is a white to creamy white powder obtained from the seed endosperm of the fruit pod of the carob tree. The high molecular weight polysaccharide composed of galactomannan, which represented 85% of carob seed components which are galactose and mannose **(Rizzo *et al*., 2004)**.

7.4. Polyphenol

The main categories of phenolic compounds found in carob fruit are phenolic acids, gallotannins and flavonoids. The concentration of polyphenols in carob fruits depends strongly on genetic, environmental and extraction methods **(Papagiannopoulos *et al*., 2004).** Some studies reported that carob phenolics are covalently bound to the dietary fibers. In addition, carob germ and carob seed are rich source of phenolic compounds **(Makris *et al*., 2007)**.

Phenolics subdivided into benzoic and cinnamic acids are the most abundant class of polyphenols in carob fruits. Carob fruits are particularly rich in flavonols such as quercetin, myricetin, kaempferol and their glucosidic derivatives. Quercetin and myricetin rhamnosides

are usually the most abundant flavonoids in carob whereas flavones, flavonones, isoflavones are of low abundance **(Owen *et al*., 2003)**.

The most characteristic group of polyphenols in carob fruits are tannins and contribute to their astringency. **Avallone *et al*. (1997)** reported the presence of hydrolysable and condensed tannins in different parts of carob fruit. From a chemical point of view, carob tannins are mainly condensed tannins (proanhocyanidins).

7.5. Amino acids

According to the World Health Organization (WHO), carob can be considered a good source of amino acids. More specifically, the carob fruit contains all seven essential amino acids (threonine, methionine, valine, isoleucine, leucine, phenylalanine and lysine) at concentrations that meet the WHO standards **(Ayaz *et al*., 2009).**

7.6. Minerals

Carob fruits are excellent reservoir of potassium and calcium. Macrominerals such as phosphorus and magnesium have been also found in carob fruits at lower concentration **(Sigge *et al*., 2011)**. It contain also many microminerals including iron, copper, zinc, manganese, nickel, barium and cobalt, etc. Generally, the carob seeds contain higher macro- and microminerals than the pods **(Oziyci *et al*., 2014).**

8. Biological activities of carob or health benefits

Numerous studies have revealed physiological responses to carob fruit and its products that may be considered health beneficial by healing and preventing chronic diseases **(Brassesco *et al*., 2021).** It has demonstrated interesting findings concerning the bioactivity of carob pulp constituents **(Goulas *et al*., 2016)**. Fiber, cyclitols, polyphenols and tannins have mainly attracted scientific attention. These groups of bioactive compounds have been linked with the health-promoting effects of carob in different therapeutic areas **(Bates *et al*., 2000).**

The carob pods were reported to exert anti-inflammatory, antimicrobial, anti-diarrheal, antioxidant, anti-ulcer, anti-constipation and anti-absorptive of glucose activities in the gastrointestinal tract **(Makris & Kefalas, 2004; Rtibi *et al*., 2017)**. The carob leaves and barks were also reported to have an outstanding potential to be used as antidiabetic agents **(Custódio *et al*., 2015)**. Besides, the bark part proved to exert an anti-inflammatory and antioxidant effect **(Lachkar *et al*., 2016)**, the leaves were found to inhibit tumor cell proliferation **(Corsi *et al*., 2002)**. Furthermore, **Ben Hsouna *et al*. (2011)** demonstrated a marked hepatoprotective and nephroprotective effect of leaf extract. These findings have

rendered carob fruit an excellent ingredient for the development of functional food and herbal supplements. The valorization of these bioactive constituents is more attractive if we consider that they are usually discarded as LBG and simple sugars are used by the industry **(Goulas *et al.*, 2016)**. An emphasis on the clinical trials that carob has been subjected to is also provided. **Table 1** highlights the chemical components of carob and their associated evaluation in human health.

Table 1: The chemical components of carob and their biological evaluation

Group of chemical constituents/ Individual substances	**Biological evaluation of constituents/ Disease**	**Carob parts fraction**	**References**
LBG/ galactomannan	Gastrointestinal effects	Seed endosperm	**(Miyazawa *et al*., 2006)**
D-Pinitol	Anti-diabetic activity	Carob pulp	**(Bates *et al*., 2000; Tetik, & Yuksel, 2014)**
Soluble and insoluble dietary fiber polyphenols/ gallic acid, gallotannins, flavonol glycosides	Glycemic control , Enhanced lipid metabolism	Carob pulp	**(Zunft *et al*., 2003 ; Gruendel *et al*., 2007)**
Insoluble dietary fiber polyphenols/tannins, cellulose, semicelllose, lignin, pectin	Cholesterol metabolism, enhances lipid oxidation, lowers postprandial acylated ghrelin	Carob fiber	**(Gruendel *et al*., 2006)**
Polyphenols/gallic acid, catechin, myricetin rhamnoside, eriodictyol rhamnoside	Anticancer effects	Carob fiber	**(Klenow *et al*., 2008)**
Polyphenols gentisic acid, chlorogenic acid, catechol, ferric acid, gallic acid, myricetin, methyl gallate, quercetin, rutin, syringic acid, theophylline, vanillin	Cytotoxic activities	Germ flour extracts seed	**(Zunft *et al*., 2003)**
Fiber	Nutritional utilization, induction of lipodemia	Carob fiber	**(Perez-Olleros *et al*., 1999)**
Fiber	Hyperlipidemia effects	Carob fiber	**(Zunft *et al*., 2003)**
Tannins-polyphenols	Anti-diarrheal effects	Carob pod	**(Guggenbichler, 1983)**
Tannins-Pectin	Anti-diarrheal effects	Carob bean juice	**(Aksit *et al*., 1998)**

2. Food preservation

Food Preservation

1. Introduction

From early history**,** when people have surplus foods and predict potential food insecurity in the future, food preservation needs to be adapted. Humans felt the necessity of food storage and preservation. Human's inquest mind has innovated and discovered different food preservation systems throughout history. Most of the preservation techniques practiced by the early humans were based on daily experiences. Utilization of natural energy including solar, biomass, and natural phenomena such as evaporation cooling, spontaneous reactions like fermentation are some of the common features of these food preservation techniques. Many traditional food preservation techniques in developing countries still follow this approach extensively. However, a wide variation prevails in each preservation technique in different regions of the globe **(Joardder & Masud, 2019).**

During production, processing, distribution, and storage, food undergoes deterioration due to a wide range of reactions including some that are physical, chemical, enzymatic and microbiological **(Sahraee *et al*., 2019).** For this reason, modern society requires modern solutions, especially in the area of food safety and thus food packaging **(Wrona, 2017).**

2. Food deterioration

Food spoilage results when microbiological, chemical, or physical changes occur, rendering the food product unacceptable to the consumer **(Petruzzi *et al*., 2017)**. The highly sensitive organic and inorganic compounds which make up food and the balance between these compounds, and the uniquely organized structures and dispersions that contribute to texture and consistency of unprocessed and manufactured products are affected by nearly every variable in the environment **(Potter & Hotchkiss, 1995)**. **Figure 5** shows the main factors contributing to food deterioration.

Heat, cold, light and other radiation, oxygen, moisture, dryness, natural food enzymes, microorganisms and macro-organisms, industrial contaminants, some foods in the presence of others, and time-all can adversely affect foods. This range of potentially destructive factors and the great diversity of natural and processed foods is why so many variations of several basic food preservation methods find application in modern food technology **(Wrona, 2017).**

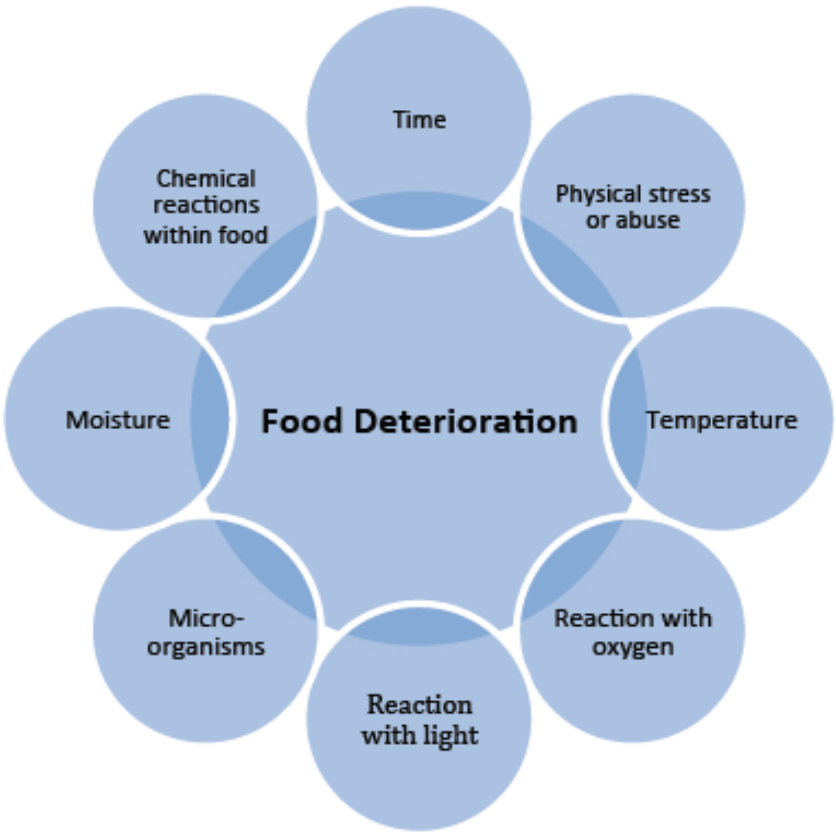

Figure 5: Diagram of factors contributing to food deterioration **(Wrona, 2017)**.

3. Food storage issues

Food spoilage results when microbiological, chemical, or physical changes occur, rendering the food product unacceptable to the consumer **(Benner, 2014)**. Food degradation is a complex phenomenon involving a wide variety of reactions. It depends on the type of food and its constituents, such as carbohydrates, lipids, proteins, vitamins and water, and the concentration of these constituents. Many of the reactions involved in food degradation are still unknown **(Nerin, 2010)**.

3.1. Lipid oxidation

Chemical food spoilage occurs when different components in the food react with each other or with some added component which alter the food's sensory characteristics **(Benner, 2014)**. A wide variety of organic molecules are susceptible to chemical attack by oxygen and more attention has been paid to lipids due to the importance of their oxidative damage **(Mcclements & Decker, 2000; Shahidi & Zhong, 2005; Waraho *et al.*, 2011).** Lipid oxidation is considered among the reactions involved in food degradation **(Pokorny *et al.*,**

2001), and has been considered as an important challenge for manufacturers and researchers to avoid food deterioration **(Kanner & Rosenthal, 1992; Shahidi & Zhong, 2005).** It is an oxidative deterioration of lipids containing any number of carbon–carbon double bonds. It takes place through multiple stages that include initiation, propagation and termination stages, and produces unhealthy compounds such as free radicals and reactive aldehyde products **(Huang & Ahn, 2019),** limiting the shelf life of many food products such as fish **(Jacobsen, 2010)**, and can be detrimental to the health of consumers **(Ahmad *et al*., 2020)**.

These consequences of lipid oxidation are not immediately detected by the consumer. However, it will also lead to a significant changes in the sensory properties including odour, flavour, colour and texture, which are easily detected by the consumer and may determine the shelf-life of the product **(Jacobsen, 2010)**.

The lipid oxidation can result from several reaction pathways depending on the medium and the initiating agents:

3.1.1. Process of free radical oxidation (autooxidation)

Free radical oxidation, also called autooxidation is the most important form of food lipid oxidation, especially in fish, and results in the production of off-odor and the formation of toxic compounds, causes loss of functional properties and nutritional value, and changes the color of food **(Soladoye *et al*., 2015)**. Free radical oxidation reaction leads to lipids breakdown and the formation of wide range of oxidation products **(Kubow, 1992).** Lipids spontaneously react with oxygen by means of autooxidation, which is a reaction in chain through the formation of free radicals. In fish, autooxidation can be initiated by light, heat and the presence of metal ions and radicals **(Sampels, 2013)**, and such a reaction consists of three main steps: initiation, propagation and termination as shown in **figure 6.**

Autooxidation leads to the formation of the primary oxidation products, hydroperoxides (ROOH). Once the oxidation process has started, a cascade of reactions will occur with each new molecule increasing the reaction speed and variability **(Heinonen *et al*., 1998)**. ROOH from lipid oxidation decompose easily at a high temperature or in the presence of metals to secondary products such as aldehydes, short chain hydrocarbons, alcohols, esters, acids and ketones **(Choe & Min, 2006).** Finally, termination products can be crosslinking products, for example, where two radicals react with each other, thereby terminating the chain reaction caused by radical reaction **(Nerin, 2010).**

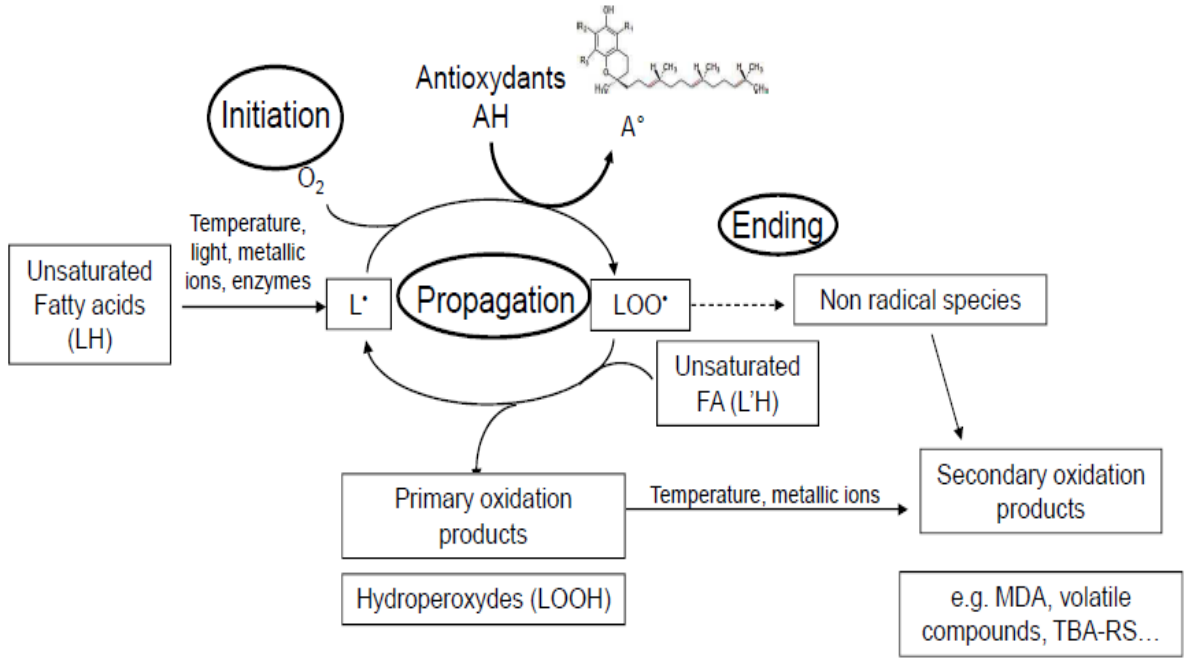

Figure 6: A general overview of the different steps in lipid oxidation **(Guyon *et al*., 2016)**.

3.1.2. Photooxidation

The development of photooxidation requires light, photosensitizer and oxygen **(Lee & Min, 1990)**. Photooxidation can degrade unsaturated fatty acid as well as proteins and generate cabbage or burnt feather odor **(Sucan, 2004)**. Direct photooxidation is due to free radicals produced by ultraviolet light irradiation, which catalyses the decomposition of hydroperoxides (ROOH) and other compounds such as peroxides (ROOR), carbonyl compounds (RCOR), or other oxygen complexes of unsaturated lipids **(Frankel, 2012)**. Photooxidation produce aliphatic and aromatic oxidized compounds, such as ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxies, sulfoxides, sulfones, phenols, anhydrides, quinones and alcohols **(Lee, 2003)**.

3.1.3. Enzymatic oxidation

Enzyme-catalyzed oxidation is related to lipoxygenase and cyclooxygenase. It is important in biological systems in the eicosanoids from the long-chain n-3 and n-6 fatty acids. Enzyme-catalyzed oxidation is also key to the production of eicosanoids (prostacyclins and leukotrienes) important for various biological activities such as vasoconstriction, vasodilation, platelets aggregation, gastric production, hyperalgesia, and inflammatory reactions (**Huang & Ahn, 2019).**

3.2. Protein oxidation

Foods are constantly exposed to reactive oxygen species (ROS) and this will not only cause lipid oxidation but also protein oxidation. For several decades proteins were ignored as a target for ROS. Protein oxidation depends on the origin of the fish, the muscle used, the treatment (crude, cooked, etc.) and other intrinsic and extrinsic factors. When oxidation occurs, it leads to various changes in the proteins, such as the changes in food texture, WHC, digestibility and juiciness **(Baron *et al*., 2007; Davies & Delsignore, 1987)**. Functional groups located on the side chains of amino acids are the main targets of this attack. Further reactions lead to the formation of different protein radicals and hydroxyl derivatives and cause protein carbonylation. Peptide backbone scission, lipid oxidation, and direct oxidation of amino acid side chains and carbonyl derivatives generated by reducing sugars are the major pathways of this carbonylation process **(Fu *et al*., 2014).**

Protein oxidation is initiated when a hydrogen atom is abstracted from the protein to generate a carbon-centered radical (C·) and in the presence of oxygen is converted to an alkylperoxy radical (COO·). The following reaction of (COO·) with hydrogen atom abstraction from another molecule leads to alkyl peroxide (COOH) formation. Subsequent reactions lead to the formation of the alkoxy radical (CO·) and hydroxyl compounds (COH). In addition, two carbon-centered radicals (alkyl-radical such as ethane, methane and propane) can react with each other in the absence of oxygen to generate carbon–carbon cross-linked derivatives **(Papuc *et al*., 2017).** The termination reaction, which in relation to food quality such as fish may affect tenderness **(Soladoye *et al*., 2015).**

3.3. Microbial contamination

In addition to the chemical alterations through the oxidation process, most of the deterioration processes are due to the biological reasons. Microbial contamination from pathogenic or spoilage bacteria can occur during improper processing, or when package integrity is compromised **(Alegbeleye *et al*., 2018)**, and may take diverse forms, which are a consequence of microbial growth, and manifests as changes in the sensory characteristics **(Gram & Huss, 1996).**

The range of spoilage microorganisms is wide. Bacteria are responsible for some of the most rapid and evident spoilage events of proteinaceous foods such as fish. The growth of yeasts and molds is generally slower than that of bacteria, but the wide variety of ecological niches they can exploit, the ability to utilize a variety of substrates, and tolerance of more

extreme conditions than (vegetative) bacteria makes them formidable spoilage agents **(Blackburn, 2006)**. Several factors are related and affect microbial spoilage of foods:

3.3.1. Food structure

Food structure is mainly related to the spoilage of different foods. Raw food for example is often protected from the attack by microorganisms by means of structure that are not easily degradable (fruit, skin, collagen muscle tissue, egg shells, etc.), but species with appropriate enzymes (cellulase, pectinase, protease, etc.) can attack these structures **(Baron & Gautier, 2016).** Fluid foods spoil rapidly because the organisms can easily spread throughout the food by means of their own mobility or by convection. Semi-solid foods such as fish can spoil as rapidly as fluid foods. Solid foods contaminate at first from their outside surface **(Modi, 2009)**.

3.3.2. Food composition

The growth of the most species of microorganisms can be also influenced by the nutrient composition. These nutrients are used as a sources of energy, carbon, nitrogen, minerals, vitamins, and other growth factors **(Hamad, 2012).** Protein foods such as meat, fish and eggs are liable to be attacked by proteolytic organisms, whereas, fat are liable to be attacked by lipolytic organisms **(Modi, 2009).** Many bacterial species, especially Gram-negative rods such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella*, and *Aeromonas*, as well as pathogenic spore formers like *Clostridium botulinum*, are proteolytic and can grow well in protein-rich foods and spoil them quickly or cause disease **(Hamad, 2012)**.

3.3.3. Water activity

The water activity (a_w), which is the ratio of the vapor pressure of the food to the vapor pressure of pure water, has an important influence on the growth of the microorganisms since an aqueous phase is a main requirement for their metabolic activity **(Kreyenschmidt & Ibald, 2012)**.

The optimum water activity for the growth of the large majority of food spoilage microorganisms is above 0.90, and the minimum is in the range 0.80-0.90. Each organism has a maximum, an optimum, and a minimum water activity for growth **(Hamad, 2012).** In general, bacteria need higher water activity for growth than yeasts and molds. There are some groups of microorganisms that can grow at water activity values of 0.60-0.75. These groups are termed xerophiles (dry-loving), halophiles (salt-loving), and osmophiles (preferring high osmotic

pressure). Most halophiles are bacteria, xerophiles are molds, and osmophiles are yeasts. Gram-negative bacteria are the most water-loving microorganisms, followed by Gram-positive bacteria, followed by yeasts and then molds. Bacteria are generally fast growing and hence they are the most important spoilers of fresh foods with high water content **(Hamad, 2012)**.

Table 2: Minimal water activity required for the growth of foodborne microbes at 25° C **(Jay, 2000; Banwart, 2004; Montville & Matthews, 2005)**

Group of microorganisms	**Minimal a_w required**
Most bacteria	0.88–0.91
Most yeasts	0.87–0.94
Most molds	0.70–0.80
Halophilic bacteria	0.75
Xerotolerant molds	0.71
Osmophilic yeasts	0.60–0.78
Xerophilic molds	0.60–0.70
Specific spoilage organisms	
Pseudomonas aeruginosa	0.96–0.98
Pseudomonas fluorescens	0.94–0.97
Lactobacillus	0.90–0.96
Leuconostoc	0.96–0.98
Hansenula	0.89–0.90
Zygosaccharomyces rouxii	0.62–0.81
Aspergillus niger	0.80–0.84
Xeromyces bisporus	0.60–0.61
Specific pathogenic organisms	
Escherichia coli	0.94–0.97
Clostridium botulinum	0.90–0.98
Staphylococcus aureus	0.83–0.92
Vibrio parahaemolyticus	0.94–0.98
Salmonella	0.93–0.96
Bacillus cereus	0.92–0.95
Aspergillus flavus	0.78–0.90

3.3.4. pH

The pH value of a food limits the range of organisms which it can support **(Modi, 2009)**. Most microorganisms develop around neutral pH, which corresponds to the pH of bacterial cytoplasm and it is optimal for bacterial enzyme activities. However, there are bacteria that can tolerate a pH outside these ranges. In general, microorganisms have established response mechanisms to deal with stress, enabling them to survive in certain pH conditions **(Baron & Gautier, 2016)**.

Table 3: Minimum pH values for the growth of some microorganisms associated with foods **(Jay, 2000; Lund & Eklund, 2000; Ray, 2004)**

Microorganisms	Minimum pH
Pathogenic bacteria	
Salmonella spp.	3.8-4.05
Campylobacter jejuni/coli	4.9
Escherichia coli O157:H7	4.5
Vibrio parahaemolytics	4.8
Yersinia enterocolitica	4.2
Clostidium botulinum	4.6-5.0
Clostridium perfringens	5.0
Staphylococcus aureus	4.0
Listeria monocytogenes	4.1-4.5
Bacillus cereus	4.9
Non-pathogenic bacteria	
Pseudomonas spp.	5.6
Bacillus stearothermophilus	5.2
Clostridium pasteurianum	4.2
Clostridium butyricum	4.2
Lactobacillus brevis	3.2
Yeasts	
Candida krusei	1.3
Saccharomyces cerevisiae	1.6
Pichia membranifaciens	1.9
Zygosaccharomyces bailii	1.8
Molds	
Aspergillus spp.	1.6
Penicillium spp.	1.6-1.9
Fusarium spp.	1.8

3.3.5. Temperature

Microbial growth is a result of a series of chemical reactions, hence it is strongly affected by temperature. For these chemical reactions to take place, the cell membranes and the enzymes, which are also strongly affected by temperature, must remain intact, and water must be available in liquid form. Microorganisms in general grow over a wide range of temperatures, extending from below 0 to above 100 °C, but the range for each individual microorganism is much narrower **(Hamad, 2012)**.

Temperature conditions during processing, transport, and storage are one of the most important factors that influence the spoilage process. Increasing temperature conditions lead to a decrease in the length of the lag phase and generation times and thus to an increase in the

growth rate. They also influence protein synthesis, enzyme activity, solute uptake, and thus the length of shelf life **(Kreyenschmidt & Ibald, 2012)**. Every type of organism is able to grow within a certain temperature range, maximum growth occurring at and around the optimum temperature. So organisms are divided on the basis of their temperature preferences into rough categories: psychrophiles, mesophiles, and thermophiles **(Modi, 2009)**.

3.3.6. Gaseous conditions

The oxygen tension and the oxidation reduction (O-R) of the food influence the type of the organisms which can grow in it. Most fresh animal foods have a low O-R throughout and they are aerobic at the surface only. Facultative organisms grow on the surface and within the food. Anaerobic organisms grow within foods held anaerobic conditions **(Modi, 2009)**.

3.3.7. Interaction phenomena

With regard to the growth of microorganisms, the combined action from the interaction between the different physicochemical parameters is important to take in consideration **(Modi, 2009)**.

3.3.8. Humidity

Relative humidity of the storage environment of foods may affect their quality, because it can lead to change in their water activity. This may lead to conditions where water activity on surfaces can reach values that allow microbial growth, leading to food spoilage or even poisoning. Microorganisms that may cause spoilage under humid storage conditions include molds, yeasts, and certain bacteria **(Hamad, 2012)**.

4. Storage of foodstuffs

The bioconservation of food has been the subject of much research for many years. It consists of an increase in the shelf life and an improvement in the food safety, using bioactive molecules natural of plant or microbial origin using microorganisms and/or their metabolites **(Stiles, 1997)**.

Food safety has now become a constant concern for the food industry. Product recalls are more and more recurrent and the multiplication of the number of inspections seems hardly able to solve the problem.

The degradation of food products affects the nutritional qualities and the sensory aspects of the products and may have effects on human health. In this context, various means of prevention are available to limit these phenomena such as the antioxidants and the

antimicrobial agents used in the food industry **(Ribeiro *et al*., 2001; Marongiu *et al*., 2004).** The role of these active agents in the food industry is to reduce and minimize the food spoilage during storage.

4.1. The use of antioxidants

In the fields of food science and nutrition, research on the natural functional biological activity of food is essential. In particular, antioxidants are one of the biggest topics of concern **(Comert & Gokmen, 2018; Yeung *et al*., 2018)**, because antioxidants play a vital role in balancing the physiological state and preventing oxidation of food **(Alexiou & Demopoulos, 2010; Pesic *et al*., 2019)**.

Synthetic antioxidants are frequently used to stabilize fats, oils, and lipids containing foods, and these antioxidants are also included in polymer processing to improve the properties of these materials **(Wanasundara & Shahidi, 2005; Byun *et al*., 2010).** However, the use of some synthetic antioxidants in food has been questioned by the scientific community **(Wanasundara & Shahidi, 2005; Gomez-Estaca *et al*., 2014)** because of the potential toxicity over the foodstuff. The alternative approach is the use of natural antioxidants, such as tocopherol, plant extracts, and essential oils from herbs and spices **(Moudache *et al*., 2017).**

4.1.1. Classification of antioxidants

Antioxidants can be classified into two major types based on their source which are: natural and synthetic antioxidants. Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action, whereas, synthetic antioxidants are artificially produced or synthesized using various techniques **(Mamta *et al*., 2013).** Schematic representation of the classification of antioxidants is shown in **figure 7**

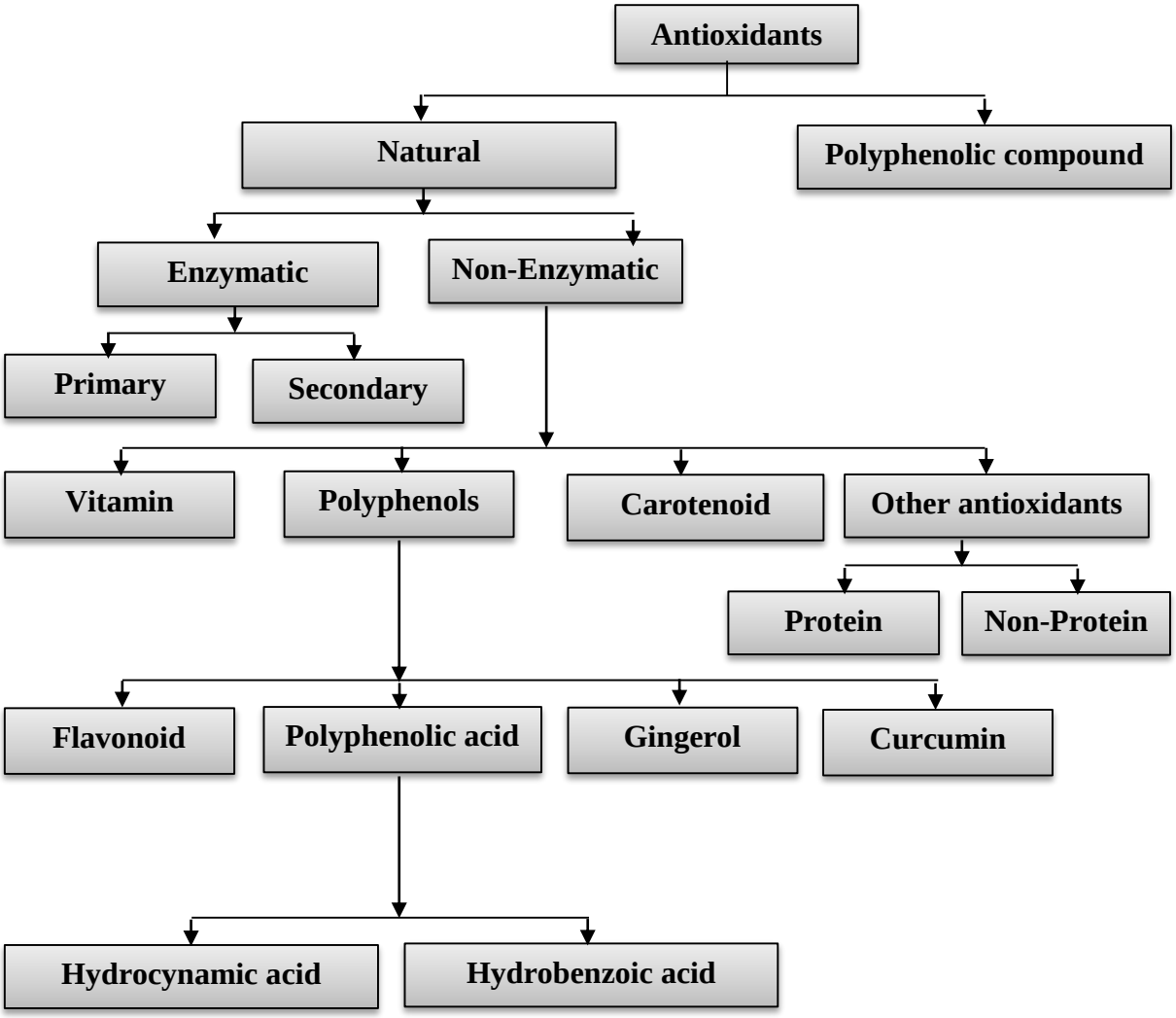

Figure 7: Schematic representation of the classification of antioxidants **(Mamta *et al*., 2013).**

4.1.2. Mechanism of antioxidant activity

There are mainly three types of mechanism known for the antioxidant activity: chain breaking, preventive, and synergetic. Schematic representation of these mechanisms is given in **figure 8**.

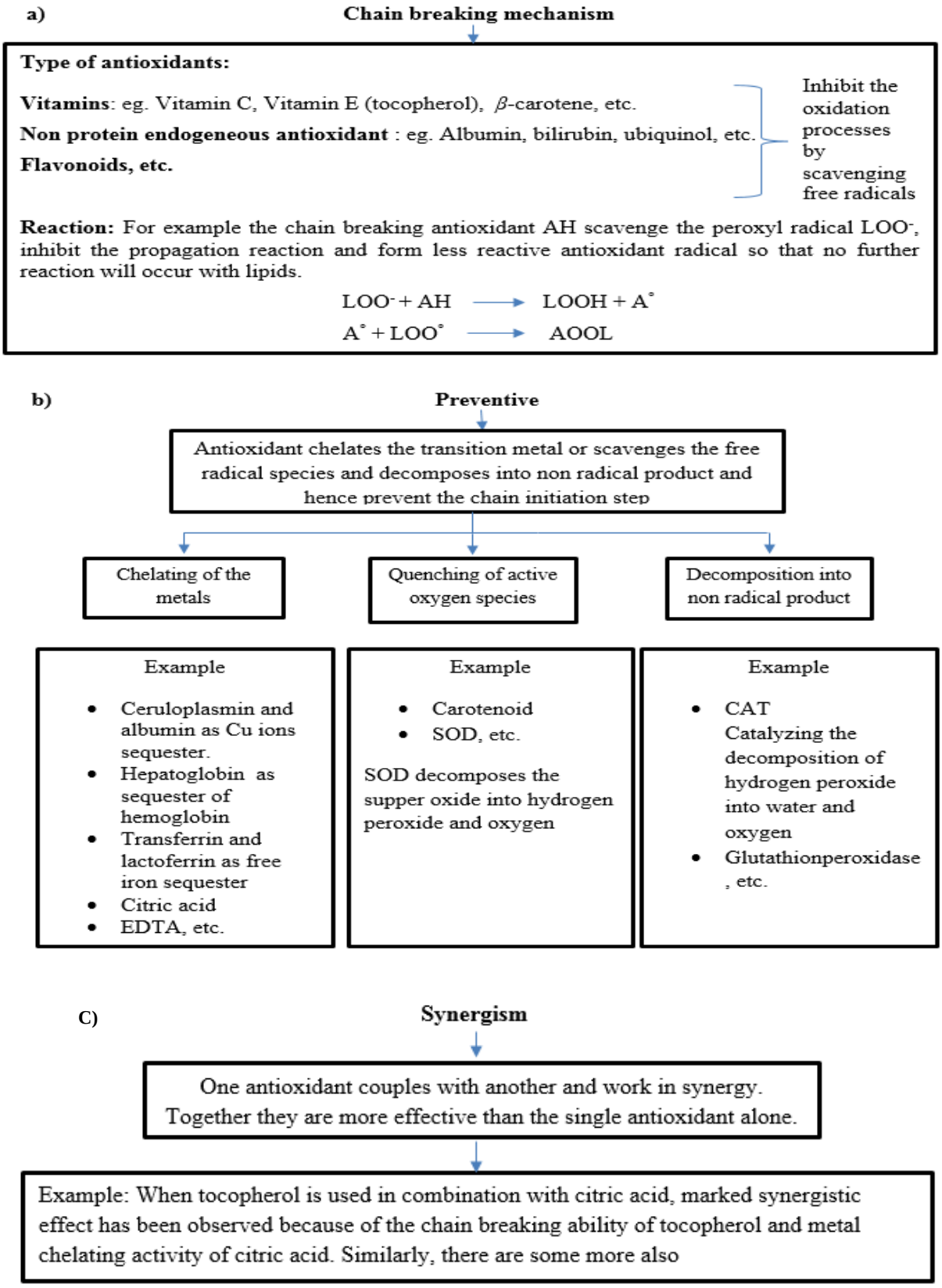

Figure 8: Schematic representation of mechanism of chain breaking, preventive and synergetic action of antioxidants respectively **(Mamta *et al.*, 2013)**.

4.2. Antimicrobial agent for food preservation

The risk of food contamination is all the higher as current market trends substantially limit the use of conventional microbiological barriers such as freezing, salting, etc., due to their negative impact on health. In this context, the biopreservation, including the use of natural antimicrobial molecules, is without doubt the most promising alternative to ensure product safety and reduce the incidence of dreaded pathogenic bacteria **(Lorenzo *et al*., 2018).**

Food antimicrobials are chemical compounds that are naturally present in food or are directly added in order to inhibit the growth of pathogenic or spoilage micro-organisms with the aim of ensuring food safety and quality **(Davidson, *et al*., 2013).**

4.2.1. Synthetic antimicrobial

A wide range of synthetic preservatives are used to extend the shelf-life of food by inhibiting bacterial growth **(Chipley, 2005; Tongnuanchan & Benjakul, 2014)**. Some examples for their use are:

- The use of sodium chloride in combination with sodium lactate reduces microbial growth, maintains chemical quality and prolongs the shelf life of ground beef during refrigerated storage **(Sallam *et al*., 2004)**.
- Nitrites such as sodium nitrite or potassium nitrite provide a stabilized red meat color, dried meat flavor and delayed rancidity **(Jay *et al*., 2005).**
- Sulfites are used against aerobic Gram negative bacilli, molds and yeasts in meats and meat products **(Ray, 2004)**.
- Sorbic acid and its salts are widely used worldwide as meat preservatives to inhibit bacteria and fungi **(Feiner, 2006)**.

Nevertheless, Consumers are concerned about the safety of synthetic preservatives used in food. Therefore, a growing demand for natural products that can serve as alternative has appeared **(Tajkarimi *et al*., 2010)**. This, in turn, has led to the search for antimicrobials derived from various natural sources.

4.2.2. Natural antimicrobial agents

The search of natural antimicrobial agents from natural sources in surrounding area has achieved great importance in the field of agro-food industry **(Paul *et al*., 2019)**.

Natural antimicrobials can come from several sources: plants, animals, bacteria, algae. Several studies of plant antimicrobials have demonstrated the efficacy of plant-derived

compounds in food applications **(Tajkarimi *et al*., 2010; Hayek *et al*., 2013)**. Certain fruits like jackfruit, papaya, plum, guava, and tamarind and their seed extracts have shown antimicrobial activity against gram-positive (*S. aureus, B. subtilis*) and gram-negative (*E. coli, P. aeruginosa*) **(Debnath *et al*., 2011)**. Pomegranate extracts inhibit the growth of several foodborne pathogenic bacteria, including *L. monocytogenes S. aureus, E. coli, Y. enterocolitica*, and *B. cereus* **(Al-Zoreky, 2009; Kanatt *et al*., 2010; Agourram *et al*., 2013)**.

The study by **Fattouch *et al*. (2007)** demonstrated an antibacterial effect of quince extract against Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa*) bacteria and yeast (*C. albicans*). A tannin-rich fraction of pomegranate peel extract showed inhibitory activity against different strains of *L. monocytogenes*. The tannin moiety alters the membrane structure of the bacterial cell, resulting in a loss of cell homeostasis **(Li *et al*., 2014).**

4.3. Food applications of carob (*Ceratonia siliqua* L.)

Great challenges for the food industry are the clear demonstration of the health benefits of natural ingredients from carob before they can be successfully incorporated into functional food products with regulatory compliance and consumer acceptance **(McClements & Xiao, 2014)**. Several studies in recent years have demonstrated the versatility and functionality of different carob products used in the production of functional foods **(Brassesco *et al*., 2021).**

The carob can be used as preservative food ingredient in the food industry due to its active agent's effects that improve the final characteristics of food products. Some examples of foodstuffs with the addition of carob extracts are presented in **Table 4**. The enhanced properties of the final products are highlighted.

Table 4: Carob based functional food and main benefits ascribed

Functional food	**Positive effects**	**References**
Water cream with carob pod	High antioxidant activity Positive effect on physicochemical and sensory properties	**(Habibzadeh *et al*., 2019)**
Muffin with carob powder	High antioxidant activity High total phenolics content	**(Pawłowska *et al*., 2018; Cervenka *et al*., 2019)**
Bread with carob flour (pods and germ flours)	Improved texture and sensory properties Antimicrobial activity High total phenolics content High antioxidant activity	**(Rico *et al*., 2018; Hoehnel *et al*., 2019)**
Carob fruit wastes in bifunctional coating for packaged salmon	Antimicrobial activity Reduced lipid oxidation	**(Goulas *et al*., 2019)**
Pasta enriched with carob flour	Good colour, firmness and hardness high antioxidant activity High total phenols content	(**Biernacka *et al*., 2017)**
Rice-based extruded snacks-like fortified with pea, bean and carob fruit	High phenolic compounds content High antioxidant activity Improved textural attributes	**(Arribas *et al*., 2019a; 2019b)**

3. Food active packaging as a new technique

Food active packaging as a new technique

1. Introduction

With the introduction of agriculture, the storage of harvest surpluses became critically important for the viability of society. Storage techniques evolved to keep that surplus suitable for consumption or viable as seed for the next season's crop. Naturally occurring decay mechanisms had to be identified and understood **(BOPP, 2019)**. With the development of cities and trade, a need also arose to accommodate those food items transported from rural to urban areas. In modern times, food security has also become a growing issue. Packaging has coevolved with societal expectations as a visible means of not only displaying and preserving food but also keeping it secure and free of contamination **(BOPP, 2019)**.

During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbiological processes **(Sahraeea *et al*., 2019).** The demand for healthy, nutritious, safe, and more natural products has recently increased the search for new food production, processing, and preservation technologies **(Castro *et al*., 2019)**. Food safety is being challenged nowadays by strong consumer demands. Food is expected to be primarily safe, then wholesome and nutritious. This led researchers to address to this consumer concern **(Wrona & Nerin, 2019).** One of the solutions is to use active packaging which can provide several benefits for foods such as preservation, maintaining foods quality and safety, and transferring information to consumers **(Djenane *et al*., 2016).**

2. Food packaging concept

It is popularly known that the basic role of packaging is to isolate products from the external environment and ensure food protection against the stagnation caused by the actions of mechanical forces, odors, dust, gases, moisture, and microorganisms, also the protection against radiation/light and insects. The contact of these destructive agents appears to decrease the shelf life of products. Moreover, it also impacts the health of consumers when contamination of food is being caused by foodborne diseases related to microorganisms **(Al-Tayar *et al*., 2019).**

Contamination can occur at any place when food is kept in an external environment such as stages of retail display, storage, shipping, distribution, and slaughtering. Hence, effective packaging should play the role of an obstacle to decrease the path of nearby food

contaminants. Therefore, the packaging is a highly important feature for increasing the shelf life of perishable food products. It decreases post contamination and provides food safety assurance during marketing **(Youssef *et al*., 2020)**.

In contemporary world, food packaging plays a vital role in preserving food quality and it became an integral part of our everyday life (Food packaging material requirements are represented in **figure 9**). Nevertheless, traditional packages provide only a passive barrier to retard the negative effect of environmental conditions on packaged foods **(Hicks, 2002)**. The traditional packaging can no longer effectively and sufficiently meet today's needs. Strong consumers' demand and market trends enforce more advanced and creative forms of food packaging **(Nerin, 2010)**.

Figure 9: Food packaging material requirements **(Lavoine, 2013)**.

3. Historical overview of food packaging

During nomadic times in human history, people ate what they could find and collect food from the environment, without worrying about protecting and store their food. However, when mankind began to settle in the communities, regions and cities, people ate what they hunted with utensils, weapons and tools manufactured. At this point, the need to enclose and storing food has become essential **(Bampouli, 2014).**

Until the 19th century, there was little sophistication in packaging materials, and packaging as we know it did not exist. Containers made from natural materials were utilized for transportation and storage. Grasses, reeds, and thin strips of wood were often woven into baskets for solid food transportation, and storage and earthenware pots were created to store and transport liquids **(BOPP, 2019).**

Oxygen absorbers are the first active packaging systems to emerge in the early 1970s in Japan. They appeared in the United States at the beginning of the 1980s, notably with significant use by the military market **(Coles, 2003)**. After, the company "Mark and Spencer" greatly contributed to the launch of the use of oxygen absorbers in Europe, by offering an innovative product on its shelves in London: fresh smoked fish in packaging containing an oxygen absorber. Interest in active packaging has grown considerably in recent years and has given rise to numerous patents on the subject **(Gontard, 2000).**

4. Active packaging

The concept of active packaging is not really new: the use of lemon to prevent apples or mushrooms from oxidation, or the use of leaves to cover products and transfer them the aroma compounds or enzymes that are responsible of their preservation, are examples of ancestral active packaging. By instinct, each of us employs the basics to preserve better and longer the food products **(Lavoine, 2013)**. As said by **Catalá and Gavara (2001)**, an active packaging is a "food/package/environment" system that works in a coordinated way to improve the quality and safety of the packaged product and increase its shelf-life. Its purpose is to protect the food against agents that cause physical, chemical, microbial or enzymatic changes **(Jamshidan *et al*., 2010).**

Active packaging is an innovative concept emerging in the field of food packaging to meet market demands, such as consumers' preference for safe, healthy and high-quality food **(Kuai *et al*., 2021)**. It is an emerging technology compared with traditional "inert packaging", incorporates active components such as oxygen scavengers, antioxidants, and antimicrobial agents **(Yildirim *et al*., 2017)**.

Active packaging's change the environmental conditions to maintain the sensory properties and ensure the safety of the product. They may act either by progressively releasing active agents to the surrounding atmosphere or by absorbing the compounds that deteriorate the food, such as oxygen or free radicals **(Montero-Prado *et al*., 2011)**.

4.1. Antioxidant active packaging

Oxidation of lipids in food not only leads to the development of rancidity, but also the potential formation of toxic aldehydes and loss of nutritional quality **(Djenane, 2015)**. In order to protect the oxidation of lipids, antioxidants are traditionally added in the initial food formulation **(Oudjedi *et al*., 2019)**. However, the direct addition of antioxidants to foods is limited by their participation in complex reactions sometimes becoming pro-oxidative and the lack of selectivity to target the surface of foods where most oxidation reactions occur **(Moudache *et al*., 2017)**. However, the alternative approach is to incorporate antioxidant agents in the polymer packaging, which prevent oxidative processes and extend the shelf life of food products **(Bolumar *et al*., 2011).** Antioxidant performance can be achieved either by adding antioxidants to the packaging material that are released from it to the food or incorporating free radical scavengers in the packaging material. This second approach does not require direct contact to the foodstuff to exhibit antioxidant properties but only direct action from the packaging material **(Wrona *et al*., 2015),** and has been demonstrated as very efficient, as the antioxidant performance takes place without direct contact with the food and doesn't affect the organoleptic quality of packaged food **(Montero-Prado *et al*., 2011).**

There is a wide range of advantages of application of active packaging: small amounts of active substances needed, localized activity, controlled release of antioxidants and elimination of processing steps such as mixing, immersion or spraying of food comparing to direct addition of antioxidants into food products **(Wrona, 2017)**. According to **Nerin (2010)**, there are several ways to create antioxidant active packaging materials:

- To incorporate antioxidants into the melted polymer, and then extrude the material. Most of the antioxidants are lost or degraded, as the process requires an increase of temperature.
- To produce the active material as a coating on other packaging materials. This is one of the most promising technologies, although some problems still exist.
- To introduce the antioxidant as an internal layer in a multilayer structure. Usually, the antioxidant layer contains an oxygen absorber. This technology is commercially available, but expensive, as it requires a special activation when used in the food industry.
- To apply the coating directly to the food; this requires the use of edible packaging materials. This is another approach in which the applications are more restricted, as not all the foodstuffs can be coated. One disadvantage is the likelihood of over-packaging,

as the coated food will still require normal packaging material for health and safety reasons.

4.2. Antimicrobial active packaging

Antimicrobial packaging is an innovative means resulting from food-borne problems. This option helps inhibit microbial growth while maintaining the quality, freshness and safety of the food **(Han, 2005)**. Antimicrobial food packaging works to reduce, inhibit or retard the growth of microorganisms that may be present in packaged foods or the packaging materials themselves **(Jung & Zhao, 2016).**

The use of packaging films containing antimicrobial agents may be the best packaging performance, by slowly migrating agents from the packaging material to the surface of the product, thus helping to maintain high concentrations where they are needed **(Han, 2005)**. If an antimicrobial agent can be released from the packaging for an extended period of time, the activity can also be extended to the stage of storage, transport and food distribution **(Avila-Sosa *et al*., 2012).**

The incorporation of active compounds into natural polymers is a valuable strategies for increasing the shelf life of packaged food products **(Mousavi Khaneghah *et al*., 2018).** From a theoretical perspective, antimicrobial agents should be delivered at a controlled rate. Additionally, the concentration of released antimicrobial agent should be neither too high nor too low to avoid adverse effects on sensorial and toxicological properties **(Mastromatteo *et al*., 2010)**. In other words, a balance between the microbial growth kinetics and the controlled release rate should be established to guarantee the proper protective function during the expected shelf-life. Therefore, one of the most interesting challenges in the field of antimicrobial systems is controlling the release rate of the antimicrobial agents from the packaging and their subsequent transfer into the food products **(Mousavi Khaneghah *et al*., 2018).** The method of incorporation, the nature of the matrix containing the antimicrobial agent, the mechanism of the release and the food properties are the most critical factors. Moreover, there are several methods for incorporating antimicrobial agents into polymeric materials including the direct incorporation of antimicrobial agents into the polymers, deposition through coating or the application of very thin layers, spraying onto the polymer surfaces, and immobilization by chemical grafting or the use of polymers that exhibit intrinsic antimicrobial properties **(Shemesh *et al*., 2015).**

5. Food packaging materials

The materials used to make food packaging include a group heterogeneous, including glass, metals, plastics, wood, cardboard and paper, with a corresponding range of performance characteristics. The most applied materials in food packaging are plastics, which provide varying degrees of protection, depending on the nature of the polymers used in their manufacture **(Nerin, 2016).**

The traditional materials of food packaging are extracted from non-renewable resources of fossil fuels and they face hardship in disposal **(Gan & Chow, 2018)**. As a result, plastic recycling has turned out to be a motivating factor to decrease plastic waste accumulation in the external environment. But this system of recycling does not operate on the food packaging systems due to the reason that organic materials are contaminated and are present on food packaging plastics **(Ingrao *et al*., 2017)**. The large absorption of plastic derived fossil fuel material has given rise to frequent waste production problems that cause pollution in the environment **(Han *et al*., 2017)**.

To reduce the environmental contamination, materials with faster degradation process are being researched and developed as substitutes to the traditional polymers **(Al-Tayar *et al*., 2019).** Bio-based polymers, derived from lipids, polysaccharides, proteins and their composites, can be produced from renewable biomass sources, such as cornstarch, vegetable fats, oils, and even microorganisms **(Mousavi Khaneghah *et al*., 2018)**

Biodegradable polymers are defined as materials that are capable of decomposition into carbon dioxide, methane, water, inorganic compounds, or biomass predominantly by the enzymatic action of microorganisms **(Verma *et al*., 2021).** The development of biopolymers (bioplastic) from renewable sources has become the interest of industries food packaging. These bioplastic materials must have durability sufficient to maintain their mechanical and physical properties for the quality of the product, and also biodegrade rapidly on disposal **(Al-Tayyar *et al*., 2019).** These biopolymers can be grouped according to the production method (**Figure 10**):

1. Polymers directly extracted from natural materials (example: starch, cellulose, casein and wheat gluten).

2. Polymers produced by chemical synthesis from renewable monomers (example: polylactate polymerized from lactic acid monomers).

3. Polymers produced by microbial fermentation (example: polyhydroxyalkanoates).

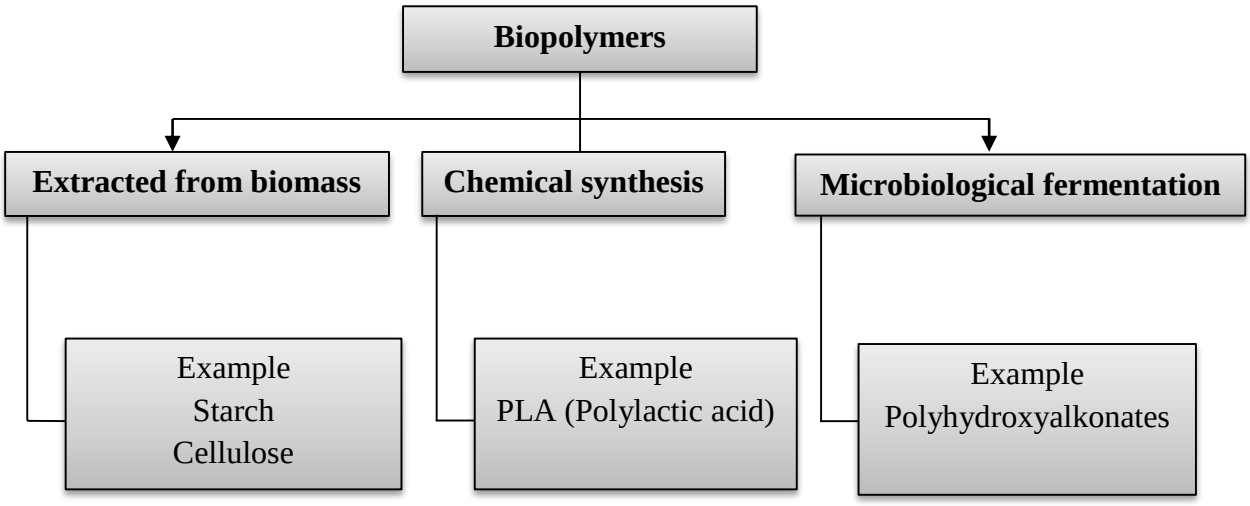

Figure 10: Schematic overview of the classification of biopolymers **(Van Tuil *et al*., 2000)**.

6. Migration

Food packaging protects foodstuffs, but it can also constitute a source of chemical food contamination. Those substances in some cases are considered as toxic for human organism **(Coles, 2003)**. In recent years, special attention has been focused on food safety issues due to the potential migration of high-risk chemicals from food contact materials. There are some factors such as the packaging material, the type and nature of food in contact and the storage conditions, which strongly influence the level of migrated substances (**Nerín *et al*., 2013; Úbeda *et al*., 2017; Qian *et al*., 2018; Alberto Lopes *et al*., 2019).**

Migration is defined as the mass transfer between the packaging material and the packaged food **(Muncke, 2016)**. Various additives might be added to polymeric packaging materials, in order to improve their properties or facilitate the manufacturing process **(Guart *et al*., 2011).** These additives, particularly those with molecules and ions of small size, may be susceptible to migration from the plastic into the foodstuff **(Simoneau *et al*., 2012; Qian *et al*., 2018)**. Moreover, materials for food packaging must not under any circumstances cause unacceptable changes in the composition, taste and odour of the product, nor may release substances in quantities that are dangerous to the health of consumers.

In the case of multilayer packaging materials, where the material is made of multiple polymer layers bonded by adhesives, migration can occur not only from the material that is in direct contact with food, but also from internal layers of the material including the adhesives. This process is due to diffusion and partition processes of the compounds between the different layers **(Tehrany & Desobry, 2004; Aznar *et al*., 2011).**

In order to guarantee the safety of food packaging, the materials must fulfill the Europe national legislation and community level legislation that continue to coexist. At the Union level, food contact materials are regulated under the EU Framework Regulation (EC) No 1935/2004 **(EU, 2004)** and the Specific Regulation (EU) No 10/2011 **(EU, 2011)** and its amendments applies on plastics **(Gavril *et al.*, 2019).** A positive list of additives containing their specific migration limits (SML), as well as the conditions for migration assay (temperature and simulants) are included in this Regulation.

In practice, there are two types of migrations: global migration and specific migration. Global migration represents the total amount of components, known or not, that have been transferred from the packaging material to food during the preparation and/or storage. It is a measurement of inertness of the packaging material. While, specific migration refers to the amount of a specific and identifiable substance that is transferred to the food **(Tovar *et al.*, 2005).** The migration does not occur equally in all packaging materials for food contact. In polymeric materials, including plastics and cellulose derivatives, the interaction occurs through a mechanism based on mass transfer through the polymer matrix, where the components, additives and residues present in the material are incorporated into the packaged product **(Garde *et al.*, 1998; Nerín *et al.*, 2012)**. **Figure 11** presents a scheme of the migration process in a polymer system/food.

The migration of additive components or residues in polymeric packaging materials to food is a phenomenon of mass transfer, consequence of the tendency to equilibrium of all chemical systems, and it is controlled by two mechanisms: diffusion and sorption. The overall process is developed in three stages: diffusion of the migrants into the polymer matrix, solvation or dissolution of migrants in the food-packaging interface and dispersion of migrants in food **(Till *et al.*, 1987)**.

Migrants are compounds of low molecular weight that are in the polymer matrix. Possible chemical migrants include plasticizers, antioxidants, non-intentionally added substances (NIAS), thermal stabilizers, slip compounds, and monomers (**Bhunia *et al.*, 2013)**. When these compounds reach a specified limit, food quality and safety may be jeopardised. Therefore, study of the migration of food-packaging compounds by exposing food or food-simulating liquids to storage conditions is of great importance **(Nerín *et al.*, 2013).**

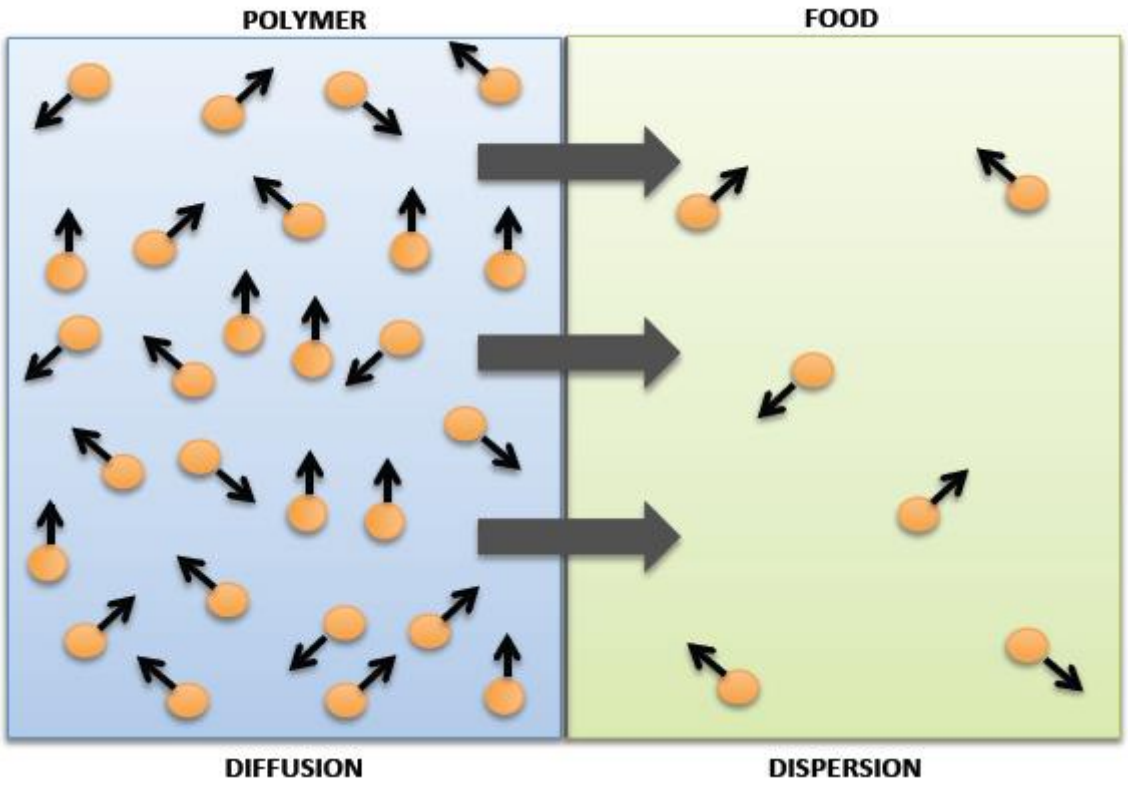

Figure 11: Scheme of a migration process in a polymer/food system **(Wrona, 2017)**.

7. Legislation

Food contact materials protect food from external contamination and preserve the nutritional value as well as the physical and sensory quality of food. However, it is important to control the migration of compounds from packaging materials to foods, as it may lead to the transference of unwanted substances that can make food less safe for consumption or that may alter its sensory and nutritional characteristics **(Ubeda *et al.*, 2019)**.

To accomplish the legislation of food contact materials, any packaging material has to follow the general established limits of migration, which means both the overall and the specific migration limits **(Tovar *et al.*, 2005)**. It is necessary, therefore, to identify the compounds that are present in the different packaging materials and that can be potential migrants **(Wrona & Nerín, 2019)**.

In Europe, it is regulated by Regulation (EU) No 10/2011 **(EU, 2011)** on plastic materials and articles intended to come into contact with food. This legislation specifies rules of migration experiments and also contains a list of substances, together with their specific migration limits (SML), that may be intentionally added to the plastic materials during its

manufacture. It also indicates how to evaluate not listed substances (NLS). If the substances are not included in this list, their migration should not be higher than 0.01 mg/kg (ratio 6:1) of food or food simulant **(Wrona, 2017)**. Moreover, for NLS, a procedure of toxicity determination can be provided by the threshold of toxicological concern (TTC), including classification of analytes to Cramer classes. TTC approach has been recommended by the European Food Safety Authority (EFSA) **(Wrona & Nerin, 2020)**.

III. Material and methods

1. Development of a new multilayer active packaging

The first part of the study aimed to explore the potential of using carob leaves, seeds and kibbles macerates, and incorporate them in the adhesive between polymer layers to design, optimize and prepare a new multilayer active biopackaging film based on cellulose.

The profile of volatile bioactive compounds was studied, and the compounds were identified and quantified. The antioxidant and antimicrobial activities of the macerates were measured. The antioxidant capacity of active packaging materials was tested. A migration test of both volatile and non-volatile compounds was carried out.

1. Material and methods

1.1. Chemicals

Acetic acid (≥ 99.7%, CAS 64-19-7), ascorbic acid (≥ 99.0%, CAS 50- 81-7), ammonium heptamolybdate tetrahydrate (≥ 99.0%, CAS 12054- 85-2), 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS 1898-66-4), hydrogen peroxide (30%, CAS 7722-84-1) and sodium salicylate (> 99.5%, CAS 54-21-7) were supplied by Sigma-Aldrich (Madrid, Spain). Sodium dihydrogen phosphate monohydrate (99%, CAS 7558-80-7) was acquired from Merck (Madrid, Spain). Sulphuric acid (H2SO4, 96%, CAS 7664-93-9) was from Panreac Química SLU (Barcelona, Spain). Phosphoric acid (85%, CAS 7664-38-2) was purchased from Scharlab (Barcelona, Spain). The plants were macerated using ethanol (99.9%, CAS 64-17-5) and acetone (99.9%, CAS 67-64-1) from Panreac. Methanol (> 99.9%, CAS 67-56-1) was from Honeywell (Madrid, Spain). Butylated hydroxyanisole (BHA, ≥ 98.5%, CAS 25013-16-5) was purchased from Sigma Aldrich, represented by the Algerian Chemical Society. Ultrapure water was produced in a Wasserlab Ultramatic GR system (Barbatáin, Spain).

1.2. Plant material

Plant material studied in this study (carob by-products) was harvested in Tizi-Ouzou (Algeria, Coordinates: 36° 43’ N, 4° 3’ E) in December 2018.The carob seeds are separated from the pods and the kibbles has been recuperated. The leaves are plucked from the carob tree **(Figure 12)**. After harvesting, the three parts of *Ceratonia siliqua* L. used are cleaned with water to remove dust. The samples were spread and dried in a ventiled place (out of the sun) and at room temperature for 10 days. The powders obtained was stored in a glass jars (previously dried) and protected from light.

Figure 12: Photograph of carob seeds, leaves and kibbles.

1.3. Maceration of samples

The extraction of the phenolic compounds was carried out by maceration according to the method developed by **Adilah *et al*. (2018)** with a slight modifications.

Briefly, 30 g of plant material powder was macerated with 80% aqueous solution of ethanol and 80% aqueous solution of acetone at room temperature. The process was sequentially repeated three times with the renewal of the solvent each 24 h. The macerates were filtered through Whatman filter paper of 0.22 μm pore size and stored in the dark at 4 °C until further use.

Different abbreviations were used for the different macerates as follow: CLE, CSE and CKE, for leaves, seeds and kibbles respectively, macerated in 80% ethanol, and CLA, CSA and CKA for leaves, seeds and kibbles macerated in 80% acetone.

1.4. Active packaging material

Solutions (w/w) of *Ceratonia siliqua* L. leaves, seeds and kibbles macerates at a concentrations of 5%, 8%, and 10% in water-based adhesive were prepared and vortexed during 1 min. 8 % was the maximum possible concentration that did not compromise the properties of adhesive (homogeneity, adhesion to substrates and high cohesive strength). The material selected as substrate was cellulose film NK from the Natureflex product range supplied by Futamura UK Ltd (Burgos, Spain). A multilayer active material was prepared using two cellulose polymer sheets glued together using a water-based biodegradable adhesive for food packaging from Samtack (Barcelona, Spain) **(Figure 13)**. Details composition of the adhesive are confidential and was approved for food packaging applications. It means that migration of components of adhesive to food simulants and/or packaged food is below the established limits

by European Union according to Commission Regulation 10/2011 **(EU, 2011)**. Therefore, the used adhesive is non-toxic, and no increased risks are expected at all. Films were prepared on laboratory scale by K Control Coater from RK Print Coat Instruments (Litlington, UK). The coating was performed by wire wound bar number 4 (colour code: black; wire diameter: 0.51 mm; wet film deposit: 40 μm). After air-drying of the solvent, the cellulose sheet with adhesive was covered by another cellulose layer. Finally, the multilayer biofilm was pressed at 40 °C and speed set to 5, using BiO 330 A3 Heavy Duty Laminator (South Korea). In addition, a blank biomaterial was prepared under identical conditions but without active macerate.

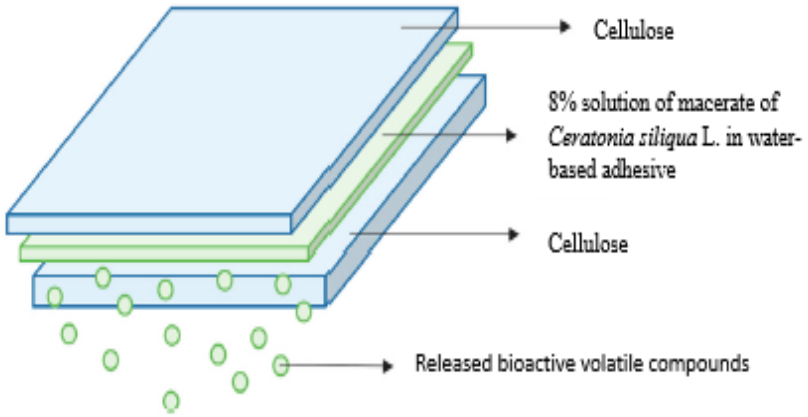

Figure 13: Scheme of developed active bio-material: CL/AA/CL.

1.5. Antioxidant activity evaluation of active macerates

1.5.1. DPPH radical scavenging activity

It is based on the capacity of antioxidants to trap the radical DPPH. The latter is reduced to the form of hydrazine (non-radical) by accepting a hydrogen atom. The greater the loss of color, the more the hydrogen donor is considered to be a strong antioxidant. Antioxidant capacity of each extract was measured by the procedure described by **Brand-Williams *et al*. (Brand-Williams *et al*., 1995).**

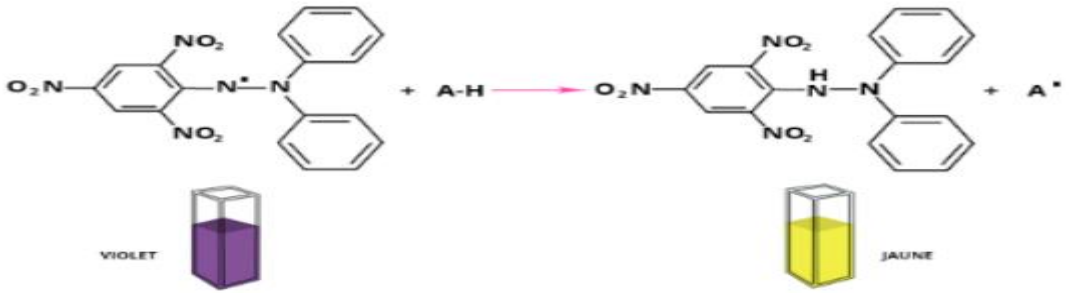

Figure 14: Reaction mechanism intervening during the DPPH test between the species radical (DPPH °) and an antioxidant (AH) **(Moudache *et al*., 2017**).

Antioxidant capacity of each extract was measured by the procedure described by Brand-Williams et al. **(Brand-Williams *et al*., 1995)**. 50 µL of different concentrations of each macerate were added to 1950 mL of the methanolic solution of DPPH. Methanol was used as blank. The absorbance (517 nm) was read against blank using a Shimadzu UV-1700 PharmaSpec spectrophotometer (Duisburg, Germany) after incubation of solutions in the darkness for 30 min at room temperature. Antioxidant capacity was expressed as the percentages of inhibition of the radical IC50.

Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and calculated according to the equation bellow **(Taylor & Todd, 1995)**.

$$\textbf{Scavenging effect (\% inhibition)} = (A_{control} - A_{sample})/ (A_{control}) \times 100\% \quad (1)$$

Where $A_{control}$ corresponds to the absorbance of the blank (1950 µL of DPPH with 50 µL of methanol), and A_{sample} corresponds to the absorbance of the sample (1950 µL of DPPH with 50 µL of the sample).

1.5.2. Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the macerates was evaluated by adapting the method used by **(Mekhoukhe *et al*., 2019).** A volume of 400 µL (25-85 µg/mL) of each macerate were mixed with 1 mL of reagent solution consisting of a mixture of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Then, it was incubated at 90 °C for 90 min and was cooled down at room temperature. The absorbance of the solution was read at 695 nm. The total antioxidant activity was expressed as ascorbic acid equivalent from a calibration curve (y = 0.0146 x + 0.0097).

1.6. Antibacterial activity of carob macerates

The antibacterial activity of macerates was tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 by the disk diffusion method. The selection of these bacterial strains was based in the potential bacteria causing spoilage of fresh salmon. Briefly, 100 µL of a suspension of the tested microorganism (10^8 CFU/mL) was spread on Mueller-Hinton agar plates. Sterile paper disks (9 mm) were impregnated with 75 µL of the macerated solution and placed on inoculated plates. After 24 h at 37 °C the diameters of the inhibition zones were measured. Each test was performed in triplicate.

1.7. Detection of volatile compounds

Each macerate was diluted 10 times using 20% ethanol and 18 mL of it were placed in a glass vial and directly analyzed by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC–MS). For this purpose, a CTC Analytics CombiPal coupled to an Agilent Technologies 6890N gas chromatograph with an MS 5975B mass spectrometer (Madrid, Spain) was used. HS-SPME-GC-MS analyses were carried out with a 100 µm polydimethylsiloxane (PDMS) fibre from Supelco (Bellefonte, PA, USA) previously conditioned before the first extraction according to the manufacturer specifications. The extraction conditions were as follows: 80 °C extraction temperature, 15 min extraction time, and 2 min desorption time. The injection was performed in splitless mode. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was as follows: 40 °C for 2 min, with a rate of 10 °C min up to 300 °C held for 2 min. Volatile compounds were separated on HP-5MS GC column (30 m × 0.25 mm × 0.25 µm film thickness, Agilent). The mass detector was used in scan mode in the *m/z* range from 45 to 350. The inlet, ion source, and quadrupole temperatures were 250, 230 and 150 °C, respectively. Analysis of blanks was also performed. All samples were analyzed in triplicate. Bioactive volatile compounds identification was based on the GC-MS spectrum interpretation compared with the spectrum database of the National Institute Standard and Technology (NIST) library. It allowed the confirmation of the name, molecular weight and structure of the chemical compounds from *Ceratonia siliqua* L. macerates. Compounds with experimental retention index close to those reported in the literature and match values higher than 80% were considered as correctly identified.

Bioactive volatile compounds semi-quantitative analysis was based on the percentage analysis. The percentage abundance of compounds in the analyzed samples was expressed as

the integrated area of each compound divided by the total area of all compounds. The sum of areas of all determined compounds was considered as 100% **(Ruiz-Hernández *et al*., 2018).**

1.8. Migration test

Migration tests were performed with two different food simulants (10% and 95% solutions of ethanol) for 3 days at 5 ºC. The simulants and parameters were based on recommendations of legislation for food contact materials EU /10/2011 and its amendments **(" (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food," 2011**). The selection of migration assays conditions was under the future purpose of using developed active bio-material such as the conservation of fresh, processed or marinated meat, fish or seafood. Also, it could be applied for preserved vegetables, cheese and meat in the oil medium, fried or roasted foods of animal origin and fried potatoes, cakes and the like, sandwiches, toasted bread, pizza and the like containing any kind of foodstuff with fatty substances on the surface. Contact time between food and active bio-packaging in worst foreseeable use should be between 1 and 3 days, and the worst foreseeable contact temperature of stored food should be ≤ 5 ºC.

Two sets of multilayer materials containing CSE and CSA macerate (8%) were studied. Three replicates of each sample were prepared. Blank samples consisting only of pure simulants were prepared. Also, blank material (material without an active agent) has been studied. Qualitative analysis of volatile and non-volatile compounds has been performed **(Figure 15)**, using the method describes below.

1.8.1. Volatile compounds

To determine the volatile migrants from the active bio-packaging HS-SPME-GC-MS method has been used to analyze 10% ethanol. In contrast, the direct injection gas chromatography-mass spectroscopy (DI-GC-MS) method has been used to analyze 95% ethanol. HS-SPME-GC-MS method has been previously described (I.1.6). In the DI-GC-MS method, 95% ethanol was injected directly in splitless mode (2 min) into GC-MS. The solvent delay was 5 min, injection volume was 1 µL. Applied chromatographic conditions were the same as for the HS-SPME-GC-MS method.

1.8.2. Non-volatile compounds

Both simulants 10% and 95% ethanol were analyzed by the UPLC-ESI-Q-TOF-MS^E method, to determine non-volatile migrants. Acquity UPLC system (Waters, Milford, MA) equipped with a quadrupole time of flight mass spectrometer (Xevo G2-XS QTOF, Waters)

was used. Chromatography was carried out in an Acquity TM system using an Acquity UPLC BEH C18 column of 1.7 μm particle size (2.1 mm × 100 mm), both from Waters (Milford, MA, USA). The Autosampler temperature was 10 °C. The Column flow was 0.3 mL/min, and the column temperature was 35 °C. Water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B) was used as mobile phase. The gradient used here was 5–95% methanol (0–10min), and the volume of sample injected was 10 μL. The optimized conditions of UPLC elution were as follows: 0 – 6 min 5% A – 95% B; 6–8 min 5% A – 95% B; 8–8.1 min, 95% A – 5% B; 8.1–10 min, 95% A – 5% B. The electrospray ionization interface was used in positive (ESI+) and negative (ESI-) modes. Sensitivity mode was used to operate the mass spectrometer with a capillary voltage of 3 kV and a sampling cone voltage of 30 V. source temperature of 150 °C, and the flow rate of desolvation gas (nitrogen) was 450 L/h at 400 °C. MS^E mode was selected for the acquisition. This data acquisition method involves the fast alternation between two energy conditions (low energy without collision ramp and high energy with collision ramp); the collision ramp energy from 5 to 30 eV was used. The mass range considered was 10–1200 Da. Centroid mode was used for data collection, and also the sensitivity analyzer mode was selected. The software Masslynx (version 4.1 waters, Milford, US) is used for data acquisition and processing in the migration of compounds.

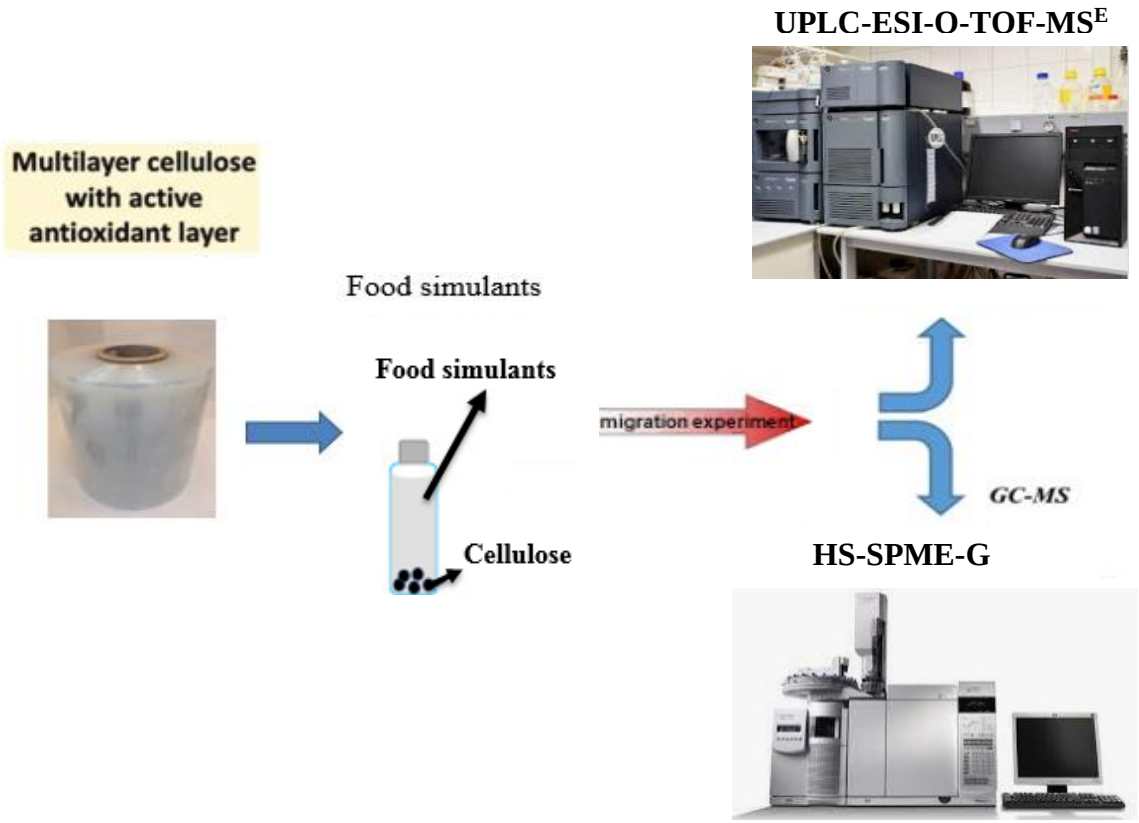

Figure 15: Migration assay process.

1.9. Antioxidant activity of the film

1.9.1. DPPH radical scavenging capacity

The DPPH radical scavenging capacity of multilayer films was determined according to the method described by **Wrona *et al*. (2017).** Sonication was performed for 30 min to extract active substances from 1 dm^2 of each active and blank film that was previously placed in an 18 mL vial with 6 g of methanol. Then 50 µL of different concentrations of macerates (50, 100, 150, 200, 250 µg/g) were mixed with 1950 µL of DPPH methanolic solution (60 µM) and kept in the dark at room temperature for 30 min. The absorbance of the solution was measured against methanol as blank at 515 nm using a spectrophotometer. The results were expressed as $IC_{50,}$ meaning the concentration providing 50 % inhibition.

1.9.2. Hydroxyl radical assay

The multilayer bio-films containing 8 % of each macerate of *Ceratonia siliqua* L. were exposed to an atmosphere enriched in free radicals passed through the plastic bags according to the procedure previously optimized **(Pezo *et al*., 2006, 2008).** The experimental assembly used for antioxidant capacity assessment of active packaging's are presented in **Figure 16**. The method consists of salicylic acid hydroxylation by the free radicals generated from aqueous hydrogen peroxide (0.29 $mol.L^{-1}$) under UV-light irradiation. The generated atmosphere enriched in free radicals is carried by an inert gas through the plastic bag made of active material and bubbles into a salicylic acid solution. Salicylic acid reacts with the not scavenged free radicals and forms 2,5-dihydroxybenzoic acid (2,5-DHB) as a primary compound. Quantitative analysis of 2,5-DHB and the residual salicylic acid was performed by high-performance liquid chromatography (Alliance 2695 Separations Module (Waters, Milford, MA, USA) with a 474 Scanning Fluorescence Detector (Waters, Milford, MA, USA). A Waters reversed-phase column (100 mm long, 4.6 mm i.d., 3 µm) Atlantis dC18 was used. The mobile phase was a mixture of acetate buffer (40 mM, pH = 5.9) and methanol (90:10, v/v). The injection volume was 20 µL. Excitation and emission wavelengths were set at 324 and 448 nm, respectively. Radical scavenging activity was calculated using equation as follow:

$$\textbf{Radical scavenging activity (\%)} = \text{Area}_{\text{2.5-DHB AOX}} / \text{Area}_{\text{2.5-DHB BK}} \times 100\% \quad \textbf{(2)}$$

Where: $\text{Area}_{\text{2,5-DHB AOX}}$ is the area of peak of 2,5-DHB in case of active bio-film, and $\text{Area}_{\text{2,5-DHB BK}}$ is the area of peak of 2,5-DHB in case of a blank film

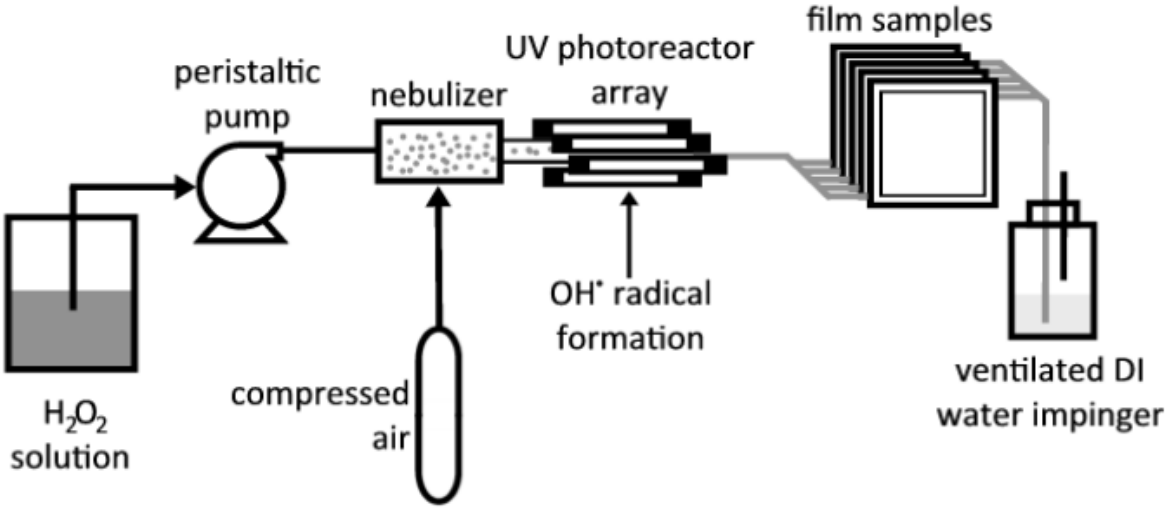

Figure 16: Experimental assembly used for antioxidant capacity assessment of active packaging's **(Wrona, 2017)**.

1.10. Characterization of the packaging colour

The colour of the active bio-materials containing CL, CS and CK macerates, and the control material, was determined according to the following method. Instrumental colour measurement was performed using a Chroma Meter CR-400 from Konica Minolta (Osaka, Japan) as was applied by **Marcos *et al*. (2014)**. C illuminant and 2° standard observer conditions were chosen. L* represents lightness (L*= 100) and darkness (L*= 0), a* represents redness and greenness for positive/negative values, and b* represent yellowness and blueness colour for positive/negative values. The values were determined in the 1976 CIELAB system. The chroma meter was calibrated with a standard whiteboard before each series of measurements (Y = 93, 70, x = 0, 3130, y = 0, 3191). The mean of 18 measurements was recorded for each film. Three replicates were tested. The total colour difference (ΔE) was determined as an estimate of colour changes and calculated by the in equation as represented bellow.

$$\mathbf{\Delta E} = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \mathbf{(3)}$$

The colour values of control films (CL/adhesive/CL) were used as reference values for ΔE calculation (L_0^*, a_0^*, b_0^*).*

2. The effectiveness study on the stored fresh salmon fillet

The second part of this research study aimed to evaluate the antioxidant effectiveness of macerated carob seeds to extend the shelf-life of stored FAS, during long term storage at 4 ± 1 °C. Organoleptic assay, color, drip loss, and WHC, pH, TBA-RS, and TVB-N were evaluated.

1. Material and methods

1. 1. Chemicals

Trichloroacetic acid (99%, CAS 76-03-9) was provided by Sigma Aldrich (Madrid, Spain); malondialdehyde-tetrabutylammonium salt (98%, CAS 100683-54-3) and 2-thiobarbituric acid (TBA ≥ 98%, CAS 504-17-6) were purchased from Fluka (Madrid, Spain). Ethanol (high-performance liquid chromatography (HPLC grade, CAS 64-17-5) and acetone (UV, IR, HPLC, GPC, APS, CAS 67-64-1) were from PanReac, AppliChem (Germany). Sodium hydroxide (NaOH, 0.25 N, CAS 1310-73-2) and sulfuric acid (H_2SO_4, 96%, CAS 7664-93-9) were from PanReac Quimica SLU (Barcelona, Spain). Methyl blue (CAS 28983-56-4), methyl red (CAS: 493-52-7) and potassium carbonate (K_2CO_3 ≥ 99%, CAS: 584-08-7) were obtained from Sigma-Aldrich Química S.A. (Madrid, Spain). Ultrapure water was obtained from a Wasserlab Ultramatic GR system (Barbatáin, Spain).

1.2. Antioxidant agents

Samples of carob fruit (*Ceratonia siliqua* L.) were collected during December 2018 from a carob tree located in Tizi-Ouzou (Algeria, Coordinates: 36°43′N 4°3′E), and the carob seeds (CS) were manually separated from the fruits and air-dried at room temperature (≈ 27 °C) for one month. Then, dried seeds were ground with an electric grinder. As extracting solvent 80 % aqueous solution of ethanol and 80 % aqueous solution of acetone by maceration method from the seeds at room temperature were used according to **Adilah *et al*. (2018)** with slight modifications. This process was successively repeated three times with the renewal of the solvent each 24 h. The macerates were filtered using Whatman filter paper (porosity 0.22 µm) and were stored in glass bottles in the dark at 4 ± 1° C until further use. The following abbreviations of macerates were applied: CSE means seeds macerated with 80% ethanol, CSA means seeds macerated with 80% acetone.

1.3. Sample preparation of Atlantic salmon fillet

Fresh salmon fillet (FSF) was selected to be used in the antioxidant effectiveness assays. The product was purchased at a local market in Zaragoza (Spain) next to the university (10 min driving distance) before each experiment. The fish was transported in a closed box with ice to the analytical chemistry laboratory (Zaragoza University, Campus Rio Ebro, Spain). The skin was removed from the flesh in aseptic conditions, and fillet samples were cut on slices of 22 g using a sterile knife. All the prepared samples were kept on ice until utilization to avoid deterioration. All the experiments were carried out using the same initial fresh salmon to ensure the same product quality.

1.4. Biopackaging active material for Atlantic salmon

The developed packaging material was based on two cellulose (CL) polymer layers from the Nutraflex (45NK) product range supplied by Futamura UK Ltd (Burgos, Spain) laminated together with a water-based biodegradable adhesive for food packaging applications from Samtack (Barcelona, Spain). Solution (w/w) of CS macerates at a concentration of 8% in water-based biodegradable adhesive were prepared and vortexed for 1 min until complete homogenization. The active adhesive was spread on the CL sheet using the coating machine K control coater from RK print coat instruments (Litlington, UK). Wire close wound bar (Bar number: 4; Color code: black; wire diameter: 0.51 mm; wet film deposit: 40 µM) was used for coating. Then CL sheet was air-dried to get rid of the solvent. The CL sheet with dry adhesive was cover by another CL layer. The developed multilayer biomaterial was placed in BiO 330 A3 Heavy Duty Laminator (South Korea), and it was pressed at 40 °C with velocity number 5. A blank biomaterial was prepared in the same way but without CS active macerate.

An amount of 22 g of FSF was placed in the Petri dish (Ø =10 cm) covered with a 10 cm × 10 cm sheet of each active biopackaging. The experiments were carried out without direct contact between the food sample and the active agent to simulate the most real conditions for fish packaging. Each petri dish was then carefully introduced in a cellulose bag. Also, the salmon with blank biomaterial without an active agent was prepared to compare the effect of fish spoilage. The samples were hermetically thermo-sealed and kept at 4 ± 1 °C for 13 days. All samples were prepared in triplicated and analyzed after 0, 3, 5, 8 and 13 days.

a) b) c)

Figure 17: a) Atlantic salmon fish, b) salmon fillet samples, c) sample of Petri dish with slice of FAS placed in active packaging with incorporated carob seed macerate.

1.5. Fresh salmon fillet (FSF) quality assessment

The quality of the FSF was tested during the experiments by evaluating different organoleptic properties and physicochemical parameters: pH, drip loss and WHC, colour measurement, TBA-RS test and TVB-N. All the measurements were done in triplicate and performed after 0, 3, 5, 8 and 13 days of samples storage.

1.5.1. Sensory analysis

The sensory analysis of FSF was done by a five-member trained panelists, selected among the workers of the analytical chemistry laboratory (University of Zaragoza, Spain). They were trained according to the method described by **(Shahidi & Botta, 1994).** The evaluation was based on two sensory attributes, such as off-odor and overall acceptability of FSF. The analysis consisted of 24 evaluations of salmon samples coded with random numbers. The off-odor and overall acceptability were evaluated using a 5-point scale according to ***(*Djenane *et al*., 2001)**. Scores for off-odour referred to the intensity of odors associated to fish oxidation were as follows: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme and were evaluated immediately after opening the package with sample. While evaluating the acceptability, 5-point hedonic scale was used, where 1 = dislike extremely; 2 = dislike; 3= nor like or dislike; 4 = like; 5= like extremely. Results are expressed as the predominant score given by panelists.

1.5.2. Colour measurement

A colorimeter Chroma Meter CR-400 from Konica Minolta (Tokyo, Japan) with D65 as the light source was used to measure the colour of the surface of salmon fillets. Each packaging was opened, samples were removed and left for blooming for 15 min. Eighteen colour determinations for each replicate were performed to cover all surface. CIE L*

(lightness), a* (redness) and b* (yellowness) were used for the characterization of the colour. The equipment was calibrated daily with white chroma meter standard plate (Y= 93.7; x = 0.3130; y= 0.3191). For each sample, the total colour difference (ΔE) as an estimate of colour changes was determined following the equation:

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)2]^{1/2} \quad \textbf{(3)}$$

The colour values of fresh salmon at day 0 of storage were used as reference values for ΔE calculation (L_0^*, a_0^*, b_0^*).

1.5.3. pH measurement

The pH of the salmon samples was determined according to the method described by **(Sallam, 2007).** A 5.0 g fish sample were cut into small pieces and then homogenized with 10 mL of distilled water in a cup-blender for 30 s. Then, the pH the resulting homogenate was measured at room temperature 25 ◦C using a digital pH-meter GLP 22 from Crison Instruments (Barcelona, Spain) previously calibrated at pH 4 and 7.

1.5.4. Drip loss and water holding capacity

Drip loss in salmon samples for the different days of storage was expressed as the difference in fillet weight between day 0 (m_0) and day x (m_x) and was calculated according to the following equation:

$$DL = (m_0 - m_x/m_0) \times 100 \quad \textbf{(4)}$$

WHC was measured in the dorsal muscle from all salmon fillets at 0, 3, 5, 8 and 13 days of storage. The dry content (D_0) of the muscle was determined by drying 2 g of each sample for 24 h at 105 °C until the equilibrium weight was obtained; hence water content (V_0) was determined **(ISO-6496, 1999)**. Meanwhile, a piece of salmon was weighed (2 g) and placed in a tube with a pre-weighed filter paper (Whatman N °1). The tubes were then centrifuged using CENTROMIX model S-549 from JP Selecta (Barcelona, Spain) for 15 min, and the exudate drained through the filter paper was collected at the bottom of the centrifuge tube. The samples were weighed before and after this procedure. The results were expressed as the amount of sample remaining after centrifugation and were calculated according to the following equation:

$$WHC = (W_0 - W_1/W_0) \times 100 \quad \textbf{(5)}$$

Where

$$W_0 = V_0/ (V_0 + D_0) \times 100$$

$$\Delta W = \Delta V_0/ (V_0 + D_0) \times 100$$

V_0 = the water content of the muscle.

D_0 = dry matter of the muscle.

ΔV_0 = the weight of the liquid separated from the sample during centrifugation

1.5.5. Oxidative stability

The lipid oxidation study of salmon samples was performed by TBA-RS method as described by **(Djenane *et al.*, 2019) .**Briefly, 10 g of FSF were mixed with 40 mL of a 10% aqueous solution of trichloroacetic acid (TCA) until a homogeneous suspension was obtained. The supernatant was filtered using Whatman N°1 filter paper. Then, 2 ml of the filtrate were mixed with 2 ml of an aqueous solution of thiobarbituric acid at a concentration of 20 mM. The mixture was heated to 97 °C for 20 min and then cooled to room temperature. The absorbance was measured at 532 nm using a spectrophotometer UV-1700 (Shimadzu Pharmaspec Iberica, Madrid, Spain) against a reference blank containing the thiobarbituric acid (TBA) reagent. All the measurements were prepared in triplicate. To calculate the concentration of secondary lipid oxidation product, a calibration curve was prepared using a malondialdehyde (MDA) solution in the range 0.1 – 0.8 µg/g. Results were expressed as mg of MDA per kg of fish.

1.5.6. Determination of TVB-N content

The TVB-N content was determined using the Conway micro diffusion method. A slice of salmon (4 g) was weighed, transferred to the stomacher bag and homogenized with 15 mL of water for 2 min at 265. Then, 10 mL of 10% TCA (w/v) were added and homogenized for 4 min to eliminate the protein content. The slurry was collected, filtered, and centrifuged. The test was performed using the micro-diffusion chamber of Conway. Briefly, 1 ml of a saturated potassium carbonate solution was placed in the outermost area of the chamber and mixed with 1 ml of the supernatant. 1 mL of sulfuric acid (H_2SO_4) was added to the central compartment of the chamber. Finally, the chamber was closed, sealed, and carefully mixed by circular movement, avoiding mixing the liquids in different compartments. After that, incubation was performed at 35 °C for 1h. The sulfuric acid was titrated by a 0.1 N solution of sodium

hydroxide (NaOH) using a micro-burette. Two drops of indicator solution were added (methyl red-methylene blue indicator).

The amount of TVB-N was calculated by the following equation:

$$TVB\text{-}N = [(Vac - Vba) \times 0.14 \times 25/ (Vm \times PM)] \times 100 \quad \textbf{(6)}$$

Where Vac (Acid volume) is the volume of sulfuric acid (1 mL), Vba (Base volume) is the volume of NaOH consumed in the titration, Vm is the volume of sample added to the Conway cell (1 mL), and PM represents the salmon weight (4 g). The TVB-N was expressed as mg N/100 g fish.

Results and discussion

1. Ceratonia siliqua L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications (Article 1)

Food Packaging and Shelf Life 31 (2022) 100764

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Ceratonia siliqua L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications

Lidia Ait Ouahioune [a], Magdalena Wrona [b], Raquel Becerril [b], Jesús Salafranca [b], Cristina Nerín [b,*], Djamel Djenane [a]

[a] Department of Food Science, Laboratory of Food Quality and Food Safety, Mouloud Mammery University, P.O. Box. 17, Tizi-Ouzou RP 15000, Algeria
[b] Department of Analytical Chemistry, Aragon Institute of Engineering Research I3A, EINA - University of Zaragoza, Torres Quevedo Building, María de Luna 3, 50015 Zaragoza, Spain

ARTICLE INFO

Keywords:
Ceratonia siliqua L.
Antioxidants
Biopackaging
Cellulose
Active packaging
Food safety

ABSTRACT

Macerates of kibbles, seeds and leaves of *Ceratonia siliqua* L. were prepared and analysed to apply them for the development of a new multilayer active packaging. The profile of forty-three different bioactive volatile compounds was obtained employing headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). The antioxidant capacity was investigated using the following methods: 2,2-diphenyl-1-picrylhydrazyl and phosphomolybdenum method, which demonstrated stronger antioxidant capacity in the case of seeds macerates. However, the analysis of the antimicrobial properties of the different macerates versus *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* revealed a weak antibacterial activity. Additionally, the different macerates were incorporated into an adhesive used to build a new biopackaging multilayer film based on cellulose polymer, whose antioxidant capacity was evaluated by two different methods (2,2-diphenyl-1-picrylhydrazyl and hydroxyl radical assay). The films with the best antioxidant activity, containing carob seeds macerates, were selected for the food safety assessment through migration assay. The obtained results showed no migrants, neither in the case of the volatile compounds nor non-volatile ones. Besides, the CIE $L^*a^*b^*$ colour of the active films was evaluated. The obtained results are therefore promising for future production of active biopackaging at an industrial scale.

1. Introduction

The demand for healthy, nutritious, safe, and more natural products has recently increased the search for new food production, processing, and preservation technologies. Consumer concerns about the quality of foods are reflected in an awareness of nutritional composition, bioactive components, and safety issues (Castro, Andrade, Silva, Vaz, & Vilarinho, 2019).

Food oxidation is a significant cause of food quality drop, affecting both the nutritional and sensory properties and its safety. Thus, it is a challenge for the food preservation industry to maintain refrigerated foods fresh (Carrizo, Taborda, Nerín, & Bosetti, 2016). Consequently, considerable research has been directed toward various preservation technologies (Sallam, 2007), such as the addition of synthetic antioxidants directly into food (Mohan, Ravishankar, Lalitha, & Srinivasa Gopal, 2012). As a result, health concerns about food ingredients have led to an increase in the request for more natural foods free of additives (Djenane, 2015). In this sense, the use of natural antioxidants is emerging as an effective alternative for product preservation (Sabeena Farvin, Grejsen, & Jacobsen, 2012). To this end, many sources of natural antioxidants have been investigated, such as herbs, plants, fruits, essential oils, natural pigments, and apiculture products (Oudjedi, Manso, Nerin, Hassissen, & Zaidi, 2019). Nevertheless, the direct addition of natural antioxidants into food formulations is challenging because they tend to be less potent than synthetic additives and therefore must be added in more significant amounts (Belasli et al., 2020; Carrizo, Gullo, Bosetti, & Nerín, 2014; Echegoyen & Nerín, 2015; Nerin, Astudillo, Covían, & Mujika, 2006; Nerín, Tovar, & Salafranca, 2008). This fact presents some disadvantages from the technological point of view, due to the intense flavour and smell, causing the alteration of the sensory characteristics of the product (El-Sayed, 2014). To overcome this challenge, the alternative approach is to incorporate them into the

* Corresponding author.
E-mail addresses: lidiaaitouahioune93@gmail.com (L. Ait Ouahioune), magdalenka.wrona@gmail.com (M. Wrona), raquel@unizar.es (R. Becerril), fjsl@unizar.es (J. Salafranca), cnerin@unizar.es (C. Nerín), djenane6@yahoo.es (D. Djenane).

https://doi.org/10.1016/j.fpsl.2021.100764
Received 12 May 2021; Received in revised form 31 August 2021; Accepted 19 October 2021

polymeric packaging instead of food (Akrami et al., 2015; Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011; Djenane, Beltrán, Camo, & Roncalés, 2016; López, Sánchez, Batlle, & Nerín, 2007). Active antioxidant packaging is a novel concept and emerging packaging technology that acts by releasing, in a controlled way, the active agents to the surface or the inner atmosphere of the product, or just by scavenging radicals responsible for deleterious effects on food quality (Nerin et al., 2006). Several antioxidant packaging materials have been proposed (Carrizo et al., 2014, 2016; Tovar, Salafranca, Sánchez, & Nerín, 2005). They have proven that the addition of natural antioxidants into packaging present significant advantages, due to the improvement of the safety and the sensory properties, delay of the food spoilage, slowdown of the oxidation, and extension the shelf life of packaged foods without compromising their quality (Borzi, Torrieri, Wrona, & Nerín, 2019; Dong, Xu, Ahmed, Li, & Lin, 2018; Sanches-Silva et al., 2014). Moreover, there is an increasing interest in the use of bio-based polymer films (Al-Tayyar, Youssef, & Al-Hindi, 2020a, 2020b), which are produced from the waste of the food industry or from underutilized means of polysaccharides, lipids, and proteins. This innovative approach has been originated from environmental concerns, the increasing burden of disposing of plastic waste and the industrial use of food waste. In a recent review (Al-Tayyar et al., 2020a, 2020b), the use of carboxymethyl cellulose/polyvinyl alcohol/CuO bionanocomposites in the coating of processed cheese demonstrated a reduction in the growth of microbial contaminants, and noticeably extended and enhanced its shelf-life. Also, a covered cheese with a probiotic, edible coating based on chitosan, alginate and carboxymethyl cellulose gained sensory properties during storage (El-Sayed, El-Sayed, Mabrouk, Nawwar, & Youssef, 2021). Another study on coating fruits with a combined chitosan-beeswax pollen grain emulsion demonstrated to be the best treatment for maintaining the quality of Le Conte pears during storage life and marketing periods (Sultan, Hafez, Saleh, & Youssef, 2021).

Several approaches have been proposed to manufacture active packaging without modifying the production line or the characteristics of the packaged product, but all of them fail by one or another reason. Extrusion of polymers involves high temperature, causing the decomposition of active agents. Coating systems could affect the sensory properties of the packaged food. However, in all these approaches, the antioxidant agent is incorporated in the layer in contact with food (Carrizo et al., 2016). A new system has been explored by eliminating the free radicals responsible for the initiation of the oxidation phenomenon. Since the free radicals can efficiently diffuse through the polymer, they can be trapped by the antioxidant agents incorporated in the adhesive between polymer films of a multilayer packaging, thus avoiding the direct contact of active agents with the packaged food (Djebari et al., 2021; Moudache, Colon, Nerín, & Zaidi, 2016; Oudjedi et al., 2019).

Carob (*Ceratonia siliqua* L.) is a flowering evergreen tree grown in the Mediterranean area (Rtibi et al., 2015). The scientific name of this tree came from the Greek word “kera” connected with the shape of the carob fruit, and the Latin word “siliqua”, associated with the hardness of the pods (Papaefstathiou, Agapiou, Giannopoulos, & Kokkinofta, 2018). Carob fruits consist of pods and seeds. They are considered powerful antioxidants due to the high content of bioactive phytochemicals (Santonocito et al., 2020). Moreover, their antimicrobial activity has been recently demonstrated against different bacteria (Ben Othmen, Garcia-Beltrán, Elfalleh, Haddad, & Esteban, 2021; Fidan et al., 2019; Goulas et al., 2019). Deseeded broken carob fruits, commonly known as kibble, are also a source of natural bioactive compounds. The effective utilization of this industrial by-product is becoming the focus of researchers (Nasar-Abbas et al., 2016; Owen et al., 2003). Furthermore, carob leaves are rich in polyphenols and flavonoids (Rtibi et al., 2015).

Various in vitro studies have shown that extracts of the carob tree have a potent antioxidant effect (Roseiro, Tavares, Roseiro, & Rauter, 2013; Sebai et al., 2013). They also have strong scavenging activity on reactive oxygen and free radicals, thus reducing oxidative damage (Roseiro, Duarte, et al., 2013; Roseiro, Tavares, et al., 2013).

To the best of our knowledge, macerates of *Ceratonia siliqua* L. kibbles, seeds and leaves have not been tested as potentially active agents in antioxidant multilayer biopackaging for food applications. Moreover, a complex profile of volatile bioactive compounds from the Algerian carob variety has been determined.

The main aim of this investigation was to design, optimize and prepare new multilayer active biopackaging films based on cellulose and the macerates of leaves, seeds, and kibbles of *Ceratonia siliqua* L. from Algeria incorporated in the adhesive between polymer layers. First of all, the profile of volatile bioactive compounds was studied, and the compounds were identified and quantified. Then, the antioxidant and antimicrobial activities of the macerates were measured. Afterwards, the antioxidant capacity of active packaging materials was tested using the in-situ hydroxyl radical generation method developed by Pezo, Salafranca, and Nerín, (2006), Pezo, Salafranca, and Nerín (2008) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Finally, the safety of the new antioxidant films for biopackaging applications has been evaluated by migration tests. The material selected as the substrate is from natural resources, which is interesting and emerges as a response to growing attention to environmental pollution and environmental footprint.

2. Material and methods

2.1. Chemicals

Acetic acid ($\geq$ 99.7%, CAS 64-19-7), ascorbic acid ($\geq$ 99.0%, CAS 50-81-7), ammonium heptamolybdate tetrahydrate ($\geq$ 99.0%, CAS 12054-85-2), 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS 1898-66-4), hydrogen peroxide (30%, CAS 7722-84-1) and sodium salicylate ($>$ 99.5%, CAS 54-21-7) were supplied by Sigma-Aldrich (Madrid, Spain). Sodium dihydrogen phosphate monohydrate (99%, CAS 7558-80-7) was acquired from Merck (Madrid, Spain). Sulphuric acid (H_2SO_4, 96%, CAS 7664-93-9) was from Panreac Química SLU (Barcelona, Spain). Phosphoric acid (85%, CAS 7664-38-2) was purchased from Scharlab (Barcelona, Spain). The plants were macerated using ethanol (99.9%, CAS 64-17-5) and acetone (99.9%, CAS 67-64-1) from Panreac. Methanol ($>$ 99.9%, CAS 67-56-1) was from Honeywell (Madrid, Spain). Butylated hydroxyanisole (BHA, $\geq$ 98.5%, CAS 25013-16-5) was purchased from Sigma Aldrich, represented by the Algerian Chemical Society. Ultrapure water was produced in a Wasserlab Ultramatic GR system (Barbatáin, Spain).

2.2. Samples

The fruits and leaves of carob (*Ceratonia siliqua* L.) were collected after fruit harvesting during December 2018 from carob trees located in the region of Tizi-Ouzou (Algeria, coordinates: 36°43′ N, 4°3′ E), where they are naturally grown.

2.3. Sample preparation

Carob pods (CP) and leaves (CL) were cleaned with tap water, the carob seeds (CS) were separated manually from the fruits, and the carob kibble (CK) were recuperated. The photograph of the samples is shown in Fig. 1.

The samples were air-dried at room temperature for ten days. Then, dried samples were ground by an electric grinder to obtain a mean particle size below 0.5 mm. The grinded samples were stored in airtight glass containers in the dark until being macerated as described below.

2.4. Maceration of samples

An amount of 30 g of powdered samples was macerated with 500 mL of 80% aqueous solution of ethanol and 80% aqueous solution of acetone at room temperature. The process was sequentially repeated

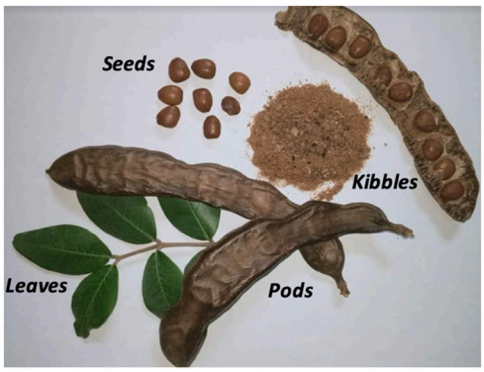

Fig. 1. Photograph of *Ceratonia siliqua* L. samples such as pods, leaves, seeds and kibbles.

three times with the renewal of the solvent each 24 h. The macerates were filtered through Whatman filter paper of 0.22 μm pore size and stored in the dark at 4 °C until further use. The following abbreviations of macerates were applied: CLE, CSE and CKE, for leaves, seeds and kibbles respectively, macerated in 80% ethanol, and CLA, CSA and CKA for leaves, seeds and kibbles macerated in 80% acetone.

2.5. Active packaging material

The material selected as substrate was cellulose film NK from the Natureflex product range supplied by Futamura UK Ltd (Burgos, Spain). A multilayer active material was prepared using two cellulose polymer sheets glued together using a water-based biodegradable adhesive for food packaging from Samtack (Barcelona, Spain). Details about the adhesive formula cannot be disclosed because of confidential reasons. Different concentrations of 5%, 8%, and 10% (w/w) of each macerate were prepared in the adhesive and tested. The mixture was vortexed for 1 min and allowed to stand at room temperature until total homogenization. The maximum concentration chosen for further preparation of active biopackaging was 8%. The selected concentration was optimum as it did not affect the adhesion properties of adhesive.

The active adhesive was spread on the cellulose sheet using a K Control Coater from RK Print Coat Instruments (Litlington, UK). The coating was performed by wire wound bar number 4 (colour code: black; wire diameter: 0.51 mm; wet film deposit: 40 μm). After air-drying of the solvent, the cellulose sheet with adhesive was covered by another cellulose layer. Finally, multilayer biofilm was pressed at 40 °C and speed set to 5, using BiO 330 A3 Heavy Duty Laminator (South Korea). In addition, a blank biomaterial was prepared under identical conditions but without active macerate.

2.6. Analysis of macerates

2.6.1. Identification of volatile compounds

The volatile compounds of macerates of leaves, seeds and kibbles of *Ceratonia siliqua* L. were analysed by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). Each macerate was diluted 5 times with ultrapure water. Chromatographic analysis was performed using a CTC Analytics autosampler from Agilent technologies (Madrid, Spain). A GC Agilent 6890N coupled to a mass-selective detector Agilent MS 5975B was used to analyse the samples. HS-SPME-GC-MS analyses were carried out with a 100 μm polydimethylsiloxane (PDMS) fibre from Supelco (Bellefonte, PA, USA) previously conditioned before the first extraction according to the manufacturer specifications. The extraction conditions were as follows: 80 °C extraction temperature, 15 min extraction time, and 2 min desorption time. The injection was performed in splitless mode. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was as follows: 40 °C for 2 min, with a rate of 10 °C min up to 300 °C held for 2 min. Volatile compounds were separated on HP-5MS GC column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent). The mass detector was used in scan mode in the *m/z* range from 45 to 350. The inlet, ion source, and quadrupole temperatures were 250, 230 and 150 °C, respectively. Analysis of blanks was also performed. All samples were analysed in triplicate.

Bioactive volatile compounds identification was based on the GC-MS spectrum interpretation compared with the spectrum database of the National Institute Standard and Technology (NIST) library. It allowed the confirmation of the name, molecular weight and structure of the chemical compounds from *Ceratonia siliqua* L. macerates. Compounds with experimental retention index close to those reported in the literature and match values higher than 80% were considered as correctly identified.

Bioactive volatile compounds semi-quantitative analysis was based on the percentage analysis. The percentage abundance of compounds in the analysed samples was expressed as the integrated area of each compound divided by the total area of all compounds. The sum of areas of all determined compounds was considered as 100% (Ruiz-Hernández, Roca, Egea-Cortines, & Weiss, 2018).

2.6.2. Antioxidant activity – DPPH method

The scavenging activity of carob leaves, seeds and kibble macerates against DPPH radical was examined (Brand-Williams, Cuvelier, & Berset, 1995). Before the test, a new 60 μM methanolic solution of DPPH was prepared. Briefly, 50 μL of each macerate were added to 1950 μL of DPPH solution. The absorbance of the mixture was determined at 515 nm with a Shimadzu UV-1700 PharmaSpec spectrophotometer (Duisburg, Germany) after 30-min incubation. Five concentrations of ascorbic acid and BHA (40, 80, 120, 160, 200 μg/mL) were prepared and used as a positive control. All the measurements were performed in triplicate. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and calculated according to Eq. (1) (Taylor & Todd, 1995).

$$\text{Scavenging effect}(\%\ \text{inhibition}) = (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}}) \cdot 100\% \quad (1)$$

Where $A_{control}$ corresponds to the absorbance of the blank (1950 μL of DPPH with 50 μL of methanol), and A_{sample} corresponds to the absorbance of the sample (1950 μL of DPPH with 50 μL of the sample). The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentration of the samples.

2.6.3. Total antioxidant capacity (TAC)

The total antioxidant capacity of the macerates was evaluated by adapting the method used by Mekhoukhe et al. (2019). A volume of 400 μL (25–85 μg/mL) of each macerate were mixed with 1 mL of reagent solution consisting of a mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Then, it was incubated at 90 °C for 90 min and was cooled down at room temperature. The absorbance of the solution was read at 695 nm. The total antioxidant activity was expressed as ascorbic acid equivalent from a calibration curve ($y = 0.0146\ x + 0.0097$).

2.6.4. Antimicrobial activity

Antimicrobial activity of macerates was tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 by the disk diffusion method. Briefly, 100 μL of a suspension of the tested microorganism (10^8 CFU/mL) was spread on Mueller-Hinton agar plates. Sterile paper disks (9 mm) were impregnated with 75 μL of the macerated solution and placed on inoculated plates. After 24 h at 37 °C the diameters of the inhibition zones were measured. Each test was performed in triplicate.

2.7. Analysis of active biopackaging

2.7.1. Migration tests

One-side migration tests have been performed using two different food simulants (10% and 95% solutions of ethanol) for 3 days at 5 °C. The simulants and parameters were based on recommendations of legislation for food contact materials, Commission Regulation (EU) No 10/2011, and its amendments (Union Europea, 2011). The conditions of migration tests were selected according to the intended use of the developed active biofilm such as the conservation of fresh, processed or marinated meat, fish or seafood. Also, it could be applied for preserved vegetables, cheese and meat in the oil medium; fried or roasted foods of animal origin and fried potatoes, cakes and the like; sandwiches, toasted bread, pizza and similar containing any kind of foodstuff with fat matter on the surface. Contact time between food and active biopackaging in worst foreseeable use should be between 1 and 3 days, and the worst foreseeable contact temperature of stored food should be > 5 °C.

Two sets of multilayer materials containing CSE and CSA macerate (8%) were studied. Three replicates of each sample were prepared. Blank samples consisting of only pure simulants were prepared. In addition, blank material without any active agent was considered. Qualitative analysis of volatile and non-volatile compounds was performed using the method described below.

2.7.2. Volatile compounds

To determine the volatile compounds migrated from the active biofilms into 10% ethanol food simulant, HS-SPME-GC-MS already described in Section 2.6.1. has been used. In contrast, samples of 95% ethanol simulant were analysed in splitless mode (2 min) by direct injection-gas chromatography-mass spectroscopy (DI-GC-MS). In this case, the solvent delay was 5 min, and injection volume was 1 μL. The chromatographic conditions were the same as those used for the HS-SPME-GC-MS method.

2.7.3. Non-volatile compounds

Both simulants, 10% and 95% ethanol, were analysed by the ultra-high performance liquid chromatography coupled to quadruple time-of-flight with MSE technology (UPLC-ESI-Q-TOF-MS^E) to determine the non-volatile migrants. An Acquity UPLC system (Waters, Milford, MA) equipped with a quadrupole time-of-flight mass spectrometer (Xevo G2-XS QTOF, Waters) was used. Chromatography was carried out using an Acquity UPLC BEH C18 column (100 mm × 2.1 mm × 1.7 μm particle size). The autosampler temperature was 10 °C, the column flow was 0.3 mL/min, and the column temperature was 35 °C. Water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B) were used as mobile phase, and the volume of sample injected was 10 μL. The optimized UPLC conditions were as follows: 0–6 min 5% A/95% B; 6–8 min 5% A/95% B; 8–8.1 min, 95% A/5% B; 8.1–10 min, 95% A – 5% B. The electrospray ionization interface was used in both positive (ESI+) and negative (ESI-) modes. Sensitivity mode was used to operate the mass spectrometer with a capillary voltage of 3 kV and a sampling cone voltage of 30 V. Source temperature was set at 150 °C, and the flow rate of desolvation gas (nitrogen) was 450 L/h at 400 °C. MS^E mode selected for the data acquisition involves the fast alternation between two energy conditions: low energy without collision ramp, and high energy with collision ramp from 5 to 30 eV. The mass range considered was 10–1200 Da. Centroid mode was used for data collection. The software Masslynx (version 4.1, Waters) was used for data acquisition and processing.

2.7.4. DPPH radical scavenging capacity

The DPPH radical scavenging capacity of multilayer films was determined according to the method described by Wrona, Cran, Nerín, and Bigger (2017). Sonication was performed for 30 min to extract active substances from 1 dm^2 of film, both active and blank, previously placed in an 18 mL glass vial with 6 g of methanol. Then, 50 μL of different concentrations of macerates (40, 80, 120, 160, 200 μg/mL) were mixed with 1950 μL of DPPH methanolic solution (60 μM) and kept in the dark at room temperature for 30 min. The absorbance of the solution was measured against methanol as blank at 515 nm using a spectrophotometer. The results were expressed as IC_{50}, meaning the concentration providing 50% inhibition.

2.7.5. Hydroxyl radical assay

The multilayer biofilms containing 8% of each macerate of *Ceratonia siliqua* L. were exposed to an atmosphere enriched in OH· free radicals passed through bag-shaped samples prepared according to the procedure previously developed by Pezo et al. (2008). The method consists of salicylic acid hydroxylation by the OH· radicals generated from aqueous hydrogen peroxide (0.29 mol/L) in vapour phase under UV-light irradiation. The generated atmosphere enriched in OH· radicals is carried by air through the bag made of active material, and finally it bubbles into a salicylic acid solution. Salicylic acid reacts with the not scavenged OH· radicals and forms 2,5-dihydroxybenzoic acid (2,5-DHB) as a primary compound. Quantitative analysis of 2,5-DHB and the residual salicylic acid was performed by high-performance liquid chromatography (Waters Alliance 2695 Separations Module with a 474 Scanning Fluorescence Detector). A Waters reversed-phase column (100 mm × 4.6 mm × 3 μm particle size) Atlantis dC18 was used. The mobile phase was a mixture of acetate buffer (40 mM, pH = 5.9) and methanol (90:10, v/v), with an injection volume of 20 μL. Excitation and emission wavelengths were set at 324 and 448 nm, respectively. Radical scavenging activity was calculated using Eq. (2).

$$\text{Radical scavenging activity } (\%) = \text{Area}_{\text{2,5-DHB AOX}}/\text{Area}_{\text{2,5-DHB BK}} \cdot 100\% \quad (2)$$

Where: $\text{Area}_{\text{2,5-DHB AOX}}$ is the area of peak of 2,5-DHB in case of active biofilm, and $\text{Area}_{\text{2,5-DHB BK}}$ is the area of peak of 2,5-DHB in case of a blank film.

2.7.6. Characterization of the packaging colour

The colour of the active biofilms containing CL, CS and CK macerates, and the control material, was determined by using a Chroma Meter CR-400 from Konica Minolta (Osaka, Japan) as described by Marcos et al. (2014). C illuminant and 2° standard observer conditions were chosen. L* represents lightness (L* = 100) and darkness (L* = 0), a* represents redness and greenness for positive/negative values, and b* represent yellowness and blueness colour for positive/negative values. The values were determined in the 1976 CIELAB colour space system. The chroma meter was calibrated with a standard whiteboard before each series of measurements (Y = 93.70, x = 0.3130, y = 0.3191). The mean of 18 measurements was recorded for each film. Three replicates were tested.

The total colour difference (ΔE) was determined as an estimate of colour changes according to Eq. (3).

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

The colour values of control films (cellulose/adhesive/cellulose) were used as reference values for ΔE calculation (L_0^*, a_0^*, b_0^*).

2.8. Statistics

Three replicates of all samples were performed and mean ± standard deviation was obtained. Therefore, error bars on the figures are standard deviations. Significant differences ($p \leq 0.05$) between samples in the case of all performed analytical tests were determined using *t*-test. At the beginning, the null hypothesis assumption was considered: analysed samples are equal. To confirm it, the experimental *t*-test statistic value was calculated compared to the theoretical value *t*-test statistic from tables. Null hypothesis was true if compared values were equal or theoretical *t*-test statistic value was higher than the experimental one.

Otherwise, analysed samples were significantly different ($p \leq 0.05$).

3. Results and discussion

3.1. Identification of volatile compounds

The volatile compounds of *Ceratonia siliqua* L. were extracted by SPME and analysed by GC-MS. This technique is solvent-free, cheap, easy, fast, and sensitive. In addition, it needs lower volume of sample than hydrodistillation, the most common method for plant extraction, which is time-consuming and requires a large amount of plant material (Zouaoui, Chenchouni, Bouguerra, Massouras, & Barkat, 2020).

Table 1 shows the identified volatile bioactive compounds, numbered according to their elution order. Also, their relative retention time (RT), retention index (RI), relative area (%), chemical formula and class are provided. A total of forty-three compounds were identified, but only a few of them were similar to those described in previous works (Ben Ayache et al., 2020; Farag & El-Kersh, 2017). This dissimilarity can be certainly explained by the differences in cultivars and the ecological factors affecting the growth process. Moreover, different extraction methods and sampling techniques may influence qualitative analysis (Ben Ayache et al., 2020). CLE was the sample that contained more volatile compounds (21), followed by CKE (18), CKA (15), CLA (13), CSE (9) and CSA (6). The same compounds were identified at trace level or with lesser relative abundance in the other macerates. Besides, in some cases, no compounds were detected. All determined substances were classified according to their chemical structure, and the distribution of volatile compounds represented by a pie chart is depicted in Fig. 2.

According to the results from Table 1, thirteen different chemical classes were determined, which indicates that the obtained extracts have a different composition of volatile compounds. Semi-quantitative analysis of the volatile bioactive compounds was based on the percentage analysis, where the abundance of compounds in each macerate was expressed as the integrated area of each compound divided by the total area of all compounds and results were represented in Fig. 2, which helped us to determine the predominant volatile compounds contained in the different macerates. Esters had a higher abundance in CSE, CKE and CLE samples. They reached 96.56%, 48.07% and 18.09% of the total area, respectively, where the ethyl hexadecanoate was recorded as the main component in such samples (53.45%, 27.19% and 14.32%, respectively). In contrast, the relative peak areas for terpenoid alcohols and sesquiterpenes were much higher in CLE (33.18% and 21.15%, respectively). A significant level of sesquiterpenes was determined in CLA (7.55%). At the same time, the CKE had the opposite tendency (0.15%). Carboxylic acids were found mainly in CKE and CKA, particularly n-hexadecanoic acid (5.70% and 24.79%, respectively). Nevertheless, the main compounds of CLA, CKA and CSA were diterpenoids (68.09%), ketones (47.14%) and sesquiterpenoids (39.48%), respectively. Several other less abundant volatile compounds were detected including monoterpenes (0.28–0.52%), alkanes (0.59–9.24%), alkenes (0.65–1.29%), alkylbenzenes (0.48–10.29%) alcohols (4.71%) and aldehydes (1.43–2.86%).

Natural antioxidants can work as scavengers, either by preventing the formation of reactive species or by removing them before they start damaging food processes. According to the literature (Djebari et al., 2021; Pinto et al., 2021), the antioxidant properties can be related to various bioactive volatile compounds.

Monoterpenes and sesquiterpenes are widely present in plants because they act as allelopathic agents. Their antioxidant capacity has been studied and proven. They can act as free radical quenchers and function through either the hydrogen donor or electron donor mechanism (Djebari et al., 2021). In fact, it has been previously shown that species rich in these compounds possessed appreciable antioxidant activity. Kelen and Tepe (2008), Mimica-Dukic, Bozin, Sokovic, and Simin (2004), Wei and Shibamoto (2007) demonstrated the antioxidant activity of the monoterpenes, especially the limonene. Singh et al. (2010) reported that the antioxidant activity of essential oils in terms of free radical scavenger was related to the antioxidant activity of DL-limonene. Previous studies have demonstrated that the species rich in caryophyllene possessed appreciable antioxidant activity (Nafis et al., 2019; Salleh, Kammil, Ahmad, & Sirat, 2015; Sarikurkcu, Ozer, Calli, & Popović-Djordjević, 2018). In addition, it was reported that the sesquiterpene (caryophyllene) showed an antioxidant activity concerning the neutralization of the DPPH radical (Mimica-Dukic et al., 2004). The sesquiterpene germacrene D was demonstrated as a strong antioxidant due to its extra cyclic methylene chemical structure (Victoria et al., 2012). Badr, Badawy, and Taktak (2021) reported that camphene, as the most significant component of *Lavandula spica* essential oil, exhibited a moderate scavenging capacity of the radical DPPH. In contrast, a study of the antiradical activities of six camphene-based thiosemicarbazones was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and peroxyl radical scavenging capacity (PSC) assays, respectively, and the results revealed that this compound exhibited a good ability for scavenging free radicals in a dose-dependent way (Yang, Liu, Xia, & Wang, 2020). Bakkali, Averbeck, Averbeck, and Idaomar (2008) found that camphene showed a significant antioxidant property against oxidative stress in murine alveolar macrophage induced by tert-butyl hydroperoxide, causing a significant decrement in the lipid peroxidation.

It is known that the antioxidant properties of active extracts cannot be related to only their major constituents (Bakkali et al., 2008). Other minor components could also contribute by synergistic effects to the obtained antioxidative activity, as well as by the interaction among the compounds (Kasrati et al., 2015; Pereira, Severino, Santos, Silva, & Souto, 2018). The analyses revealed that carob leaves, seeds, and kibbles were rich in volatile bioactive compounds characterized by antioxidant properties. Therefore, *Ceratonia siliqua* L. macerates can be considered as a good source of active agents for being used in active biopackaging films.

3.2. Antioxidant capacity of macerates

3.2.1. DPPH radical assay

The antioxidant activity of *Ceratonia siliqua* L. macerates was performed using the DPPH method. This in vitro test was chosen due to its simplicity, stability, and reproducibility (Benchikh, Louaileche, George, & Merlin, 2014). It is mainly associated with the ability of bioactive compounds to adsorb and neutralize free radicals (Popovici & Saykova, 2009). As given in Fig. 3a, the obtained results showed that CL, CS and CK macerates have powerful antioxidant activity. The antioxidant activity of the different macerates was evidenced from the obtained results compared to the performances of the standards used.

This activity was higher in CSE and CSA. All obtained values were significantly different ($p \leq 0.05$). Additionally, very high reproducibility of analysed replicated was obtained. Indeed, powerful antioxidant activity depends on the part of the plant and the solubility of bioactive compounds in the chosen solvent. In this sense, our results agree with previous findings. Mekhoukhe et al. (2019) demonstrated that the antioxidant activity of CS depended on the solvent polarity (aqueous acetone 70% > aqueous ethanol 80% > aqueous methanol 80%). In another study, the ethanolic extract of CL showed a promising antiradical effect (Hajaji et al., 2011). However, the acetonic extract of CK provided the strongest activity, according to Makris and Kefalas (2004). Benchikh et al. (2014) demonstrated that all assayed extracts of carob pulp varieties from Algeria can scavenge hydrogen- or electron-donating mechanisms and were highly correlated ($p < 0.001$) with their bioactive phytochemical contents. The efficacy of solvents on the antioxidant activity of the extracts was also studied by Goulas et al. (2019) with DPPH assay, and the results demonstrated that the ethyl acetate and acetone extracts had the lowest antioxidant activity, while it was revealed that the most promising solvents for the recovery of carob antioxidants were acetone–water (80:20, v/v), acidic acetone, acidic methanol, and water. As a result of the literature search, the antioxidant

Table 1
Results of identification and semi-quantification (expressed as percentage) of bioactive volatile compounds of CL, CS and CK of *Ceratonia siliqua* L. macerates. Compounds not detected in the different samples are marked with the symbol "–".

No.	Retention time (min)	Retention index	Compound	Formula	CAS	Chemical class	Relative percentage (%)					
							CLE	CLA	CKE	CKA	CSE	CSA
1	8.531	920	Camphene	$C_{10}H_{16}$	79-92-5	Monoterpene	–	0.61 ± 0.07	–	–	–	–
2	8.778	952	4-Ethyl-octane	$C_{10}H_{22}$	15869-86-0	Alkane	–	–	0.28 ± 0.03	–	–	–
3	9.755	1000	Decane	$C_{10}H_{22}$	1Z24-18-5	Alkane	–	0.52 ± 0.07	–	0.26 ± 0.03	–	–
4	10.197	1022	o-Cymene	$C_{10}H_{14}$	527-84-4	Alkybenzene	–	–	–	–	0.48 ± 0.22	10.29 ± 1.07
5	10.285	1030	DL-Limonene	$C_{10}H_{16}$	138-86-3	Monoterpene	–	–	0.28 ± 0.07	–	0.52 ± 0.22	–
6	13.237	1200	Dodecane	$C_{12}H_{26}$	112-40-3	Alkane	–	0.65 ± 0.08	0.78 ± 0.28	2.06 ± 0.09	–	16.17 ± 4.12
7	14.707	1300	Tridecane	$C_{13}H_{28}$	629-50-5	Alkane	–	0.40 ± 0.04	–	–	–	–
8	15.049	1322	Isocetane	$C_{16}H_{34}$	224-506-8	Alkane	–	0.87 ± 0.08	–	–	–	–
9	15.199	1325	4,6-dimethyl-dodecane	$C_{14}H_{30}$	61141-72-8	Alkane	–	–	–	0.83 ± 0.07	–	–
10	15.442	1351	α-Cubebene	$C_{15}H_{24}$	17699-14-8	Sesquiterpene	0.34 ± 0.12	–	–	–	–	–
11	15.943	1392	1-Tetradecene	$C_{14}H_{30}O$	1120-36-1	Alkene	0.65 ± 0.29	1.29 ± 0.29	–		–	–
12	16.047	1400	Tetradecane	$C_{14}H_{30}$	629-59-4	Alkane	0.67 ± 0.19	2.76 ± 0.13	1.97 ± 0.69	3.87 ± 2.54	0.59 ± 0.23	20.19 ± 3.63
13	16.406	1419	Caryophyllene	$C_{15}H_{24}$	87-44-5	Sesquiterpene	11.04 ± 3.45	6.88 ± 0.09	–	–	–	–
14	16.602	1427	Nonyl 2-methylpropanoate	$C_{13}H_{26}O_2$	10522-34-6	Ester	–	–	–	0.35 ± 0.04	–	–
15	16.849	1454	Humulene	$C_{15}H_{24}$	6753-98-6	Sesquiterpene	0.83 ± 0.26	–	–	–	–	–
16	17.103	1477	γ-Muurolene	$C_{15}H_{24}$	30021-74-0	Sesquiterpene	1.74 ± 0.61	–	–	–	–	–
17	17.187	1481	Germacrene D	$C_{15}H_{24}$	23986-74-5	Sesquiterpene	3.17 ± 0.87	–	–	–	–	–
18	17.575	1485	α-Amorphene	$C_{15}H_{24}$	483-75-0	Sesquiterpene	1.09 ± 0.39	0.67 ± 0.02	–	–	–	–
19	17.622	1497	2-Tridecanone	$C_{13}H_{26}O$	593-08-8	Ketone	–	–	0.05 ± 0.02	–	–	–
20	17.675	1524	δ-Cadinene	$C_{15}H_{24}$	483-76-1	Sesquiterpene	2.94 ± 1.02	–	0.15 ± 0.02	–	–	–
21	17.680	1531	cis-Calamenene	$C_{15}H_{22}$	72937-55-4	Sesquiterpenoid	–	–	–	–	–	17.92 ± 7.33
22	18.427	1595	Ethyl dodecanoate	$C_{14}H_{28}O_2$	106-33-2	Ester	–	–	–	–	1.66 ± 0.83	–
23	18.498	1600	Hexadecane	$C_{16}H_{34}$	544-76-3	Alkane	0.46 ± 0.14	–	1.32 ± 0.11	2.22 ± 0.29	–	–
24	18.844	1623	α-Corocalene	$C_{15}H_{20}$	20129-39-9	Sesquiterpenoid	0.27 ± 0.08	–	–	–	–	–
25	19.450	1674	Cadalene	$C_{15}H_{18}$	483-78-3	Sesquiterpenoid	0.66 ± 0.23	–	1.53 ± 0.19	1.72 ± 0.03	0.77 ± 0.50	21.56 ± 2.21
26	19.613	1698	2-Pentadecanone	$C_{15}H_{30}O$	2345-28-0	Ketone	–	–	20.87 ± 2.28	31.80 ± 1.39	–	–
27	19.671	1710	2-Pentadecanol	$C_{15}H_{32}O$	1653-34-5	Alcohol	–	–	–	4.71 ± 0.23	–	–
28	19.792	1715	Pentadecanal	$C_{15}H_{30}O$	2765-11-9	Aldehyde	1.43 ± 0.50	2.45 ± 0.19	2.86 ± 0.30	2.49 ± 0.09	–	–
29	20.185	1780	Dodecyl butyrate	$C_{16}H_{32}O_2$	3724-61-6	Ester	–	–	4.27 ± 0.36	3.49 ± 0.25	–	–
30	20.636	1794	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	124-06-1	Ester	0.51 ± 0.16	–	–	–	–	–
31	21.128	1837	Neophytadiene	$C_{20}H_{38}$	504-96-1	Diterpenoid	8.75 ± 1.34	68.09 ± 2.79	–	–	–	–
32	21.174	1844	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	502-69-2	Ketone	–	–	10.28 ± 0.47	11.63 ± 0.53	1.20 ± 0.24	14.67 ± 0.11
33	21.746	1902	2-Heptadecanone	$C_{17}H_{34}O$	2922-51-2	Ketone	–	–	4.93 ± 0.28	3.71 ± 0.19	–	–
34	21.984	1926	Methyl hexadecanoate	$C_{17}H_{34}O_2$	112-39-0	Ester	–	–	4.33 ± 0.38	3.96 ± 0.31	–	–
35	22.214	1948	Isophytol	$C_{20}H_{40}O$	505-32-8	Terpenoid alcohol	0.78 ± 0.10	0.79 ± 0.09	–	–	–	–
36	22.331	1968	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	57-10-3	Carboxylic acid	–	–	5.70 ± 6.23	24.79 ± 3.69	–	–
37	22.640	1993	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	628-97-7	Ester	14.32 ± 4.20	–	27.19 ± 1.83	–	53.45 ± 13.55	–
38	23.650	2110	(*E*)-Methyl-9-octadecenoate	$C_{19}H_{36}O_2$	1937-62-8	Ester	–	–	0.88 ± 0.11	–	–	–
39	23.771	2114	Phytol	$C_{20}H_{40}O$	150-86-7	Terpenoid alcohol	32.40 ± 4.60	32.40 ± 4.60	–	–	–	–
40	24.047	2139	9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-	$C_{18}H_{30}O_2$	463-40-1	Carboxylic acid	8.96 ± 10.95	–	–	–	–	–
41	24.205	2162	Ethyl linoleate	$C_{20}H_{36}O_2$	544-35-4	Ester	1.79 ± 1.42	–	–	–	36.55 ± 20.43	–
42	24.251	2174	(*E*)-Ethyl-9-octadecenoate	$C_{20}H_{38}O_2$	6114-18-7	Ester	–	–	11.40 ± 1.18	–	24.90 ± 5.14	–
43	24.477	2175	Methyl-17-methyl octadecanoate	$C_{20}H_{40}O_2$	55124-97-5	Ester	1.47 ± 1.42	–	–	–	–	–

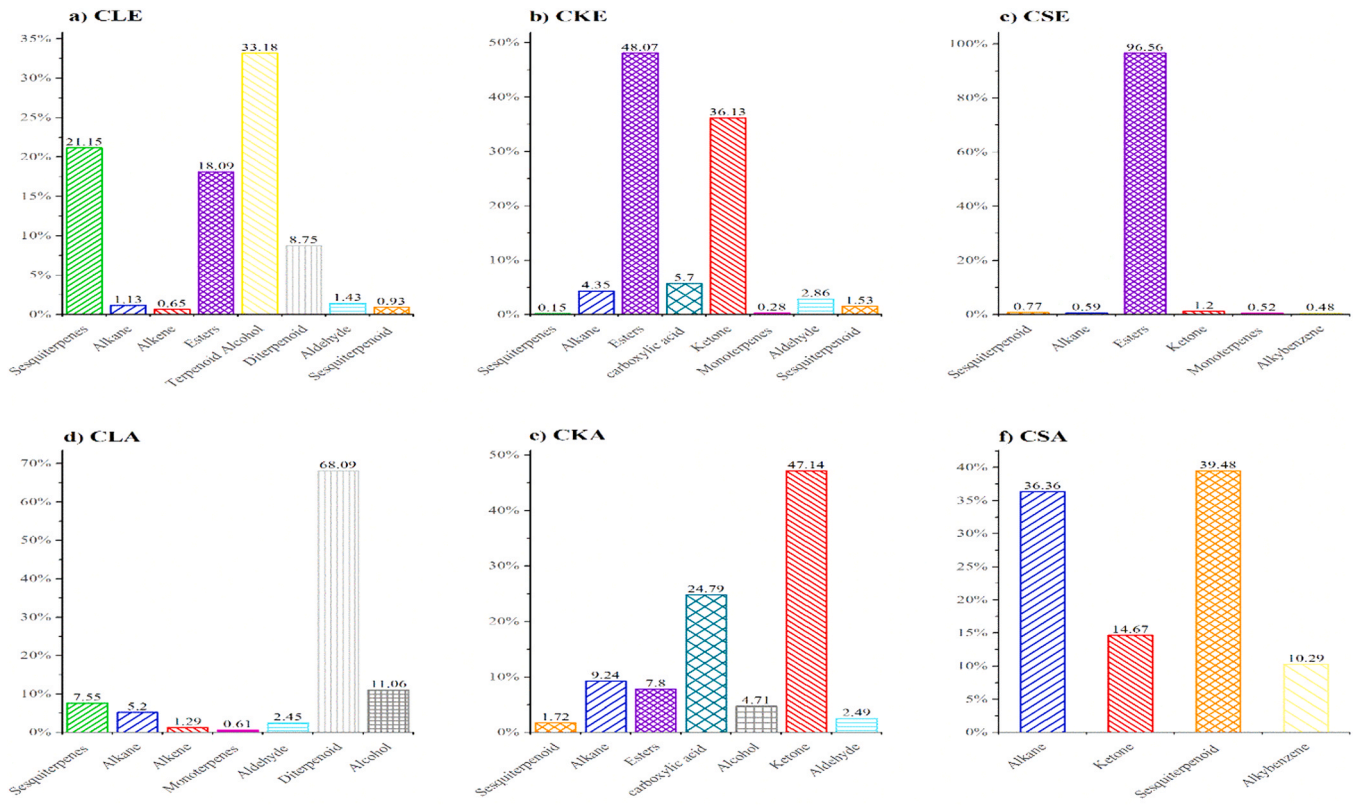

Fig. 2. Column graph representing percentage of determined compounds grouped into different chemical classes in various samples such as a) CKE; b) CLA; c) CSE; d) CKA; e) CLE; f) CSA.

activity of the different extracts is loosely related to their chemical composition, the determined major compounds and the possible synergistic effects established between them (De Saint Laumer, Frérot, & Herrmann, 2003). Bozin, Mimica-Dukic, Samojlik, and Jovin (2007) noted that the mixture of mono and sesquiterpene showed notable scavenging activity by a synergistic effect, which may explain the strong antioxidant activity that was observed through our results. Mimica-Dukic et al. (2004) reported that the monoterpenes (limonene) and sesquiterpenes (caryophyllene) are responsible for the neutralization of the DPPH radical. Wei and Shibamoto (2007) noted significant antioxidant activity of essential oils rich in monoterpenes (limonene). However, Kelen and Tepe (2008) reported that the monoterpene (limonene) individually tested did not have a significant antioxidant activity compared to the same constituent when tested together with α-pinene and β-pinene. Another in vitro study on the evaluation of the antioxidant activity of the D-Limonene confirmed a strong antioxidant activity by reducing the free radical formation in the different antioxidant assays (Shah & Mehta, 2018). DPPH scavenging capacity increased when the level of the OH- groupings present in the aromatic rings raises. However, other phenolic compounds that are present but were not determined may also have an antioxidant effect (Abidar et al., 2020). From our results, we can deduce that *Ceratonia siliqua* L. seeds, leaves and kibbles could be used as a potential source of antioxidants what can be seen in Fig. 3a.

3.2.2. *Phosphomolybdenum assay*

The phosphomolybdenum reduction is a quantitative method expressed as equivalents of ascorbic acid. It gives a direct estimation of the reducing capacity of the sample of interest. It is based on the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V) and measurement of the absorbance at 695 nm due to the reduced green molybdenum complex (Sharadanand Phatak, Subhash Hendre, & Rohan Sharadanand Phatak, 2014). The total antioxidant capacity of CS, CL and CK macerates is shown in Fig. 3b. It decreases in the following order: CSE > CSA > CLE > CLA > CKE > CKA. Their respective total antioxidant capacities were 72.81 ± 0.09; 67.22 ± 3.91; 53.52 ± 0.09; 51.76 ± 0.20; 26.96 ± 2.14 and 12.35 ± 0.46 μg/mL (expressed as ascorbic acid equivalents).

Several in vitro studies showed that the carob tree extracts have potent antioxidant effects (Rtibi et al., 2015; Sebai et al., 2013). A study reported by Mekhoukhe et al. (2019) on the antioxidant activity of CS extracts demonstrated that TAC decreased in the following order: aqueous acetonic extract > aqueous ethanolic extract > aqueous methanolic extract. Their respective TAC were 112.59, 62.73, and 41.83 μg/mL. Another study reported that TAC decreased in this order: acetonic extract > methanolic extract > ethanolic extract (Lakkab et al., 2019). Hajaji et al. (2011) reported that the extracts from three varieties of *Ceratonia siliqua* L. leaves from Morocco showed significant antioxidant capacity compared to the reference antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid (AA), in a dose dependent manner.

Therefore, it can be concluded that the solvent had a significant effect on the estimated antioxidant activity as it determined the rates and mechanism of the reaction of the phenolics with reagents (Nenadis & Tsimidou, 2002). It also confirms the highest antiradical effect of CS macerate determined by the DPPH assay. The slight variation detected among carob parts can be explained by the fact that the phosphomolybdenum reduction method is based on an estimation of antioxidant activity of polyphenols and other non-volatile antioxidant compounds, which were not the object of this study.

3.3. *Antioxidant capacity of active multilayer biopackaging*

As shown in Fig. 3c, the highest antioxidant capacity was obtained in active biofilm with CSE followed by CSA, CLE, CLA, CKE and CKA with a value of 180.67, 182.34, 185.06, 227.83, 343.57, and 548.26 μg/mL,

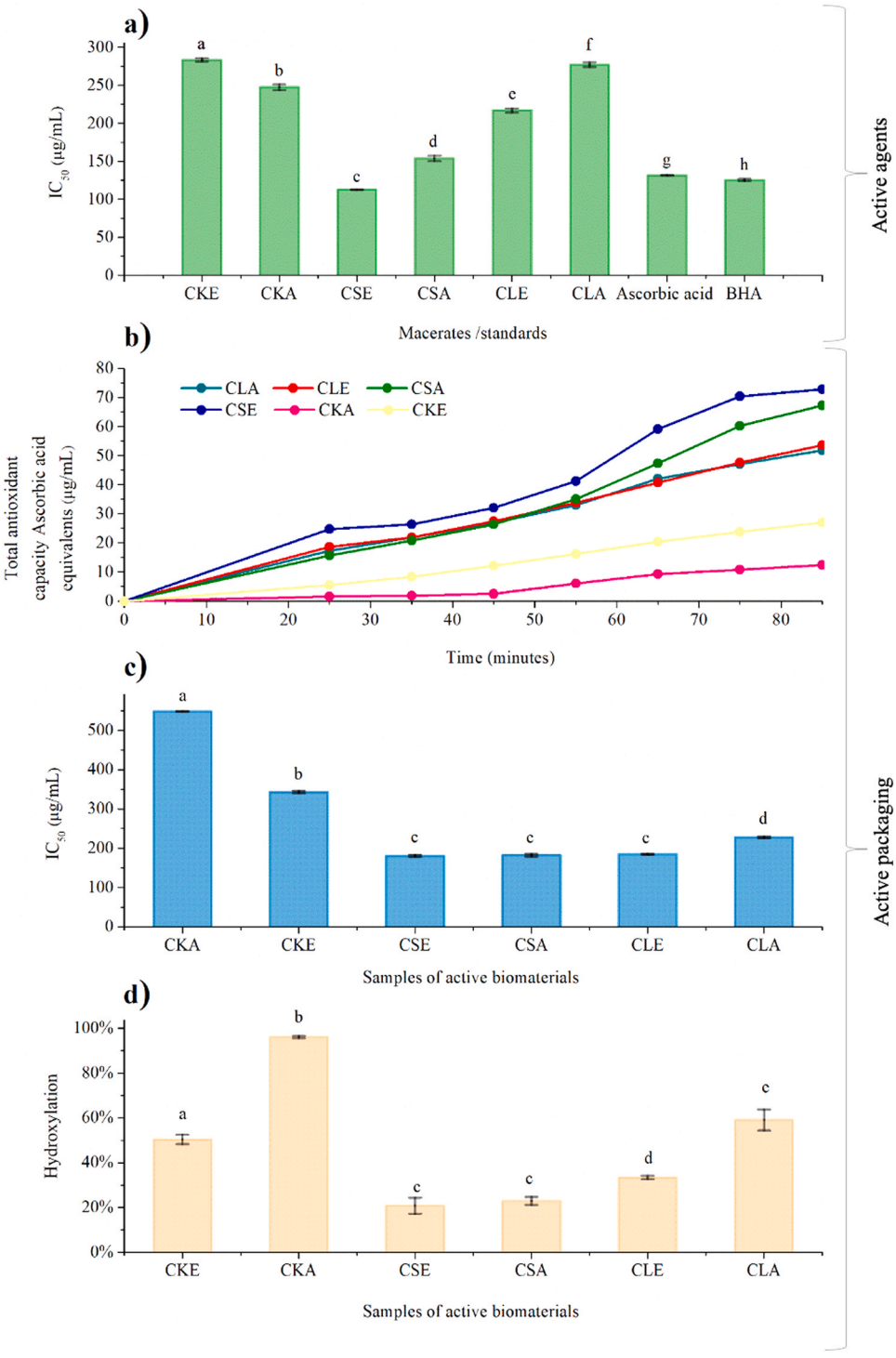

Fig. 3. Comparison of antioxidant activity of macerates and active biofilms, where: a) DPPH radical scavenging activity of *Ceratonia siliqua* L. macerates and standards; b) Total antioxidant capacity of macerates; c) IC_{50} values of active biomaterial packaging containing CL, CS and CK macerates; d) free OH· radical assay of different active biopackaging subjected to hydroxylation during 24 h (blank = 100%). Different letters (a–h) indicate statistically significant differences between samples ($p \leq 0.05$). All samples were performed in triplicate.

respectively. There was no significant difference among samples such as CSE, CSA and CLE. Blank biofilm without macerates did not change the absorbance of DPPH, which shows its absence of antioxidant properties.

The antioxidant capacities of multilayer biofilms were based on the scavenging of gas-phase OH·radicals, generated from an aqueous peroxide solution, by the carob extracts incorporated into biofilms. The obtained results after 24 h of hydroxylation are shown in Fig. 3d. As can be seen, significant differences were observed among almost all antioxidant biofilms. Samples of carob seeds extracted with ethanol and acetone and incorporated into polymer matrix provided similar results. The active biofilm containing CS macerates showed the best results with values of 20.91 ± 3.59% and 23.04 ± 1.79% for CSE and CSA, respectively, in good agreement with the DPPH test, followed by CLE (33.49 ± 0.78%), CKE (50.43 ± 2.08%), CLA (59.12 ± 4.72%), and CKA (96.12 ± 0.52%). This effect is associated with the presence of scavengers of free radicals and can be explained by the different chemical reactions taking part in each method for the determination of the antioxidant activity. Finally, based on these results, the active biopackaging with CS macerates incorporated was selected for the migration assay.

It should be highlighted that Fig. 3a–d compare the antioxidant capacity of pure macerates, as well as antioxidant packaging with

incorporated extracts to assess the behaviour of the active agents. The obtained results confirm that active films eliminate the free radicals responsible for the initiation of the oxidation phenomenon. Since free radicals can efficiently diffuse through the polymer, they can be trapped by the antioxidant agents fixed in the multilayer packaging as components of the adhesive among polymer films, thus avoiding the direct contact with the packaged food. Consequently, the results of the antioxidant activity obtained for active films are weaker than those obtained in the macerates.

3.4. Antibacterial activity of macerates

The inhibition zones of macerates, expressed in mm, are given in Table 2. The macerates generated larger inhibition against the *Staphylococcus aureus*, compared to the *Escherichia coli* and *Pseudomonas aeruginosa*, suggesting that macerates have better antimicrobial activity against gram-positive bacteria than against gram-negative bacteria. This behaviour can be due to the outer lipopolysaccharide membrane of gram-negative strains, which restricts the diffusion of hydrophobic compounds (Burt, 2004).

Macerates such as CKE, CLE and CLA demonstrated the highest antimicrobial activity against the three microorganisms tested. However, from a practical point of view this antimicrobial activity is not enough for the use of extracts in antimicrobial active packaging applications. Antimicrobials typically used in active packaging such as cinnamon essential oil or clove essential oil showed similar inhibition zones by applying 25 times less antimicrobial in the disk (López, Sánchez, Batlle, & Nerín, 2005).

3.5. Migration tests and risk assessment

Biopackaging and active antioxidant packaging are new areas of technology with protective effect against oxidative damage with the advantage of its sustainability. Nevertheless, to accomplish the legislation of food contact materials, packaging must not transfer their chemical components into the foods through the migration process. A migration test and risk assessment of new multilayer active biopackaging films based on cellulose were performed to assess their safety in the present investigation. Two sets of food packaging biofilms containing CSE and CSA (8%) were studied in this work, and 10% and 95% aqueous ethanol solutions were used as food simulants with a contact time of 3 days at temperature < 5 °C.

The obtained chromatograms of both simulants after the exposure to active biofilms were compared to those of the blank biofilm and those of pure simulants. No peaks were detected, neither in the case of the volatile compounds nor non-volatile ones. Similar results were obtained by Oudjedi et al. (2019) on a new antioxidant active packaging which incorporated Algerian extracts of sage and bay leaves, where no migration of compounds was noticed from both kinds of packaging. These results could be expected, as probably the compounds are grafted in the adhesive behind the cellulose layer in contact with the simulant, as demonstrated by previous studies (Carrizo et al., 2016). Another research on the migration of compounds from cellulose-based trays, coated with an active filler, by using the acetic acid at 3% (v/v), ethanol at 50% (v/v) and vegetable oil as food simulants, resulted in compliance with the migration limits imposed from EU regulation, thus demonstrating the suitability of the prepared material for food contact (Bugatti, Viscusi, & Gorrasi, 2020).

Therefore, the experimental results here obtained confirmed that the prepared active biofilm is safe and can be used as antioxidant food biopackaging for future preservation, according to experiment described in Section 2.7.1.

Table 2
Inhibition zones of macerates (results expressed in mm).

Sample	*E. coli*	*P. aeruginosa*	*S. aureus*
CSA	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	22.1 ± 1.0^{a}
CSE	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	22.4 ± 0.5^{a}
CKA	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	11.6 ± 1.4^{b}
CKE	16.4 ± 2.0^{b}	20.3 ± 1.0^{b}	26.3 ± 1.1^{c}
CLA	20.8 ± 1.0^{c}	22.1 ± 0.2^{c}	27.9 ± 1.9^{c}
CLE	$19.4 \pm 1.4^{b,c}$	22.9 ± 0.4^{d}	27.8 ± 1.6^{c}

Different letters (a–d) indicate statistically significant differences between samples ($p \leq 0.05$).

3.6. Colour measurement

In food packaging applications, the appearance of films is an essential issue (Urbina, Eceiza, Gabilondo, Corcuera, & Retegi, 2019). The colour of the film is often the factor determining its production on an industrial scale, introducing the product to the market and purchasing the product by the consumer.

To evaluate the influence of active agents on the polymer matrix, colour parameters of the active biofilms containing CL, CS, and CK macerates were measured. Moreover, total colour changes (ΔE) were calculated, and the obtained results are shown in Table 3. The cellulose sheet material used in the present work was colourless and translucent. The visual examination of all the biofilms containing the different macerates of leaves, seeds, and kibbles from *Ceratonia siliqua* L. showed a light painted colour compared to the blank biofilm without macerates. The instrumental colour analysis results presented in Table 3 show that L^*, a^*, b^* increased after the incorporation of the macerates. Concerning lightness, a higher value (L^*) was recorded in the CKE, followed by CKA > CLE > CSA > CLA > CSE. Nevertheless, statistical analysis showed that there were no significant differences ($p \leq 0.05$) among the L* values of all samples. Little changes were noticed (Student *t*-test, $p \leq 0.05$), in the positive and negative values of a^* and b^* parameters in all bioactive materials in comparison to the blank biofilm. Almost all values were recorded with only a slight increase. The fundamental differences in colour (ΔE) were calculated, and results showed that the highest value corresponded to CKE ($\Delta E = 2.87$), and the lowest one was found in CSE ($\Delta E = 0.76$) as the film less affected by the addition of the macerate in the adhesive formulation. Our results can be explained by the application of the minimum possible concentration of macerate (8%). The obtained results of total colour change (values from 0 to 100) should be interpreted according to the following key: $\Delta E < 1$ means that the colour change is not perceptible by human eyes; if $\Delta E = 1$–2, the change is perceptible after very close observation; when $\Delta E = 2$–10, the change is perceptible at first sight; in the range $\Delta E = 11$–49, colours are more likely similar than dissimilar, whereas $\Delta E = 50$–100 are perceived as opposite colours.

4. Conclusion

In this work, forty-three volatile compounds were identified in *Ceratonia siliqua* L. macerates and, among them, natural antioxidants have been noticed. The antioxidant activity was carried out with two different methods, and the best results corresponded to macerates of *Ceratonia siliqua* L. seeds with IC_{50} values of 112.75 ± 0.32, and 153.61 ± 3.65 μg/mL for the CSE and CSA extracts, respectively using DPPH test, while values of 72.81 ± 0.09, and 67.22 ± 3.91 μg/mL were recorded in the case of CSE and CSA extracts, respectively by the phosphomolybdenum assay.

Besides, a new antioxidant biofilm based on cellulose as the matrix was successfully developed and was evaluated for its antioxidant capacity by a method based on in situ hydroxyl radical generator and DPPH test. The one containing *Ceratonia siliqua* L. seeds macerated with 80% ethanol and 80% acetone demonstrated the strongest antioxidant activity with percentages of hydroxylation of $20.91 \pm 3.59\%$ and $23.04 \pm 1.79\%$, respectively, followed by CLE, CKE, CLA, and CKA with percentages of hydroxylation of $33.49 \pm 0.78\%$; $50.43 \pm 2.08\%$; $59.12 \pm 4.72\%$ and $96.12 \pm 0.52\%$, respectively (100% hydroxylation

Table 3
CIELAB colour parameters of the films and total colour change (ΔE).

	Biomaterials with ethanolic macerates				Biomaterials with acetonic macerates			
	Control	CL	CS	CK	Control	CL	CS	CK
L^*	88.09 ± 0.29^a	87.29 ± 0.80^a	87.81 ± 0.69^a	88.44 ± 0.18^a	88.03 ± 0.34^a	87.72 ± 0.50^a	87.86 ± 0.37^a	88.01 ± 0.58^a
a^*	2.76 ± 0.03^a	3.23 ± 0.06^b	3.31 ± 0.04^b	3.46 ± 0.03^c	2.80 ± 0.02^a	3.03 ± 0.06^d	3.28 ± 0.04^b	3.55 ± 0.21^b
b^*	-3.33 ± 0.08^a	-4.70 ± 0.08^b	-3.78 ± 3.21^c	-6.09 ± 0.04^c	-3.42 ± 0.05^a	-4.11 ± 0.14^e	-4.79 ± 0.09^b	-5.80 ± 0.58^d
ΔE	0.00	1.71	0.76	2.87	0.00	0.79	1.46	2.50

Different letters (a–e) indicate statistically significant differences between samples ($p \leq 0.05$).

means no antioxidant properties). In contrast, the antioxidant capacity was also shown in the case of seeds macerates biofilms with DPPH test (180.67 ± 2.5 and 182.34 ± 3.21 μg/mL for CSE and CSA extracts, respectively). The following macerates CKE, CLE and CLA demonstrated the highest antimicrobial activity against all microorganisms that were tested. Nevertheless, the evaluation of the antimicrobial activity of the different macerates against bacterial strains showed a weak antibacterial activity, limiting our study to evaluate the antioxidant performance of the new biofilm.

Finally, a migration test of both volatile and non-volatile compounds was carried out, and the obtained results showed the full absence of migrants in all the cases. Therefore, it can be concluded that the developed material complies with Commission Regulation (EU) No 10/2011 and all its amendments for food contact materials. These results are promising for future production of developed active biofilms at industrial scale. Nevertheless, before that, subsequent trials on real food samples are necessary.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Ministry of Higher Education and Scientific Research (MESRS) and the Government of Algeria for the doctoral scholarship for Lidia Ait Ouahioune (Process 2019/2020). The authors also wish to thank the Government of Aragon and the European Social Fund for financial support (T53-20R) to the GUIA Group.

References

Abidar, S., Boiangiu, R., Dumitru, G., Todirascu-Ciornea, E., Amakran, A., Cioanca, O., … Nhiri, M. (2020). The aqueous extract from Ceratonia siliqua leaves protects against 6-hydroxydopamine in zebrafish: Understanding the underlying mechanism. *Antioxidants, 9*(4), 304. https://doi.org/10.3390/antiox9040304

Akrami, F., Rodríguez-Lafuente, A., Bentayeb, K., Pezo, D., Ghalebi, S. R., & Nerín, C. (2015). Antioxidant and antimicrobial active paper based on Zataria (Zataria multiflora) and two cumin cultivars (Cuminum cyminum). *LWT - Food Science and Technology, 60*(2), 929–933. https://doi.org/10.1016/j.lwt.2014.09.051

Al-Tayyar, N. A., Youssef, A. M., & Al-hindi, R. (2020a). Antimicrobial food packaging based on sustainable Bio-based materials for reducing foodborne pathogens: A review. *Food Chemistry, 310*, Article 125915. https://doi.org/10.1016/j.foodchem.2019.125915

Al-Tayyar, N. A., Youssef, A. M., & Al-Hindi, R. R. (2020b). Edible coatings and antimicrobial nanoemulsions for enhancing shelf life and reducing foodborne pathogens of fruits and vegetables: A review. *Sustainable Materials and Technologies, 26*, 1–12. https://doi.org/10.1016/j.susmat.2020.e00215

Badr, M. M., Badawy, M. E. I., & Taktak, N. E. M. (2021). Characterization, antimicrobial activity, and antioxidant activity of the nanoemulsions of Lavandula spica essential oil and its main monoterpenes. *Journal of Drug Delivery Science and Technology, 65*, Article 102732. https://doi.org/10.1016/j.jddst.2021.102732

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology, 46*, 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Belasli, A., Ben Miri, Y., Aboudaou, M., Aït Ouahioune, L., Montañes, L., Ariño, A., & Djenane, D. (2020). Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (Laurus nobilis L.): Potential use as wheat preservative. *Food Science and Nutrition, 8*(9), 4717–4729. https://doi.org/10.1002/fsn3.1650

Ben Ayache, S., Behija Saafi, E., Emhemmed, F., Flamini, G., Achour, L., & Muller, C. D. (2020). Biological activities of aqueous extracts from carob plant (Ceratonia siliqua L.) by antioxidant, analgesic and proapoptotic properties evaluation. *Molecules, 25*(14), 3120. https://doi.org/10.3390/molecules25143120

Ben Othmen, K., Garcia-Beltrán, J. M., Elfalleh, W., Haddad, M., & Esteban, M.Á. (2021). Phytochemical compounds and biological properties of carob pods (Ceratonia siliqua L.) extracts at different ripening stages. *Waste and Biomass Valorization, 12*(9), 4975–4990. https://doi.org/10.1007/s12649-021-01352-x

Benchikh, Y., Louaileche, H., George, B., & Merlin, A. (2014). Changes in bioactive phytochemical content and in vitro antioxidant activity of carob (Ceratonia siliqua L.) as influenced by fruit ripening. *Industrial Crops and Products, 60*, 298–303. https://doi.org/10.1016/j.indcrop.2014.05.048

Borzi, F., Torrieri, E., Wrona, M., & Nerín, C. (2019). Polyamide modified with green tea extract for fresh minced meat active packaging applications. *Food Chemistry, 300*, 125–242. https://doi.org/10.1016/j.foodchem.2019.125242

Bozin, B., Mimica-Dukic, N., Samojlik, I., & Jovin, E. (2007). Antimicrobial and antioxidant properties of Rosemary and Sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. *Journal of Agricultural and Food Chemistry, 55*(19), 7879–7885. https://doi.org/10.1021/jf0715323

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bugatti, V., Viscusi, G., & Gorrasi, G. (2020). Formulation of a bio-packaging based on pure cellulose coupled with cellulose acetate treated with active coating: Evaluation of shelf life of pasta ready to eat. *Foods, 9*(10), 1–14. https://doi.org/10.3390/foods9101414

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. *International Journal of Food Microbiology, 94*, 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Camo, J., Lorés, A., Djenane, D., Beltrán, J. A., & Roncalés, P. (2011). Display life of beef packaged with an antioxidant active film as a function of the concentration of oregano extract. *Meat Science, 88*(1), 174–178. https://doi.org/10.1016/j.meatsci.2010.12.019

Carrizo, D., Gullo, G., Bosetti, O., & Nerín, C. (2014). Development of an active food packaging system with antioxidant properties based on green tea extract. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 31*(3), 364–373. https://doi.org/10.1080/19440049.2013.869361

Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2016). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science and Emerging Technologies, 33*, 534–541. https://doi.org/10.1016/j.ifset.2015.10.018

Castro, F. V. R., Andrade, M. A., Silva, A. S., Vaz, M. F., & Vilarinho, F. (2019). The contribution of a whey protein film incorporated with green tea extract to minimize the lipid oxidation of salmon (Salmo salar L.). *Foods, 8*(8), 327. https://doi.org/10.3390/foods8080327

De Saint Laumer, J. Y., Frérot, E., & Herrmann, A. (2003). Controlled release of perfumery alcohols by neighboring-group participation. Comparison of the rate constants for the alkaline hydrolysis of 2-acyl-, 2-(hydroxymethyl)-, and 2-carbamoylbenzoates. *Helvetica Chimica Acta, 86*(8), 2871–2899. https://doi.org/10.1002/hlca.200390236

Djebari, S., Wrona, M., Boudria, A., Salafranca, J., Nerin, C., Bedjaoui, K., & Madani, K. (2021). Study of bioactive volatile compounds from different parts of Pistacia lentiscus L. extracts and their antioxidant and antibacterial activities for new active packaging application. *Food Control, 120*, Article 107514. https://doi.org/10.1016/j.foodcont.2020.107514

Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of algerian citrus essential oils and their application in Sardina pilchardus. *Foods, 4*(2), 208–228. https://doi.org/10.3390/foods4020208

Djenane, D., Beltrán, J. A., Camo, J., & Roncalés, P. (2016). Influence of vacuum-ageing duration of whole beef on retail shelf life of steaks packaged with oregano (Origanum vulgare L.) active film under high O_2. *Journal of Food Science and Technology, 53*(12), 4244–4257. https://doi.org/10.1007/s13197-016-2419-1

Dong, Z., Xu, F., Ahmed, I., Li, Z., & Lin, H. (2018). Characterization and preservation performance of active polyethylene films containing rosemary and cinnamon essential oils for Pacific white shrimp packaging. *Food Control, 92*, 37–46. https://doi.org/10.1016/j.foodcont.2018.04.052

Echegoyen, Y., & Nerín, C. (2015). Performance of an active paper based on cinnamon essential oil in mushrooms quality. *Food Chemistry, 170*, 30–36. https://doi.org/10.1016/j.foodchem.2014.08.032

El-Sayed, A.M. (2014). *The Pherobase: Database of pheromones and semiochemicals*. The Pherobase. Retrieved from ⟨http://www.pherobase.com⟩.

El-Sayed, H. S., El-Sayed, S. M., Mabrouk, A. M. M., Nawwar, G. A., & Youssef, A. M. (2021). Development of eco-friendly probiotic edible coatings based on chitosan, alginate and carboxymethyl cellulose for improving the shelf life of UF soft cheese. *Journal of Polymers and the Environment, 29*(6), 1941–1953. https://doi.org/10.1007/s10924-020-02003-3

Farag, M. A., & El-Kersh, D. M. (2017). Volatiles profiling in Ceratonia siliqua (Carob bean) from Egypt and in response to roasting as analyzed via solid-phase microextraction coupled to chemometrics. *Journal of Advanced Research, 8*(4), 379–385. https://doi.org/10.1016/j.jare.2017.05.002

Fidan, H., Mihaylova, D., Petkova, N., Sapoundzhieva, T., Slavov, A., & Krastev, L. (2019). Determination of chemical composition, antibacterial and antioxidant properties of products obtained from carob and honey locust. *Turkish Journal of Biochemistry, 44*(3), 316–322. https://doi.org/10.1515/tjb-2018-0113

Goulas, V., Hadjivasileiou, L., Primikyri, A., Michael, C., Botsaris, G., Tzakos, A. G., & Gerothanassis, I. P. (2019). Valorization of carob fruit residues for the preparation of novel bi-functional polyphenolic coating for food packaging applications. *Molecules, 24*(17), 1–10. https://doi.org/10.3390/molecules24173162

Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., … Lachkar, M. (2011). Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob tree barks growing in Morocco. *Arabian Journal of Chemistry, 4*(3), 321–324. https://doi.org/10.1016/j.arabjc.2010.06.053

Kasrati, A., Alaoui Jamali, C., Bekkouche, K., Spooner-Hart, R., Leach, D., & Abbad, A. (2015). Chemical characterization and insecticidal properties of essential oils from different wild populations of Mentha suaveolens subsp. timija (BRIQ.) HARLEY from Morocco. *Chemistry and Biodiversity, 12*(5), 823–831. https://doi.org/10.1002/cbdv.201400236

Kelen, M., & Tepe, B. (2008). Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. *Bioresource Technology, 99*(10), 4096–4104. https://doi.org/10.1016/j.biortech.2007.09.002

Lakkab, I., El Hajaji, H., Lachkar, N., Lefter, R., Ciobica, A., El Bali, B., & Lachkar, M. (2019). Ceratonia siliqua L. seed peels: Phytochemical profile, antioxidant activity, and effect on mood disorders. *Journal of Functional Foods, 54*, 457–465. https://doi.org/10.1016/j.jff.2019.01.041

López, P., Sánchez, C., Batlle, R., & Nerín, C. (2005). Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry, 53*(17), 6939–6946. https://doi.org/10.1021/jf050709v

López, P., Sánchez, C., Batlle, R., & Nerín, C. (2007). Development of flexible antimicrobial films using essential oils as active agents. *Journal of Agricultural and Food Chemistry, 55*(21), 8814–8824. https://doi.org/10.1021/jf071737b

Makris, D. P., & Kefalas, P. (2004). Carob pods (Ceratonia siliqua L.) as a source of polyphenolic antioxidants. *Food Technology and Biotechnology, 42*(2), 105–108.

Marcos, B., Sárraga, C., Castellari, M., Kappen, F., Schennink, G., & Arnau, J. (2014). Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications. *Food Packaging and Shelf Life, 1*(2), 140–150. https://doi.org/10.1016/j.fpsl.2014.04.002

Mekhoukhe, A., Kicher, H., Ladjouzi, A., Medouni-Haroune, L., Brahmi, F., Medouni-Adrar, S., & Madani, K. (2019). Antioxidant activity of carob seeds and chemical composition of their bean gum by- products. *Journal of Complementary and Integrative Medicine, 16*(1), 1–11. https://doi.org/10.1515/jcim-2017-0158

Mimica-Dukic, N., Bozin, B., Sokovic, M., & Simin, N. (2004). Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. *Journal of Agricultural and Food Chemistry, 52*(9), 2485–2489. https://doi.org/10.1021/jf030698a

Mohan, C. O., Ravishankar, C. N., Lalitha, K. V., & Srinivasa Gopal, T. K. (2012). Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (Sardinella longiceps) during chilled storage. *Food Hydrocolloids, 26*(1), 167–174. https://doi.org/10.1016/j.foodhyd.2011.05.005

Moudache, M., Colon, M., Nerín, C., & Zaidi, F. (2016). Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. *Food Chemistry, 212*, 521–527. https://doi.org/10.1016/j.foodchem.2016.06.001

Nafis, A., Kasrati, A., Jamali, C. A., Mezrioui, N., Setzer, W., Abbad, A., & Hassani, L. (2019). Antioxidant activity and evidence for synergism of Cannabis sativa (L.) essential oil with antimicrobial standards. *Industrial Crops and Products, 137*, 396–400. https://doi.org/10.1016/j.indcrop.2019.05.032

Nasar-Abbas, S. M., E-Huma, Z., Vu, T. H., Khan, M. K., Esbenshade, H., & Jayasena, V. (2016). Carob kibble: A bioactive-rich food ingredient. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 63–72. https://doi.org/10.1111/1541-4337.12177

Nenadis, N., & Tsimidou, M. (2002). Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH.) tests. *JAOCS Journal of the American Oil Chemists' Society, 79*(12), 1191–1195. https://doi.org/10.1007/s11746-002-0626-z

Nerin, C., Astudillo, M., Covían, I., & Mujika, R. (2006). *WO2007144444A1 Active packaging that inhibits food pathogens*.

Nerín, C., Tovar, L., & Salafranca, J. (2008). Behaviour of a new antioxidant active film versus oxidizable model compounds. *Journal of Food Engineering, 84*(2), 313–320. https://doi.org/10.1016/j.jfoodeng.2007.05.027

Oudjedi, K., Manso, S., Nerin, C., Hassissen, N., & Zaidi, F. (2019). New active antioxidant multilayer food packaging films containing Algerian Sage and Bay leaves extracts and their application for oxidative stability of fried potatoes. *Food Control, 98*, 216–226. https://doi.org/10.1016/j.foodcont.2018.11.018

Owen, R. W., Haubner, R., Hull, W. E., Erben, G., Spiegelhalder, B., Bartsch, H., & Haber, B. (2003). Isolation and structure elucidation of the major individual polyphenols in carob fibre. *Food and Chemical Toxicology, 41*(12), 1727–1738. https://doi.org/10.1016/S0278-6915(03)00200-X

Papaefstathiou, E., Agapiou, A., Giannopoulos, S., & Kokkinofta, R. (2018). Nutritional characterization of carobs and traditional carob products. *Food Science and Nutrition, 6*(8), 2151–2161. https://doi.org/10.1002/fsn3.776

Pereira, I., Severino, P., Santos, A. C., Silva, A. M., & Souto, E. B. (2018). Linalool bioactive properties and potential applicability in drug delivery systems. *Colloids and Surfaces B: Biointerfaces, 171*, 566–578. https://doi.org/10.1016/j.colsurfb.2018.08.001

Pezo, D., Salafranca, J., & Nerín, C. (2006). Design of a method for generation of gas-phase hydroxyl radicals, and use of HPLC with fluorescence detection to assess the antioxidant capacity of natural essential oils. *Analytical and Bioanalytical Chemistry, 385*, 1241–1246. https://doi.org/10.1007/s00216-006-0395-4

Pezo, Davinson, Salafranca, J., & Nerín, C. (2008). Determination of the antioxidant capacity of active food packagings by in situ gas-phase hydroxyl radical generation and high-performance liquid chromatography–fluorescence detection. *Journal of Chromatography A, 1178*(1–2), 126–133. https://doi.org/10.1016/j.chroma.2007.11.062

Pinto, T., Aires, A., Cosme, F., Bacelar, E., Morais, M. C., Oliveira, I., … Gonçalves, B. (2021). Bioactive (poly)phenols, volatile compounds from vegetables, medicinal and aromatic plants. *Foods, 10*(1), 106. https://doi.org/10.3390/foods10010106

Popovici, C., & Saykova, I. (2009). Evaluation de l ' activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. *Génie Industriel, 4*(16), 26–39.

Roseiro, L. B., Duarte, L. C., Oliveira, D. L., Roque, R., Bernardo-Gil, M. G., Martins, A. I., & Rauter, A. P. (2013). Supercritical, ultrasound and conventional extracts from carob (Ceratonia siliqua L.) biomass: Effect on the phenolic profile and antiproliferative activity. *Industrial Crops and Products, 47*, 132–138. https://doi.org/10.1016/j.indcrop.2013.02.026

Roseiro, L. B., Tavares, C. S., Roseiro, J. C., & Rauter, A. P. (2013). Antioxidants from aqueous decoction of carob pods biomass (Ceretonia siliqua L.): Optimisation using response surface methodology and phenolic profile by capillary electrophoresis. *Industrial Crops and Products, 44*, 119–126. https://doi.org/10.1016/j.indcrop.2012.11.006

Rtibi, K., Jabri, M. A., Selmi, S., Souli, A., Sebai, H., El-Benna, J., … Marzouki, L. (2015). Gastroprotective effect of carob (Ceratonia siliqua L.) against ethanol-induced oxidative stress in rat. *BMC Complementary and Alternative Medicine, 15*(1), 292. https://doi.org/10.1186/s12906-015-0819-9

Ruiz-Hernández, V., Roca, M. J., Egea-Cortines, M., & Weiss, J. (2018). A comparison of semi-quantitative methods suitable for establishing volatile profiles. *Plant Methods, 14*(1), 1–15. https://doi.org/10.1186/s13007-018-0335-2

Sabeena Farvin, K. H., Grejsen, H. D., & Jacobsen, C. (2012). Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (Trachurus trachurus): Effect on lipid and protein oxidation. *Food Chemistry, 131*(3), 843–851. https://doi.org/10.1016/j.foodchem.2011.09.056

Sallam, K. I. (2007). Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chemistry, 101*(2), 592–600. https://doi.org/10.1016/j.foodchem.2006.02.019

Salleh, W. M. N. H., Kammil, M. F., Ahmad, F., & Sirat, H. M. (2015). Antioxidant and anti-inflammatory activities of essential oil and extracts of Piper miniatum. *Natural Product Communications, 10*(11), 2005–2008. https://doi.org/10.1177/1934578×1501001151

Sanches-Silva, A., Costa, D., Albuquerque, T. G., Buonocore, G. G., Ramos, F., Castilho, M. C., … Costa, H. S. (2014). Trends in the use of natural antioxidants in active food packaging: A review. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 31*(3), 374–395. https://doi.org/10.1080/19440049.2013.879215

Santonocito, D., Granata, G., Geraci, C., Panico, A., Siciliano, E. A., Raciti, G., & Puglia, C. (2020). Carob seeds: Food waste or source of bioactive compounds? *Pharmaceutics, 12*(11), 1–12. https://doi.org/10.3390/pharmaceutics12111090

Sarikurkcu, C., Ozer, M. S., Calli, N., & Popović-Djordjević, J. (2018). Essential oil composition and antioxidant activity of endemic Marrubium parviflorum subsp. oligodon. *Industrial Crops and Products, 119*, 209–213. https://doi.org/10.1016/j.indcrop.2018.04.023

Sebai, H., Souli, A., Chehimi, L., Rtibi, K., Amri, M., El-Benna, J., & Sakly, M. (2013). In vitro and in vivo antioxidant properties of Tunisian carob (Ceratonia siliqua L.). *Journal of Medicinal Plants Research, 7*(2), 85–90. https://doi.org/10.5897/JMPR12.915

Shah, B. B., & Mehta, A. A. (2018). In vitro evaluation of antioxidant activity of D-Limonene. *Asian Journal of Pharmacy and Pharmacology, 4*(6), 883–887. https://doi.org/10.31024/ajpp.2018.4.6.25

Sharadanand Phatak, R., Subhash Hendre, A., & Rohan Sharadanand Phatak, C. (2014). Total antioxidant capacity (TAC) of fresh leaves of Kalanchoe pinnata. *Journal of Pharmacognosy and Phytochemistry JPP, 2*(25), 32–35.

Singh, P., Shukla, R., Prakash, B., Kumar, A., Singh, S., Mishra, P. K., & Dubey, N. K. (2010). Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food and Chemical Toxicology, 48*(6), 1734–1740. https://doi.org/10.1016/j.fct.2010.04.001

Sultan, M., Hafez, O. M., Saleh, M. A., & Youssef, A. M. (2021). Smart edible coating films based on chitosan and beeswax-pollen grains for the postharvest preservation of Le Conte pear. *RSC Advances, 11*(16), 9572–9585. https://doi.org/10.1039/d0ra10671b

Taylor, S., & Todd, P. A. (1995). Understanding information technology usage: A test of competing models. *Information Systems Research, 6*(2), 144–176. https://doi.org/10.1287/isre.6.2.144

Tovar, L., Salafranca, J., Sánchez, C., & Nerín, C. (2005). Migration studies to assess the safety in use of a new antioxidant active packaging. *Journal of Agricultural and Food Chemistry, 53*(13), 5270–5275. https://doi.org/10.1021/jf050076k

Union Europea. (2011). Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Offical Journal of the European Union, 21*, 1–136.

Urbina, L., Eceiza, A., Gabilondo, N., Corcuera, M.Á., & Retegi, A. (2019). Valorization of apple waste for active packaging: multicomponent polyhydroxyalkanoate coated nanopapers with improved hydrophobicity and antioxidant capacity. *Food Packaging and Shelf Life, 21*, Article 100356. https://doi.org/10.1016/j.fpsl.2019.100356

Victoria, F. N., Lenardão, E. J., Savegnago, L., Perin, G., Jacob, R. G., Alves, D., … Nascente, P. da S. (2012). Essential oil of the leaves of Eugenia uniflora L.: Antioxidant and antimicrobial properties. *Food and Chemical Toxicology, 50*(8), 2668–2674. https://doi.org/10.1016/j.fct.2012.05.002

Wei, A., & Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils. *Journal of Agricultural and Food Chemistry, 55*(5), 1737–1742. https://doi.org/10.1021/jf062959x

Wrona, M., Cran, M. J., Nerín, C., & Bigger, S. W. (2017). Development and characterisation of HPMC films containing PLA nanoparticles loaded with green tea extract for food packaging applications. *Carbohydrate Polymers, 156*, 108–117. https://doi.org/10.1016/j.carbpol.2016.08.094

Yang, L., Liu, H., Xia, D., & Wang, S. (2020). Antioxidant properties of camphene-based thiosemicarbazones: Experimental and theoretical evaluation. *Molecules, 25*(5), 1–16. https://doi.org/10.3390/molecules25051192

Zouaoui, N., Chenchouni, H., Bouguerra, A., Massouras, T., & Barkat, M. (2020). Characterization of volatile organic compounds from six aromatic and medicinal plant species growing wild in North African drylands. *NFS Journal, 18*, 19–28. https://doi.org/10.1016/j.nfs.2019.12.001

2. Novel active biopackaging incorporated with macerate of carob (Ceratonia siliqua L.) to extend shelf-life of stored Atlantic salmon fillets (Salmo salar L.).
(Article 2)

LWT - Food Science and Technology 156 (2022) 113015

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Novel active biopackaging incorporated with macerate of carob (*Ceratonia siliqua* L.) to extend shelf-life of stored Atlantic salmon fillets (*Salmo salar* L.).

Lidia Ait Ouahioune [a], Magdalena Wrona [b], Cristina Nerín [b,*], Djamel Djenane [a]

[a] Department of Food Science, Laboratory of Food Quality and Food Safety, Mouloud Mammeri University, P.O. Box. 17, Tizi-Ouzou RP, 15000, Algeria
[b] Department of Analytical Chemistry, Aragon Institute of Engineering Research I3A, EINA - University of Zaragoza, Torres Quevedo Building, María de Luna 3, 50018, Zaragoza, Spain

ARTICLE INFO

Keywords:
Fresh salmon fillet
Carob seeds
Active biopackaging
Shelf-life
Food quality

ABSTRACT

Two antioxidant bio-based packaging materials incorporated with an 8% aqueous solution of carob seed ethanol macerate (CSE) or 8% aqueous solution of carob seed acetone macerate (CSA) were developed. Fresh salmon fillets were packaged in active and control films and stored at refrigeration temperature at 4 ± 1 °C. The quality was evaluated by sensory analysis, color, pH, water holding capacity and drip loss, thiobarbituric acid reactive substances, and total volatile basic nitrogen. The CSE and CSA samples presented satisfactory off-odor and overall acceptability results than the control samples until the 5th storage day. For color analysis, active samples preserved better the characteristics of fresh salmon than the controls during the first storage days. The fresh salmon fillet covered with active packaging presented on the 5th storage day a lower values of pH (6.54 ± 0.05 and 6.60 ± 0.11), drip loss (3.17 ± 0.76 and 2.83 ± 0.29), thiobarbituric acid reactive substances (0.056 ± 0.033 and 0.088 ± 0.054) and total volatile basic nitrogen (30.04 ± 3.54 and 32.67 ± 4.81), whereas, the highest water holding capacity values (92.23 ± 1.09 and 92.91 ± 3.07) for CSE and CSA respectively, as compared to those of blank biopackaging.

1. Introduction

Fresh Atlantic salmon fillets (*Salmo salar* L.) are popular seafood consumed worldwide because of their household convenience, high nutritional value, and beneficial health effects primarily due to the presence of omega-3 long-chain fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential and vital nutrients (Bell, Henderson, Tocher, & Sargent, 2004). However, due to their polyunsaturated fatty acids, fresh salmon fillets are easily susceptible to lipid oxidation, even at refrigeration temperatures. The oxidation process decreases the salmon quality and modifies the taste, odor, texture, and consistency. At the same time, its nutritional value decreases (Ghaly, Dave, Budge, & Brooks, 2010). Over the last decades, different searches for technologies that favor fresh salmon utilization have been intensified due to the increasing demand for high-quality fresh salmon (Fernández & Roeckel, 2009).

The improvement in preservation techniques to bring the fresh salmon safely to the consumer, simultaneously maintaining its organoleptic characteristics, are the main allies of fish industries (Soares, Silva, Barbosa, Pinheiro, & Vicente, 2017). Various works have been published applying natural antioxidants to fish preservation (Abidar et al., 2020; Djenane, 2015). Nevertheless, the direct addition of natural antioxidants into food formulations is challenging because they tend to be less potent than synthetic additives and therefore must be added in more significant amounts, which may negatively affect the organoleptic properties of the product (Nerín, Aznar, & Carrizo, 2016). Natural antioxidants could be added indirectly into the biodegradable polymer matrix. This novel strategy is known as active biopackaging (Djebari et al., 2021; Wrona, Vera, Pezo, & Nerín, 2017). It appears to be a pivotal blueprint for reducing the usage of synthetic antioxidants and consequently minimizing the astringency and bitterness of these compounds. Moreover, the application of biopackaging reduces the environmental impact of conventional packaging (Khwaldia, Ferez, Banon, Desobry, & Hardy, 2004).

* Corresponding author.
E-mail addresses: lidiaaitouahioune93@gmail.com (L.A. Ouahioune), magdalenka.wrona@gmail.com (M. Wrona), cnerin@unizar.es (C. Nerín), djenane6@yahoo.es (D. Djenane).

https://doi.org/10.1016/j.lwt.2021.113015
Received 12 September 2021; Received in revised form 29 November 2021; Accepted 21 December 2021
Available online 23 December 2021

The food processing industry generates large amounts of waste, which are considered a source of natural bioactive compounds (Zhao, Xiong, & McNear, 2013). In this context, efforts are focused on their valorization as a source of natural antioxidants (Lammi et al., 2017; Lammi, Le Moigne, Djenane, Gontard, & Angellier-Coussy, 2018).

Native to the Middle East, the carob tree (*Ceratonia siliqua* L.) is found naturally in Algeria, Spain, and other Mediterranean countries (Quezel & Santa, 1963). Carob seeds makeup 10% of total fruit weight and are considered an essential by-product that can be used in active biopackaging materials for the food industry applications. Carob seeds are a source of tocopherols and organic acids, including phenolic compounds. Antioxidant properties attributed to their bioactive compounds have been shown (Ben Ayache et al., 2020; Fidan et al., 2020).

Various *in vivo* studies have shown the antioxidant effectiveness of active antioxidant biopackaging based on biodegradable polymer materials on the shelf-life of Atlantic salmon (Cao & Song, 2020; Lan et al., 2021). Goulas et al. (2019) showed that the application of carob polyphenolic coating on the salmon produced an antioxidant effect by reducing the oxidation phenomena. It has been previously reported that when the primary free radicals are eliminated, the release of antioxidants is not required. Thus, owing to its strong radical scavenging activity, carob seed extract can be an efficient antioxidant applied without direct contact with the food matrix. Furthermore, Ait Ouahioune et al. (2022) demonstrated the strong antioxidant activities of new antioxidant biopackaging based on cellulose material incorporated with carob seeds macerates. Moreover, a migration study of antioxidants from the materials was performed according to the European Regulation EU/10/2011 for food contact materials. The obtained results showed no migrants, either in the case of the volatile or non-volatile compounds. Also, high antioxidant capacity as a free radical scavenger based on the study of Pezo, Salafranca, and Nerín (2006); Pezo, Salafranca, and Nerín (2008) has been demonstrated.

To the best of our knowledge, macerated carob seeds have not been tested as a potentially active agent in antioxidant biopackaging to extend the shelf-life of stored fresh Atlantic salmon (FAS).

The purpose of this study was to determine the antioxidant efficiency of the new antioxidant active biopackaging material containing carob seeds macerates for the stored FAS, and therefore extend the shelf-life of this product during long term storage at 4 ± 1 °C. Organoleptic assay, color, drip loss, and water holding capacity (WHC), pH, thiobarbituric acid reactive substances (TBARS), and the total volatile basic nitrogen (TVB-N) will be evaluated.

2. Material and methods

2.1. Chemicals

Trichloroacetic acid (99%, CAS 76-03-9) was provided by Sigma Aldrich (Madrid, Spain); malondialdehyde-tetrabutylammonium salt (98%, CAS 100683-54-3) and 2-thiobarbituric acid (TBA ≥98%, CAS 504-17-6) were purchased from Fluka (Madrid, Spain). Ethanol (high-performance liquid chromatography (HPLC grade, CAS 64-17-5) and acetone (UV, IR, HPLC, GPC, APS, CAS 67-64-1)) were from PanReac, AppliChem (Germany). Sodium hydroxide (NaOH, 0.25 N, CAS 1310-73-2) and sulfuric acid (H_2SO_4, 96%, CAS 7664-93-9) were from PanReac Quimica SLU (Barcelona, Spain). Methyl blue (CAS 28983-56-4), methyl red (CAS: 493-52-7), and potassium carbonate ($K_2CO_3 \geq 99\%$, CAS: 584-08-7) were obtained from Sigma-Aldrich Química S.A. (Madrid, Spain). Ultrapure water was obtained from a Wasserlab Ultramatic GR system (Barbatáin, Spain).

2.2. Antioxidant agents

Samples of carob fruit (*Ceratonia siliqua* L.) were collected during December 2018 from a carob tree located in Tizi-Ouzou (Algeria, Coordinates: 36°43′N 4°3′E), and an amount of 60 g of carob seeds (CS) was manually separated from the fruits and air-dried at room temperature (≈27 °C) for one month. Then, dried seeds were ground with an electric grinder. As extracting solvent, 80% aqueous solution of ethanol or 80% aqueous solution of acetone by maceration method from the seeds at room temperature were used according to Adilah, Jamilah, Noranizan, and Hanani (2018) with slight modifications. This process was successively repeated three times with the renewal of the solvent each 24 h. The macerates were filtered using Whatman filter paper (porosity 0.22 μm) and were stored in glass bottles in the dark at 4 ± 1 °C until further use. The following abbreviations of macerates were applied: CSE means seeds macerated with 80% ethanol; CSA means seeds macerated with 80% acetone.

2.3. Sample preparation fillet

Fresh salmon fillet (FSF) was selected to be used in the antioxidant effectiveness assays. The product was purchased whole within 24 h post-harvesting from a local seafood market in Zaragoza, Spain. Salmon was beheaded, and the bones were removed from it immediately. After that, it was cut into 23 identical fillets that weighed approximately 1500 g. The salmon slices were then transported to the analytical chemistry laboratory (Zaragoza University, Campus Rio Ebro, Spain) in a polystyrene closed box with appropriate flake ice within 10 min of arrival. The salmon slices were divided into four batches (each batch for one treatment). The skin was removed from the flesh in aseptic conditions. Slices of 22 g were prepared in triplicate for each treatment during the different storage days. A sterile knife was used for samples preparation. All the prepared samples were kept on ice until utilization to avoid deterioration. All the experiments were carried out using the same initial fresh salmon to ensure the same product quality.

2.4. Biopackaging active material

The developed packaging material was based on two cellulose (CL) polymer layers from the Nutraflex (45NK) product range supplied by Futamura UK Ltd (Burgos, Spain) laminated together with a water-based biodegradable adhesive for food packaging applications from Samtack (Barcelona, Spain). Solution (w/w) of CS macerates at a concentration of 8% in water-based biodegradable adhesive were prepared and vortexed for 1 min until complete homogenization. The active adhesive was spread on the CL sheet using the coating machine K control coater from RK print coat instruments (Litlington, UK). Wire close wound bar (Bar number: 4; color code: black; wire diameter: 0.51 mm; wet film deposit: 40 μM) was used for coating. The CL sheet was air-dried to get rid of the solvent. The CL sheet with dry adhesive was covered by another CL layer. The developed multilayer biomaterial was placed in BiO 330 A3 Heavy Duty Laminator (South Korea), and it was pressed at 40 °C with velocity number 5.

Solution (w/w) of ethanol (C1) and acetone (C2) were prepared at a concentration of 8% in water-based biodegradable adhesive as the blank biomaterial.

An amount of 22 g of FSF was placed in the Petri dish (Ø = 10 cm) covered with a 10 cm × 10 cm sheet of each active biopackaging. The sample size (n-value) for each treatment was fifteen (n = 15). The experiments were carried out without direct contact between the food sample and the active agent to simulate the most real conditions for fish packaging. Each petri dish was then carefully introduced in a cellulose bag. Also, the salmon with blank biomaterial without an active agent (C1 and C2) was prepared to compare the effect of fish spoilage. The samples were hermetically thermo-sealed and kept at 4 ± 1 °C for 13 days. All samples were prepared in triplicate and analyzed after 0, 3, 5, 8, and 13 days.

2.5. Quality assessment

The quality of the FSF was tested during the experiments by

evaluating different organoleptic properties and physicochemical parameters: pH, drip loss and WHC, color measurement, TBARS test, and TVB-N values. All the measurements were done in triplicate and performed after 0, 3, 5, 8, and 13 days of samples storage.

2.5.1. Sensory analysis

The sensory properties that were considered to discriminate between the samples for a grade of acceptability were the visual appearance of the salmon, such as color and texture, and the odor attribute, which are directly related to the consumers salmon acceptability, according to the method described in a previous study (Wrona, Vera, et al., 2017). Fresh salmon should have a bright pink or orange color. If salmon fish has a pale, dull color, means that it is likely spoiled. Salmon fish should also have fine white lines running through it, which indicate freshness.

The sensory analysis of FSF was done by five-member trained panelists selected among the workers of the analytical chemistry laboratory (University of Zaragoza, Spain). They were trained according to the method described by (Shahidi & Botta, 1994). The evaluation was based on two sensory attributes, namely off-odor and overall acceptability of FSF. The analysis consisted of 24 evaluations of salmon samples coded with random numbers. The off-odor and overall acceptability were evaluated using a 5-point scale according to Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2001). Scores for off-odor referred to the intensity of odors associated to fish oxidation were as follows: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme and were evaluated immediately after opening the package with sample. While evaluating the acceptability, 5-point hedonic scale was used, where 1 = dislike extremely; 2 = dislike; 3 = nor like or dislike; 4 = like; 5 = like extremely. Results are expressed as the predominant score given by panelists.

2.5.2. Color measurement

A colorimeter Chroma Meter CR-400 from Konica Minolta (Tokyo, Japan) with D65 as the light source was used to measure the color on the surface of salmon fillets. Each packaging was opened, samples were removed, and left for blooming for 15 min. Eighteen color determinations for each replicate were performed to cover the whole surface. CIE L* (lightness), a* (redness), and b* (yellowness) were used for the characterization of the color. The equipment was calibrated daily with white chroma meter standard plate (Y = 93.7; x = 0.3130; y = 0.3191). For each sample, the total color difference (ΔE) as an estimate of color changes was determined following equation (1):

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

The color values of fresh salmon on 0 storage day were used as reference values for ΔE calculation (L_0^*, a_0^*, b_0^*).

2.5.3. pH measurement

The pH of the salmon samples was determined according to the method described by Sallam (2007). A 5.0 g of fish sample were cut into small pieces and then homogenized with 10 mL of distilled water in a cup-blender for 30 s. Then, the pH of the resulting homogenate was measured at room temperature 25 °C using a digital pH-meter GLP 22 from Crison Instruments (Barcelona, Spain) previously calibrated at pH 4 and 7.

2.5.4. Drip loss and water holding capacity

Drip loss in salmon samples for the different storage days was expressed as the difference in fillet weight between day 0 (m_0) and day x (m_x) and was calculated according to equation (2)

$$DL = (m_0 - m_x/m_0) \times 100 \quad (2)$$

WHC was measured in the dorsal muscle from all salmon fillets at 0, 3, 5, 8, and 13 storage days. The dry content (D_0) of the muscle was determined by drying 2 g of each sample for 24 h at 105 °C until the equilibrium weight was obtained; hence water content (V_0) was determined (ISO-6496, 1999). Meanwhile, a piece of salmon was weighed (2 g) and placed in a tube with a pre-weighed filter paper (Whatman N °1). The tubes were then centrifuged using CENTROMIX model S-549 from JP Selecta (Barcelona, Spain) at rpm 30*100 for 15 min at room temperature. The exudate filtered through the filter paper was collected at the bottom of the centrifuge tube. The samples were weighed before and after this procedure. The results were expressed as the amount of sample remaining after centrifugation and were calculated according to equation (3):

$$WHC = (W_0 - \Delta W / W_0) \times 100 \quad (3)$$

where

$$W_0 = V_0/(V_0 + D_0) \times 100 \quad (4)$$

$$\Delta W = \Delta V_0/(V_0 + D_0) \times 100 \quad (5)$$

V_0 = the initial water content of the muscle.

D_0 = the initial dry matter of the muscle.

ΔV_0 = the weight of the liquid separated from the sample during centrifugation

2.5.5. Oxidative stability

The lipid oxidation study of salmon samples was performed by the TBARS method as described by Djenane, Aboudaou, Ferhat, Ouelhadj, and Ariño (2019). Briefly, 10 g of FSF were mixed with 40 mL of a 10% aqueous solution of trichloroacetic acid (TCA) until a homogeneous suspension was obtained. The supernatant was filtered using Whatman N°1 filter paper. Then, 2 mL of the filtrate were mixed with 2 mL of an aqueous solution of thiobarbituric acid (TBA) at a concentration of 20 mM. The mixture was heated to 97 °C for 20 min and then cooled to room temperature. The absorbance was measured at 532 nm using a spectrophotometer UV-1700 (Shimadzu Pharmaspec Iberica, Madrid, Spain) against a reference blank containing the TBA reagent. All the measurements were prepared in triplicate. To calculate the concentration of secondary lipid oxidation product, a calibration curve was prepared using a malondialdehyde solution (MDA) in the range 0.1 − 0.8 μg/g. Results were expressed as mg of MDA per kg of fish.

2.5.6. TVB-N content

The TVB-N content was determined using the Conway micro diffusion method. A slice of salmon (4 g) was weighed, transferred to the stomacher bag, and homogenized with 15 mL of water for 2 min at 265 rpm. Then, 10 mL of 10% TCA (w/v) were added and homogenized for 4 min to eliminate the protein content. The slurry was collected, filtered, and centrifuged. The test was performed using the micro-diffusion chamber of Conway. Briefly, 1 mL of a saturated potassium carbonate solution was placed in the outermost area of the chamber and mixed with 1 mL of the supernatant. One mL of sulfuric acid was added to the central compartment of the chamber. Finally, the chamber was closed, sealed, and carefully mixed by circular movement, avoiding mixing the liquids in different compartments. After that, incubation was performed at 35 °C for 1 h. The sulfuric acid was titrated by a 0.1 N solution of sodium hydroxide using a micro-burette. Two drops of indicator solution were added (methyl red-methylene blue indicator).

The amount of TVB-N was calculated by equation (6).

$$TVB\text{-}N = [(Vac - Vba) \times 0.14 \times 25/ (Vm \times PM)] \times 100 \quad (6)$$

where Vac is the volume of sulfuric acid (1 mL), Vba is the volume of NaOH consumed in the titration, Vm is the volume of sample added to the Conway cell (1 mL), and PM represents the salmon weight (4 g). The TVB-N was expressed as mg N/100 g fish.

2.6. Statistics

Experiments were performed at least in triplicate. The results were expressed as mean ± standard deviations. The statistical significance of differences among different treatment and storage periods was evaluated by one-way analysis of variance (ANOVA) followed by the Post Hoc HSD Tukey test with significance at $p < 0.05$. The sample size was n = 15. A correlation matrix (CM) of data was performed, which shows the quantitative assessment classes of severability. The normality and homogeneity of variance assumptions were tested and checked using Cochran's C test, Harley and Bartlett, with P < 0.0063.

Principal component analysis (PCA) was performed to reduce the multivariate data's dimensionality, visualize them graphically, with minimal loss of information, and identify different groups of FAS samples. This multivariate analysis allowed to summarize the information included in the variables studied into a few principal components or factors, providing a simplified interpretation of data variance through mathematical methods. All data were statistically evaluated using STATISTICA version 7.1 (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Sensory analysis

In the present study, the cellulose biopolymer selected as the substrate is from natural resources and emerges as a response to growing attention to environmental pollution and environmental footprint. It was combined with active agents from carob seeds macerates that have antioxidant effects for the development of active bio-based packaging systems to improve the shelf-life of FAS. These compounds were considered agro-food waste (Santonocito et al., 2020). This approach is very interesting, as it creates an added value to this industrial by-product and reduces the total price of the final packaging system (Quiles-Carrillo, Mellinas, Garrigos, Balart, & Torres-Giner, 2019).

The results of the evaluation of the sensory characteristics such as smell and overall acceptability in the salmon fillet influenced by the developed multilayer active biopackaging on 0, 3, 5, 8, and 13 storage days at 4 ± 1 °C are shown in Fig. 1.

On the 3rd and 5th storage days, samples wrapped with both antioxidant biopackaging material were acceptable and had a pleasant odor, characteristic of fresh fish compared to samples covered with blank biopackaging material, which were directly rejected on the 5th storage day. Regarding the results after the 5th storage day, it can be observed that in all cases, samples packaged with CSE and CSA biomaterial presented lower scores of off-odor attributes and higher scores of the acceptability attributes than the corresponding salmon fillet packaged with blank biopackaging material. However, the scores of off-odor attributes increased significantly. In contrast, overall acceptability scores decreased significantly ($p < 0.05$) as the storage time increased. All samples presented off-odor and not acceptable characteristics and were considered spoiled, where the rancidity and ammonia fishy smell were the highest in the case of salmon covered with blank biomaterial. That might be caused by lipid and protein oxidation (Guyon, Meynier, & de Lamballerie, 2016). The scores recorded for smell and overall acceptability displayed that the addition of carob seeds macerates considerably protected the sensory characteristics of the salmon throughout the storage period.

This fact may be due to the presence of antioxidant compounds, particularly volatile compounds and non-volatile ones. Ait Ouahioune et al. (2022) identified the volatile compounds composition of carob seeds macerates and the results revealed that they were rich in volatile bioactive compounds characterized by antioxidant properties such as monoterpenes and sesquiterpenes, which have been proven to have an antioxidant capacity as free radical quenchers and act through either the hydrogen donor or electron donor mechanism (Djebari et al., 2021).

The odor effect of carob agent incorporated in the active biomaterial on samples packaged with CSE and CSA biomaterial were evaluated by comparing them to the blank samples, and no odor effect of carob seeds was detected in the samples of both kinds of active packaging, especially in the 3rd and 5th storage day.

3.2. Color analysis

The color of the salmon fillet surface is an important quality attribute for consumers' acceptability (Merlo et al., 2019). The data of the evaluation of color *CIE L*a*b** throughout the storage time showed significant interaction ($p < 0.05$) between treatments and storage time (Table 1). The initial color parameters values obtained in the FSF were as follows: 49.55 ± 1.73 for L^* (lightness), 12.43 ± 1.52 for a^* (redness), and 15.41 ± 0.99 for b^* (yellowness). According to the literature, there are several reasons for color differences of salmon muscle, which different contents could cause in astaxanthin carotenoid and the haem pigments (Yagiz et al., 2010).

Fig. 2 shows the photos of the salmon samples on the 5th storage day. The three replicates of each sample look similar, and satisfactory reproducibility was obtained for them. Spoilage color resulting in the change of salmon color can be observed in the case of samples from blank packaging (C1 and C2), whereas the samples from active packaging (CSE and CSA) retained the reddish color of fresh salmon. After the 5th storage day, all samples exhibited a slight apparent increase in the lightness and yellowness parameters, whereas the redness parameter decreased during the storage period.

In contrast, as the storage time increased, the samples covered with CSE and CSA active packaging retained the color characteristics of fresh salmon (lower L^* and b^*-value and higher a^*-value) than the samples from blank packaging. This can be explained by the structural changes caused by protein denaturation, which increases the light absorption and scattering on the surface of the salmon fillet (Lerfall, Bendiksen, Olsen, & Østerlie, 2016; Merlo et al., 2019; Van Haute, Raes, Devlieghere, & Sampers, 2017).

Regarding the change in a^*-value parameter, this can be mainly attributed to the potential oxidation of the salmon pigments, responsible for the typical red-orange color of Atlantic salmon caused by carotenoids (mainly astaxanthin and canthaxanthin) besides haem proteins (Giménez, Roncalés, & Beltrán, 2005).

According to Ruff, FitzGerald, Cross, and Kerry (2002), the color muscle becomes more yellow as the malondialdehyde concentration resulting from lipid oxidation increases. In parallel, the yellowish color is related to the interaction between the aldehydes as products from a lipid auto-oxidation with amino groups of proteins. Similar phenomenon was obtained in previous studies (Giménez et al., 2005; Merlo et al., 2019). This is reflected in the value of the calculated color change (ΔE) parameter between fresh salmon and samples from different storage days. As described by Tiwari, Muthukumarappan, O'Donnell, and Cullen (2008), the total color difference can be perceived as small for $\Delta E < 1.5$, distinct for ΔE between 1.5 and 3, and very distinct for $\Delta E > 3$. Thus, the changes in salmon color could be classified as small for the active samples on the 5th storage day, whereas very distinct and visible for the human eyes on the last storage day, being similar to control samples (Table 1). Therefore, the developed active bio-based packaging incorporated with macerates of carob seeds as agro-food waste had a certain preservative effect on the color by minimizing the oxidation of pigment of salmon, which may be due to the presence of active compounds present in CSE and CSA that eliminate the free radicals responsible for the initiation of the oxidation phenomenon from the package headspace, thus avoiding the direct contact of active agents with the packaged FSF (Carrizo et al., 2015).

3.3. pH

The pH measurement is used as a spoilage indicator in fishery products and can provide interesting information on the state of

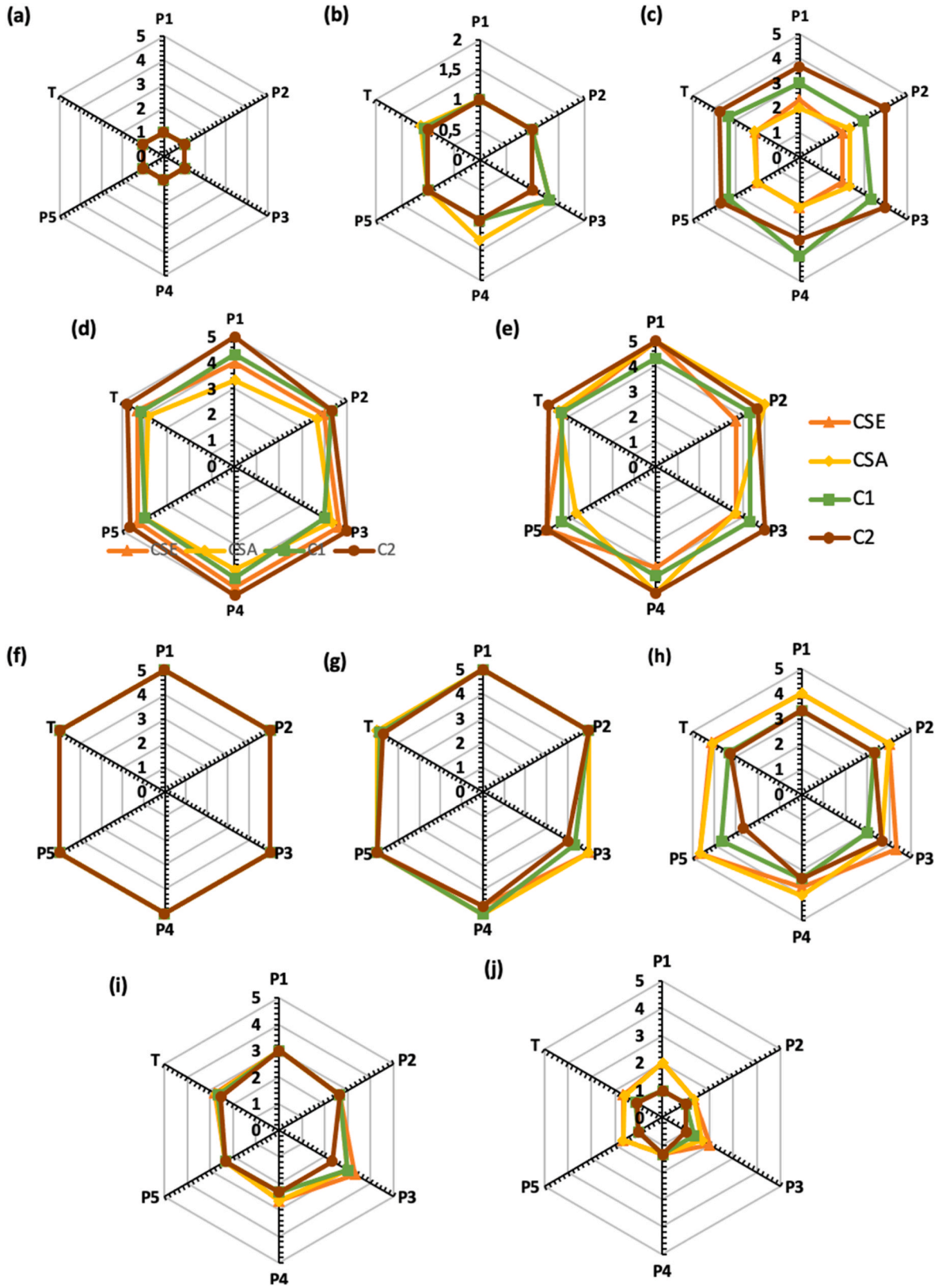

Fig. 1. Sensorial characteristics of CSE, CSA, C1 and C2 samples. Scores (1–5) are means of three evaluations of five panellists. Where: (a), (b), (c), (d) and (e): Off-Odor parameter of samples according to storage time (Day 0, Day 3, Day 5, Day 8 and Day 13), respectively. Where: (f), (g), (h), (i) and (j): Overall acceptability parameter of samples according to storage time (Day 0, Day 3, Day 5, Day 8 and Day 13), respectively.

Table 1
Instrumental color (L^*, a*, b*) and total color change ΔE during refrigerated storage (4 °C) of FAS fillets*.

Parameters	Treatments	Storage time				
		Day 0	Day 3	Day 5	Day 8	Day 13
L*	C1	49.55 ± 1.73^{Aa}	49.48 ± 0.25^{Aa}	50.85 ± 1.29^{Aa}	53.19 ± 1.72^{Ab}	53.76 ± 1.82^{Ab}
	C2	49.55 ± 1.73^{Aa}	49.75 ± 0.47^{Aa}	52.29 ± 1.40^{Ab}	52.74 ± 1.79^{Ab}	53.97 ± 1.46^{Ab}
	CSA	49.55 ± 1.73^{Aa}	49.79 ± 1.74^{Aa}	50.63 ± 1.80^{Aab}	52.45 ± 1.16^{Abc}	53.68 ± 1.61^{Ac}
	CSE	49.55 ± 1.73^{Aa}	49.81 ± 1.16^{Aa}	50.73 ± 1.62^{Aa}	52.74 ± 1.79^{Ab}	53.03 ± 1.54^{Ab}
a*	C1	12.43 ± 1.52^{Ab}	12.23 ± 0.44^{ABab}	11.97 ± 1.13^{Aab}	11.92 ± 0.81^{Aab}	11.06 ± 0.9^{Aa}
	C2	12.43 ± 1.52^{Aa}	12.01 ± 0.58^{Aa}	12.60 ± 1.49^{Aa}	11.51 ± 1.06^{Aa}	11.29 ± 1.20^{Aa}
	CSA	12.43 ± 1.52^{Aa}	12.64 ± 1.16^{ABa}	12.68 ± 1.07^{Aa}	12.19 ± 0.71^{Aa}	11.46 ± 0.41^{Aa}
	CSE	12.43 ± 1.52^{Aa}	13 ± 0.62^{Ba}	12.40 ± 0.59^{Aa}	12.20 ± 1.67^{Aa}	11.74 ± 0.72^{Aa}
b*	C1	15.41 ± 0.99^{Aa}	15.88 ± 0.47^{ABa}	16.03 ± 0.92^{ABa}	16.09 ± 0.88^{Aa}	16.37 ± 0.72^{Aa}
	C2	15.41 ± 0.99^{Aa}	15.82 ± 0.93^{ABab}	15.87 ± 0.87^{ABab}	16.16 ± 1.25^{Aab}	17.01 ± 1.66^{Ab}
	CSA	15.41 ± 0.99^{Aa}	16.22 ± 0.82^{Bab}	16.40 ± 0.44^{Bab}	16.61 ± 0.79^{Ab}	16.90 ± 1.16^{Ab}
	CSE	15.41 ± 0.99^{Aa}	15.03 ± 0.71^{Aa}	15.30 ± 1.17^{Aa}	15.66 ± 1.08^{Aa}	15.86 ± 1.12^{Aa}
ΔE	C1	0	0.52	1.51	3.74	4.53
	C2	0	0.62	2.77	3.39	4.83
	CSA	0	0.86	1.49	2.28	4.50
	CSE	0	0.63	1.19	3.21	3.58

*Values are mean ± SD of six replicates (n = 6). Different uppercase letters indicate statistically significant differences between analyzed samples (A < B < C) (column), whereas different lowercase letters indicate statistically significant differences between storage time (a<b < c < d) (lines), using Post-hoc HSD Tukey test ($p < 0.05$). CSE: carob seed ethanol; CSA: carob seed acetone; C1: control 1 and C2: control 2.

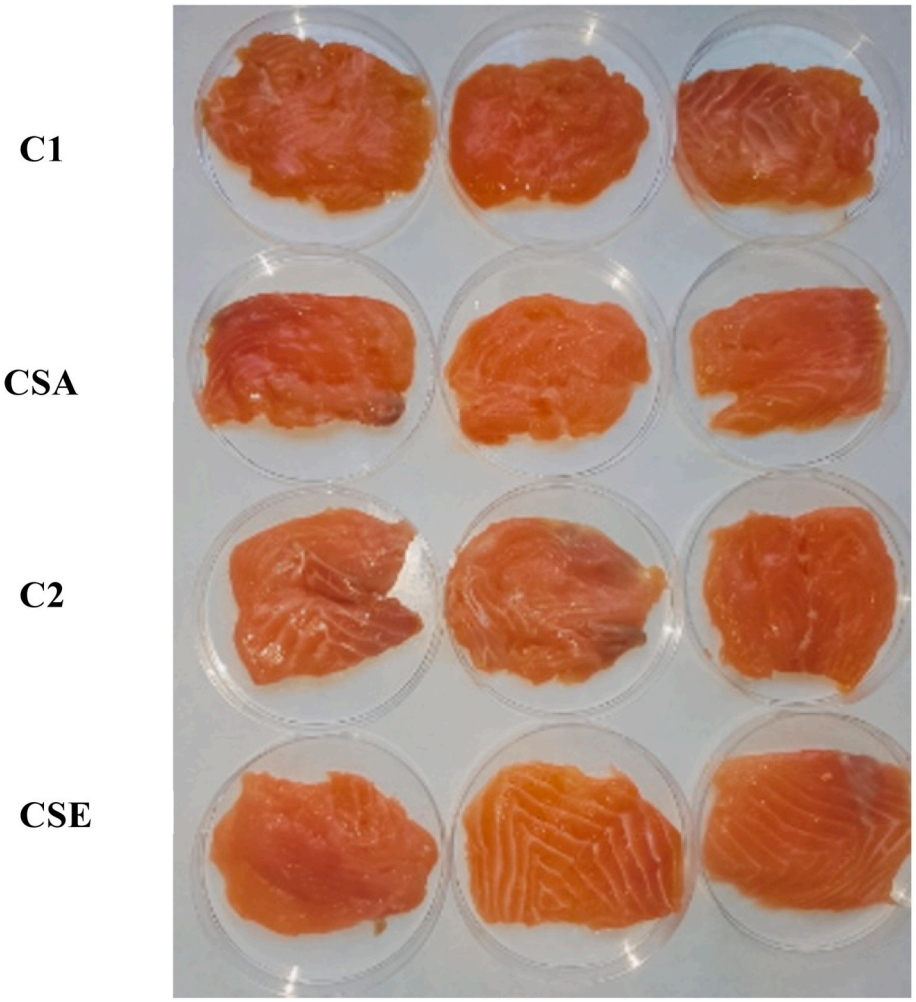

Fig. 2. Sample of Petri dish with sliced salmon fish placed in blank packaging C1 and C2 (without active agent) and active packaging consisted of CSA and CSE active film at the 5th day of experiment.

freshness and quality of these products (Freitas, Vaz-Pires, & Câmara, 2019). Values of the pH changes of FAS samples are shown in Fig. 3a. As it can be seen, the initial pH value (day 0) of fresh salmon fillet obtained in this study was 6.40 ± 0.01, similar to this obtained in the previous work of Xiong, Kamboj, Ajlouni, and Fang (2021) on the FSF with values of 6.02 ± 0.02 and 6.22, respectively. These results are within the

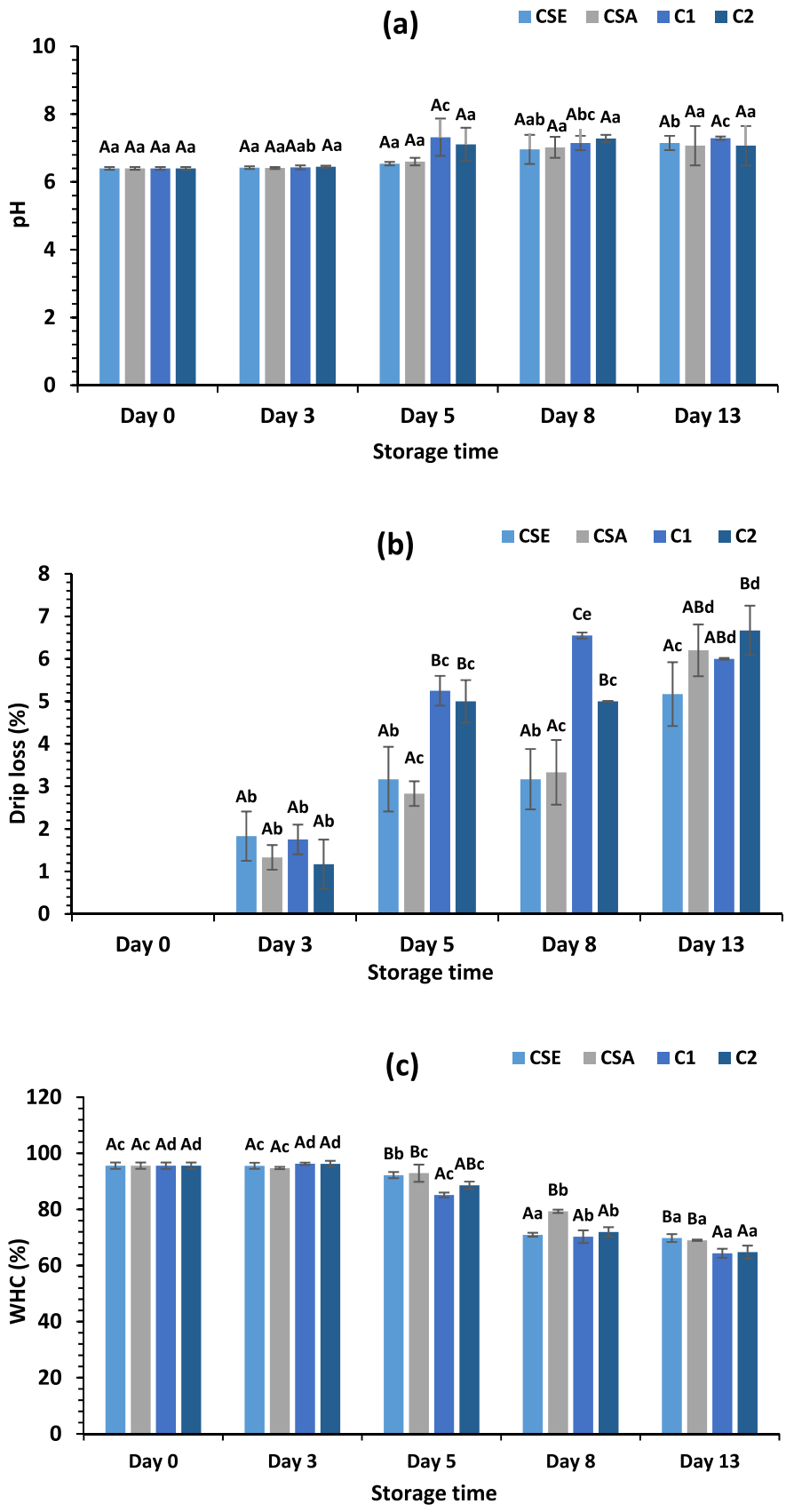

Fig. 3. Results of a) pH measurement of salmon samples at different day of storage; b) drip loss measurement of samples during salmon samples during storage time; c) WHC measurement of the different salmon samples. The results are the mean ± SD of 3 replicates (n = 3). Different uppercase letters indicate significant differences between treatments analyzed (A < B < C), whereas different lowercase letters indicate significant differences between storage time (a<b < c < d). CSE: carob seed ethanol; CSA: carob seed acetone; C1: control 1 and C2: control 2.

normal pH range for fresh salmon (~6.2) (FDA U.S. Food and Drug Administration, 2009). Throughout storage, blank samples showed a faster increase in pH values, which reached 7.32 ± 0.55 and 7.11 ± 0.49 for C1 and C2 on the 5th storage day, respectively. These results confirmed that the blank samples were degrading at a faster rate during the storage time. However, results with mean values of 6.60 ± 0.11 and 6.54 ± 0.05 were obtained in the case of samples packaged with active materials in the same storage day, which are within limits required by legislation (Júnior & de Oshiro, 2017) and validated as the threshold for fish food freshness and safety (pH ≤ 7.0), indicating that these samples were degrading more slowly than the control samples. The reason for such difference between samples from active bio-based packaging and controls can be related to the antimicrobial action of the natural agents present in the carob seeds macerates, which inhibit the bacteria that cause protein degradation and the production of basic compounds responsible for the pH increase (Vatavali, Karakosta, Nathanailides, Georgantelis, & Kontominas, 2013). After the 5th storage day, the pH significantly increased ($p < 0.05$) in the course of the refrigerated storage for all treatments, which is associated with the production of basic amines through protein breakdown by the action of spoilage bacteria (Karabagias, Badeka, & Kontominas, 2011). It was reported that the pH increases with microbial spoilage. The reason for this is related to the formation of nitrogenous compounds such as ammonium and biogenic amines as a result of enzymatic activity and the proteolytic activity of psychrophilic bacteria (Debevere & Boskou, 1996).

3.4. Drip loss and WHC

The changes in protein-protein conformation and denaturation of important proteins, leading to the exudation from muscles of what is collected as drip loss (Fidalgo et al., 2019). The drip loss directly quantifies the loss of saleable weight and/or the deterioration of appearance and further facilitates surface microbial growth (Duun & Rustad, 2008). The result from Fig. 3b shows a significant ($p < 0.05$) increase of liquid loss through storage time. As it can be seen, samples covered with active bio-based packaging presented lower values than the control, which can be explained by the presence of antimicrobial agents in the CSE and CSA macerates that inhibit the microbial growth responsible for the structural changes in the muscle, such as proteolytic degradation of myofibrillar proteins and increased extracellular space (Kaale, Eikevik, Rustad, & Nordtvedt, 2014). Similar results were reported by Rollini et al. (2016), who demonstrated that the carvacrol-coextruded multilayer film effectively prevented microbial spoilage in fresh salmon. Another study on the valorization of carob fruit wastes to prepare bifunctional coating showed a preservative effect on salmon fillet quality by reducing bacterial growth (Goulas et al., 2019). As the storage time increased, the values increased until the last storage day and reached values of 6.00 ± 0.00%; 6.67 ± 0.58%; 5.17 ± 0.75%, and 6.20 ± 0.61% for C1, C2, CSE, and CSA, respectively, indicating the degradation of FSF samples for all treatments.

A useful tool for describing the quality of muscle foods *post-mortem* is to measure the WHC of the muscle, which is the ability of a muscle to resist water loss. It is an important quality parameter for raw Atlantic salmon, as it affects both profitability and quality by affecting the weight change during transport and storage (Lakshmanan, Parkinson, & Piggott, 2007).

In the current study, the initial mean value WHC of 95.59 ± 1.12% was found in fresh salmon, whereas values decreased significantly during refrigerated storage for all kinds of samples. However, the C1 and C2 samples showed the lowest WHC on the 13th storage day, differing significantly from CSA and CSE samples (Fig. 3c). These induced effects could be explained by the structural change in the muscle and denaturation of important myofibrillar and/or sarcoplasmic proteins. These results confirmed that the control samples were degrading at a faster rate during the storage time. However, samples covered with active bio-based packaging degraded slowly, which was demonstrated by the highest WHC. These results may be related to the active compounds of CSE and CSA macerates, such as antimicrobial agents, as previously explained, which retained better the characteristics of FSF than the control samples. A similar effect was reported elsewhere (Hultmann & Rustad, 2002; Lerfall, Bendiksen, Olsen, & Østerlie, 2015). There are indications that proteolytic enzymes, possibly originating from bacteria, are responsible for the postmortal degradation of extracellular matrix components and influence the WHC (Olsson, Seppola, & Olsen, 2007).

3.5. TBARS

TBARS is a measure of MDA, which is a good indicator for determining the progress of lipid oxidation and carbonyl and aldehyde production (Azizi-Lalabadi, Rafiei, Divband, & Ehsani, 2020). An external calibration curve was carried out to quantify the MDA in the range between 0.1 and 0.8 μg/g of MDA solution.

The TBA values for all salmon slices samples are shown in Fig. 4a. The initial value of TBA was 0.02 ± 0.03 μg MDA/g of salmon. In general, the results showed a significant ($p \leq 0.05$) increase of TBA values for all samples after the 3rd storage day, where at the 5th day, the rate of oxidation of samples packaged with CSE and CSA active films (0.056 ± 0.033 μg MDA/g of salmon and 0.088 ± 0.054 μg MDA/g of salmon, respectively) were lower than the corresponding values of the control group, reaching values of 0.188 ± 0.011 μg MDA/g of salmon and 0.166 ± 0.027 μg MDA/g of salmon for C1 and C2, respectively. Obtained results may be attributed to the increased oxidation of unsaturated fatty acids and partial dehydration of the salmon fillet. The difference of obtained results between control and active samples is mainly due to the antioxidant activity of carob seeds macerates, based on natural antioxidant compounds immobilized in the multilayer biomaterial (Ait Ouahioune et al., 2022). The mechanism of antioxidant protection that active compounds from carob seeds macerates offer to FSF does not imply a positive migration of antioxidants to salmon but a real scavenging and non-migrating system that can take place without direct contact between the carob seeds macerates and the fresh salmon (Carrizo, Taborda, Nerín, & Bosetti, 2015; Vera et al., 2016; Wrona, Bentayeb, & Nerín, 2015).

This new kind of active packaging works differently than those usually conceived as the material supplying antioxidants or acting as oxygen absorbers. At the same time, it's a concept of a free radical scavenger (Carrizo et al., 2015). The oxidation process is a reaction of radicals initiated by OH· and O· free radicals. When they are removed, the reaction does not take place, as it was demonstrated in the previous works of Pezo, Salafranca, and Nerín (2006); Pezo et al. (2008); Colon and Nerin (2015); Carrizo et al. (2015); Nerín (2011); Dicastillo et al. (2011). The free radicals have a short life, as they are very reactive, and can easily permeate through the polymers. Thus, they will arrive at the bioactive compounds positions, where they were grafted in the adhesive formulate and trapped by the carob seeds molecules endowed with antioxidant properties as a free radical scavenger and therefore protects the FSF versus oxidation processes (Moudache, Nerin, Colon, & Zaidi, 2017). There are a few studies regarding the active antioxidant packaging formulated with natural antioxidants from the carob (Ait Ouahioune et al., 2022; Goulas et al., 2019). It was reported that the packaged salmon with carob polyphenolic coating retains the quality of salmon by reducing lipid oxidation, avoiding the presence of undesirable off-flavors in salmon fillets during refrigerated storage at 6 °C (Goulas et al., 2019). According to Castro, Andrade, Silva, Vaz, and Vilarinho (2019), the TBA values of 0.5 μg MDA/g are the acceptable levels of MDA in the fish flesh, which normally correspond to a development of an objectionable odor. Therefore, the TBA values of our samples covered with CSE and CSA active films during all the storage period was lower than that allowed for the fresh fish for the perception of lipid oxidation, except for the C1 on the last storage day. Thus, these findings indicate that both active bio-based films incorporated with the carob seeds macerates, considered agro-food waste, had the most

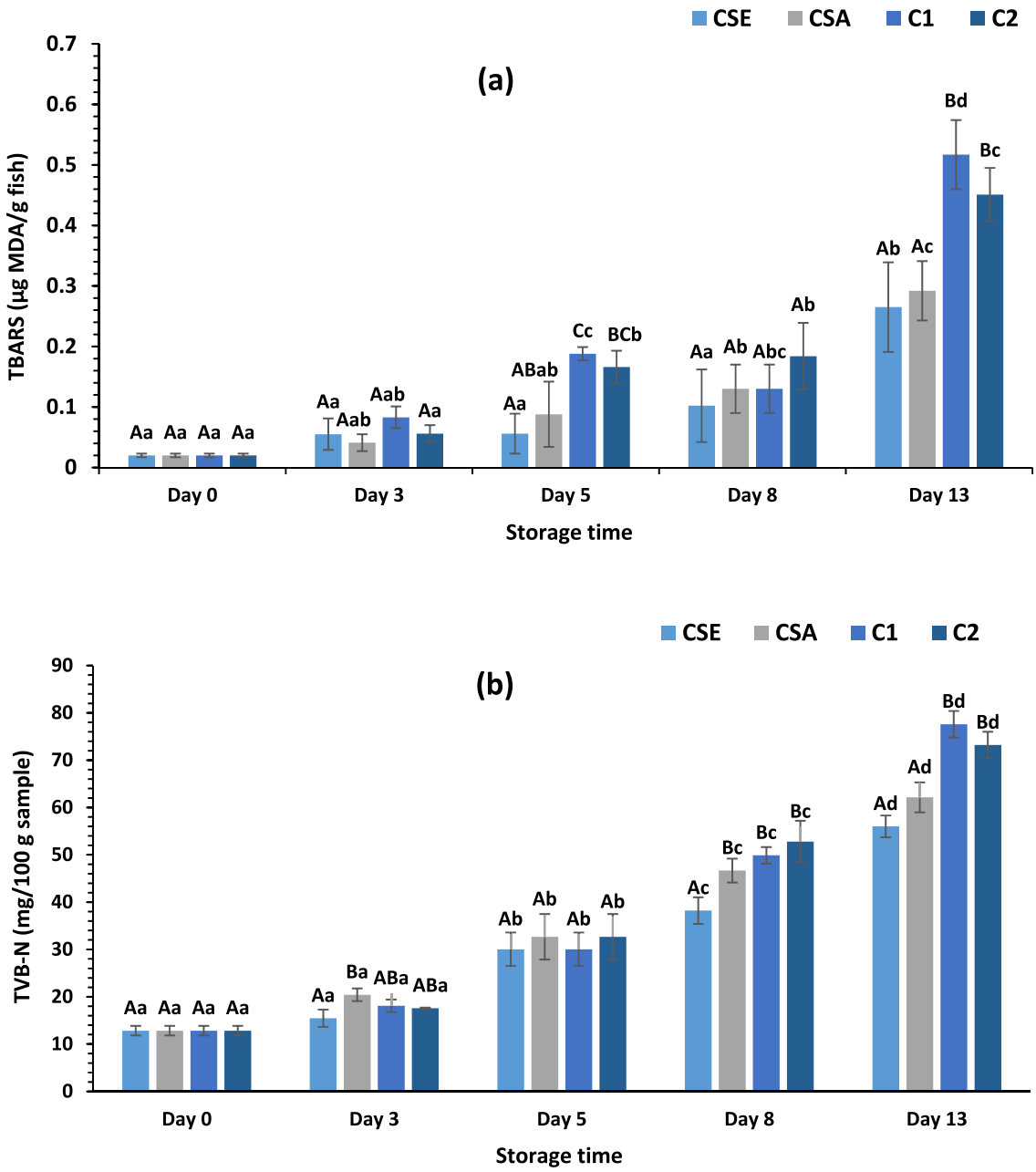

Fig. 4. Results of a) Concentration of thiobarbituric acid (TBA) (mg of malondialdehyde/100 g of sample) in the different salmon samples (*Salmo salar* L.) stored at 4 °C; b) TVB-N values for salmon samples during 14 days of storage at 5 °C. The results are the mean ± SD of three replicates (n = 3). Different uppercase letters indicate statistically significant differences between samples analyzed (A < B < C) (column), whereas different lowercase letters indicate statistically significant differences between storage time (a<b < c < d) (lines), using Post-hoc HSD Tukey test ($p < 0.05$). CSE: carob seed ethanol; CSA: carob seed acetone; C1: control 1 and C2: control 2.

protective effect on the delay of fat oxidation in the FSF. Moreover, they could also be suitable for packaging other types of food such as meat. Nevertheless, scale-up tests regarding this type of packaging are mandatory to assess its commercial and economic viability and add value to several industries' by-products.

3.6. TVB-N

TVB-N is one of the most widely used indices of seafood quality. It is a term that includes measurement of trimethylamine, dimethylamine, ammonia, and other compounds associated with seafood spoilage, which increases as spoilage progresses (Mohan, Ravishankar, Lalitha, & Srinivasa Gopal, 2012). Fig. 4b shows the evolution of TVB-N values versus the storage period. As can be seen at the beginning of storage, FSF showed a TVB-N value of 12.83 ± 1.01 mg N/100 g of salmon, which is slightly higher than that reported by Alves et al. (2018). The values of TVB-N increased significantly ($p < 0.05$) throughout the storage time for all studied samples, showing on day 13 values of 77.59 ± 2.81; 73.21 ± 2.81; 56 ± 2.31 and 62.13 ± 3.16 mg N/100 g of salmon for C1, C2, CSE, and CSA samples, respectively. TVB-N increase is related to spoilage by bacteria and the activity of endogenous enzymes. CSE and CSA active biopackaging showed lower values than the control samples, mainly between the 3rd and 5th storage days. Reasons for these differences include the enhanced microbial load or the ability of the carob seeds

extract to de-aminate non-protein nitrogen compounds (Ojagh, Núñez-Flores, López-Caballero, Montero, & Gómez-Guillén, 2011).

High TVB-N values are undesirable since they indicate that salmon fillets are spoiled. Based on the sensory attributes of the current study, the more realistic TVB-N limit of 35 mg N/100 g of fish was proposed as the upper limit above, which the salmon is considered spoiled and not suitable for human consumption and within the value that was also established by Regulation (EC) No 2074/2005. As can be seen, a significant increase in the TVB-N values of the different samples after the 5th storage day was observed (Fig. 4b), exceeding the maximum limit of acceptability, indicating a good correlation with the sensory analysis results.

3.7. Correlation matrix and multivariate statistical analysis

Table S1 in supplementary material shows the correlation matrix among the different parameters used to evaluate the FSF quality. Different values corresponded to the discrimination indexes between parameters. The results showed a highly significant correlation between pH, TBA, TVB-N, WHC, sensory attributes, and L^* parameters, whereas a significant correlation with a^* and b^* parameters was obtained.

Multivariate statistical analysis was applied to compare C1, C2, CSE, and CSA salmon samples stored. The technique most used is PCA, which allows the reduction of a large set of multivariate data into a small number of principal components and gives a simplified interpretation of data. In the current study, the observations are the different samples of packaged FSF, and the variables are the different parameters used to evaluate the sample quality. The PCA score biplot with the projection of the individuals on the factorial plane (1 × 2) and projection of the variables on the factorial plane (1 × 2) based on storage time are displayed in Fig. 5a and b, respectively.

As shown in Fig. 5b, the correlation between the results of the different parameters used in the salmon quality assessment was obtained, agreeing with the results of the correlation matrix. PC1 and PC2 (85.21% and 5.65%, respectively) explained 90.86% of the total variation. Thus, they can be used as an adequate explanation of the data.

In total, 10 standardized variables were introduced to create the covariance matrix. The first principal component (PC1) that represented 85.21% of the variance was found to be highly correlated with all the variables, with a positive correlation coefficient for drip loss (0.931), pH (0.909), TBA (0,901), TVB-N (0.979), off-odor (0.956), L^* (0.969) and b^* (0.756), whereas negative with WHC (−0.961), overall acceptability (−0.990) and a^* (−0.853). The PC2 (5.65% of the variance) had a positive correlation coefficient with drip loss (0.165), pH (0.286), off-odor (0.231), L^* (0.143) and a^* (0.287) and negative with TBA (−0.233), TVB-N (−0.084), WHC (−0.095), overall acceptability (−0.034) and b^* (−0.478). Some of the variables are highly correlated and appeared together in the biplot (Fig. 5b). At the right, pH, off-odor drip loss, TVB-N, TBA and b^*. At the left, are overall acceptability, WHC and a^*.

Four groups were observed in the score plot (Fig. 5a). As it can be seen, it is possible to observe that all the clusters appear distinct, but, in some cases, there is not a clear separation between the samples of the same cluster. The CSAD3, CSAD5, C1D3, C2D3 emerged into a cluster with the samples of day 0 showing the similar characteristic of FAS. CSAD5 and CSED5 samples were clustered with samples of day 0 and samples of day 3, respectively. This phenomenon can be explained by the effectiveness of the active biopackaging incorporated with CSA and CSE macerates in the deterioration delay of the FAS compared with C1D5 and C2D5, which degraded at a faster rate (clustered together C1D8, C2D8, CSED8, CSED13). Along with the storage time, CSED13 and CSAD8 emerged with samples of day 8 and day 13, respectively, showing that samples from CSE retain better the characteristic of salmon samples than CSA on the last storage day. The results of PCA demonstrated that there was some useful information on the effect of active biopackaging on the shelf-life extension of salmon samples compared to the control samples.

4. Conclusions

A new antioxidant cellulose biofilm based on the incorporation of carob seeds macerates was successfully developed. This study reports the beneficial effect of CSA and CSE biopackaging on FSF samples stored at 4 ± 1 °C, especially in maintaining the color values closer to those of fresh salmon at the beginning of storage and keeping salmon samples with lower pH, TBA, TVB-N, drip losses values, and higher WHC values. Moreover, satisfactory sensory evaluation results were obtained with samples acceptable between the 3rd and 5th days compared to the control samples. In conclusion, the developed active biopackaging can scavenge the free radicals and inhibit lipids and proteins' oxidation. It is

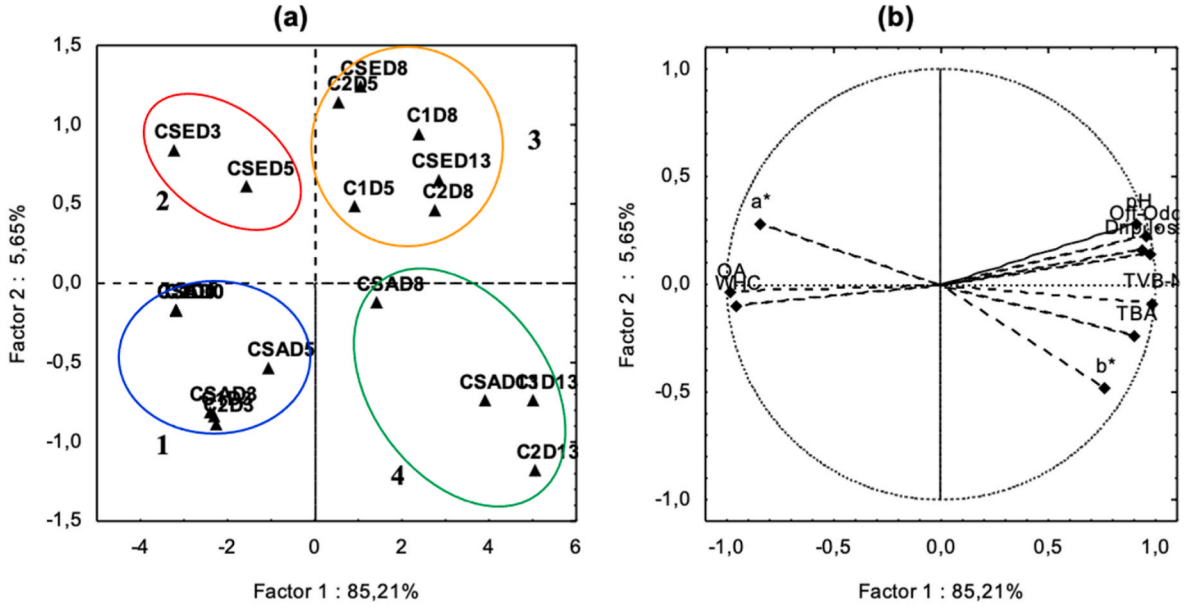

Fig. 5. Principal component analysis (PCA) biplot; (a): projection of the individuals on the factorial plane (1 × 2). (b): projection of the variables on the factorial plane (1 × 2). CSE: carob seed ethanol; CSA: carob seed acetone; C1: control 1 and C2: control 2. D1; D3; D5; D8 and D13: storage days.

an effective, promising, and efficient method for increasing the shelf-life quality of fresh salmon fish, suggesting its application in the food industry. It is considered a sustainable approach to synthetic plastic material, allowing an added value to the wastes of food industries.

CRediT authorship contribution statement

Lidia Ait Ouahioune: Methodology, Conceptualization, Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Magdalena Wrona:** Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Cristina Nerín:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Djamel Djenane:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Ministry of Higher Education and Scientific Research (MESRS) and the Government of Algeria for the doctoral scholarship for Lidia Ait Ouahioune (Process 2019/2020). The authors also wish to thank the Project RTI2018-097805-B-I00 financed by Ministerio de Ciencia e Innovación, Spain; Government of Aragon and the European Social Fund for financial support (T53-20R) to the GUIA group. Special thanks are given to our colleague from the University of Bejaia (Algeria): Mr. Otmani Amar, for his valuable help with the Statistical analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.113015.

References

Abidar, S., Boiangiu, R. S., Dumitru, G., Todirascu-Ciornea, E., Amakran, A., Cioanca, O., et al. (2020). The aqueous extract from ceratonia siliqua leaves protects against 6-hydroxydopamine in zebrafish: Understanding the underlying mechanism. *Antioxidants, 9*(4), 304. https://doi.org/10.3390/antiox9040304

Adilah, A. N., Jamilah, B., Noranizan, M. A., & Hanani, Z. A. N. (2018). Utilization of mango peel extracts on the biodegradable films for active packaging. *Food Packaging and Shelf Life, 16*, 1–7. https://doi.org/10.1016/j.fpsl.2018.01.006, 2018.

Ait Ouahioune, L., Wrona, M., Becerril, R., Salafranca, J., Nerin, C., & Djenane, D. (2022). Ceratonia siliqua L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications. *Food Packaging and Shelf Life, 31*. https://doi.org/10.1016/j.fpsl.2021.100764, 100764.

Alves, V. L. C. D., Rico, B. P. M., Cruz, R. M. S., Vicente, A. A., Khmelinskii, I., & Vieira, M. C. (2018). Preparation and characterization of a chitosan film with grape seed extract-carvacrol microcapsules and its effect on the shelf-life of refrigerated Salmon (Salmo salar). *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 89*, 525–534. https://doi.org/10.1016/j.lwt.2017.11.013, 2018.

Azizi-Lalabadi, M., Rafiei, L., Divband, B., & Ehsani, A. (2020). Active packaging for Salmon stored at refrigerator with Polypropylene nanocomposites containing 4A zeolite, ZnO nanoparticles, and green tea extract. *Food Sciences and Nutrition, 8*(12), 6445–6456. https://doi.org/10.1002/fsn3.1934

Bell, J. G., Henderson, R. J., Tocher, D. R., & Sargent, J. R. (2004). Replacement of dietary fish oil with increasing linseed oil levels: Modify flesh fatty acid compositions in atlantic salmon (Salmo salar) using a fish oil finishing diet. *Lipids, 39*(3), 223–232. https://doi.org/10.1007/s11745-004-1223-5

Ben Ayache, S., Behija Saafi, E., Emhemmed, F., Flamini, G., Achour, L., & Muller, C. D. (2020). Biological activities of aqueous extracts from carob plant (ceratonia siliqua L.) by antioxidant, analgesic and proapoptotic properties evaluation. *Molecules, 25*(14), 3120. https://doi.org/10.3390/molecules25143120

Cao, T. L., & Song, K. B. (2020). Development of bioactive Bombacaceae gum films containing cinnamon leaf essential oil and their application in packaging of fresh salmon fillets. *Lebensmittel-Wissenschaft & Technologie, 131*, 1–8. https://doi.org/10.1016/j.lwt.2020.109647, 109647.

Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2015). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science And Emerging Technologies, 33*, 534–541. https://doi.org/10.1016/j.ifset.2015.10.018

Castro, F. V. R., Andrade, M. A., Silva, A. S., Vaz, M. F., & Vilarinho, F. (2019). The contribution of a whey protein film incorporated with green tea extract to minimize the lipid oxidation of salmon (Salmo salar L.). *Foods, 8*(8), 327. https://doi.org/10.3390/foods8080327

Colon, M., & Nerin, C. (2015). Development and application of an analytical procedure for specific migration of green tea compounds in IV gamma nectarine active packaging. *Food Control, 57*, 419–425. https://doi.org/10.1016/j.foodcont.2015.04.035

Debevere, J., & Boskou, G. (1996). Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *International Journal of Food Microbiology, 31*(1–3), 221–229. https://doi.org/10.1016/0168-1605(96)01001-X

Dicastillo, C. L., Nerín, C., Alfaro, P., Catalá, R., Gavara, R., & Hernandez-Muñoz, P. (2011). Development of new antioxidant active packaging films base on ethylene vinyl alcohol copolymer (EVOH) and green tea extract. *Journal of Agricultural and Food Chemistry, 59*, 7832–7840. https://doi.org/10.1021/jf201246g

Djebari, S., Wrona, M., Boudria, A., Salafranca, J., Nerin, C., Bedjaoui, K., et al. (2021). Study of bioactive volatile compounds from different parts of Pistacia lentiscus L. extracts and their antioxidant and antibacterial activities for new active packaging application. *Food Control, 120*, 1–8. https://doi.org/10.1016/j.foodcont.2020.107514, 107514.

Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of algerian citrus essential oils and their application in sardina pilchardus. *Foods, 4*(2), 208–228. https://doi.org/10.3390/foods4020208

Djenane, D., Aboudaou, M., Ferhat, M. A., Ouelhadj, A., & Ariño, A. (2019). Effect of the aromatisation with summer savory (Satureja hortensis L.) essential oil on the oxidative and microbial stabilities of liquid whole eggs during storage. *Journal of Essential Oil Research, 31*(5). https://doi.org/10.1080/10412905.2019.1610516

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2001). Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. *Journal of Food Science, 66*(1), 181–186. https://doi.org/10.1111/j.1365-2621.2001.tb15603.x

Duun, A. S., & Rustad, T. (2008). Quality of superchilled vacuum packed Atlantic salmon (Salmo salar) fillets stored at _1.4 and _3.6 _C. *Food Chemistry, 106*(1), 122e131.

FDA U.S. Food and Drug Administration. (2009). CFSAN—bad bug book—pH values of various foods. Available from: www.fda.gov. (Accessed 29 June 2009).

Fernández, K., Aspe, E., & Roeckel, M. (2009). Shelf-life extension on fillets of Atlantic Salmon (*Salmo salar*) using natural additives, superchilling and modified atmosphere packaging. *Food Control, 20*(11), 1036–1042. https://doi.org/10.1016/j.foodcont.2008.12.010

Fidalgo, L. G., Castro, R., Trigo, M., Aubourg, S. P., Delgadillo, I., & Saraiva, J. A. (2019). Quality of fresh atlantic salmon (Salmo salar) under hyperbaric storage at low temperature by evaluation of microbial and physicochemical quality indicators. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-019-02346-3

Fidan, H., Stankov, S., Petkova, N., Petkova, Z., Iliev, A., Stoyanova, M., et al. (2020). Evaluation of chemical composition, antioxidant potential and *functional* properties of carob (Ceratonia siliqua L.) seeds. *Journal of Food Science & Technology, 57*(7), 2404–2413. https://doi.org/10.1007/s13197-020-04274-z

Freitas, J., Vaz-Pires, P., & Câmara, J. S. (2019). Freshness assessment and shelf-life prediction for Seriola dumerili from aquaculture based on the quality index method. *Molecules, 24*(19), 3530. https://doi.org/10.3390/molecules24193530

Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences, 7*, 846–864. https://doi.org/10.3844/ajassp.2010.859.877

Giménez, B., Roncalés, P., & Beltrán, J. A. (2005). The effects of natural antioxidants and lighting conditions on the quality of salmon (Salmo salar) fillets packaged in modified atmosphere. *Journal of the Science of Food and Agriculture, 85*(6), 1033–1040. https://doi.org/10.1002/jsfa.2055

Goulas, V., Hadjivasileiou, L., Primikyri, A., Michael, C., Botsaris, G., Tzakos, A. G., et al. (2019). Valorization of carob fruit residues for the preparation of novel bi-functional polyphenolic coating for food packaging applications. *Molecules, 24*(17), 1–10. https://doi.org/10.3390/molecules24173162

Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology, 50*, 131–143. https://doi.org/10.1016/j.tifs.2016.01.026

Hultmann, L., & Rustad, T. (2002). Textural changes during iced storage of salmon (Salmo salar) and cod (Gadus morhua). *Journal of Aquatic Food Product Technology, 11*(3/4), 105e123. https://doi.org/10.1300/J030v11n03_09

ISO-6496. (1999). No Determination of moisture and other volatile matter content. In *Animal feeding stuffs*.

Júnior, J. M., & de Oshiro, M. (2017). Atualizações importantes introduzidas pelo novo Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal: Decreto n° 9.013 de 29 de março de 2017. *Vigilância Sanitária em Debate. 5. Vigilância Sanitária em Debate*, 73–80. https://doi.org/10.22239/2317-269x.01019

Kaale, L. D., Eikevik, T. M., Rustad, T., & Nordtvedt, T. S. (2014). Changes in water holding capacity and drip loss of Atlantic salmon (Salmo salar) muscle during superchilled storage. *LWT – Food Science and Technology, 55*, 528–535. https://doi.org/10.1016/j.lwt.2013.10.021

Karabagias, I., Badeka, A., & Kontominas, M. G. (2011). Shelf life extension of lamb meat using thyme or orégano essential oils and modified atmosphere packaging. *Meat Science, 88*, 109–116. https://doi.org/10.1016/j.meatsci.2010.12.010

Khwaldia, K., Ferez, C., Banon, S., Desobry, S., & Hardy, J. (2004). Milk proteins for edible films and coatings. *Critical Reviews in Food Science and Nutrition, 44*(4), 239–251. https://doi.org/10.1080/10408690490464906

Lakshmanan, R., Parkinson, J. A., & Piggott, J. R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (Salmo salar). *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 40*(3), 544–551. https://doi.org/10.1016/j.lwt.2005.12.003

Lammi, S., Barakat, A., Mayer-Laigle, C., Djenane, D., Gontard, N., & Angellier-Coussy, H. (2017). Dry fractionation of olive pomace as a sustainable process to produce fillers for biocomposites. *Powder Technology, 326*, 44–53. https://doi.org/10.1016/j.powtec.2017.11.060, 2018.

Lammi, S., Le Moigne, N., Djenane, D., Gontard, N., & Angellier-Coussy, H. (2018). Dry fractionation of olive pomace for the development of food packaging biocomposites. *Industrial Crops and Products, 120*, 250–261. https://doi.org/10.1016/j.indcrop.2018.04.052, 2018.

Lan, W., Wang, S., Zhang, Z., Liang, X., Liu, X., & Zhang, J. (2021). Development of red apple pomace extract/chitosan-based films reinforced by TiO2 nanoparticles as a multifunctional packaging material. *International Journal of Biological Macromolecules, 168*, 105–115. https://doi.org/10.1016/j.ijbiomac.2020.12.051, 2021.

Lerfall, J., Bendiksen, E.Å., Olsen, J. V., & Østerlie, M. (2016). A comparative study of organic- versus conventional Atlantic salmon. II. Fillet color, carotenoid- and fatty acid composition as affected by dry salting, cold smoking and storage. *Aquaculture, 451*, 369–376. https://doi.org/10.1016/j.aquaculture.2015.10.004

Lerfall, J., Roth, B., Skare, E. F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M. A., et al. (2015). Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon *(Salmo salar L.)* during ice storage. *Food Chemistry, 175*, 157–165. https://doi.org/10.1016/j.foodchem.2014.11.111

Merlo, T. C., Contreras-Castillo, C. J., Saldaña, E., Barancelli, G. V., Dargelio, M. D. B., Yoshida, C. M. P., et al. (2019). Incorporation of pink pepper residue extract into chitosan film combined with a modified atmosphere packaging: Effects on the shelf life of salmon fillets. *Food Research International, 125*, 1–10. https://doi.org/10.1016/j.foodres.2019.108633, 2019.

Mohan, C. O., Ravishankar, C. N., Lalitha, K. V., & Srinivasa Gopal, T. K. (2012). Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (Sardinella longiceps) during chilled storage. *Food Hydrocolloids, 26*(1), 167–174. https://doi.org/10.1016/j.foodhyd.2011.05.005

Moudache, M., Nerin, C., Colon, M., & Zaidi, F. (2017). Antioxidant effect of an innovative active plastic film containing olive leaves extract on fresh pork meat and its evaluation by Raman spectroscopy. *Food Chemistry, 229*, 98–103. https://doi.org/10.1016/j.foodchem.2016.06.001

Nerín, C. (2011). Antioxidant active packaging and antioxidant edible films Chapter 31 in the book "Oxidation in foods and beverages and antioxidant applications". In E. A. Decker, R. J. Elias, & D. J. McClements (Eds.), *Oxidation in foods and beverages and antioxidant applications* (1st ed., pp. 496–515). Cambridge, United Kingdom: Woodhead Publishing. Management in Different Industry Sectors, 2.

Nerín, C., Aznar, M., & Carrizo, D. (2016). Food contamination during food process. *Trends in Food Science & Technology, 48*, 63–68. https://doi.org/10.1016/j.tifs.2015.12.004

Ojagh, S. M., Núñez-Flores, R., López-Caballero, M. E., Montero, M. P., & Gómez-Guillén, M. C. (2011). Lessening of high-pressure-induced changes in Atlantic salmon muscle by the combined use of a fish gelatin-lignin film. *Food Chemistry, 125*(2), 595–606. https://doi.org/10.1016/j.foodchem.2010.08.072

Olsson, G. B., Seppola, M. A., & Olsen, R. L. (2007). Water-holding capacity of wild and farmed cod (Gadus morhua) and haddock (Melanogrammus aeglefinus) muscle during ice storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 40*(5), 793–799. https://doi.org/10.1016/j.lwt.2006.04.004

Pezo, D., Salafranca, J., & Nerín, C. (2006). Design of a method for generation of gas-phase hydroxyl radicals, and use of HPLC with fluorescence detection to assess the antioxidant capacity of natural essential oils. *Analytical and Bioanalytical Chemistry, 385*, 1241–1246. https://doi.org/10.1007/s00216-006-0395-4

Pezo, D., Salafranca, J., & Nerín, C. (2008). Determination of the antioxidant capacity of active food packagings by in situ gas-phase hydroxyl radical generation and high-performance liquid chromatography–fluorescence detection. *Journal of Chromatography A, 1178*(1–2), 126–133. https://doi.org/10.1016/j.chroma.2007.11.062

Quezel, P., & Santa, S. (1963). *Nouvelle flore de l'Algérie et des régions désertiques méridionales (tome 1) (Editions d)*.

Quiles-Carrillo, L., Mellinas, C., Garrigos, M. C., Balart, R., & Torres-Giner, S. (2019). Optimization of microwave-assisted extraction of phenolic compounds with antioxidant activity from carob pods. *Food Analytical Methods. Food Analytical Methods*. https://doi.org/10.1007/s12161-019-01596-3

Regulation (EC) No 2074/2005. Official Journal of the European union, 27–59.

Rollini, M., Nielsen, T., Musatti, A., Limbo, S., Piergiovanni, L., Hernandez Munoz, P., et al. (2016). Antimicrobial performance of two different packaging materials on the microbiological quality of fresh salmon. *Coatings, 6*(1), 6. https://doi.org/10.3390/coatings6010006

Ruff, N., FitzGerald, R. D., Cross, T. F., & Kerry, J. P. (2002). Fillet shelf-life of atlantic halibut Hippoglossus hippoglossus L. fed elevated levels of α-tocopheryl acetate. *Aquaculture Research, 33*(13), 1059–1071. https://doi.org/10.1046/j.1365-2109.2002.00770.x

Sallam, K. I. (2007). Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chemistry, 101*(2), 592–600. https://doi.org/10.1016/j.foodchem.2006.02.019

Santonocito, D., Granata, G., Geraci, C., Panico, A., Siciliano, E. A., Raciti, G., et al. (2020). Carob seeds: Food waste or source of bioactive compounds? *Pharmaceutics, 12*(11), 1–12. https://doi.org/10.3390/pharmaceutics12111090

Shahidi, F., & Botta, J. R. (1994). Seafoods: Chemistry, processing technology and quality. *In Seafoods: Chemistry, Processing Technology and Quality*. https://doi.org/10.1007/978-1-4615-2181-5

Soares, N., Silva, P., Barbosa, C., Pinheiro, R., & Vicente, A. A. (2017). Comparing the effects of glazing and chitosan-based coating applied on frozen salmon on its organoleptic and physicochemical characteristics over six-months storage. *Journal of Food Engineering, 194*, 79–86. https://doi.org/10.1016/j.jfoodeng.2016.07.021, 2017.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2008). Kinetics of freshly squeezed orange juice quality changes during ozone processing. *Journal of Agricultural and Food Chemistry, 56*(15), 6416–6422. https://doi.org/10.1021/jf800515e

Union Europea. (2011). In *, 21. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food Offical Journal of the European Union* (pp. 1–136).

Van Haute, S., Raes, K., Devlieghere, F., & Sampers, I. (2017). Combined use of cinnamon essential oil and MAP/vacuum packaging to increase the microbial and sensorial shelf life of lean pork and salmon. *Food Packaging and Shelf Life, 12*, 51–58. https://doi.org/10.1016/j.fpsl.2017.02.004

Vatavali, K., Karakosta, L., Nathanailides, C., Georgantelis, D., & Kontominas, M. G. (2013). Combined effect of chitosan and oregano essential oil dip on the microbiological, chemical, and sensory attributes of red porgy (*Pagrus pagrus*) stored in ice. *Food and Bioprocess Technology, 6*, 3510–3521. https://doi.org/10.1007/s11947-012-1034-z

Vera, P., Echegoyen, Y., Canellas, E., Nerin, C., Palomo, M., Madrid, Y., et al. (2016). Nano selenium as antioxidant agent in a multilayer food packaging material. *Analytical and Bioanalytical Chemistry*, 1–12. https://doi.org/10.1007/s00216-016-9780-9

Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). *Food Control, 54*, 200–207. https://doi.org/10.1016/j.foodcont.2015.02.008

Wrona, M., Nerin, C., Alfonso, M. J., & Caballero, M. A. (2017). Antioxidant packaging with encapsulated green tea for fresh minced meat. *Innovative Food Science & Emerging Technologies, 41*, 307–313. https://doi.org/10.1016/j.ifset.2017.04.001

Wrona, M., Vera, P., Pezo, D., & Nerín, C. (2017). Identification and quantification of odours from oxobiodegradable polyethylene oxidised under a free radical flow by headspace solid-phase microextraction followed by gas chromatography-olfactometry-mass spectrometry. *Talanta, 172*, 37–44. https://doi.org/10.1016/j.talanta.2017.05.022

Xiong, Y., Kamboj, M., Ajlouni, S., & Fang, Z. (2021). Incorporation of salmon bone gelatine with chitosan, gallic acid and clove oil as edible coating for the cold storage of fresh salmon fillet. *Food Control, 125*, 1–9. https://doi.org/10.1016/j.foodcont.2021.107994, 107994.

Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Raghavan, S., & Marshall, M. R. (2010). Correlation between astaxanthin amount and a* value in fresh Atlantic salmon (Salmo salar) muscle during different irradiation doses. *Food Chemistry, 120*(1), 121–127. https://doi.org/10.1016/j.foodchem.2009.09.086

Zhao, J., Xiong, Y. L., & McNear, D. H. (2013). Changes in structural characteristics of antioxidative soy protein hydrolysates resulting from scavenging of hydroxyl radicals. *Journal of Food Science, 78*(2), 152–159. https://doi.org/10.1111/1750-3841.12030

V. General discussion

This research is a part of a project "Active packaging for food preservations " for the development of active packaging systems from natural resources such as cellulose for packaging of fresh food (project code: RTI2018-097805-B-100, funded by Ministry of Science and Innovation, Spain). The target was to increase the storage period of foods and maintain their organoleptic and nutritional properties during storage. The package that would be biodegradable at the end of its life and therefore environmentally friendly was highly sought after.

The objective of this thesis consisted to study the antioxidant and the antimicrobial properties of extracts from plants with a potential application in the food industry. The scientific challenge of this work was to understand the impact of antioxidant bio-packaging incorporated with active agents on the extension of the shelf life of a food product.

In order to respond for the purpose of our study, we opted for the valorization of *Ceratonia siliqua* L. seeds, leaves and kibbles. The choice of these by-products was done for the availability and abundance of these biomasses in the study region, also to remedy to an environmental problem which is pollution by eliminating the conventional packaging based on fossil resources.

1. Valorization and analysis of carob active macerates for their application in active packaging

In recent years, development of eco-friendly alternatives such as bio-based food packaging materials is one of the current research trends to face the environmental problems associated to the accumulation of wastes from food industry **(Al-Tayar *et al.*, 2019)**. Since the present study is recorded in a context of reduction of environmental pollution. For this, we first recovered the three parts of the carob which are: leaves, seeds and kibbles, which were the subject of our study. Macerates of kibbles, seeds and leaves of *Ceratonia siliqua* L. were prepared and analyzed to apply them for the development of a new multilayer active packaging (Article 1).

Different tests were chosen based on previous studies to confirm the compounds responsible for the antioxidant activity of the different extracts.

The different macerates were analyzed for the determination of their volatile compounds composition, employing HS-SPME-GC-MS. The profile of forty-three (43) different bioactive volatile compounds was obtained.

Thirteen (13) different chemical classes of bioactive volatile compounds, characterized by different antioxidant mechanisms, were identified, such as monoterpenes and sesquiterpenes, which can act as free radical quenchers and function through either the hydrogen (H) donor or electron (e^-) donor mechanism **(Djebari *et al.*, 2021).** Comparison with data from the literature is not easy, so diverse factors (Plant, season, climate, variety, extraction process, etc.) can influence **(Moudache *et al.*, 2017)**. According to the literature, studies have identified different chemical classes of volatile compounds. In this context, **Ben Ayache *et al.* (2020)**, described the volatile compounds profile and phytochemical content of *Ceratonia siliqua* L., where fifty (50) different components have been identified. **Krokou *et al.* (2019)** found 163 molecules as volatile components in Spanish carob deseeded pods, mainly aliphatic acids (77.5%) and aliphatic esters (10.52%). In contrast, a Tunisian report **(Ben Hsouna *et al.*, 2011)** has identified 25 different compounds in the essential oil of carob pods, with a complex mixture of hydrocarbons, terpenoids, esters, alcohols, ketones, fatty acids and aldehydes. The differences may be explained by the dissimilarity in cultivars and the ecological factors affecting the growth process, also because of the different types of extract and sampling techniques (**Ben Ayache *et al.*, 2020).** In the present work, the solvent also intervenes in the variability of the results.

The antioxidant activity of the different macerates was investigated using the following methods: DPPH and phosphomolybdenum method, which demonstrated the best antioxidant capacity in the case of seeds macerates. This strong activity can be explained by the greater number of bioactive volatile and non-volatiles compounds in the carob seed macerates endowed with antioxidant activity. According to **Santonocito *et al.* (2020),** the strong antioxidant activity of carob seed macerates can be related to the phytochemical composition of the seeds extracts, especially to its volatile and non-volatile compounds composition. Another studies have shown the antioxidant activity of carob extracts **(Benchikh *et al.*, 2014; Goulas & Georgiou, 2019; Mekhoukhe *et al.*, 2018; Quiles-Carrillo *et al.*, 2019)**. Most of these works have studied different phenolics extraction conditions, and they have directly related the content of phenolic compounds with the antioxidant activity. **Benchikh *et al.* (2014)** showed that carob pods have the highest total phenolic, total flavonoid and ascorbic acid content in the unripe stage. Therefore, the antioxidant activity decreases significantly throughout the ripening process. Similar results were obtained by **Saci *et al.* (2019)**, suggesting that the extract of unripe carob may provide a substantial source of secondary metabolites, which act as natural antioxidants.

The determination of the non-volatile compounds which may comprise phenolic compounds with antioxidant activity are not discussed in the present thesis (results under analysis). However, we are intensively working with data analysis of the non-volatile compounds analyzed by UPLC-ESI-Q-TOF-MS^E with software Masslynx used for the data acquisition and the identification of the non-volatile compounds. The results of the analysis of non-volatile compounds will be included in another manuscript that will be published after.

The *Ceratonia siliqua* L. leaves, seeds and kibbles were incorporated into a water-based adhesive layer and its antioxidant capacity was evaluated by two methods: DPPH assay and the hydroxyl radical generation. The applied procedure of DPPH test is commonly used during the investigation of new active packaging, including multilayer systems. However, it is not a direct analysis method of the antioxidant capacity in the case of analysis of films. Therefore, additional analysis of the antioxidant capacity should be done to have more extensive data. Because of it, we performed parallel analysis that introduces the samples into an in-situ generator of hydroxyl radicals. This method has been developed in the analytical chemistry laboratory (University of Zaragoza, Spain) and is more realistic, as it measures the real antioxidant capacity of the film itself containing the antioxidants, without extraction of active substances.

Food packaging protects foodstuffs, but it can also constitute a source of chemical food contamination due to migration processes. Migration is defined as the mass transfer between the packaging material and the packaged food. Materials for food packaging must not under any circumstances cause unacceptable changes in the composition, taste and odour of the product, nor may release substances in quantities that are dangerous to the health of consumers. Especially plastics must meet strict formal requirements. In Europe national legislation and community level legislation continue to coexist. At the Union level, food contact materials are regulated under the EU Framework Regulation **(EC) No 1935/2004 (EU, 2004)** and the Specific Regulation **(EU) No 10/2011 (EU, 2011)** applies on plastics. In the present work, the films with the best antioxidant activity, containing carob seeds macerates, were selected for the food safety assessment through migration assay, which have been performed using two different food simulants (10% and 95% solutions of ethanol) for 3 days at 5 ºC. The simulants and parameters were based on recommendations of legislation for food contact materials, already mentioned above. The conditions of migration tests were selected according to the intended use of the developed active biofilm such as the

conservation of fresh fish. Therefore, qualitative analysis of volatile and non-volatile compounds was performed and it was noticed that no migrants were detected.

Consequently, the absence of the migration process means that this new kind of active packaging works differently than those usually conceived either as the material supplying antioxidants or acting as oxygen absorber, while, it's a concept of free radical scavenger. In this point, it is important to understand the oxidation process, which is a radical reaction initiated by the presence of $OH^{\cdot}$ and $O^{\cdot}$ free radicals. It is a chain reaction including initiation, propagation and termination. Therefore, when free radicals are removed, the reaction does not take place (**Pezo *et al*., 2006; Pezo *et al*., 2008; Nerín, 2011; Dicastillo *et al*., 2011; Colón & Nerín, 2015; Carrizo *et al*., 2015)**. The free radicals have short-life, are very reactive and can easily permeate through the polymers. Thus, they will arrive at the bioactive compounds positions, where they were grafted in the adhesive formulate and trapped by the molecules endowed with antioxidant properties as free radical scavenger. Therefore, the approach is not due to the migration of bioactive compounds or to the oxygen absorption but a real scavenger system that protects the food versus oxidation processes **(Djebari *et al*., 2021)**.

Antibacterial activity were performed for the various macerates studied against some bacterial strains. The results obtained were unsatisfactory in terms of zone of inhibition, which makes the use of these extracts in the bioactive packaging for the prevention of microbial alterations ineffective, limiting our study only to evaluate the antioxidant performance of the new biofilm. Whereas, the study of **Goulas *et al*. (2019)** on the formation of polyphenolic coatings as an alternative strategy to produce sustainable packaging materials for foods, showed that the polyphenols from carob can produce antimicrobial materials for food packaging, which opens new horizons for the use of green polyphenolic coatings in food packaging. In contrast, **Ben Othmen *et al*. (2020**), demonstrated that the ethanolic extract of carob leaf exhibited a strong antibacterial activity against tested bacteria at higher concentrations. The leaf extracts of carob (*Ceratonia siliqua*) showed an antimicrobial activity in vitro against *P. astrosepticum* **(Meziani *et al*., 2015)**.

The influence of active agents on the polymer matrix was evaluated by measuring the colour parameters, which is very important factor that determine its production on an industrial scale, introducing the product to the market and purchasing the product by the consumer.

The visual examination of all the biofilms showed a light painted colour compared to the blank biofilms without macerates. Almost all values were recorded with only a slight increase. The fundamental differences in colour (ΔE) were calculated, and results showed that the highest value corresponded to CKE (ΔE =2.87), and the lowest one was found in CSE (ΔE = 0.76) as the film less affected by the addition of the macerate in the adhesive formulation. Our results can be explained by the application of the minimum possible concentration of macerate (8%).

2. The antioxidant efficiency of the antioxidant active biopackaging for the stored Fresh salmon fillet

The second part of the thesis was devoted to the study of the effect of the antioxidant active packaging incorporated with the carob seed macerates on the shelf-life of the FSF (Article 2). The choice of the FSF is due to its nutritional and economic importance and for its high consumer consumption.

For this purpose, two antioxidant bio-based packaging materials incorporated with an 8% aqueous solution of carob seed ethanol macerate (CSE) or 8% aqueous solution of carob seed acetone macerate (CSA) were developed. FSF were packaged in active and control films and stored at refrigeration temperature at 4 ± 1°C and were evaluated for its quality.

Fresh salmon is a highly perishable product and has a very short storage life, which limits its distribution and marketing (**Gómez-Estaca *et al.*, 2018).** The spoilage of fresh salmon during storage is mainly caused by enzymatic reactions (microbial and autolytic degradation), thus generating off-flavors **(Powell *et al.*, 2015).** Additionally, lipid oxidation of polyunsaturated fatty acids is one of the limiting factors of shelf life. The oxidation produces volatiles compounds that affect sensory properties **(Jacobsen *et al.*, 2019),** influencing the acceptance of fresh salmon. Different quality parameters were used in the present study to evaluate the salmon samples of each treatments.

Two sensorial attributes were used in the salmon sensorial analysis such as: overall acceptability and smell. To discriminate the samples from the grade of acceptability, the visual appearance of the salmon, such as color and texture, and the odor attribute were used. If salmon fish has a pale, dull color, this means that it is spoiled. The odor effect of active agents from carob seeds macerates was evaluated in comparison with control and no odor was detected, since the new material developed as an alternative to thus modifying the production line or the characteristics of the packaged product, where the active agents were incorporated

in the layer in contact with food. But, it is a real scavenger system, as the free radicals can efficiently diffuse through the polymer, they can be trapped by the antioxidant agents incorporated in the adhesive between polymer films of a multilayer packaging, thus avoiding the direct contact of active agents with the packaged salmon **(Ait Ouahioune *et al*., 2021).**

On the other hand, the color of the salmon fillet surface is an important quality attribute for consumers' acceptability. The reddish color in fresh salmon is associated with market acceptance and receives different prices depending on perceived color **(Lerfall *et al*., 2015)**. The structural changes of muscle proteins increase the light scatter and the lightening (L*) of the surface salmon fillet over time. These structural changes were more evident in the control samples, which exhibited a greater muscle proteolysis (lower WHC and lower hardness) and, consequently, greater light scattering and higher L* values at the end of storage. In parallel, the lipid auto-oxidation products, the aldehydes, can interact with the amino groups of proteins and lead to the development of yellowish color **(Zhang *et al*., 2013)**.

The pH measurement demonstrated a significant increase for all the samples. Whereas, lower pH values were recorded in the case of samples covered with active packaging. The reason for such difference may be due to the antimicrobial action of some natural active agents present in the carob seeds macerates, which inhibit the bacteria that cause protein degradation and the production of basic compounds responsible for the pH increase **(Vatavali *et al*., 2013).**

Furthermore, drip loss and WHC are also an important quality parameters, and they are closely related to textural properties. High WHC has an effect of post-mortem structural changes in the muscle, inducing drip loss that implies nutrients loss. The results of the present study demonstrated a significant decrease in WHC during storage time, while a significant increase was showed in the case of drip loss. The comparison between results from samples covered with active packaging demonstrated lower values of drip loss and higher values of WHC than the control samples, indicating that blank samples were degraded at faster rate during storage, which confirmed that active agents of carob macerates inhibited the microbial growth in FSF.

The evaluation of the lipid oxidation is the essential point of this study. TBA-RS was used to assess the liberation of MDA concentration in the different samples. The results demonstrated a significant increase in the MDA concentration in each kind of samples. The CSE and CSA were the samples that recorded lower values of MDA than the control samples.

It is important to underline that the new multilayer active biopackaging works differently than the other kinds of packaging, as it is a new approach of free radical scavenging system and not a migration of active compounds. In these multilayer films, active agents were not in direct contact with salmon samples, but in indirect contact, as they were grafted on the internal layer of the multilayer by the adhesive used to build the laminate.

Total volatile basic nitrogen (TVB-N) is often used as a biomarker protein and amine degradation, which is the results of the spoilage of mechanisms that cause accumulation of organic amines **(Bekhit *et al*., 2021**). Regarding TVB-N of the present study, results demonstrated an increased values of each treatments, where lower values were recorded in the case of active samples ($p < 0.05$). The obtained results can be explained by the antimicrobial action of active agents from carob macerates. Similar results were obtained by **Alves *et al*. (2018)**, which may be attributed to the antimicrobial activity of chitosan and the extracts present in the microcapsules, contributing to the reduction of the bacterial population or the reduction of the capacity of the bacteria to perform oxidative deamination.

Finally, a statistical tool was used to give a simplified interpretation of data, which confirmed the correlation between the results of the different parameters used in the salmon quality assessment. Moreover, the positive effect of active biopackaging on the shelf life extension of salmon samples compared to control samples.

Therefore, the bioactive packaging based on natural antioxidant compounds immobilized in the multilayer plastic and without being in contact with food, is very effective in protecting the salmon against oxidation. It could extend its shelf-life, and represented an important advantage to conventional packaging, which is in agreement with previous studies **(Nerin, 2010, Carrizo *et al*., 2015, Moudache *et al*., 2017).**

VI. Conclusion and perspectives

Petroleum-based plastics are widely used in food packaging. However, they represent a serious health and environmental problem since they are made from non-renewable resources and could carry dangerous and toxic substances **(Al-Tayar *et al*., 2019)**. For these reasons, the search for new packaging materials is of great importance **(Borzi *et al*., 2019)**. Films based on biopolymers such as cellulose could constitute an interesting and ecological alternative to conventional packaging, if their biological and functional characteristics are improved by a combination with natural active agents, such as the carob (*Ceratonia siliqua* L.) by-products used in the present study, which contain a composition lends them to a multitude of uses.

At the end of this thesis, we can retain the following points:

- This work is an alternative approach to synthetic plastic material and opens a new promising way to transform by-products with higher added value (design of active antioxidant packaging), through clean technology allowing better ecological valorization.
- The combination of carob leaves, seeds and kibbles macerates with thermoplastic polymers in the development of functional and biodegradable food packaging at low cost is a relevant and sustainable approach for the exploration of this industrial waste.
- The evaluation of the free radical scavenging activity of the different biofilms incorporated with the carob macerates, demonstrated the best results in the case of carob seeds, which confirms their possible use for the trapping of free radicals in real food.
- The extended shelf life of FSF stored have shown the antioxidant effectiveness of the new antioxidant packaging in the inhibition of lipids and proteins oxidation.

Perspectives

The oxidation of unsaturated lipids is a major cause of deterioration of food quality, giving rise to the appearance of bad taste and loss of nutritional value of foodstuffs. Societal concerns about health and antioxidants used in food are constantly increasing. In order to minimize the amount of antioxidants directly added to food, active packaging containing antioxidants are promising solutions. The relevance of using cellulose, a biodegradable industrial polymer, to create an active antioxidant packaging was studied in this thesis. To consider a real industrial application and for a better understanding of the effectiveness of the new active packaging in the delay of food spoilage, certain aspects still need to be studied:

- It would be also interesting to study the composition in non-volatile compounds analyzed by UPLC-ESI-Q-TOF-MS^E with software Masslynx used for the data acquisition and the identification of the non-volatile compounds.
- Analysis of mechanical performance of the developed packaging are essential and must be considered and planned during the trials with real food, before the application at industrial scale.
- An extension of the developed model to other antioxidants or active agents as well as other similar polymers to cellulose may improve the applications to ensure better food safety.
- Blends of cellulose with other biopolymers can be developed to provide other types of active antioxidant packaging or in combination with other polymers with antimicrobial properties such as nisin.

Bibliographic references

- Abidar, S., Boiangiu, R. S., Dumitru, G., Todirascu-Ciornea, E., Amakran, A., Cioanca, O., … Nhiri, M. (2020). The aqueous extract from *Ceratonia siliqua* leaves protects against 6-hydroxydopamine in zebrafish: Understanding the underlying mechanism. *Antioxidants*, 9(4), 304. https://doi.org/10.3390/antiox9040304.
- Adilah, A. N., Jamilah, B., Noranizan, M. A., & Hanani, Z. A. N. (2018). Utilization of mango peel extracts on the biodegradable films for active packaging. *Food Packaging and Shelf Life*, 16(2018), 1–7. https://doi.org/10.1016/j.fpsl.2018.01.006.
- Agourram, A., Ghirardello, D., Rantsiou, K., Zeppa, G., Belviso, S., Romane, A., Oufdou, K., & Giordano, M. (2013). Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts. *International Journal of Food Properties*, 16(5), 1092–1104. https://doi.org/10.1080/10942912.2011.576446.
- Ahmad, M.I., Umair ljaz, M., Ul Haq, I., & Li, C. (2020).The role of meat protein in generation of oxidative stress and pathophysiology of metabolic syndromes, *Food Science of Animal Resources*, 40(1), 1–10. https://doi.org/10.5851/kosfa.2019.e96.
- Ait Ouahioune, L., Wrona, M., Becerril, R., Salafranca, J., Nerin, C., & Djenane, D. (2021). *Ceratonia siliqua* L. kibbles, seeds and leaves as a source of volatile bioactive compounds for antioxidant food biopackaging applications. *Food Packaging and Shelf Life*, 31 100764. https://doi.org/10.1016/j.fpsl.2021.100764.
- Akrami, F., Rodríguez-Lafuente, A., Bentayeb, K., Pezo, D., Ghalebi, S. R., & Nerín, C. (2015). Antioxidant and antimicrobial active paper based on Zataria (*Zataria multiflora*) and two cumin cultivars (*Cuminum cyminum*). *LWT - Food Science and Technology*, 60(2), 929–933. https://doi.org/10.1016/j.lwt.2014.09.051.
- Aksit, S., Caglayan, S., Cukan, R, & Yaprak, I. (1998). Carob bean juice: a powerful adjunct to oral rehydration solution treatment in diarrhoea. *Paediatric Perinatal Epidemiology*, 12(2), 176–81. https://doi.org/10.1046/j.1365-3016.1998.00093.x.
- Albanell, E., Caja, G., & Plaixats, J. (1991). Characteristics of Spanish carob pods and nutritive value of carob kibbles. Fourrages et sous-produits méditerranéens. Zaragoza: CIHEAM, 135–136 (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 16). http://om.ciheam.org/om/pdf/a16/91605058.pdf.
- Alberto Lopes, J., Tsochatzis, E. D., Robouch, P., & Hoekstra, E. (2019). Influence of preheating of food contact polypropylene cups on its physical structure and on the migration of additives. *Food Packaging and Shelf Life*, 20, 100305. https://doi.org/10.1016/j.fpsl.2019.100305.

- Albertos, I., Jaime, I., Diez, A. M., González-Arnáiz, L., & Rico, D. (2015). Carob seed peel as natural antioxidant in minced and refrigerated (4° C) Atlantic horse mackerel (*Trachurus trachurus*). *LWT-Food Science and Technology*, 64(2), 650–656. https://*doi*.org/*10.1016*/*j*.*lwt*.2015.06.037.
- Alegbeleye, O. O., Singleton, I., & Sant'Ana, A. S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiology*, 73, 177–208. https://doi.org/10.1016/j.fm.2018.01.003.
- Alexiou, P., & Demopoulos, V. J. (2010). A diverse series of substituted benzenesulfonamides as aldose reductase inhibitors with antioxidant activity: design, synthesis, and in vitro activity. *Journal of Medicinal Chemistry*, 53(21), 7756–7766. https://doi.org/10.1021/jm101008m.
- Al-Tayyar, N. A., Youssef, A. M., & Al-hindi, R. (2019). Antimicrobial food packaging based on sustainable bio-based materials for reducing foodborne pathogens: A review. *Food Chemistry*, 125915. https://doi.org/10.1016/j.foodchem.2019.125915.
- Al-Tayyar, N. A., Youssef, A. M., & Al-Hindi, R. R. (2020). Edible coatings and antimicrobial nanoemulsions for enhancing shelf life and reducing foodborne pathogens of fruits and vegetables: A review. *Sustainable Materials and Technologies*, 26, 1–12. https://doi.org/10.1016/j.susmat.2020.e00215.
- Alves, V. L. C. D., Rico, B. P. M., Cruz, R. M. S., Vicente, A. A., Khmelinskii, I., & Vieira, M. C. (2018). Preparation and characterization of a chitosan film with grape seed extract-carvacrol microcapsules and its effect on the shelf-life of refrigerated Salmon (*Salmo salar*). *LWT - Food Science and Technology*, 89(2018), 525–534. https://doi.org/10.1016/j.lwt.2017.11.013.
- Al-Zoreky, N. S. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *International Journal of Food Microbiology*, 134(3), 244–248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002.
- Amico, F.P., & Source, E.G. (1997). Medical plants and phytotherapy in mussomeli area (Caltanisseta, sicily Italy), *Fitoterapia*, 68(2), 143–159.
- Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2019a). Bioactive compounds, antioxidant activity, and sensory analysis of rice-based extruded snacks-like fortified with bean and carob fruit flours. *Foods*, *8*(9), 381. https://doi.org/10.3390/foods8090381.

- Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2019b). The effect of extrusion on the bioactive compounds and antioxidant capacity of novel gluten-free expanded products based on carob fruit, pea and rice blends. *Innovative Food Science & Emerging Technologies*, 52, 100–107. https://doi.org/10.1016/j.ifset.2018.12.003.
- Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2020). Cooking effect on the bioactive compounds, texture, and color properties of cold-extruded rice/bean-based pasta supplemented with whole carob fruit. *Foods*, 9(4), 415. https://doi.org/10.3390/foods9040415.
- Avallone, R., Plessi, M., Baraldi, M., & Monzani, A. (1997). Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. *Journal of Food Composition and Analysis*, 10(2), 166–72. https://doi.org/10.1006/jfca.1997.0528.
- Avila-Sosa, R., Palou, E., Jiménez Munguía, M. T., Nevárez-Moorillón, G. V., Navarro Cruz, A. R., & López-Malo, A. (2012). Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. *International Journal of Food Microbiology*, 153(1-2), 66–72. https://doi.org/10.1016/j.ijfoodmicro.2011.10.017.
- Ayaz, F.A., Torun, H., Ayaz, S., Correia, P.J., Alaiz, M., Sanz, C., Grúz, J., & Strnad, M. (2007). Determination of chemical composition of Anatolian carob pod (*Ceratonia siliqua* L.): sugars, amino and organic acids, minerals and phenolic compounds. *Journal of Food Quality*, 30(6), 1040–1055. https://doi.org/10.1111/j.1745-4557.2007.00176.x.
- Aydın, S., & Özdemir, Y. (2017). Development and characterization of carob flour based functional spread for increasing use as nutritious snack for children. *Journal of Food Quality*, *2017*, 1–7. https://doi.org/10.1155/2017/5028150.
- Azizi-Lalabadi, M., Rafiei, L., Divband, B., & Ehsani, A. (2020). Active packaging for salmon stored at refrigerator with polypropylene nanocomposites containing 4A zeolite, ZnO nanoparticles, and green tea extract. *Food Science and Nutrition*, 8(12), 6445–6456. https://doi.org/10.1002/fsn3.1934.
- Aznar, M., Vera, P., Canellas, E., Nerín, C., Mercea, P., & Störmer, A. (2011). Composition of the adhesives used in food packaging multilayer materials and migration studies from packaging to food. *Journal of Materials Chemistry*, 21(12), 4358. https://doi.org/10.1039/C0JM04136J.
- Badr, M. M., Badawy, M. E. I., & Taktak, N. E. M. (2021). Characterization, antimicrobial activity, and antioxidant activity of the nanoemulsions of *Lavandula spica*

essential oil and its main monoterpenes. *Journal of Drug Delivery Science and Technology*, *65*, 102732. https://doi.org/10.1016/j.jddst.2021.102732**.**

- Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology*, 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106**.**
- Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1), 191–203. http://dx.doi.org/10.1016/j.foodchem.2005.07.042.
- Bampouli, A., Kyriakopoulou, K., Papaefstathiou, G., Louli, V., Krokida, M., & Magoulas, K. (2014). Comparison of different extraction methods of *Pistacia lentiscus* var. chia leaves: yield, antioxidant activity and essential oil chemical composition. *Journal of Applied Research on Medicinal and Aromatic Plants*, 1(3), 81–91. http://dx.doi.org/10.1016/j.jarmap.2014.07.001.
- Banwart, J.G. (2004). Basic food microbiology, 2nd ed. Daryaganj, New Delhi.
- Barak, S., & Mudgil, D. (2014). Locust bean gum: Processing, properties and food applications—A review. *International Journal of Biological Macromolecules*, 66, 74–80. http://dx.doi.org/10.1016/j.ijbiomac.2014.02.017.
- Barbosa-Pereira, L., Aurrekoetxea, G. P., Angulo, I., Paseiro-Losada, P., & Cruz, J. M. (2014). Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef. *Meat Science*, 97(2), 249–254. http://dx.doi.org/10.1016/j.meatsci.2014.02.006.
- Baron, C. P., KjÆrsgård, I. V. H., Jessen, F., & Jacobsen, C. (2007). Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*). *Journal of Agricultural and Food Chemistry*, 55(20), 8118–8125. https://doi.org/10.1021/jf070686f.
- Baron, F., & Gautier, M. (2016). Microbial spoilage. *Handbook of Food Science and Technology 1*. 53–97. https://doi.org/10.1002/9781119268659.ch3.
- Bates, S. H., Jones, R. B., & Bailey, C. J. (2000). Insulin-like effect of pinitol. *British Journal of Pharmacology*, 130(8), 1944–1948. https://doi.org/*10.1038/sj.bjp.0703523.*
- Batlle, I., & Tous, J. (1997). Carob tree. *Ceratonia siliqua* L. promoting the conservation and use of underutilized and neglected crops. 17. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
- Baytop, T. (1984) Therapy with medicinal plant in Turkey (Past and present), Publication of the Istanbul University, 3255, Istanbul.

- Bekhit, A. E. A., Giteru, S. G., Holman, B. W. B., & Hopkins, D. L. (2021). Total volatile basic nitrogen and trimethylamine in muscle foods: Potential formation pathways and effects on human health. *Comprehensive Reviews in Food Science and Food Safety*, 20(4), 3620–3666. https://doi.org/10.1111/1541-4337.12764.
- Belasli, A., Ben Miri, Y., Aboudaou, M., Ait Ouahioune, L., Montañes, L., Ariño, A., & Djenane, D. (2020). Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (*Laurus nobilis* L.): Potential use as wheat preservative. *Food Science and Nutrition*, *8*(9), 4717–4729. https://doi.org/10.1002/fsn3.1650**.**
- Bell, J. G., Henderson, R. J., Tocher, D. R., & Sargent, J. R. (2004). Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids*, 39(3), 223–232. https://doi.org/10.1007/s11745-004-1223-5.
- Ben Ayache, S., Behija Saafi, E., Emhemmed, F., Flamini, G., Achour, L., & Muller, C. D. (2020). Biological activities of aqueous extracts from carob plant (*Ceratonia siliqua* L.) by antioxidant, analgesic and proapoptotic properties evaluation. *Molecules*, 25(14), 3120. https://doi.org/10.3390/molecules25143120.
- Ben Hsouna, A., Trigui, M., Ben Mansour, R., Mansour, R. B., Jarraya, R. M., Damak, M., & Jaoua, S. (2011). Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential oil with preservative effects against *Listeria* inoculated in minced beef meat. *International Journal of Food Microbiology*, 148, 66–72. https://doi.org/10.1016/j.ijfoodmicro.2011.04.028.
- Ben Othmen, K., Garcia-Beltrán, J. M., Elfalleh, W., Haddad, M., & Esteban, M. Á. (2021). Phytochemical compounds and biological properties of carob pods (*Ceratonia siliqua* L.) extracts at different ripening stages. *Waste and Biomass Valorization*, 12(9), 4975–4990. https://doi.org/10.1007/s12649-021-01352-x.
- Benchikh, Y., Louaileche, H., George, B., & Merlin, A. (2014). Changes in bioactive phytochemical content and in vitro antioxidant activity of carob (*Ceratonia siliqua* L.) as influenced by fruit ripening. *Industrial Crops and Products*, 60, 298–303. https://doi.org/10.1016/j.indcrop.2014.05.048**.**
- Benito-Peña, E., González-Vallejo, V., Rico-Yuste, A., Barbosa-Pereira, L., Cruz, J. M., Bilbao, A., Alvarez-Lorenzo, C., & Moreno-Bondi, M. C. (2016). Molecularly imprinted hydrogels as functional active packaging materials. *Food Chemistry*, 190, 487–494. http://dx.doi.org/10.1016/j.foodchem.2015.05.128.

- Benner, R. A. (2014). Organisms of concern but not foodborne or confirmed foodborne: spoilage microorganisms. *Encyclopedia of Food Safety*, 245–250. https://doi.org/10.1016/B978-0-12-378612-8.00169-4.
- Bhunia, K., Sablani, S. S., & Tang, J. (2013). Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. *Comprehensive Reviews in Food Science and Food Safety*, 12(5), 523545. https://doi.org/10.1111/1541-4337.12028.
- Biernacka, B., Dziki, D., Gawlik-Dziki, U., Różyło, R., & Siastała, M. (2017). Physical, sensorial, and antioxidant properties of common wheat pasta enriched with carob fiber. *LWT - Food Science and Technology*, 77, 186–192. http://dx.doi.org/10.1016/j.lwt.2016.11.042.
- Biner, B., Gubbuk, H., Karhan, M., Aksu, M., & Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey. *Food Chemistry*, 100(4), 1453–1455. https://doi.org/10.1016/j.foodchem.2005.11.037.
- Blackburn, C. de W. (2006). Managing microbial food spoilage: an overview. *Food Spoilage Microorganisms*, 147–170. https://doi.org/10.1533/9781845691417.2.147.
- Bolumar, T., Andersen, M.L., & Orlien, V. (2011) Antioxidant active packaging for chicken meat processed by high pressure treatment. *Food Chemistry*, 129, 1406–1412. http://dx.doi.org/10.1016/j.foodchem.2011.05.082.
- Bopp, A. F. (2019). The evolution of food preservation and packaging. *Chemistry's Role in Food Production and Sustainability: Past and Present*, 209–226. https://doi.org/10.1021/bk-2019-1314.ch015.
- Borzi, F., Torrieri, E., Wrona, M., & Nerín, C. (2019). Polyamide modified with green tea extract for fresh minced meat active packaging applications. *Food Chemistry*, 300, 125–242. https://doi.org/10.1016/j.foodchem.2019.125242**.**
- Bozin, B., Mimica-Dukic, N., Samojlik, I., & Jovin, E. (2007). Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*) essential oils. *Journal of Agricultural and Food Chemistry*, 55(19), 7879–7885. https://doi.org/10.1021/jf0715323**.**
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. https://doi.org/https://doi.org/10.1016/S0023-6438(95)80008-5**.**

- Brassesco, M. E., Brandão, T. R. S., Silva, C. L. M., & Pintado, M. (2021). Carob bean (*Ceratonia siliqua* L.): a new perspective for functional food. *Trends in Food Science & Technology*, 114, 310–322. https://doi.org/10.1016/j.tifs.2021.05.037.
- Bugatti, V., Viscusi, G., & Gorrasi, G. (2020). Formulation of a bio-packaging based on pure cellulose coupled with cellulose acetate treated with active coating: evaluation of shelf life of pasta ready to eat. *Foods*, 9(10), 1–14. https://doi.org/10.3390/foods9101414**.**
- Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, 94, 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022**.**
- Byun, Y., Kim, Y. T., & Whiteside, S. (2010). Characterization of an antioxidant polylactic acid (PLA) film prepared with α-tocopherol, BHT and polyethylene glycol using film cast extruder. *Journal of Food Engineering*, 100(2), 239–244. https://doi.org/10.1016/j.jfoodeng.2010.04.005.
- Calixto, F. S., & Cañellas, J. (1982). Components of nutritional interest in carob pods (*Ceratonia siliqua*). *Journal of the Science of Food and Agriculture*, 33(12), 1319–1323. https://doi.org/10.1002/jsfa.2740331219.
- Camo, J., Lorés, A., Djenane, D., Beltrán, J. A., & Roncalés, P. (2011). Display life of beef packaged with an antioxidant active film as a function of the concentration of oregano extract. *Meat Science*, 88(1), 174–178. https://doi.org/10.1016/j.meatsci.2010.12.019**.**
- Campos, D. A., Gómez-García, R., Vilas-Bóas, A. A., Madureira, A. R., & Pintado, M. M. (2020). Management of fruit industrial by-products—a case study on circular economy approach. *Molecules*, 25(2), 320. https://doi.org/10.3390/molecules25020320.
- Cao, T. L., & Song, K. Bin. (2020). Development of bioactive bombacaceae gum films containing cinnamon leaf essential oil and their application in packaging of fresh salmon fillets. *LWT*, 131(109647), 1–8. https://doi.org/10.1016/j.lwt.2020.109647.
- Carrizo, D., Gullo, G., Bosetti, O., & Nerín, C. (2014). Development of an active food packaging system with antioxidant properties based on green tea extract. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 31(3), 364–373. https://doi.org/10.1080/19440049.2013.869361.
- Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2015). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. I*nnovative Food Science and Emerging Technologies*, 33, 534–541. https://doi.org/10.1016/j.ifset.2015.10.018.

- Carrizo, D., Taborda, G., Nerín, C., & Bosetti, O. (2016). Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. *Innovative Food Science and Emerging Technologies*, 33, 534–541. https://doi.org/10.1016/j.ifset.2015.10.018**.**
- Castro, F. V. R., Andrade, M. A., Silva, A. S., Vaz, M. F., & Vilarinho, F. (2019). The contribution of a whey protein film incorporated with green tea extract to minimize the lipid oxidation of salmon (*Salmo salar* L.). *Foods*, 8(8), 327. https://doi.org/10.3390/foods8080327.
- Catalá, R., & Gavara, R. (2001). Nuevos envases. De la protección pasiva a la defensa activa de los alimentos envasados. *Arbor*, 168(661), 109–127. https://doi.org/10.3989/arbor.2001.i661.825.
- Cattaneo, A., Federighi, G., & Vaz, S. (2020). The environmental impact of reducing food loss and waste: a critical assessment. *Food Policy*, 101890. https://doi.org/10.1016/j.foodpol.2020.101890.
- Cervenka, L., Frühbauerov´a, M., & Velichov´a, H. (2019). Functional properties of muffin as affected by substituing wheat flour with carob powder. *Potravinarstvo Slovak Journal of Food Sciences*, 13(1), 212–217. https://doi.org/10.5219/1033.
- Coles, R., McDowell, D., & Kirwan, M. (2003). Food packaging technology: a critical assessment. *Food Policy*, 101890.
- Colon, M., & Nerin, C. (2015). Development and application of an analytical procedure for specific migration of green tea compounds in IV gamma nectarine active packaging. *Food Control*, 57, 419–425. https://doi.org/10.1016/j.foodcont.2015.04.035.
- Cömert, E. D., & Gökmen, V. (2018). Evolution of food antioxidants as a core topic of food science for a century. *Food Research International*, 105, 76–93. https://doi.org/10.1016/j.foodres.2017.10.056.
- Corsi, L., Avallone, R., Cosenza, F., Farina, F., Baraldi, C., Baraldi, M. (2002). Antiproliferative effects of *Ceratonia siliqua* L. on mouse hepatocellular carcinoma cell line. *Fitopoterpia*, 73, 674–684. https://*doi*.org/*10.1016/S0367-326X*(*02*)*00227-7*.
- Cronquist, A. (1981) An integrated system of classification of flowering plants. Columbia University Press, New York, 248-250.
- Custódio, L., Patarra, J., Alberício, F., Neng, N. R., Nogueira, J. M. F., & Romano, A. (2015). In vitro antioxidant and inhibitory activity of water decoctions of carob tree (*Ceratonia siliqua* L.) on cholinesterases, α-amylase and α-glucosidase. *Natural Product Research*, 29(22), 2155–2159. http://dx.doi.org/10.1080/14786419.2014.996147.

- Cutter, C. N. (2002). Microbial control by packaging: a review. *Critical Reviews in Food Science and Nutrition*, 42(2), 151–161. https://doi.org/10.1080/10408690290825493.
- Chipley, J.R. (2005). Sodium benzoate and benzoic acid. In Antimicrobials in Foods ed. Davidson, P.M. and Branen, A.L. 1993, 11-48. New York: Marcel Dekker.
- Choe, E., & Min, D.B. (2006) Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, 5, 169–186.https://doi.org/10.1111/j.1541-4337.2006.00009.x.
- Dakia, P. A. (2011). Carob (*Ceratonia siliqua* L.) seeds, endosperm and germ composition, and application to health. *Nuts and Seeds in Health and Disease Prevention*, 293–299. https://*doi*.org/*10.1016*/*b978*-0-12-375688-6.10035-0.
- Dakia, P. A., Wathelet, B., & Paquot, M. (2007). Isolation and chemical evaluation of carob (*Ceratonia siliqua* L.) seed germ. *Food Chemistry*, 102(4), 1368–1374. https://*doi*.org/*10.1016*/*j.foodchem*.2006.05.059.
- Davidson, P. M., Critzer, F. J., & Taylor, T. M. (2013). Naturally occurring antimicrobials for minimally processed foods. *Annual Review of Food Science and Technology*, 4(1), 163–190. https://doi.org/10.1146/annurev-food-030212-182535.
- Davies, K.J., & Delsignore, M. E. (1987). Protein damage and degradation by oxygen radicals. III. Modification of secondary and tertiary structure. *Journal of Biological Chemistry*. 262(20), 9908–13.
- De Saint Laumer, J. Y., Frérot, E., & Herrmann, A. (2003). Controlled release of perfumery alcohols by neighboring-group participation. Comparison of the rate constants for the alkaline hydrolysis of 2-acyl-, 2-(hydroxymethyl)-, and 2-carbamoylbenzoates. *Helvetica Chimica Acta*, 86(8), 2871–2899. https://doi.org/10.1002/hlca.200390236.
- Debevere, J., & Boskou, G. (1996). Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *International Journal of Food Microbiology*, 31(1–3), 221–229. https://doi.org/10.1016/0168-1605(96)01001-X.
- Debnath, S., Habibur Rahman, S. M., Deshmukh, G., Duganath, N., Pranitha, C., & Chiranjeevi, A. (2011). Antimicrobial screening of various fruit seed extracts. *Pharmacognosy Journal*, 3(19), 83–86. https://doi.org/10.5530/pj.2011.19.15.
- Diaz, C.S. (1997). Syrup of natural carob sugars and a process for its production. U.S. Patent 5, 624,500 A.
- Dicastillo, C.L., Nerín, C., Alfaro, P., Catalá, R., Gavara, R., & Hernandez-Muñoz, P. (2011). Development of new antioxidant active packaging films base on ethylene vinyl alcohol

copolymer (EVOH) and green tea extract. *Journal of Agricultural and Food Chemistry*, 59, 7832–7840. https://doi.org/10.1021/jf201246g.

- Djebari, S., Wrona, M., Boudria, A., Salafranca, J., Nerin, C., Bedjaoui, K., & Madani, K. (2021). Study of bioactive volatile compounds from different parts of *Pistacia lentiscus* L. extracts and their antioxidant and antibacterial activities for new active packaging application. *Food Control*, 120(107514), 1–8. https://doi.org/10.1016/j.foodcont.2020.107514.
- Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of Algerian citrus essential oils and their application in *sardina pilchardus*. *Foods*, 4(4), 208–228. https://doi.org/10.3390/foods4020208.
- Djenane, D., Aboudaou, M., Ferhat, M. A., Ouelhadj, A., & Ariño, A. (2019). Effect of the aromatisation with summer savory (*Satureja hortensis* L.) essential oil on the oxidative and microbial stabilities of liquid whole eggs during storage. *Journal of Essential Oil Research*, *31*(5). https://doi.org/10.1080/10412905.2019.1610516.
- Djenane, D., Beltrán, J. A., Camo, J., & Roncalés, P. (2016). Influence of vacuum-ageing duration of whole beef on retail shelf life of steaks packaged with oregano (*Origanum vulgare* L.) active film under high O2. *Journal of Food Science and Technology*, *53*(12), 4244–4257. https://doi.org/10.1007/s13197-016-2419-1.
- Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2001). Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. *Journal of Food Science*, 66(1), 181–186. https://doi.org/10.1111/j.1365-2621.2001.tb15603.x.
- Dong, Z., Xu, F., Ahmed, I., Li, Z., & Lin, H. (2018). Characterization and preservation performance of active polyethylene films containing rosemary and cinnamon essential oils for pacific white shrimp packaging. *Food Control*, *92*, 37–46. https://doi.org/10.1016/j.foodcont.2018.04.052.
- Duun, A. S., & Rustad, T. (2008). Quality of superchilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at -1.4 and -3.6 °C. *Food Chemistry*, 106(1), 122–131.
- Echegoyen, Y., & Nerín, C. (2015). Performance of an active paper based on cinnamon essential oil in mushrooms quality. *Food Chemistry*, *170*, 30–36. https://doi.org/10.1016/j.foodchem.2014.08.032.
- El-Sayed, A. M. (2014). The pherobase: Database of pheromones and semiochemicals. *The Pherobase*. Retrieved from http://www.pherobase.com.

- El-Sayed, H. S., El-Sayed, S. M., Mabrouk, A. M. M., Nawwar, G. A., & Youssef, A. M. (2021). Development of eco-friendly probiotic edible coatings based on chitosan, alginate and carboxymethyl cellulose for improving the shelf life of uf soft cheese. *Journal of Polymers and the Environment*, 29(6), 1941–1953. https://doi.org/10.1007/s10924-020-02003-3.
- EU, 2004. Regulation (EC) 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food. *Official Journal of the European Union*, 338, 4–17.
- EU, 2011. Comission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union*, 12, 1–89.
- Fang, Z., Zhao, Y., Warner, R. D., & Johnson, S. K. (2017). Active and intelligent packaging in meat industry. *Trends in Food Science & Technology*, 61, 60–71. http://dx.doi.org/10.1016/j.tifs.2017.01.002.
- FAO (2011). Food and Agriculture Organization of the United Nations. Global food losses and food waste – Extent, causes and prevention.Rome, Italy.
- Farag, M. A., & El-Kersh, D. M. (2017). Volatiles profiling in *Ceratonia siliqua* (Carob bean) from Egypt and in response to roasting as analyzed via solid-phase microextraction coupled to chemometrics. *Journal of Advanced Research*, *8*(4), 379–385. https://doi.org/10.1016/j.jare.2017.05.002.
- Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C. I. G., Angioni, A., Dessi, S., … Cabras, P. (2007). Antimicrobial activity of tunisian quince (*cydonia oblongamiller*) pulp and peel polyphenolic extracts. *Journal of Agricultural and Food Chemistry*, 55(3), 963–969. https://*doi*.org/*10.1021/jf062614e.*
- Faustino, M., Veiga, M., Sousa, P., Costa, E., Silva, S., & Pintado, M. (2019). Agro-food byproducts as a new source of natural food additives. *Molecules*, 24(6), 1056. https://doi.org/10.3390/molecules24061056.
- FDA U.S. Food and Drug Administration (2009). CFSAN—Bad Bug Book—pH values of various foods. Available from: www.fda.gov. (Accessed Jun 29, 2009).
- Feiner (2006). 6 - Additives: proteins, carbohydrates, fillers and other additives. *Meat Products Handbook.Woodhead Publishing Series in Food Science, Technology and Nutrition*, 89–141. https://doi.org/10.1533/9781845691721.1.89.
- Fernández, K., Aspe, E., & Roeckel, M. (2009). Shelf-life extension on fillets of Atlantic Salmon (*Salmo salar*) using natural additives, superchilling and modified atmosphere

packaging. *Food Control*, 20(11), 1036–1042. http://dx.doi.org/10.1016/j.foodcont.2008.12.010.

- Fidalgo, L. G., Castro, R., Trigo, M., Aubourg, S. P., Delgadillo, I., & Saraiva, J. A. (2019). Quality of fresh Atlantic salmon (*Salmo salar*) under hyperbaric storage at low temperature by evaluation of microbial and physicochemical quality indicators. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-019-02346-3.
- Fidan, H., Mihaylova, D., Petkova, N., Sapoundzhieva, T., Slavov, A., & Krastev, L. (2019). Determination of chemical composition, antibacterial and antioxidant properties of products obtained from carob and honey locust. *Turkish Journal of Biochemistry*, 44(3), 316–322. https://doi.org/10.1515/tjb-2018-0113**.**
- Fidan, H., Stankov, S., Petkova, N., Petkova, Z., Iliev, A., Stoyanova, M., … Ercisli, S. (2020). Evaluation of chemical composition, antioxidant potential and *functional* properties of carob (*Ceratonia siliqua* L.) seeds. *Journal of Food Science and Technology*, *57*(7), 2404–2413. https://doi.org/10.1007/s13197-020-04274-z.
- Frankel, E. N. (2012). Photooxidation of unsaturated fats. *Lipid Oxidation*, 51–66. https://doi.org/10.1533/9780857097927.51.
- Freitas, J., Vaz-Pires, P., & Câmara, J. S. (2019). Freshness assessment and shelf-life prediction for seriola dumerili from aquaculture based on the quality index method. *Molecules*, 24(19), 3530. https://doi.org/10.3390/molecules24193530.
- Fu, Q., Liu, R., Zhang, W., Li, Y., Wang, J., & Zhou, G. (2014). Effects of different packaging systems on beef tenderness through protein modifications. *Food and Bioprocess Technology*, 8(3), 580–588. https://doi.org/10.1007/s11947-014-1426-3.
- Gan, I., & Chow, W. S. (2018). Antimicrobial poly (lactic acid)/cellulose bionanocomposite for food packaging application: a review. *Food Packaging and Shelf Life*, 17, 150–161. https://doi.org/10.1016/j.fpsl.2018.06.012.
- Garde, J. A., Catalá, R., & Gavara, R. (1998). Analysis of antioxidants extracted from polypropylene by supercritical fluid extraction. *Food Additives and Contaminants*, 15(6), 701–708. http://dx.doi.org/10.1080/02652039809374700.
- Gavril, G. L., Wrona, M., Bertella, A., Świeca, M., Râpă, M., Salafranca, J., & Nerín, C. (2019). Influence of medicinal and aromatic plants into risk assessment of a new bioactive packaging based on polylactic acid (PLA). *Food and Chemical Toxicology*, 110662. https://doi.org/10.1016/j.fct.2019.110662.

- Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: review. *American Journal of Applied Sciences*, 7, 846–864. https://doi.org/10.3844/ajassp.2010.859.877.
- Giménez, B., Roncalés, P., & Beltrán, J. A. (2005). The effects of natural antioxidants and lighting conditions on the quality of salmon (*Salmo salar*) fillets packaged in modified atmosphere. *Journal of the Science of Food and Agriculture*, 85(6), 1033–1040. https://doi.org/10.1002/jsfa.2055.
- Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science & Technology*, 35(1), 42–51. https://doi.org/10.1016/j.tifs.2013.10.008.
- Gómez-Estaca, J., Alemán, A., López-Caballero, M. E., Baccan, G. C., Montero, P., & Gómez-Guillén, M. C. (2018). Bioaccessibility and antimicrobial properties of a shrimp demineralization extract blended with chitosan as wrapping material in ready-to-eat raw salmon. *Food Chemistry*. 276, 342-349. https://doi.org/10.1016/j.foodchem.2018.10.031.
- Gontard, N. (2000). Les emballages actifs. Edition Tec et Doc. Paris. ISBN: 2-7430-0387.
- Goulas, V., & Georgiou, E. (2019). Utilization of Carob Fruit as Sources of Phenolic Compounds with Antioxidant Potential: Extraction Optimization and Application in Food Models. *Foods*, 9(1), 20. https://doi.org/10.3390/foods9010020.
- Goulas, V., Hadjivasileiou, L., Primikyri, A., Michael, C., Botsaris, G., Tzakos, A. G., & Gerothanassis, I. P. (2019). Valorization of carob fruit residues for the preparation of novel bi-functional polyphenolic coating for food packaging applications. *Molecules*, 24(17), 1–10. https://doi.org/10.3390/molecules24173162.
- Goulas, V., Stylos, E., Chatziathanasiadou, M., Mavromoustakos, T., & Tzakos, A. (2016). Functional components of carob fruit: linking the chemical and biological space. *International Journal of Molecular Sciences*, 17(11), 1875. https://doi.org/*10.3390/ijms17111875*.
- Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33(1), 121–137. https://doi.org/10.1016/0168-1605(96)01134-8.
- Granato, D., Nunes, D. S., & Barba, F. J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional

foods: A proposal. *Trends in Food Science & Technology*, 62, 13–22. https://doi.org/10.1016/j.tifs.2016.12.010.

- Gruendel, S., Garcia, A. L., Otto, B., Mueller, C., Steiniger, J., Weickert, M. O., … Koebnick, C. (2006). Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. *The Journal of Nutrition*, 136(6), 1533–1538. https://doi.org/10.1093/jn/136.6.1533.
- Gruendel, S., Otto, B., Garcia, A. L., Wagner, K., Mueller, C., Weickert, M. O., … Koebnick, C. (2007). Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans. *British Journal of Nutrition*, 98(01), 101. *https://doi.org/10.1017/S0007114507701642.*
- Guart, A., Bono-Blay, F., Borrell, A., & Lacorte, S. (2011). Migration of plasticizers phthalates, bisphenol A and alkylphenols from plastic containers and evaluation of risk. *Food Additives & Contaminants: Part A*, 28(5), 676–685. http://dx.doi.org/10.1080/19440049.2011.555845.
- Guggenbichler, J. P. (1983). Adherence of enterobacteria in infantile diarrhea and its prevention. *Infection*, 11(4), 239–242. https://doi.org/10.1007/BF01641209.
- Gulay, M.S., Yildiz-Gulay, O., Ata, A., Balic, A., & Demirtas (2012). Toxicological evaluation of carob (*Ceratonia siliqua*) bean extracts in male new zealand white rabbits. Journal of Animal and Veterinary Advances, 11, 1853–1857. *http*://dx.doi.org/*10.3923/javaa.2012.1853.1857.*
- Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: a review with emphasis on high-pressure treatments. *Trends in Food Science & Technology*, 50, 131–143. http://dx.doi.org/10.1016/j.tifs.2016.01.026.
- Habibzadeh, D., & Seyedain Ardabili, S. M. (2019). Evaluation of physicochemical, rheological and sensory properties of wafer cream by replacing cocoa powder with carob pod and chicory root powders. *International Food Research Journal*, 26(3), 1059–1068.
- Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., … Lachkar, M. (2011). Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob tree barks growing in Morocco. *Arabian Journal of Chemistry*, 4(3). https://doi.org/10.1016/j.arabjc.2010.06.053**.**
- Hamad, S. H. (2012). Factors affecting the growth of microorganisms in food. *Progress in Food Preservation*, 405–427. https://*doi*.org/*10.1002/9781119962045.ch20.*

- Han, J. H. (2005). Antimicrobial packaging systems. *Innovations in Food Packaging*, 80–107. http://doi.org/10.1016/b978-012311632-1/50038-3.
- Han, Y., Yu, M., & Wang, L. (2018). Physical and antimicrobial properties of sodium alginate/carboxymethyl cellulose films incorporated with cinnamon essential oil. *Food Packaging and Shelf Life*, 15, 35-42. https://doi.org/10.1016/j.fpsl.2017.11.001.
- Hayek, S.A., Gyawali, R., Salam, A.I. (2013). Antimicrobial natural products. *Microbial pathogens and strategies for combating them: science, technology and education* (A. Méndez-Vilas, Ed.).
- Heinonen, I.M., Meyer, A.S., & Frankel, E.N. (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry*, 46, 4107-4112. http://dx.doi.org/10.1021/jf980181c.
- Hicks, A. (2002). Minimum-Packaging technology for processed foods environmental considerations. *AU JT*. 6(2), 89-94.
- Hoehnel, A., Axel, C., Bez, J., Arendt, E. K., & Zannini, E. (2019). Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread. *Journal of Cereal Science*, 89, 102816. https://doi.org/10.1016/j. jcs.2019.102816.
- Hsouna, A. B., Trigui, M., Jarraya, R. M., Damak, M., & Jaoua, S. (2015). Identification of phenolic compounds by high performance liquid chromatography/mass spectrometry (HPLC/MS) and in vitro evaluation of the antioxidant and antimicrobial activities of *Ceratonia siliqua* leaves extracts. *Journal of Medicinal Plants Research*, 9(14), 479–485. https://doi.org/10.5897/JMPR2011.685.
- Huang, X., & Ahn, D. U. (2019). Lipid oxidation and its implications to meat quality and human health. *Food Science and Biotechnology*. https://doi.org/10.1007/s10068-019-00631-7.
- Hultmann, L., & Rustad, T. (2002). Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology*, 11(3/4), 105e123. https://doi.org/10.1300/J030v11n03_09.
- Ingrao, C., Gigli, M., & Siracusa, V. (2017). An attributional life cycle assessment application experience to highlight environmental hotspots in the production of foamy polylactic acid trays for fresh-food packaging usage. *Journal of Cleaner Production*, 150, 93–103. https://doi.org/10.1016/j.jclepro.2017.03.007.
- ISO-6496. (1999). No determination of moisture and other volatile matter content. In *Animal feeding stuffs*.

- Jacobsen, C. (2010). Challenges when developing omega-3 enriched foods. Oléagineux, corps gras, *Lipides*, 17(4), 251–258. https://doi.org/10.1051/ocl.2010.0327.
- Jacobsen, C., Paiva-Martins, F., Schwarz, K., & Bochkov, V. (2019). Lipid Oxidation and Antioxidants in Food and Nutrition. *European Journal of Lipid Science and Technology*, 121(9), 1900298. https://doi.org/10.1002/ejlt.201900298.
- Jamshidian, M., Tehrany, E. A., Imran, M., Jacquot, M., & Desobry, S. (2010). Poly-lactic acid: production, applications, nanocomposites, and release studies. *Comprehensive Reviews in Food Science and Food Safety*, 9(5), 552–571. https://doi.org/10.1111/j.1541-4337.2010.00126.x.
- Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Indicators of food microbial quality and safety. In: modern food microbiology. *Food Science Text Series*. 473– 495. https://doi.org/*10.1007/0-387-23413-6_20.*
- Jay, J.M. (2000) Modern food microbiology, 6th ed. Aspen Publishers, Gaithersburg, 29–36. ISBN 0-8342-1671-X.
- Joardder, M. U. H., & Masud, M. H. (2019). A brief history of food preservation. *food Preservation in Developing Countries: Challenges and Solutions*, 57–66. https://doi.org/10.1007/978-3-030-11530-2_3.
- Jung, J., & Zhao, Y. (2016). Antimicrobial packaging for fresh and minimally processed fruits and vegetables. *Antimicrobial Food Packaging*, 243–256. https://doi.org/10.1016/B978-0-12-800723-5.00018-8.
- Júnior, J. M., & de Oshiro, M. (2017). Atualizações importantes introduzidas pelo novo regulamento de inspeção industrial e sanitária de produtos de origem animal: decreto n° 9.013 de 29 de março de 2017. vigilância sanitária em debate. 5. vigilância sanitária em debate (pp. 73–80). https://doi.org/10.22239/2317-269x.01019.
- Kaale, L. D., Eikevik, T. M., Rustad, T., & Nordtvedt, T. S. (2014). Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage. *LWT – Food Science and Technology*, 55, 528–535. https://doi.org/ 10.1016/j.lwt.2013.10.021.
- Kaderi, M., Ben Hamouda, G., Zaeir, H., Hanana, M., & Hamrouni, L. (2014). Notes ethnobotanique et phytopharmacologique sur *Ceratonia siliqua* (L.). *Phytothérapie*, 13(2), 144–147. https://doi.org/10.1007/s10298-014-0904-4.
- Kanatt, S. R., Chander, R., & Sharma, A. (2010). Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *International Journal*

of Food Science & Technology, 45(2), 216–222. https://doi.org/10.1111/j.1365-2621.2009.02124.x.

- Kanner, J., & Rosenthal, I. (1992). An assessment of lipid oxidation in foods (Technical Report). *Pure and Applied Chemistry*, 64(12), 1959–1964. *http*://dx.*doi*.org/*10.1351*/*pac199264121959*.
- Karabagias, I., Badeka, A., & Kontominas, M. G. (2011). Shelf life extension of lamb meat using thyme or orégano essential oils and modified atmosphere packaging. *Meat Science*, 88, 109–116. https://doi.org/10.1016/j.meatsci.2010.12.010.
- Kasrati, A., Alaoui Jamali, C., Bekkouche, K., Spooner-Hart, R., Leach, D., & Abbad, A. (2015). Chemical characterization and insecticidal properties of essential oils from different wild populations of *Mentha suaveolens* subsp. timija (BRIQ.) HARLEY from Morocco. *Chemistry and Biodiversity*, 12(5), 823–831. https://doi.org/10.1002/cbdv.201400236**.**
- Kelen, M., & Tepe, B. (2008). Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. *Bioresource Technology*, 99(10), 4096–4104. https://doi.org/10.1016/j.biortech.2007.09.002**.**
- Khwaldia, K., Ferez, C., Banon, S., Desobry, S., & Hardy, J. (2004). Milk proteins for edible films and coatings. *Critical Reviews in Food Science and Nutrition*, 44(4), 239–251. https://doi.org/10.1080/10408690490464906.
- Klenow, S., Glei, M., Haber, B., Owen, R., & Pool-Zobel, B. L. (2008). Carob fibre compounds modulate parameters of cell growth differently in human HT29 colon adenocarcinoma cells than in LT97 colon adenoma cells. *Food and Chemical Toxicology*, 46(4), 1389–1397.https://doi.org/10.1016/j.fct.2007.09.003.
- Koronis, G., Silva, A., & Fontul, M. (2013). Green composites: A review of adequate materials for automotive applications. *Composites Part B: Engineering*, 44(1), 120–127. http://dx.doi.org/10.1016/j.compositesb.2012.07.004.
- Kreyenschmidt, J., & Ibald, R. (2012). Modeling shelf life using microbial indicators. *Shelf Life Assessment of Food*, 127–168. https://doi.org/10.1201/b11871-7.
- Krokou, A., Stylianou, M., & Agapiou, A. (2019). Assessing the volatile profile of carob tree *(Ceratonia siliqua* L.*). Environmental Science and Pollution Research.* https://doi.org/10.1007/s11356-019-04664-7
- Ku, H., Wang, H., Pattarachaiyakoop, N., & Trada, M. (2011). A review on the tensile properties of natural fiber reinforced polymer composites. *Composites Part B: Engineering*, 42(4), 856–873. http://dx.doi.org/10.1016/j.compositesb.2011.01.010.

- Kuai, L., Liu, F., Chiou, B.-S., Avena-Bustillos, R. J., McHugh, T. H., & Zhong, F. (2021). Controlled release of antioxidants from active food packaging: a review. *Food Hydrocolloids*, 120, 106992. https://doi.org/10.1016/j.foodhyd.2021.106992.
- Kubow, S. (1992). Routes of formation and toxic consequences of lipid oxidation products in foods. *Free Radical Biology and Medicine*, 12(1), 63–81. https://doi.org/10.1016/0891-5849(92)90059-P.
- Lachkar, N., Al-Sobarry, M., El-Hajaji, H., Lamkinsi, T., Lachkar, M., Cherrah, Y., & Allaoui, K. (2016). Anti-inflammatory and antioxidant effect of *Ceratonia siliqua* L. Methanol barks extract. *Journal of Chemical and Pharmaceutical Research*, 8(3), 202-210.
- Lakkab, I., El Hajaji, H., Lachkar, N., Lefter, R., Ciobica, A., El Bali, B., & Lachkar, M. (2019). *Ceratonia siliqua* L. seed peels: Phytochemical profile, antioxidant activity, and effect on mood disorders. *Journal of Functional Foods*, *54*, 457–465. https://doi.org/10.1016/j.jff.2019.01.041**.**
- Lakshmanan, R., Parkinson, J. A., & Piggott, J. R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (Salmo salar). *LWT - Food Science and Technology*, 40(3), 544–551. https://doi.org/10.1016/j.lwt.2005.12.003.
- Lalit, R., Mayank, P., & Ankur, K. (2018). Natural fibers and biopolymers characterization: a future potential composite material. strojnícky casopis. *Journal of Mechanical Engineering*, 68(1), 33–50. https://doi.org/10.2478/scjme-2018-0004.
- Lammi, S., Barakat, A., Mayer-Laigle, C., Djenane, D., Gontard, N., & Angellier-Coussy, H. (2017). Dry fractionation of olive pomace as a sustainable process to produce fillers for biocomposites. *Powder Technology*, *326*(2018), 44–53. https://doi.org/10.1016/j.powtec.2017.11.060.
- Lammi, S., Le Moigne, N., Djenane, D., Gontard, N., & Angellier-Coussy, H. (2018). Dry fractionation of olive pomace for the development of food packaging biocomposites. *Industrial Crops and Products*, 120(2018), 250–261. https://doi.org/10.1016/j.indcrop.2018.04.052.
- Lan, W., Wang, S., Zhang, Z., Liang, X., Liu, X., & Zhang, J. (2021). Development of red apple pomace extract/chitosan-based films reinforced by TiO2 nanoparticles as a multifunctional packaging material. *International Journal of Biological Macromolecules*, 168(2021), 105–115. https://doi.org/10.1016/j.ijbiomac.2020.12.051.
- Lavoine, N. (2013). Conception, mise en œuvre et caractérisation de nouveaux bio-nano-matériaux fonctionnels. HAL.https://tel.archives-ouvertes.fr/tel-01124263.

- Lee, R. F. (2003). Photo-oxidation and photo-toxicity of crude and refined oils. *Spill Science & Technology Bulletin*, 8(2), 157–162. https://doi.org/10.1016/S1353-2561(03)00015-X.
- Lee, S. H., & Min, D. B. (1990). Effects, quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. *Journal of Agricultural and Food Chemistry*, 38(8), 1630–1634. https://doi.org/10.1021/jf00098a002.
- Lerfall, J., Bendiksen, E. Å., Olsen, J. V., & Østerlie, M. (2016). A comparative study of organic- versus conventional Atlantic salmon. II. Fillet color, carotenoid- and fatty acid composition as affected by dry salting, cold smoking and storage. *Aquaculture*, 451, 369–376. http://dx.doi.org/10.1016/j.aquaculture.2015.10.004.
- Lerfall, J., Roth, B., Skare, E. F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M. A., & Rotabakk, B. T. (2015). Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon *(Salmo salar* L.*)* during ice storage. *Food Chemistry*, 175, 157–165. http://dx.doi.org/10.1016/j.foodchem.2014.11.111.
- Li, G., Xu, Y., Wang, X., Zhang, B., Shi, C., Zhang, W., & Xia, X. (2014). Tannin-rich fraction from pomegranate rind damages membrane of listeria monocytogenes. *Foodborne Pathogens and Disease*, 11(4), 313–319. https://doi.org/10.1089/fpd.2013.1675.
- Liang, Z., Han, D., Han, F., Wu, Z., Zhao, X., Fu, W., … Niu, L. (2021). Novel strategy of natural antioxidant nutrition quality evaluation in food: oxidation resistance mechanism and synergistic effects investigation. *Food Chemistry*, 359, 129768. https://doi.org/10.1016/j.foodchem.2021.129768.
- López-de-Dicastillo, C., Alonso, J. M., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2010). Improving the antioxidant protection of packaged food by incorporating natural flavonoids into ethylene−vinyl alcohol copolymer (EVOH) films. *Journal of Agricultural and Food Chemistry*, 58(20), 10958–10964.
- López, P., Sánchez, C., Batlle, R., & Nerín, C. (2005). Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry*, 53(17), 6939–6946. https://doi.org/10.1021/jf050709v**.**
- López, P., Sánchez, C., Batlle, R., & Nerín, C. (2007). Development of flexible antimicrobial films using essential oils as active agents. *Journal of Agricultural and Food Chemistry*, 55(21), 8814–8824. https://doi.org/10.1021/jf071737b**.**

- Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., … Franco, D. (2018). Berries extracts as natural antioxidants in meat products: a review. *Food Research International*, 106, 1095–1104. https://doi.org/10.1016/j.foodres.2017.12.005.
- Lund, B.M., Eklund, T. (2000) Control of pH and use of organic acids. In the microbiological safety and quality of food, Aspen Publishers, Gaithersburg, MD, pp. 175–199.
- Makris, D. P., & Kefalas, P. (2004). Carob pods (*Ceratonia siliqua* L.) as a source of polyphenolic antioxidants. *Food Technology and Biotechnology*, 42(2), 105–108.
- Makris, D. P., Boskou, G., & Andrikopoulos, N. K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis*, 20(2), 125–132. https://*doi*.org/*10.1016/j.jfca.2006.04.010.*
- Mamta, Misra, K., Dhillon, G. S., Brar, S. K., & Verma, M. (2013). Antioxidants. In book: *Biotransformation of Waste Biomass into High Value Biochemicals*, 117–138. http://dx.doi.org/10.1007/978-1-4614-8005-1_6.
- Marakis, S. (1996). Carob bean in food and feed: current status and future potentials: a critical appraisal. *Journal of Food Science and Technology*, 33(5), 365-383.
- Marcos, B., Sárraga, C., Castellari, M., Kappen, F., Schennink, G., & Arnau, J. (2014). Development of biodegradable films with antioxidant properties based on polyesters containing α-tocopherol and olive leaf extract for food packaging applications. *Food Packaging and Shelf Life*, *1*(2), 140–150. https://doi.org/10.1016/j.fpsl.2014.04.002.
- Marongiu, B., Porcedda, S., Piras, A., Rosa, A., Deiana, M., & Dessì, M. A. (2004). Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. *officinalis* and *Melissa officinalis* subsp. *inodora*. *Phytotherapy Research*, 18(10), 789–792. https://doi.org/10.1002/ptr.1549.
- Mastromatteo, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2010). Advances in controlled release devices for food packaging applications. *Trends in Food Science & Technology*, 21(12), 591–598. https://*doi*.org/*10.1016/j.tifs.2010.07.010.*
- Mcclements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science*, 65(8), 1270–1282. https://doi.org/10.1111/j.1365-2621.2000.tb10596.x.

- McClements, D. J., & Xiao, H. (2014). Excipient foods: Designing food matrices that improve the oral bioavailability of pharmaceuticals and nutraceuticals. *Food and Function, 5*(7), 1320–1333. https://doi.org/10.1039/c4fo00100a.
- Mekhoukhe, A., Kicher, H., Ladjouzi, A., Medouni-Haroune, L., Brahmi, F., Medouni-Adrar, S., & Madani, K. (2019). Antioxidant activity of carob seeds and chemical composition of their bean gum by- products. *Journal of Complementary and Integrative Medicine*, 16(1), 1–11. https://doi.org/10.1515/jcim-2017-0158.
- Merlo, T. C., Contreras-Castillo, C. J., Saldaña, E., Barancelli, G. V., Dargelio, M. D. B., Yoshida, C. M. P., … Venturini, A. C. (2019). Incorporation of pink pepper residue extract into chitosan film combined with a modified atmosphere packaging: Effects on the shelf life of salmon fillets. *Food Research International*, 125(2019), 1–10. https://doi.org/10.1016/j.foodres.2019.108633.
- Merzouki, A., Ed-Derfoufi, F., El Aallali, A., & Molero-Mesa, J. (1997). Wild medicinal plants used by local Bouhmed population (Morocco), *Fitoter*, 68,444–460.
- Meziani, S., Oomah, B. D., Zaidi, F., Simon-Levert, A., Bertrand, C., & Zaidi-Yahiaoui, R. (2015). Antibacterial activity of carob (Ceratonia siliqua L.) extracts against phytopathogenic bacteria Pectobacterium atrosepticum. *Microbial Pathogenesis*, 78, 95–102. http://dx.doi.org/10.1016/j.micpath.2014.12.001.
- Mimica-Dukic, N., Bozin, B., Sokovic, M., & Simin, N. (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (*Lamiaceae)* essential oil. *Journal of Agricultural and Food Chemistry*, 52(9), 2485–2489. https://doi.org/10.1021/jf030698a.
- Miyazawa, R., Tomomasa. T., Kaneko. H., Morikawa, A. (2006). Effect of formula thickened with locust bean gum on gastric emptying in infants. *Journal of Paediatrics and Child Health*, 42 (12), 808–812. https://doi.org/10.1111/j.1440-1754.2006.00982.x.
- Modi, H.A. (2009). An introduction to microbial spoilage of foods. In: Modi, H.A. (Ed.), microbial spoilage of foods. Aavishkar Publishers, Jaipur, India, pp, 43–66. ISBN978-81-7910-285-5.
- Mohan, C. O., Ravishankar, C. N., Lalitha, K. V., & Srinivasa Gopal, T. K. (2012). Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. *Food Hydrocolloids*, 26(1), 167–174. https://doi.org/10.1016/j.foodhyd.2011.05.005.
- Montero-Prado, P., Rodriguez-Lafuente, A., & Nerin, C. (2011). Active label-based packaging to extend the shelf-life of "*Calanda*" peach fruit: Changes in fruit quality and

enzymatic activity. *Postharvest Biology and Technology*, 60(3), 211–219. https://dx.doi.org/*10.1016*/*j.postharvbio*.2011.01.008.

- Montville, T.J. and Matthews, K. (2005). Food microbiology: an Introduction. ASM Press, Washington DC.
- Moudache, M., Colon, M., Nerín, C., & Zaidi, F. (2016). Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. *Food Chemistry*, 212, 521–527. https://doi.org/10.1016/j.foodchem.2016.06.001.
- Moudache, M., Colon, M., Nerin, C., & Zaidi, F. (2017). Antioxidant effect of an innovative active plastic film containing olive leaves extract on fresh pork meat and its evaluation by raman spectroscopy. *Food Chemistry*, 229, 98–103. https://doi.org/ 10.1016/j.foodchem.2016.06.001.
- Mousavi Khaneghah, A., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions. *Food and Bioproducts Processing*, 111, 1–19. https://doi.org/10.1016/j.fbp.2018.05.001.
- Muncke, J. (2016). Chemical migration from food packaging to food. In book: *Reference Module in Food Science*. https://doi.org/*10.1016/B978-0-08-100596-5.03311-4*.
- Nafis, A., Kasrati, A., Jamali, C. A., Mezrioui, N., Setzer, W., Abbad, A., & Hassani, L. (2019). Antioxidant activity and evidence for synergism of *Cannabis sativa* (L.) essential oil with antimicrobial standards. *Industrial Crops and Products*, 137, 396–400. https://doi.org/10.1016/j.indcrop.2019.05.032.
- Nasar-Abbas, S. M., e-Huma, Z., Vu, T.H., Khan, M. K., Esbenshade, H., & Jayasena, V. (2015). Carob kibble: a bioactive-rich food ingredient. comprehensive, *Reviews in Food Science and Food Safety*, 15(1), 63–72. https://*doi*.org/*10.1111/1541-4337.12177*.
- Ncube, L. K., Ude, A. U., Ogunmuyiwa, E. N., Zulkifli, R., & Beas, I. N. (2020). Environmental impact of food packaging materials: a review of contemporary development from conventional plastics to polylactic acid based materials. *Materials*, 13(21), 4994. https://doi.org/*10.3390*/*ma13214994*.
- Ndir, B., Lognay, G., Wathelet B, Cornelius C., Marlier, M., Thonart, P. (2000) Composition chimique du nététu, condiment alimentaire produit par fermentation des graines du caroubier africain *Parkia biglobosa* (Jacq.) Benth. *Biotechnologie, Agronomie, Société et Environnement*, 4, 101–5.

- Nenadis, N., & Tsimidou, M. (2002). Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH.) Tests. *JAOCS, Journal of the American Oil Chemists' Society*, 79(12), 1191–1195. https://doi.org/10.1007/s11746-002-0626-z.
- Nerín, C. (2010). Antioxidant active food packaging and antioxidant edible films. *Oxidation in Foods and Beverages and Antioxidant Applications*, 496–515. https://doi.org/*10.3390*/*ma13214994.*
- Nerín, C. (2011). Antioxidant active packaging and antioxidant edible films Chapter 31 in the book "Oxidation in foods and beverages and antioxidant applications". In E.A. Decker, R.J. Elias, & D.J. McClements (Eds.), Oxidation in Foods and Beverages and Antioxidant Applications (1st edition). Management in Different Industry Sectors, 2. (pp. 496–515). Cambridge, United Kingdom: Woodhead Publishing. Management in Different Industry Sectors, 2.
- Nerin, C. (2016). Migration analysis of compounds in food packaging. *Edible Films and Coatings*.pp 8.
- Nerin, C., Alfaro, P., Aznar, M., & Domeno, C. (2013). The challenge of identifying nonintentionally added substances from food packaging materials: a review. *Analytica Chimica Acta*, 775, 14–24. https://doi.org/10.1016/j.aca.2013.02.028.
- Nerin, C., Astudillo, M., Covían, I., & Mujika, R. (2006). *WO2007144444A1 Active packaging that inhibits food pathogens*.
- Nerín, C., Aznar, M., & Carrizo, D. (2016). Food contamination during food process. *Trends in Food Science and Technology*, 48, 63–68. https://doi.org/10.1016/j.tifs.2015.12.004.
- Nerin, C., Bentayeb, K., & Rodriguez-Lafuente, A. (2012). Sampling techniques for the determination of migrants from packaging materials in food. *Comprehensive Sampling and Sample Preparation*, 357–379. https://doi.org/10.1016/B978-0-12-381373-2.00139-3.
- Nerín, C., Gaspar, J., Vera, P., Canellas, E., Aznar, M., & Mercea, P. (2013). Determination of partition and diffusion coefficients of components of two rubber adhesives in different multilayer materials. *International Journal of Adhesion and Adhesives*, 40, 56–63. http://dx.doi.org/10.1016/j.ijadhadh.2012.07.003.
- Nerín, C., Tovar, L., & Salafranca, J. (2008). Behaviour of a new antioxidant active film versus oxidizable model compounds. *Journal of Food Engineering*, 84(2), 313–320. https://doi.org/10.1016/j.jfoodeng.2007.05.027.

- Ojagh, S. M., Núñez-Flores, R., López-Caballero, M. E., Montero, M. P., & Gómez-Guillén, M. C. (2011). Lessening of high-pressure-induced changes in Atlantic salmon muscle by the combined use of a fish gelatin-lignin film. *Food Chemistry*, 125(2), 595–606. https://doi.org/10.1016/j.foodchem.2010.08.072.
- Olsson, G. B., Seppola, M. A., & Olsen, R. L. (2007). Water-holding capacity of wild and farmed cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) muscle during ice storage. *LWT - Food Science and Technology*, 40(5), 793–799. https://doi.org/10.1016/j.lwt.2006.04.004.
- Oudjedi, K., Manso, S., Nerin, C., Hassissen, N., & Zaidi, F. (2019). New active antioxidant multilayer food packaging films containing Algerian sage and bay leaves extracts and their application for oxidative stability of fried potatoes. *Food* Control, 98, 216–226. https://doi.org/https://doi.org/10.1016/j.foodcont.2018.11.018**.**
- Owen, R. W., Haubner, R., Hull, W. E., Erben, G., Spiegelhalder, B., Bartsch, H., & Haber, B. (2003). Isolation and structure elucidation of the major individual polyphenols in carob fibre. *Food and Chemical Toxicology*, 41(12), 1727-1738. http://dx.*doi*.org/*10.1016*/*S0278-6915*(*03*)*00200-X*.
- Oziyci, H. R., Tetik, N., Turhan, I., Yatmaz, E., Ucgun, K., Akgul, H., … Karhan, M. (2014). Mineral composition of pods and seeds of wild and grafted carob (*Ceratonia siliqua* L.) fruits. *Scientia Horticulturae*, 167, 149–152. https://doi.org/10.1016/j.scienta.2014.01.005.
- Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., & Napolitano, A. (2020). Bioactive phenolic compounds from agri-food wastes: an update on green and sustainable extraction methodologies. *Frontiers in Nutrition*, https://doi.org/10.3389/fnut.2020.00060.
- Papaefstathiou, E., Agapiou, A., Giannopoulos, S., & Kokkinofta, R. (2018). Nutritional characterization of carobs and traditional carob products. *Food Science and Nutrition*, 6(8), 2151–2161. https://doi.org/10.1002/fsn3.776**.**
- Papagiannopoulos, M., Wollseifen, H. R., Mellenthin, A., Haber, B., & Galensa, R. (2004). Identification and quantification of polyphenols in carob fruits (*Ceratonia siliqua* L.) and derived products by HPLC-UV-ESI/MSn. *Journal of Agricultural and Food Chemistry*, 52(12), 3784–3791. https://doi.org/*10.1021*/*jf030660y*.
- Papuc, C., Goran, G. V., Predescu, C. N., & Nicorescu, V. (2016). Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: a

review. *Comprehensive Reviews in Food Science and Food Safety*, 16(1), 96–123. https://doi.org/10.1111/1541-4337.12241.

- Paul, R.K., Dutta, D., Chakraborty, D., Nayak,, Dutta, P.K., Nag, M. (2019). Antimicrobial agents from natural sources: An overview. *Advance Pharmaceutical Journal.* 4(2), 41–51 41. https://doi.org/10.31024/apj.2019.4.2.1.
- Pawłowska, K., Kuligowski, M., Jasi´nska-Kuligowska, I., Kido´n, M., Siger, A., Rudzi´nska, M., & Nowak, J. (2018). Effect of replacing cocoa powder by carob powder in the muffins on sensory and physicochemical properties. *Plant Foods for Human Nutrition*, 73(3), 196–202. https://doi.org/10.1007/s11130-018-0675-0.
- Pereira, I., Severino, P., Santos, A. C., Silva, A. M., & Souto, E. B. (2018). Linalool bioactive properties and potential applicability in drug delivery systems. *Colloids and Surfaces B: Biointerfaces*, 171, 566–578. https://doi.org/10.1016/j.colsurfb.2018.08.001.
- Pérez-Olleros, L., Garcia-Cuevas, M., & Ruiz-Roso, B. (1999). Nota. Influencia de la pulpa y fibra natural de algarrobas sobre algunos aspectos de la utilización nutritiva de la dieta y la colesterolemia en ratas / Note. Influence of pulp and natural carob fiber on some aspects of nutritional utilization and cholesterolemia in rats. *Food Science and Technology International*, 5(5), 425–430. https://doi.org/10.1177/108201329900500508.
- Pešić, M. B., Milinčić, D. D., Kostić, A. Ž., Stanisavljević, N. S., Vukotić, G. N., Kojić, M. O., … Tešić, Ž. L. (2019). In vitro digestion of meat- and cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected?. *Food Chemistry*, 284, 28–44. https://doi.org/10.1016/j.foodchem.2019.01.107.
- Petruzzi, L., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Microbial spoilage of foods. *The Microbiological Quality of Food*, 1–21. http://dx.doi.org/10.1016/B978-0-08-100502-6.00002-9.
- Pezo, D., Salafranca, J., & Nerín, C. (2006). Design of a method for generation of gas-phase hydroxyl radicals, and use of HPLC with fluorescence detection to assess the antioxidant capacity of natural essential oils. *Analytical and Bioanalytical Chemistry*, 385, 1241–1246. https://doi.org/10.1007/s00216-006-0395-4.
- Pezo,D.,Salafranca, J., & Nerín, C. (2008). Determination of the antioxidant capacity of active food packagings by in situ gas-phase hydroxyl radical generation and high-performance liquid chromatography–fluorescence detection. *Journal of Chromatography A*, 1178(1–2), 126–133. https://doi.org/10.1016/j.chroma.2007.11.062.

- Pinto, T., Aires, A., Cosme, F., Bacelar, E., Morais, M. C., Oliveira, I., … Gonçalves, B. (2021). Bioactive (poly)phenols, volatile compounds from vegetables, medicinal and aromatic plants. *Foods, 10*(1), 106. https://doi.org/10.3390/foods10010106.
- Poojary, M. M., Putnik, P., Bursać Kovačević, D., Barba, F. J., Lorenzo, J. M., Dias, D. A., & Shpigelman, A. (2017). Stability and extraction of bioactive sulfur compounds from Allium genus processed by traditional and innovative technologies. *Journal of Food Composition and Analysis*, 61, 28–39. https://doi.org/10.1016/j.jfca.2017.04.007.
- Pokorny, J., Yanishlieva, N. V. & Gordon, M. H. (2001). Antioxidantes de los alimentos. Aplicaciones prácticas. Zaragoza (Spain): Acribia, S. A., 67.
- Popovici, C., & Saykova, I. (2009). Evaluation de l' activité antioxydante des composés phénoliques par la réactivité avec le radical libre DPPH. *Génie Industriel*, 4(16), 26–39.
- Potter, N. N., & Hotchkiss, J. H. (1995). Food deterioration and its control. *Food Science*, 113–137. https://doi.org/10.1007/978-1-4615-4985-7_7.
- Powell, S. M., Ratkowsky, D. A., & Tamplin, M. L. (2015). Predictive model for the growth of spoilage bacteria on modified atmosphere packaged Atlantic salmon produced in Australia. *Food Microbiology*, 47, 111–115. https://*doi*.org/*10.1016*/*j.fm.2014.12.001*.
- Qian, S., Ji, H., Wu, X., Li, N., Yang, Y., Bu, J., ... Zhang, C. (2018). Detection and quantification analysis of chemical migrants in plastic food contact products. *PLOS ONE*, 13(12), e0208467. https://doi.org/10.1371/journal.pone.0208467.
- Quezel, P., & Santa, S. (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales (tome 1) (Editions d).
- Quiles-Carrillo, L., Mellinas, C., Garrigos, M. C., Balart, R., & Torres-Giner, S. (2019). Optimization of microwave-assisted extraction of phenolic compounds with antioxidant activity from carob pods. Food Analytical Methods. *Food Analytical Methods*. https://doi.org/10.1007/s12161-019-01596-3.
- Ray, B. (2004). Fundamental food microbiology, 3rd Ed. CRC Press, FL, pp 439-534. ISBN: 0- 8493-1610-3.
- Regulation (EC) No 2074/2005. Official Journal of the European union, 27–59.
- Rejeb, M.N. (1995). Le caroubier en Tunisie: situations et perspectives d'amélioration-quel avenir pour l'amélioration des plantes? AUPELF-UREF Ed., John Libbey Eurotext, Paris, 79-85.

- Ribeiro, M., Bernardo-Gil, M., & Esquível, M. (2001). *Melissa officinalis*, L.: study of antioxidant activity in supercritical residues. *The Journal of Supercritical Fluids*, 21(1), 51–60. https://doi.org/10.1016/S0896-8446(01)00078-X.
- Rico, D., Alonso De Linaje, A., Herrero, A., Asensio-Vegas, C., Miranda, J., Martínez-Villaluenga, C., De Luis, D. A., & Martin-Diana, A. B. (2018). Carob by-products and seaweeds for the development of functional bread. *Journal of Food Processing and Preservation*, 42(8), 1–9. https://doi.org/10.1111/jfpp.13700.
- Rico, D., Martín-Diana, A. B., Martínez-Villaluenga, C., Aguirre, L., Silván, J. M., Dueñas, M., … Lasa, A. (2019). In vitro approach for evaluation of carob by-products as source bioactive ingredients with potential to attenuate metabolic syndrome (MetS). *Heliyon*, 5(1), e01175. https://doi.org/10.1016/j.heliyon.2019.e01175.
- Rizzo, V., Tomaselli, F., Gentile, A., La Malfa, S., & Maccarone, E. (2004). Rheological properties and sugar composition of locust bean gum from different carob varieties (*Ceratonia siliqua* L.). *Journal of Agricultural and Food Chemistry*, 52(26), 7925–7930. https://doi.org/10.1021/jf0494332.
- Rodríguez-Solana, R., Romano, A., & Moreno-Rojas, J. M. (2021). Carob pulp: a nutritional and functional by-product worldwide spread in the formulation of different food products and beverages. A review. Processes, 9(7), 1146. https://doi.org/10.3390/pr9071146.
- Rollini, M., Nielsen, T., Musatti, A., Limbo, S., Piergiovanni, L., Hernandez Munoz, P., & Gavara, R. (2016). Antimicrobial performance of two different packaging materials on the microbiological quality of fresh salmon. *Coatings*, 6(1), 6. https://doi.org/10.3390/coatings6010006.
- Roseiro, L. B., Duarte, L. C., Oliveira, D. L., Roque, R., Bernardo-Gil, M. G., Martins, A. I., … Rauter, A. P. (2013). Supercritical, ultrasound and conventional extracts from carob (*Ceratonia siliqua* L.) biomass: Effect on the phenolic profile and antiproliferative activity. *Industrial Crops and Products*, 47, 132–138. https://doi.org/10.1016/j.indcrop.2013.02.026.
- Roseiro, L. B., Tavares, C. S., Roseiro, J. C., & Rauter, A. P. (2013). Antioxidants from aqueous decoction of carob pods biomass (*Ceratonia siliqua* L.): optimisation using response surface methodology and phenolic profile by capillary electrophoresis. *Industrial Crops and Products*, 44, 119–126. https://doi.org/10.1016/j.indcrop.2012.11.006**.**
- Rtibi, K., Jabri, M. A., Selmi, S., Souli, A., Sebai, H., El-Benna, J., … Marzouki, L. (2015). Gastroprotective effect of carob (*Ceratonia siliqua* L.) against ethanol-induced

oxidative stress in rat. *BMC Complementary and Alternative Medicine*, 15(1), 292. https://doi.org/10.1186/s12906-015-0819-9.

- Rtibi, K., Selmi, S., Grami, D., Amri, M., Eto, B., El-benna, J., … Marzouki, L. (2017). Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua* L.) on the gastrointestinal tract: a review. *Biomedicine & Pharmacotherapy*, 93, 522–528. http://dx.doi.org/10.1016/j.biopha.2017.06.088 0753-3322.
- Ruff, N., FitzGerald, R. D., Cross, T. F., & Kerry, J. P. (2002). Fillet shelf-life of Atlantic halibut *Hippoglossus hippoglossus* L. fed elevated levels of α-tocopheryl acetate. *Aquaculture Research*, 33(13), 1059–1071. https://doi.org/10.1046/j.1365-2109.2002.00770.x.
- Ruiz-Hernández, V., Roca, M. J., Egea-Cortines, M., & Weiss, J. (2018). A comparison of semi-quantitative methods suitable for establishing volatile profiles. *Plant Methods*, 14(1), 1–15. https://doi.org/10.1186/s13007-018-0335-2.
- Sabeena Farvin, K. H., Grejsen, H. D., & Jacobsen, C. (2012). Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): Effect on lipid and protein oxidation. *Food Chemistry*, 131(3), 843–851. https://doi.org/10.1016/j.foodchem.2011.09.056.
- Saci, F., Bachir bey, M., Louaileche, H., Gali, L., & Bensouici, C. (2019). Changes in anticholinesterase, antioxidant activities and related bioactive compounds of carob pulp (*Ceratonia siliqua* L.) during ripening stages. *Journal of Food Measurement and Characterization*, 14(2), 937–945. https://doi.org/10.1007/s11694-019-00344-9.
- Sahraee, S., Milani, J. M., Regenstein, J. M., & Kafil, H. S. (2019). Protection of foods against oxidative deterioration using edible films and coatings: a review. *Food Bioscience*, 100451. https://doi.org/10.1016/j.fbio.2019.100451.
- Sallam, K. I. (2007). Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chemistry*, 101(2), 592–600. https://doi.org/10.1016/j.foodchem.2006.02.019.
- Sallam, K. I., Ishioroshi, M., & Samejima, K. (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. *LWT - Food Science and Technology*, 37(8), 849–855. https://doi.org/10.1016/j.lwt.2004.04.001.
- Salleh, W. M. N. H. W., Kammil, M. F., Ahmad, F., & Sirat, H. M. (2015). Antioxidant and anti-inflammatory activities of essential oil and extracts of *Piper miniatum*. *Natural Product Communications*, 10(11), 2005–2008. https://doi.org/10.1177/1934578x1501001151.

- Sampels, S. (2013). Oxidation and antioxidants in fish and meat from farm to fork. *Food Industry*. http://dx.doi.org/10.5772/53169.
- Sanches-Silva, A., Costa, D., Albuquerque, T. G., Buonocore, G. G., Ramos, F., Castilho, M. C., … Costa, H. S. (2014). Trends in the use of natural antioxidants in active food packaging: a review. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, 31(3), 374–395. https://doi.org/10.1080/19440049.2013.879215.
- Santonocito, D., Granata, G., Geraci, C., Panico, A., Siciliano, E. A., Raciti, G., & Puglia, C. (2020). Carob seeds: food waste or source of bioactive compounds?, *pharmaceutics*, 12(11), 1–12. https://doi.org/10.3390/ pharmaceutics12111090.
- Stavrou, I. J., Christou, A., & Kapnissi-Christodoulou, C. P. (2018). Polyphenols in carobs: A review on their composition, antioxidant capacity and cytotoxic effects, and health impact. *Food Chemistry*, 269, 355–374. https://doi.org/10.1016/j.foodchem.2018.06.152.
- Sarikurkcu, C., Ozer, M. S., Calli, N., & Popović-Djordjević, J. (2018). Essential oil composition and antioxidant activity of endemic *Marrubium parviflorum* subsp. *oligodon*. *Industrial Crops and Products*, 119, 209–213. https://doi.org/10.1016/j.indcrop.2018.04.023.
- Sebai, H., Souli, A., Chehimi, L., Rtibi, K., Amri, M., El-Benna, J., & Sakly, M. (2013). In vitro and in vivo antioxidant properties of Tunisian carob (*Ceratonia siliqua* L.). *Journal of Medicinal Plants Research*, 7(2), 85–90. https://doi.org/10.5897/JMPR12.915**.**
- Shah, B. B., & Mehta, A. A. (2018). In vitro evaluation of antioxidant activity of D-Limonene. *Asian Journal of Pharmacy and Pharmacology*, 4(6), 883–887. https://doi.org/10.31024/ajpp.2018.4.6.25.
- Shahidi, F., & Botta, J. R. (1994). Seafoods: chemistry, processing technology and quality. In *Seafoods: Chemistry, Processing Technology and Quality*. https://doi.org/10.1007/978-1-4615-2181-5.
- Shahidi, F., & Zhong, Y. (2005). Lipid oxidation: measurement methods. *Bailey's Industrial Oil and Fat Products*, (3) 357–385. https://doi.org/10.1002/047167849X.bio050.
- Sharadanand Phatak, R., Subhash Hendre, A., & Rohan Sharadanand Phatak, C. (2014). Total antioxidant capacity (TAC) of fresh leaves of Kalanchoe pinnata. *Journal of Pharmacognosy and Phytochemistry JPP*, 2(25), 32–35.
- Shemesh, R., Goldman, D., Krepker, M., Danin-Poleg, Y., Kashi, Y.,Vaxman, A., Segal, E. (2015). LDPE/clay/carvacrol nanocomposites with prolonged antimicrobial activity.

Journal of Applied Polymer Science. 132 (2), 41261–41269. http://dx.doi.org/10.1002/app.41261.

- Sigge, G. O., lipumbu, L., & Britz, T. J. (2011). Proximate composition of carob cultivars growing in South Africa. *South African Journal of Plant and Soil*, 28(1), 17–22. http://dx.doi.org/10.1080/02571862.2011.10640008.
- Simoneau, C., Van den Eede, L., & Valzacchi, S. (2011). Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate. *Food Additives & Contaminants: Part A*, 1–12. https://doi.org/10.1080/19440049.2011.644588.
- Singh, P., Shukla, R., Prakash, B., Kumar, A., Singh, S., Mishra, P. K., & Dubey, N. K. (2010). Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, dl-limonene. *Food and Chemical Toxicology*, 48(6), 1734–1740. https://doi.org/https://doi.org/10.1016/j.fct.2010.04.001.
- Smith, B. M., Bean, S. R., Schober, T. J., Tilley, M., Herald, T. J., & Aramouni, F. (2010). Composition and molecular weight distribution of carob germ protein fractions. *Journal of Agricultural and Food Chemistry*, 58(13), 7794-7800. https://doi.org/10.1021/jf101523p.
- Soares, N., Silva, P., Barbosa, C., Pinheiro, R., & Vicente, A. A. (2017). Comparing the effects of glazing and chitosan-based coating applied on frozen salmon on its organoleptic and physicochemical characteristics over six-months storage. *Journal of Food Engineering*, 194(2017), 79–86. https://doi.org/10.1016/j.jfoodeng.2016.07.021.
- Soladoye, O. P., Juárez, M. L., Aalhus, J. L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, 14(2), 106–122. https://doi.org/10.1111/1541-4337.12127.
- Srour, N., Daroub, H., Toufeili, I., & Olabi, A. (2015). Developing a carob-based milk beverage using different varieties of carob pods and two roasting treatments and assessing their effect on quality characteristics. *Journal of the Science of Food and Agriculture*, 96(9), 3047–3057. https://*doi*.org/*10.1002/jsfa.7476.*
- Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, 36(1), 1–29. https://doi.org/10.1016/S0168-1605(96)01233-0.

- Sucan, M.K. (2004). Identifying and preventing off-flavors. *Food Technology*. 58(11), 36–40.
- Sultan, M., Hafez, O. M., Saleh, M. A., & Youssef, A. M. (2021). Smart edible coating films based on chitosan and beeswax-pollen grains for the postharvest preservation of Le Conte pear. *RSC Advances*, 11(16), 9572–9585. https://doi.org/10.1039/d0ra10671b.
- Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, 21(9), 1199–1218. https://doi.org/10.1016/j.foodcont.2010.02.003.
- Taylor, S., & Todd, P. A. (1995). Understanding information technology usage: a test of competing models. *Information Systems Research*, 6(2), 144–176. https://doi.org/10.1287/isre.6.2.144.
- Tehrany, E. A., & Desobry, S. (2004). Partition coefficients in food/packaging systems: a review. *Food Additives and Contaminants*, 21(12), 1186–1202. http://dx.doi.org/10.1080/02652030400019380.
- Tetik, N., & Yüksel, E. (2014). Ultrasound-assisted extraction of d-pinitol from carob pods using response surface methodology. *Ultrasonics Sonochemistry*, 21(2), 860–865. http://dx.doi.org/10.1016/j.ultsonch.2013.09.008.
- Till, D., Schwope, A. D., Ehntholt, D. J., Sidman, K. R., Whelan, R. H., Schwartz, P. S., … Rainey, M. L. (1987). Indirect food additive migration from polymeric food packaging materials. *CRC Critical Reviews in Toxicology*, 18(3), 215–243. https://doi.org/10.3109/10408448709089862.
- Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2008). Kinetics of freshly squeezed orange juice quality changes during ozone processing. *Journal of Agricultural and Food Chemistry*, 56(15), 6416–6422. https://doi.org/10.1021/jf800515e.
- Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79(7), R1231–R1249. https://doi.org/10.1111/1750-3841.12492.
- Tovar, L., Salafranca, J., Sánchez, C., & Nerín, C. (2005). Migration studies to assess the safety in use of a new antioxidant active packaging. *Journal of Agricultural and Food Chemistry*, 53(13), 5270–5275. https://doi.org/10.1021/jf050076k.
- Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2013). Effect of carob flour addition on the rheological properties of gluten-free

breads. *Food and Bioprocess Technology*, 7(3), 868–876. https://doi.org/10.1007/s11947-013-1104-x.

- Ubeda, S., Aznar, M., Rosenmai, A. K., Vinggaard, A. M., & Nerín, C. (2019). Migration studies and toxicity evaluation of cyclic polyesters oligomers from food packaging adhesives. *Food Chemistry*, 125918. https://doi.org/10.1016/j.foodchem.2019.125918.
- Úbeda, S., Aznar, M., Vera, P., Nerín, C., Henríquez, L., Taborda, L., & Restrepo, C. (2017). Overall and specific migration from multilayer high barrier food contact materials – kinetic study of cyclic polyester oligomers migration. *Food Additives & Contaminants*: *Part A*, 34(10), 1784–1794. https://doi.org/10.1080/19440049.2017.1346390.
- Urbina, L., Eceiza, A., Gabilondo, N., Corcuera, M. Á., & Retegi, A. (2019). Valorization of apple waste for active packaging: multicomponent polyhydroxyalkanoate coated nanopapers with improved hydrophobicity and antioxidant capacity. *Food Packaging and Shelf Life*, 21, 100356. https://doi.org/10.1016/j.fpsl.2019.100356.
- Van Haute, S., Raes, K., Devlieghere, F., & Sampers, I. (2017). Combined use of cinnamon essential oil and MAP/vacuum packaging to increase the microbial and sensorial shelf life of lean pork and salmon. *Food Packaging and Shelf Life*, 12, 51–58. http://dx.doi.org/10.1016/j.fpsl.2017.02.004.
- Van Tuil, R., Fowler, P., Lawther, M., Weber, C.J. (2000). Properties of biobased packaging materials. Publisher: KVL, Department of Dairy and Food Science.
- Vatavali, K., Karakosta, L., Nathanailides, C., Georgantelis, D., & Kontominas, M. G. (2013). Combined effect of chitosan and oregano essential oil dip on the microbiological, chemical, and sensory attributes of red porgy (*Pagrus pagrus*) stored in ice. *Food and Bioprocess Technology*, 6, 3510–3521. https://doi.org/10.1007/s11947-012-1034-z.
- Vera, P., Echegoyen, Y., Canellas, E., Nerin, C., Palomo, M., Madrid, Y., & Camara, C. (2016). Nano selenium as antioxidant agent in a multilayer food packaging material. Analytical and Bioanalytical Chemistry, 1–12. http://dx.doi.org/10.1007/ s00216-016-9780-9.
- Verma, M. K., Shakya, S., Kumar, P., Madhavi, J., Murugaiyan, J., & Rao, M. V. R. (2021). Trends in packaging material for food products: historical background, current scenario, and future prospects. *Journal of Food Science and Technology*, 58(11), 4069–4082. https://doi.org/10.1007/s13197-021-04964-2.
- Vinceković, M., Viskić, M., Jurić, S., Giacometti, J., Bursać Kovačević, D., Putnik, P., … Režek Jambrak, A. (2017). Innovative technologies for encapsulation of Mediterranean

plants extracts. *Trends in Food Science & Technology*, 69, 1–12. http://dx.doi.org/10.1016/j.tifs.2017.08.001.

- Victoria, F. N., Lenardão, E. J., Savegnago, L., Perin, G., Jacob, R. G., Alves, D., … Nascente, P. da S. (2012). Essential oil of the leaves of *Eugenia uniflora* L.: Antioxidant and antimicrobial properties. *Food and Chemical Toxicology*, 50(8), 2668–2674. https://doi.org/10.1016/j.fct.2012.05.002.
- Wanasundra, P.K.J.P.D., & Shahidi, F. (2005). Antioxidants: science, technology, and applications. edible oil and fat products: *Chemistry, Properties, and Safety Aspects*. https://doi.org/10.1002/047167849X.bio002.
- Waraho, T., McClements, D. J., & Decker, E. A. (2011). Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions. *Food Chemistry*, 129(3), 854–859. https://doi.org/10.1016/j.foodchem.2011.05.034.
- Wei, A., & Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils. *Journal of Agricultural and Food Chemistry*, 55(5), 1737–1742. https://doi.org/10.1021/jf062959x.
- Wrona (2017). Nuevos agentes antioxidants incorporados en nuevos materiales de envase en el marco del proyecto innpacto actibiopack. Memoria presentada para optar al titulo de Doctor International. Universidad de Zaragoza. pp 159.
- Wrona, M., & Nerin, C. (2019). CHAPTER 7. Risk Assessment of Plastic Packaging for Food Applications: Mass Spectrometry Techniques. *Food Contact Materials Analysis*. https://doi.org/10.1039/9781788012973-00163.
- Wrona, M., & Nerín, C. (2020). Analytical Approaches for Analysis of Safety of Modern Food Packaging: a Review. *Molecules*, 25(3), 752. https://doi.org/10.3390/molecules25030752.
- Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). *Food Control*, 54, 200–207. https://doi.org/10.1016/j.foodcont.2015.02.008.
- Wrona, M., Cran, M. J., Nerín, C., & Bigger, S. W. (2017). Development and characterisation of HPMC films containing PLA nanoparticles loaded with green tea extract for food packaging applications. *Carbohydrate Polymers*, 156, 108–117. https://doi.org/10.1016/j.carbpol.2016.08.094.

- Wrona, M., Nerin, C., Alfonso, M. J., & Caballero, M. A. (2017). Antioxidant packaging with encapsulated green tea for fresh minced meat. *Innovative Food Science & Emerging Technologies*, 41, 307–313. http://dx.doi.org/10.1016/j.ifset.2017.04.001.
- Wrona, M., Vera, P., Pezo, D., & Nerín, C. (2017). Identification and quantification of odours from oxobiodegradable polyethylene oxidised under a free radical flow by headspace solid-phase microextraction followed by gas chromatography-olfactometry-mass spectrometry. *Talanta*, 172, 37–44. https://doi.org/10.1016/j.talanta.2017.05.022.
- Xiong, Y., Kamboj, M., Ajlouni, S., & Fang, Z. (2021). Incorporation of salmon bone gelatine with chitosan, gallic acid and clove oil as edible coating for the cold storage of fresh salmon fillet. *Food Control*, 125(107994), 1–9. https://doi.org/10.1016/j.foodcont.2021.107994.
- Yagiz, Y., Kristinsson, H. G., Balaban, M. O., Welt, B. A., Raghavan, S., & Marshall, M. R. (2010). Correlation between astaxanthin amount and *a** value in fresh Atlantic salmon (*Salmo salar*) muscle during different irradiation doses. *Food Chemistry*, 120(1), 121–127. https://doi.org/10.1016/j.foodchem.2009.09.086.
- Yang, L., Liu, H., Xia, D., & Wang, S. (2020). Antioxidant properties of camphene-based thiosemicarbazones: Experimental and theoretical evaluation. *Molecules*, 25(5), 1–16. https://doi.org/10.3390/molecules25051192.
- Yeung, A. W. K., Mocan, A., & Atanasov, A. G. (2018). Let food be thy medicine and medicine be thy food: A bibliometric analysis of the most cited papers focusing on nutraceuticals and functional foods. *Food Chemistry*, 269, 455–465. https://doi.org/10.1016/j.foodchem.2018.06.139.
- Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R.,… Coma, V. (2017). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety*, 17(1), 165–199. https://doi.org/10.1111/1541-4337.12322.
- Yousif, A. K., & Alghzawi, H. M. (2000). Processing and characterization of carob powder. *Food Chemistry*, 69(3), 283-287. http://dx.doi.org/10.1016/S0308-8146(99)00265-4.
- Youssef, A. M., Assem, F. M., El-Sayed, H. S., El-Sayed, S. M., Elaaser, M., & Abd El-Salam, M. H. (2020). Synthesis and evaluation of eco-friendly carboxymethyl cellulose/polyvinyl alcohol/CuO bionanocomposites and their use in coating processed cheese. *RSC Advances*, 10(62), 37857–37870. https://doi.org/10.1039/d0ra07898k.
- Zunft, H. J. F., Luder, W., Harde, A., Haber, B., Graubaum, H. J., Koebnick, C., & Gruenwald, J. (2003). Carob pulp preparation rich in insoluble fibre lowers total and LDL

cholesterol in hypercholesterolemic patients. *European Journal of Nutrition*, 42(5), 235–242. https://doi.org/10.1007/s00394-003-0438-y**.**

- Zouaoui, N., Chenchouni, H., Bouguerra, A., Massouras, T., & Barkat, M. (2020). Characterization of volatile organic compounds from six aromatic and medicinal plant species growing wild in North African drylands. *NFS Journal*, *18*, 19–28. https://doi.org/10.1016/j.nfs.2019.12.001.
- Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein Oxidation: Basic Principles and Implications for Meat Quality. *Critical Reviews in Food Science and Nutrition*, 53(11), 1191–1201. http://dx.doi.org/10.1080/10408398.2011.577540.
- Zhao, J., Xiong, Y. L., & McNear, D. H. (2013). Changes in structural characteristics of antioxidative soy protein hydrolysates resulting from scavenging of hydroxyl radicals. *Journal of Food Science*, 78(2), 152–159. https://doi.org/10.1111/1750-3841.12030.

Appendixes

Appendix 1 (Standard curves)

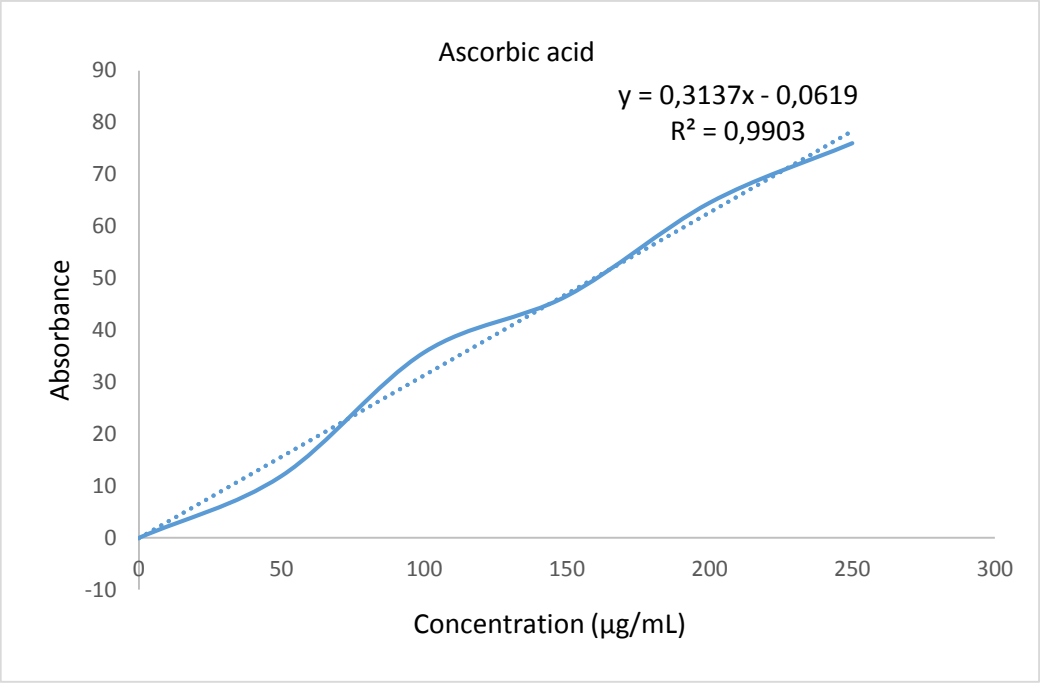

Figure 1: Ascorbic acid standard curve (DPPH).

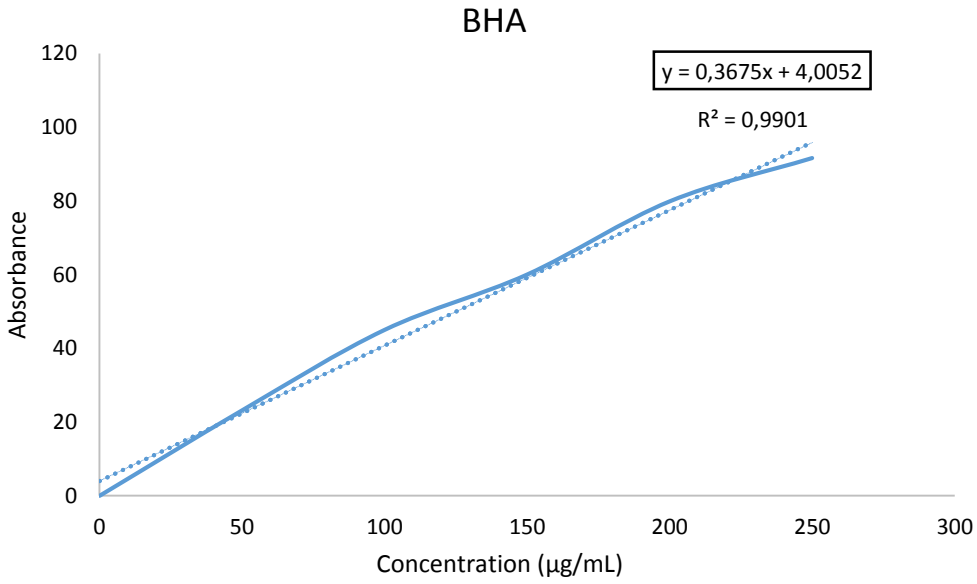

Figure 2: Butylated hydroxyanisole (BHA) standard curve.

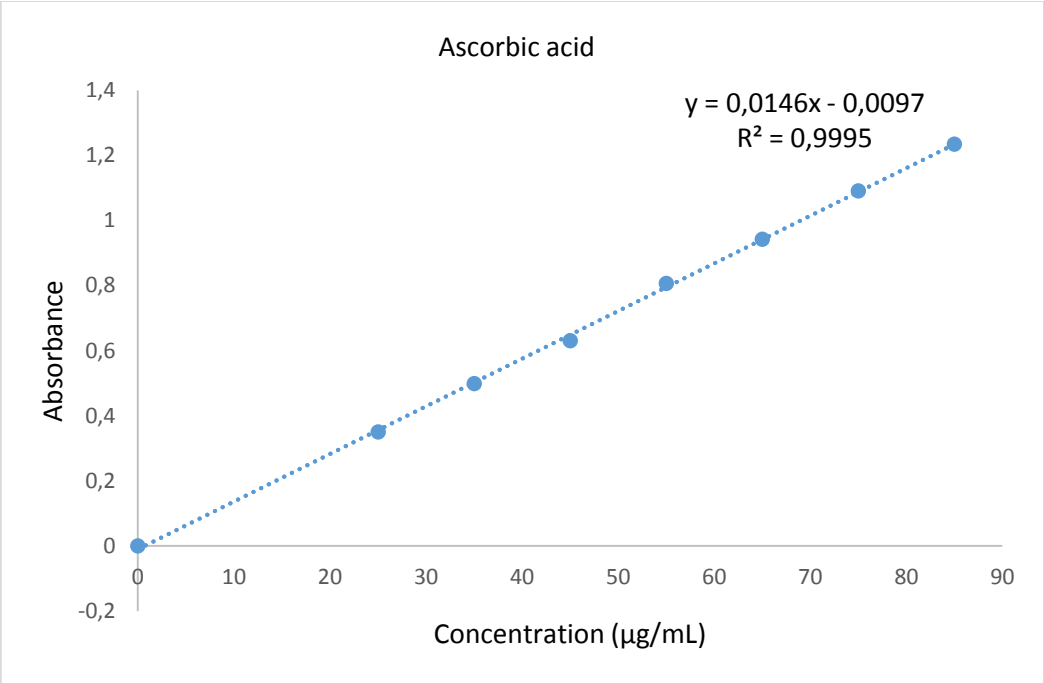

Figure 3: Ascorbic acid standard curve (TAC).

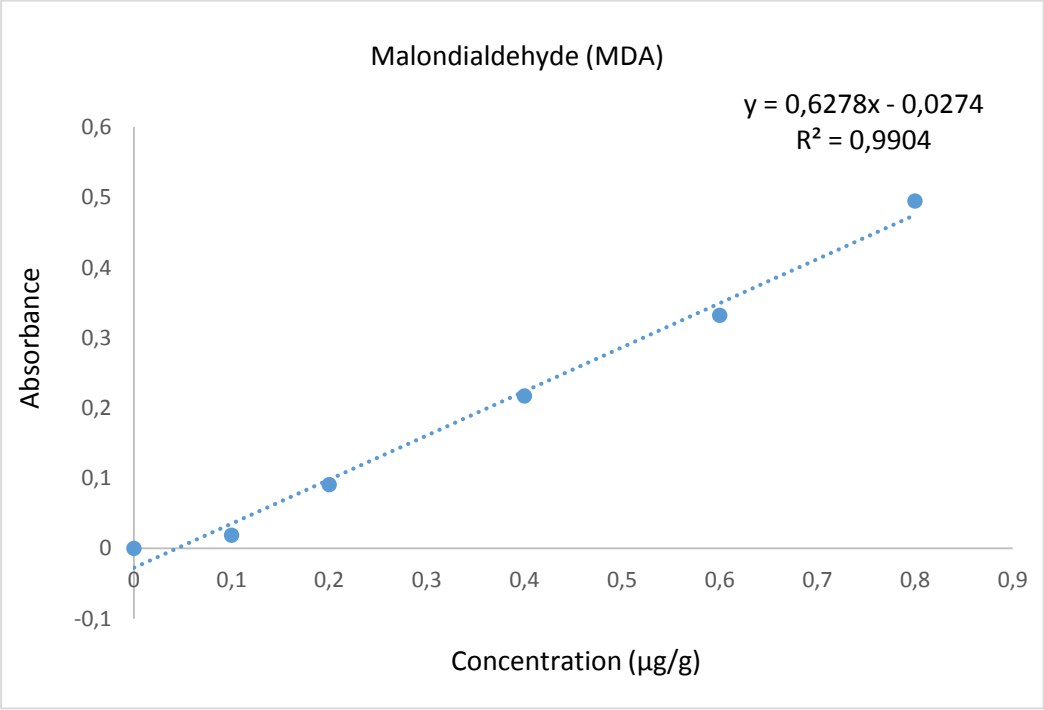

Figure 4: Malondialdehyde standard curve.

Appendix 2

Table 1: Correlation matrix among the different parameters. The numbers indicate the discrimination indexes.

	Drip loss	pH	TBA	TVB-N	WHC	Off-Odor	Overall acceptability	*L**	*a**	*b**
Drip loss	1.00****									
pH	0.76***	1.00****								
TBA	0.78***	0.63***	1.00****							
TVB-N	0.87***	0.70***	0.89***	1.00****						
WHC	-0.84***	-0.71***	-0.80***	-0.94***	1.00****					
Off-Odor	0.90***	0.79***	0.74***	0.90***	-0.94***	1.00****				
Overall acceptability	-0.89***	-0.75***	-0.88***	-0.97***	0.96***	-0.95***	1.00****			
L*	0.80***	0.72***	0.72***	0.85***	-0.85***	0.87***	-0.87***	1.00****		
a*	-0.45**	-0.45**	-0.54**	-0.53**	0.52**	-0.45**	0.51**	-0.56**	1.00****	
b*	0.48**	0.25**	0.48**	0.51**	-0.44**	0.45**	-0.48**	0.45**	-0.42*	1.00****

*: *good correlation (green)*; **: *significant correlation (yellow)*; ***: *highly significant correlation (red)*; ****: *total correlation (grey). The numbers represent the correlation coefficient between the different studied parameters.*

Appendix3

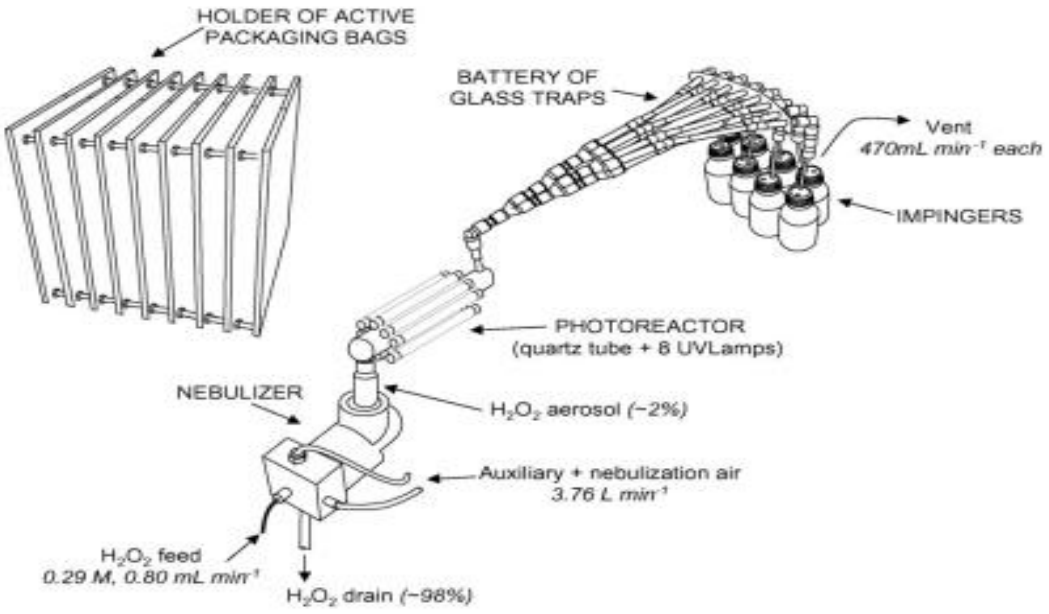

Figure 5: Experimental assembly used for antioxidant capacity assessment of essential oils and polymeric active packaging's.

Résumé

L'objectif de ce projet de thèse est de concevoir un bio-emballage actif issu de ressources naturelles comme alternative aux emballages conventionnels d'origine fossile. Le caroubier (*Ceratonia siliqua* L.) est incontestablement la végétation caractéristique du bassin méditerranéen. En plus de son importance commerciale, il possède des propriétés médicinales. Il est largement utilisé par plusieurs industries agro-alimentaires, qui génèrent plusieurs déchets industriels et causent de graves problèmes environnementaux, cependant, leur valorisation devrait être encouragée.

A travers cette étude, nous avons tenté d'une part de caractériser et d'identifier les différents composés volatils des feuilles, des graines et des résidus issus de la séparation des graines des gousses de *Ceratonia siliqua* L. et d'évaluer leur potentiel antioxydant et antimicrobien. Ensuite, les différents extraits ont été préparés et analysés pour leur application dans la fabrication d'un nouvel emballage actif multicouche à base de polymère cellulosique. D'autre part, un test de bio-conservation a été réalisé pour évaluer son efficacité sur des filets de saumon frais. Les résultats obtenus dans ce travail ont montré une forte activité antioxydante pour les macéras de graines de caroube avec deux méthodes différentes, où des valeurs d'IC_{50} de 112,75 ± 0,32 et 153,61 ± 3,65 µg/mL, respectivement, ont été obtenues en utilisant le test DPPH. Alors que CSE et CSA ont montré la meilleure activité antioxydante par le dosage du phosphomolybdène avec des valeurs de 72,81 ± 0,09 et 67,22 ± 3,91 µg/mL, respectivement. L'analyse chromatographique (HS-SPME-GC-MS) des différents macéras a mis en évidence la présence de quarante-trois composés volatils. L'activité antimicrobienne des différents extraits a montré une faible activité antimicrobienne limitant notre étude à évaluer uniquement la performance antioxydante du nouveau biofilm. Les résultats de l'évaluation de la sécurité sanitaire des aliments n'ont montré aucune migration de composés volatils, y compris non volatils, dans les simulants alimentaires utilisés.

Le filet de saumon frais recouvert du bio-emballage actif stocké à des températures de réfrigération de 4 ± 1 °C a présenté des résultats d'odeur désagréable et d'acceptabilité globale satisfaisants par rapport aux échantillons de contrôle jusqu'au 5ème jour de stockage. Pour l'analyse de la couleur, les échantillons actifs ont mieux conservé les caractéristiques du saumon frais que les témoins pendant les premiers jours de stockage. Les échantillons recouverts d'un emballage actif présentaient au 5ème jour de stockage des valeurs inférieures de pH (6,54 ± 0,05 et 6,60 ± 0,11), d'égouttement (3,17 ± 0,76 et 2,83 ± 0,29%), de substances réactives à l'acide thiobarbiturique (0,056 ± 0,033 et 0,088 ± 0,054 µg MDA/g de saumon.) et d'azote basique volatil total (30,04 ± 3,54 et 32,67 ± 4,81 mg N/100 g de saumon), tandis que les valeurs de capacité de rétention d'eau les plus élevées (92,23 ± 1,09 et 92,91 ± 3,07 %) pour CSE et CSA respectivement, par rapport à ceux des bio-emballages vierges.

Par conséquent, ce nouveau bio-emballage actif a prolongé la durée de conservation du filet de saumon frais, ce qui est prometteur pour la production future de biofilms actifs développés à l'échelle industrielle.

Mots-clés : *Ceratonia siliqua* L., bio-emballage, cellulose, emballage actif, graines de caroube, sécurité alimentaire, filets de saumon frais, durée de conservation.