Universidade do Minho
Escola de Engenharia

Débora Lemos Gadelha de Oliveira

Bigels loaded with antimicrobials and antioxidants for the extension of food shelf life

Dissertação de Mestrado

Mestrado em Tecnologia e Ciência Alimentar

Trabalho efetuado sob a orientação do

Doutor Artur Martins

Professor Doutor Armando Venâncio

December 2021

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U.PORTO
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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration. I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Bigéis carregados com antimicrobianos e antioxidantes para a extensão do tempo de prateleira do alimento

RESUMO

Há uma demanda crescente por parte das autoridades de saúde e dos consumidores para o desenvolvimento de ingredientes e produtos alimentícios mais saudáveis. Tem havido uma tentativa de substituir a gordura animal em alimentos, principal fonte de ácidos graxos saturados, por opções mais saudáveis e seguras. Uma abordagem inovadora para reduzir as gorduras saturadas da dieta é a estruturação de ontras fontes de gordura, como óleos vegetais, em estruturas semi-sólidas chamadas bigéis, que podem substituir total ou parcialmente o conteúdo lipídico original de produtos alimentícios. O objetivo desta tese foi o desenvolvimento e caracterização de bigéis com a incorporação de compostos antioxidantes e antimicrobianos para substituir parcialmente o teor de gordura de um produto cárneo. Formulações de bigeis com e sem compostos bioativos adicionados (cúrcuma, extrato de alecrim, ácido gálico, extrato de chá verde, palmitato de polifenol de chá e óleo essencial de alecrim) foram produzidas e caracterizadas quanto à microestrutura, aparência visual, capacidade de ligação de óleo, atividade antioxidante e antimicrobiana. A atividade antioxidante dos compostos foi avaliada pelo ensaio de substâncias reativas ao ácido 2-tiobarbitúrico (TBARS). A atividade antifúngica dos compostos foi analisada por meio de ensaios de inibição e difusão de fungos contra 4 fungos patogênicos naturalmente encontrados em produtos cárneos (*A. westerdijkiae*, *A. nomius*, *P. solitum* e *P. nordicum*). Os bigeis com adição de óleo essencial de alecrim foram selecionados para serem avaliados em relação à textura, e a atividade antimicotoxigênica deste composto foi medida. Os principais objetivos desta dissertação foram cumpridos, e concluiu-se que para a obtenção de formulações estáveis, um hidrogel com 1,5 % goma de alfarroba e κ-carragenano e um oleogel com 10 % GMS e óleo de girassol foi a combinação ideal. O óleo essencial de alecrim pode ser efetivamente incorporado em bigéis para melhorar a vida útil de um produto à base de carne, bem como a segurança microbiana.

Palavras-chave: bigeis, antioxidantes, antimicrobianos, alecrim.

Bigels loaded with antimicrobials and antioxidants for the extension of food shelf life

ABSTRACT

There is a growing demand for the development of healthier ingredients and food products, which is requested by both health authorities and consumers. There has been an attempt to substitute animal fat, the main source of saturated fatty acids, for healthier and safer options. An innovative approach to reduce dietary saturated fats is the structuring of other fat sources, such as vegetable oils, into semi-solid structures called bigels, that could completely or partially substitute the original lipid content of food products. The objective of this thesis was the development and characterization of bigels with the incorporation of antioxidant and antimicrobial compounds to partially substitute the fat content of a meat product. Bigel formulations with and without added bioactive compounds (turmeric, rosemary extract, gallic acid, green tea extract, tea polyphenol palmitate, and rosemary essential oil) were produced and characterized regarding microstructure, visual appearance, oil binding capacity, antioxidant, and antimicrobial activity. The antioxidant activity of the compounds was evaluated by 2-thiobarbituric acid-reactive substances (TBARS) assay. Antifungal activity of the compounds was analyzed through fungal inhibition and diffusion assays against 4 pathogenic fungi naturally found in meat products (*A. westerdijkiae*, *A. nomius*, *P. solitum,* and *P. nordicum*). Rosemary essential oil bigels were selected to be evaluated regarding texture, and this compound's anti-mycotoxigenic activity was measured. The main objectives of this dissertation were fulfilled, and it was concluded that to obtain stable formulations, a hydrogel with 1.5 % locust bean gum and κ-carrageenan and an oleogel with 10 % GMS and sunflower oil was the optimal combination. Rosemary essential oil could effectively be incorporated into bigels to potentially improve a meat product's shelf life and microbial safety.

Keywords: bigel, antioxidants, antimicrobials, rosemary.

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List of acronyms

AFB1	Aflatoxin B1
BHT	Butylated hydroxytoluene
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration
GMS	Glycerol monostearate
GRAS	Generally Recognized as Safe
LBG	Locust bean gum
MAG	Mono-acylglycerol
MEA	Malt Extract Agar
MUFA	Monounsaturated fatty acid
MUM	Micoteca da Universidade do Minho
OTA	Ochratoxin-A
PHO	Partially hydrogenated oil
PUFA	Polyunsaturated fatty acid
RE	Rosemary extract
REO	Rosemary essential oil
SFA	Saturated fatty acid
TFA	Trans fatty acid
TPP	Tea polyphenol palmitate
YES	Yeast Extract Supplemented
κ-C	κ-Carrageenan

List of symbols

(w/w) Weight by weight

(v/v) Volume by volume

(o/w) Oil-in-water

(w/o) Water-in-oil

INTRODUCTION

1.1 Research motivation

There is a growing demand for the development of healthier ingredients and food products, which is requested by both health authorities and consumers. Many scientific works demonstrate that the quality of dietary fat influences human's wellbeing, and that prioritizing the consumption of mono or polyunsaturated lipids, rather than saturated (SFAs) or trans fatty acids (TFAs), have benefits over cardiovascular health (Marangoni & Garti, 2011; Rivellese et al., 2003). TFAs are the result of the incomplete hydrogenation of edible oils, and they are present in high amounts in partially hydrogenated oils (PHOs). During the 1940s, fat hydrogenation gained focus as a way to tailor desired technological food properties, such as to develop fats that melted at a specific temperature but remained solid at room temperature (Bhandari et al., 2020). However, in 2015, PHOs were determined not Generally Recognized as Safe (GRAS) by the U.S Food and Drug Administration (FDA).

It is well known that diets rich in SFAs and TFAs are correlated with a higher incidence of cardiovascular disease and many other disfunctions, such as augmented inflammation and oxidative stress, as well as type 2 diabetes and metabolic syndrome (López-Pedrouso et al., 2021). Because of that, there has been an attempt to substitute both animal fat (main source of SFAs) and PHOs for healthier and safer options (Bhandari et al., 2020). Further, The World Health Organization is also promoting a reduction in fat intake and an increase in the consumption of unsaturated fat (WHO, 2015).

However, substitution of SFAs and TFAs for healthier options comes with technological complications. Because lipids with high saturation levels tend to be solid at room temperature, while more unsaturated fats tend to have liquid form, the replacement of the saturated fat content of foods often leads to a negative outcome in terms of processing and functionality, causing problems with food texture, spreadability, flavor or rancidity. Also, unsaturated fats are naturally further prone to oxidation, which may shorten product's shelf life. Therefore, there is an urgent need to replace unhealthy ingredients with healthier ones, while maintaining flavor and high levels of consumer acceptance (López-Pedrouso et al., 2021).

An innovative approach aiming to reduce dietary trans and saturated fats is edible oil structuring, imprisoning oil into semi-solid structures with gel-like characteristics. This process can be applied to food

products, such as meat, ice-cream, and pastries, allowing to substitute their fat content, and at the same time, preserve key technological aspects (Marangoni & Garti, 2011). Besides, when it comes to reformulating a food product, obtaining a good overall sensorial acceptability is key. So, it is important to mimic aspects of taste and flavor, since the presence of trans and saturated fats are responsible for many sensory attributes of food and the replacement of hard fats with oils usually leads to a decrease on product quality (S. Barbut et al., 2016; Martins et al., 2018).

Gels can be defined as materials with characteristics of both solids and liquids, and they can be classified as hydrogels, oleogels, bigels or structured emulsions. Oleogels are solid-like materials produced by the entrapment of oil into a three-dimensional network of structuring molecules, named oleogelators. One possible oleogel applicability is its use for the incorporation of functional molecules, such as antimicrobials and antioxidants. This is particularly important because structured oils usually have high unsaturation levels, which makes them much more unstable and prone to oxidation upon processing and storage conditions (Hwang, 2020). Thus, incorporation of antioxidant compounds can counterbalance this issue, possibly promoting a beneficial effect on product's characteristics or on consumer's health, thereby aiding in the product's value.

In this sense, oleogelation can combine the unique features of solid and unsaturated fats without the need of a high saturation level. Oleogelation consists in the conversion of liquid oil into a gel, without modifying the oil's chemical characteristics. There are few edible and bio-based alternatives for combined structuring purposes and for the delivery of bioactive compounds. Bigels are gels comprised of two structured immiscible phases, so they can carry both hydrophilic and hydrophobic compounds. Besides, because only one of its phases is oil-based, the substitution of animal fat for bigels could also be useful for the development of low-fat products. Hence, the formulation of innovative bigels carrying antimicrobials and antioxidants is aligned with ongoing consumer and industries demands (Martins et al., 2018).

1.2 Objectives

The objective of this thesis was the development and characterization of bigels with the incorporation of antioxidant and antimicrobial compounds to partially substitute the fat content of a meat product. To accomplish this, the first aim of this work was to produce and characterize several bigel samples based on

their visual appearance, microscopy analysis and oil binding capacity, so that optimal formulations could be selected for the next steps. The second objective of this work was to determine the effectiveness of the bioactive compounds as antioxidants or antimicrobial agents, evaluating their influence over gel's structure in micro and macroscale level.

1.3 Dissertation outline

This thesis was divided into 5 parts.

The first one comprises this dissertation's framework and motivation. Part 2 consists of a literature review on the types of gels and oleogelators, focusing on edible bigels. Also, this section provides an overview of the use of bioactive compounds for improved functionality in food products and presents a thorough examination of oleogels developed for meat applications. Part 3 contains the materials and methodology used throughout this work. Part 4 presents the results and a critical discussion about them. Finally, part 5 consists of the major conclusions and suggests what could be explored in future studies.

2 LITERATURE REVIEW

2.1 Hydrogels, oleogels, bigels, and structured emulsions

Based on the nature of the medium used, gel formulations can be classified into four main categories: hydrogels, oleogels, bigels and structured emulsions (Inamuddin, 2020). Hydrogels are gels that have a polar liquid phase, commonly with water as the dispersion medium, in which a gelling agent forms hydrophilic polymer chains that are held together by cross-links (Warren et al., 2017). The structure and properties of those gels are dependent on the type of biopolymers used, as well as on the type of molecular interactions between them. Also, the addition of external crosslinker molecules can highly influence the hydrogel's final characteristics (Klein & Poverenov, 2020).

For hydrogel preparation, both synthetic and natural polymers can be employed. However, utilization of the latter is preferable in terms of safety and environmental concerns, since they often have better biocompatibility and biodegradability, and cost a low price (Klein & Poverenov, 2020). The most commonly used natural biopolymers used to form edible hydrogel networks are proteins and polysaccharides, which can be used combined or separately. Polysaccharides (such as starch, pectin, gums, carrageenans, etc.) are non-toxic, biocompatible, and biodegradable polymers that contain numerous functional groups that favor chemical and physical interactions (Ali & Ahmed, 2018). Also, hydrogels can be loaded with hydrophilic bioactive compounds, therefore functioning as delivery systems for the release of molecules in a sustained manner. Also, their structural properties are very adaptable and can be controlled by altering its network density (Inamuddin, 2020).

An oleogel, on the other hand, is a three-dimensional and thermo-reversible structure that holds an apolar organic solvent (such as a vegetable oil). Edible oleogels are composed by both an edible liquid oil phase and gelator (Lampp et al., 2019). The self-assembly of different types of gelator, in connection with various types of oils, makes up the gels' bulk structure, providing distinctive rheological, physical, and sensory features. Oleogels can be be easily tailored to meet its final food application. For example, they can be designed to act as healthy substitutes for solid fats in meat products, such as hamburgers, sausages, ham, and pates (López-Pedrouso et al., 2021; Martins et al., 2018; Martins, Silva, et al., 2019). Along with that, oleogels, similarly to hydrogels, can carry bioactive compounds that can be targeted to specific sites in clinical

applications. However, given its hydrophobic nature, these gels are limited to carry lipophilic compounds, therefore narrowing its possible applications.

Conventional emulsions are systems where two or more immiscible liquid components are homogenized together, creating an arrangement where one phase is dispersed into the other in the form of droplets. There can be either oil-in-water (O/W) or water-in-oil (W/O) systems. Even though emulsions are thermodynamically unstable, it is possible to delay or inhibit their destabilization. For example, emulsifiers are typically amphiphilic compounds that can be added to emulsified systems for stabilizing purposes, due to their capacity to decrease the interfacial tension between the dispersed and continuous phase, thereby retarding droplet coalescence. It is also known that an increase on the viscosity of the continuous phase can promote better emulsion stability. Thus, the incorporation of biopolymers with this purpose can help hinder the movement of droplets and delay phase separation and emulsion breaking (Martin et al., 2018; Rodríguez-Abreu & Lazzari, 2008).

On the other hand, structured emulsions (also termed emulgels or emulsion gels) are different from conventional emulsions. They consist of a gel and a liquid phase emulsified together, so that only one of the emulsion phases (dispersed or continuous) is structured, whereas the other is not. Thus, there can be oleogel-in-water or hydrogel-in-oil systems. When compared to simple emulsions, these emulsion gels present several advantages, such as higher stability during storage (due to diminished oil movement and reduced oxygen diffusion in the gel), and better controlled release of drugs within the intestinal tract as a result of gel matrix protection. In addition to that, structured emulsions enable a great range of new applications, such as mimicking the textural effects of animal fat using vegetable oils (Lin et al., 2020).

Moreover, there are other types of emulsified systems combining hydrophilic and lipophilic features. Bigels (or hybrid gels) combine characteristics of both hydrogels and oleogels, and they are comprised of two or more immiscible liquids, but in this case, both the dispersed and continuous phases are structured and stabilized by individual gelators. There can be oleogel-in-hydrogel or hydrogel-in-oleogel hybrid systems (Martins, Silva, et al., 2019). In the former, the oleogel is present as the dispersed phase, while the hydrogel is the continuous phase; in the latter, the configuration is the opposite. These semi-solid formulations tend to exhibit better properties than the emulsion gels, since they have two gelled phases instead of one. Figure 1 represents the structural differences between oleogels, hydrogels, emulgels and bigels.

Bigels may be prepared by mixing hydrogels and oleogels using low or high shear mechanical stirring, either by cold or hot blending. Studies show that the hot emulsification technique, which consists in mixing the melted hydrogel and oleogel within high temperatures, promote better and faster homogenization when compared to cold blending. Further, in most cases the use of emulsifiers can be dismissed, since in this type of preparation both phases are gelled shortly after mixing (Samui et al., 2021; Singh et al., 2014; Wakhet et al., 2015). This is particularly useful for applications in meat products, since the presence of surfactants or other food additives in this type of food may be undesirable. Even though bigels have recognized potential for many purposes, these hybrid formulations are still recent and underexplored.

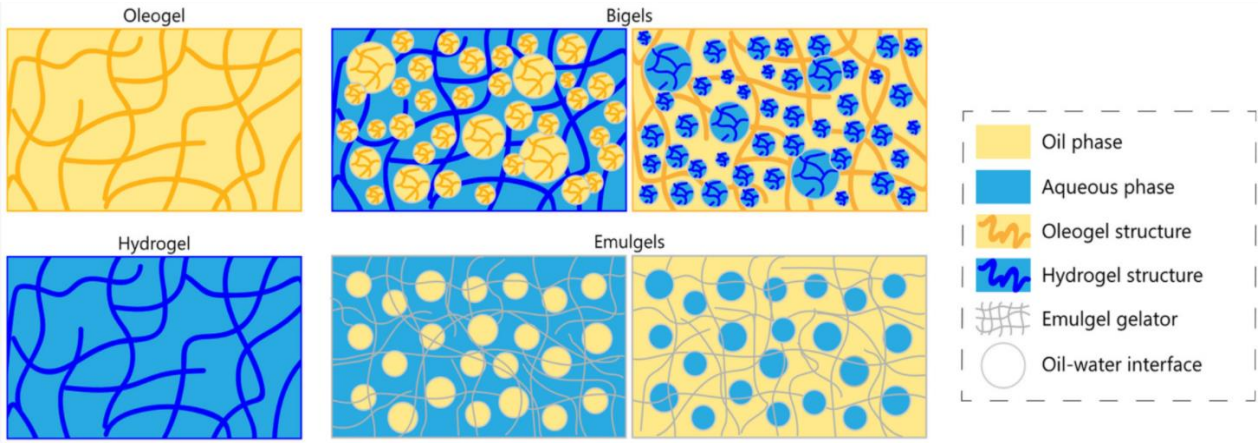

Figure 1 – Schematic representation of oleogels, hydrogels, emulgels and bigels. Reprinted with permission from previous research (Pinto et al., 2021).

When it comes to bigels, there are until this date few works describing edible hydrogel and oleogel combinations. Table 1 features a search done between 2014 and 2021, using the keywords “edible”, "bigel”, “food” and “hybrid gel” at Google Scholar.

Table 1 – Literature review on bigels developed for food applications

Oleogel		**Hydrogel**		**Emulsifiers**	**Reference**
Oil	**Gelator**	**Solvent**	**Gelator**		
Canola oil	Glycerol monostearate		Gelatin	Lecithin and glycerol	Samui et al., 2021
Soybean oil	Stearic acid		Whey protein concentrate	Soy lecithin	Bollom et al., 2020

Oleogel		Hydrogel		Emulsifiers	Reference
Oil	**Gelator**	**Solvent**	**Gelator**		
Corn oil	Glycerol monostearate	Water	κ-carrageenan	-	Zheng et al., 2020
Soybean oil	Glycerol monostearate and beeswax		Gellan gum	-	Zhu et al., 2021
Soybean oil	Stearic acid		Gelatin-agar 1:1	-	Wakhet et al., 2015
Soybean oil	Rice bran wax		Gelatin		Saffold & Acevedo, 2021
Sunflower oil	Sorbitan monopalmitate		Guar, acacia and xantham gum	-	Behera et al., 2015
Sesame oil	Sorbitan monostearate		Guar gum	-	Singh et al., 2014

2.2 Oleogelators

Based on their molecular characteristics, gelators can either be low molecular weight compounds, polymers, or inorganic compounds. By changing or combining different oleogelator types, it is possible to adjust the gel's physical properties and achieve unique structured systems (Fayaz et al., 2019; Inamuddin, 2020). Monoglycerides, natural waxes, phytosterols and ethyl cellulose are the main gelators used in oleogel development targeting meat products formulations (López-Pedrouso et al., 2021). Oleogels structured by mono-acylglycerol (MAG) are examples of self-assembled systems based on low molecular weight structuring agents. Those gels are prepared by heating both the oil and the MAG above their melting temperatures, and then cooling the mixture below MAG's crystallization temperature (Samui et al., 2021). Apart from the gelator's molecular characteristics, oleogelation can be further classified according to the chemical type of gelators, the number of gelators used and the type of building blocks and structuring principles involved in oleogelation (Patel & Dewettinck, 2016).

Oleogelation can be achieved through direct or indirect approaches (Davidovich-Pinhas, 2018). Direct dispersion is the most common technique, which may lead to either crystallite conformations or self-assembled networks, depending on the type of structurant used (Davidovich-Pinhas, 2018; Fayaz et al., 2019). Through this methodology, one or more lipophilic gelators are dispersed into the liquid oil at elevated temperatures, which is then followed by cooling under shear or quiescent conditions (Patel & Dewettinck, 2016).

Edible gels may have different textural attributes designed for various food applications, according to the solvent, the processing conditions and combination of ingredients used (Banerjee & Bhattacharya, 2012; Pernetti et al., 2007). To be relevant for food purposes, gelators should comply with certain traits, such as affinity for the liquid phase (polar or apolar), self-assembling properties, and preferably thermo-reversibility (Patel et al., 2013). Further, to make them pertinent for food applications, the structurants must be able to gel edible oils at chilled and ambient temperatures, which are the most common storage conditions (i.e., between 5 and 25 ◦C).

2.3 Bioactive compounds for improved functionality in food products

Along human history, several methods have been employed to preserve meat for longer (i.e., curing, refrigerating, etc.). Increased food quality and shelf life are desired attributes by both the consumers and the meat industry. Shelf life is highly influenced by microbial growth and oxidation by-products, that may affect food's taste, odor, color, and safety. Fresh meat products are particularly susceptible to spoilage, since they are intermediate moisture foods (with water activity values higher than 0.85) and are rich in essential nutrients that favor the rapid proliferation of fungi and bacteria (Jayasena & Jo, 2013).

Currently, consumers long for healthier products, with a preference for natural additives, like extracts and essential oils (EOs), avoiding synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), that have potentially toxic effects on human's (Agregán et al., 2019). Natural antioxidants are considered safer, have increased potential acceptability, stability and palatability, and are cognitively associated with positive aspects of health and food safety (Jayasena & Jo, 2013; Martins, Silva, et al., 2019; Mitterer-Daltoé, et al., 2020; Naveena et al., 2008). In this sense, fortification of foods with bioactive compounds can add value to the final product. For a preservative to be suitable, it must be stable

and not exert negative influences over the meat (i.e., unpleasant color, taste, or smell). It is paramount to consider properties such as solubility, volatility, and compatibility with the food matrix, so that the new ingredient does not disrupt the original physiochemical properties of the product (Pathak, 2017). Yet, it is also fundamental to consider on the commercial feasibility of the final product, which means that ingredient cost should be examined too (Pernetti et al., 2007).

One of the primary causes of undesirable economic impact in the meat industry is the contamination by fungi, which may increase production costs due to product losses. In some cases, fungal growth represents a health hazard to consumers, especially if the contaminants are toxigenic or pathogenic (Asefa et al., 2010). Further, fungal growth may also result in a decrease in product's shelf life, generating undesirable off-flavors (Bailly & Guerre, 2009). In dry-cured meats, the high salt concentration and lipid content, added to the low water activity, potentiate the growth of xerotolerant and xerophilic fungi, which are species well adapted to limited water availability. Examples of fungi that can be present in meat products are some belonging to the *Penicillium* and *Aspergillu*s genera, such as *Penicillium solitum, Penicillium nordicum, Aspergillus westerdijkiae* and *Aspergillus nomius* (Perrone et al., 2019).

Mycotoxins are toxic metabolites produced by fungi that may have hazardous effects over animals and humans. Most noteworthy mycotoxins, such as aflatoxins, ochratoxin-A (OTA) and fumonisins, are produced by the genera *Aspergillus*, *Penicillium* and *Fusarium*. Cereals and cereal-based foods are the main source of direct mycotoxin contamination for humans. Besides, animal products can be contaminated with mycotoxin-producing fungi, resulting on a health hazard to consumers. *A. nomius* and *P. nordicum* are two species that can develop in processed meat, producing aflatoxins and OTA, respectively (Vipotnik et al., 2017). Aflatoxins are the most toxic and carcinogenic group of naturally occurring mycotoxins, with aflatoxin B1 (AFB1) being the most toxic of all. OTA is also a major mycotoxin, with nephrotoxic, hepatotoxic and immunotoxic effects that affect primarily the kidneys and the liver, and it has been rated as a 2B carcinogen by the International Agency for Research on Cancer (IARC, 1993).

Apart from microbial proliferation, enzymatic autolysis and oxidation play a big role in decreasing meat's quality during storage. Polyunsaturated fatty acids are particularly susceptible to oxidative processes, since double bonds function as optimal initiators for oxidation reacting with atmospheric oxygen thus leading to the production of free radicals and hydroperoxides. Oxidation disturbs meat's typical organoleptic traits, such as tenderness, juiciness and color, and its nutritional profile, leading to food decay (Kalogianni et al.,

2020). Nowadays, meat waste due to deterioration and contamination account for approximately 20 % of the global economic costs due to food losses, worth around $150 billion USD (FAO, 2013).

In this context, the addition of natural preservatives to prevent meat oxidation and foodborne pathogens has emerged as a novel preservation technology. Phenolic compounds are considered effective antioxidants due to their ability to deactivate and stabilize free radicals by incorporating them into their aromatic ring (Kalogianni et al., 2020). Examples with valuable food industry applicability are phenolic acids, flavonoids, quinones, tannins and curcuminoids (Falowo et al., 2014; Gan et al., 2019; Hintz et al., 2015; Kalogianni et al., 2020). EOs are derived from plants and consist of a mixture of phenolic compounds. Many have been used by the food industry as antioxidant or antimicrobial additives, such as oregano, clove, ginger, coriander, sage and rosemary essential oils (Hintz et al., 2015; Jayasena & Jo, 2013).

To extract plant's bioactive compounds, various chemical processes can be employed, generating different end-products with distinct compositions and preferred food uses. EOs are usually obtained through steam distillation. Oleoresins, on the other hand, are viscous materials composed of both resins and EOs, and oleoresins are acquired when a spice is extracted by the use of a solvent (Suderman, 2011). Further, extracts can whether be in the form of a solid, a fluid or a powder. Studies show that plant EOs have higher antimicrobial activity when compared to oleoresins and crude extracts (Celiktas et al., 2007; Morsy & Abd El-Salam, 2017). When it comes to flavor, fewer odor and taste notes are destroyed during oleoresin extraction as compared to distillation of essential oils, which may be either advantageous or unfavorable depending on the desired food application (Suderman, 2011). Moreover, as compared to crude spices, EOs and oleoresins have higher stability during storage, better standardization, greater microbial safety and no seasonal variation, making them the preferred choice by the meat industry (Jayasena & Jo, 2013). Finally, functional elements such as EOs, oleoresins or crude extracts can be incorporated into oleogel systems as a mean to improve a meat product's shelf life, potentially protecting the oil against oxidation or offering improved microbial safety (Pinto et al., 2021).

2.4 Oleogel systems for meat applications

As mentioned, oil structuring is a promising solution to substitute trans and saturated fatty acids for healthier fats in various processed foods. Also, oil entrapment into the gel matrix may help extend product's

shelf life. Studies show that liquid oil tends to oxidize faster as compared to its structured form as oleogel or bigel, which suggests that oil structuring can help reduce product deterioration and increase oil resistance to oxidation (Alejandre et al., 2019; Samui et al., 2021). However, since vegetable oils are higher in MUFAs and PUFAs as compared to animal fats and those are naturally more prone to lipid oxidation, the addition of antioxidant compounds may still be valuable.

Many scientific works have explored the potential applications of oleogel systems in different meat products such as meat batters, patties, burgers, frankfurters, fermented sausages, and liver pâtés. Table 2 comprises a literature review from the last 4 years (2018 – 2021) of the most relevant oleogel systems developed for meat applications.

Table 2 – Literature review on oleogel-based systems developed for meat applications between 2018 and 2021

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Sesame oil	Beeswax	Oleogel	Beef burger	Oleogels exhibited less hardness than the animal fats which in turn decreased the hardness, gumminess, and chewiness of the raw burgers to <50 % of control sample. Cooking shrinkage and lipid oxidation increased significantly.	Moghtadaei et al., 2018
Linseed oil	ɣ-oryzanol and β-sitosterol	Oleogel	Pork patties	The incorporation of *Fucus vesiculosus* extract at the concentration of 1000 mg kg^{-1} into pork patties protected the samples against oxidation. This treatment was not as effective as the synthetic butylated hydroxytoluene at the concentration of 200 mg kg^{-1}.	(Agregán et al., 2019)
Cinnamon essential oil	Ethyl cellulose	Oleogel	Sausages	The oleogel presented antibacterial activity and this was dependent on the ethyl cellulose viscosity.	Zhang et al., 2019

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Olive oil, linseed oil and fish oil	Ethyl cellulose and beeswax	Oleogel	Pork burgers	Curcumin effectively reduced the lipid oxidation process. Samples formulated with beeswax oleogel presented adequate technological properties and good sensory acceptability. Curcumin provided a yellow color that reduced sensory acceptance.	Gómez-Estaca et al., 2020
Canola oil	Hydroxypropyl methylcellulose	Oleogel	Beef patties	The oleogel exhibited greater resistance against oxidation than the canola oil under accelerated storage conditions. Sensory evaluation showed highest overall acceptability at the 50 % fat replacement level. Textural properties (firmness and work of shear) of the oleogels were higher than those of beef tallow.	Oh et al., 2019
Sunflower oil	Glyceryl monostearate	Oleogel	Sausages	The oleogel usage as a fat substitute made the sausages lighter and caused a small increase in hardness in the sausages with 100 % fat replacement. All samples showed good acceptance by the consumers.	Ferro et al., 2021

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Linseed oil	Beeswax	Oleogel	Pork pâté	The concentration of beeswax used was enough to successfully induce oleogelation of the oil. The omega-6/omega-3 ratio demonstrated that the pork backfat replacement by oleogel resulted in healthier pâté in comparison with control. Despite some differences in hardness and cohesiveness between the control and the fat-substituted pâté, these were not enough to limit the utilization of such technology in this type of application.	Martins et al., 2020
Soybean oil	Ethyl cellulose	Oleogel	Pork batters	Meat batters with fat replacers were darker and less red, but more yellow due to the incorporation of vegetable oil. Oleogels substantially modified the fatty acid profile and decreased lipid oxidation with no detrimental effects on texture or acceptance.	Ferrer-González et al., 2019

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Linseed oil	Oryzanol-sitosterol 60:40 (w/w)	Oleogel	Pork patties	Incorporation of oleogels resulted in an improvement of the fatty acid profile. There were no differences between the patties produced with the oleogel and the control, regarding textural parameters such as hardness, cohesiveness, and chewiness. Despite the sensorial panel's preference for the control samples, the group with the least amount of oleogel is also well classified in both the acceptance and preference tests.	Martins, Lorenzo, et al., 2019
Soybean oil	Rice bran wax	Oleogel	Sausages	Texture profile analysis showed oleogel treatments were similar to control in terms of firmness, chewiness, and springiness. Sensory evaluation pointed that the fat replacement did not influence sample's aroma, but the flavor was significantly reduced.	Wolfer et al., 2018

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Olive oil	Sodium alginate and κ-carrageenan	Oleogel-based emulsion	Beef burgers	Treatments showed significantly lower TBARS compared to the control group during the storage. Control group had lower cooking yields and higher fat content. A 51 % and 35 % reduction of the total fat and energy content respectively with an increase of unsaturated fatty acids (65 %) and a significant decrease in saturated fat (44 %) were achieved in the product with the highest level of substitution.	Özer & Çelegen, 2021
Sunflower oil	Pork skin	Oleogel	Sausages	Emulsion stability increased and cooking loss decreased with higher pork back fat replacement by oleogel. The acceptance and the sensory profile of the samples were not affected by the substitution of up to 50 % of pork back fat by oleogel.	da Silva et al., 2019
Linseed	Beeswax	Oleogel	Sausages	The fatty acid profile was substantially improved. The SFA content was reduced, and more balanced ratios of omega-6/omega-3 were achieved. The sausages with linseed oleogel also had diminished cholesterol content as compared to control sausages.	Franco et al., 2019

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Olive oil and chia oil	Beeswax for the oleogel and gelatin for the emulsion gel	Oleogel and emulsion gel	Sausages	Products had an improved fatty acid profile. Reformulated samples showed a good oxidative status during chilled storage. Further studies are needed to improve the sensory attributes of the reformulated samples.	Gómez-Estaca et al., 2020
Olive oil	Sodium alginate	Oleogel	Goat meat burgers	Reformulated burgers were harder and presented a more difficult chewiness as compared to control. Ashes, protein, and collagen contents were similar in all groups. Fat substituted burgers had significantly lower fat content.	Teixeira et al., 2021
Soybean oil	Carnaúba wax (CW) and adipic acid (AA)	Oleogel	Burgers	The presence of AA improved gel strength and crystallinity. AA reinforced oleogels had the highest oil binding capacity and oxidative stability. The CW2 %/AA4 % oleogel sample was selected as an optimized sample for application in cakes and beef burgers.	Aliasl khiabani et al., 2020

Oil	Gelator	Type of structure	Meat product	Main conclusions	Reference
Canola oil	Ethyl cellulose and glycerol monostearate	Oleogel	Pâtés	Pâtés made with canola oil oleogel had similar hardness values to control. Oleogel pâtés were perceived to have similar sensory hardness, oiliness, and juiciness as the control. Light microscopy showed that fat globules were larger in pâtés with oleogel than the control treatment. Saturated fat content was reduced by 60 %.	Shai Barbut et al., 2019

Until date, there are no bigel formulations developed specifically for substituting fat in meat products. Most common oils used in meat formulations are linseed oil and sunflower oil, while monoglicerides, natural waxes and ethyl cellulose are the most used oleogelators.

Moghtadaei et al. (2018) developed sesame oil oleogels using beeswax (BW) as oleogelator and observed that although oleogels contained more unsaturated fatty acids, their melting point was higher than that of animal fats. Interestingly, in this study panelists preferred the flavor of the beef burgers that had the highest percentage of fat substitution, showing that it is possible to develop a meat product with substituted fat and still maintain acceptable sensorial traits. However, oxidation proceeded more rapidly in the burger containing the biggest amount of oleogel, which points to the necessity of adding some sort of antioxidant to counteract oxidative processes along storage.

Gómez-Estaca et al. (2019) developed fat substituted pork burgers loaded with curcumin, which presented adequate technological properties and good overall sensory acceptability. However, curcumin provided a bright yellow color that reduced sensory acceptance (Gómez-Estaca et al., 2020). Moreover, when adding a bioactive compound into the gel, it is important to consider its possible disadvantages over the final product's appearance.

3 METHODOLOGY

The first steps were performed to find the best approach and ingredient proportion for the development of stable bigels with the adequate traits for fat substitution in a meat product. For the oleogel development, glycerol monostearate (GMS) was selected as the oleogelator. GMS is a hydrophobic low-molecular weight monoglyceride, and it is able to act as an effective lipid structurant for food applications, even at relatively low concentrations (Co & Marangoni, 2012; Singh et al., 2015). For the hydrogel production, two biopolymers were selected as gelators: locust bean gum (LBG) and κ-carrageenan (κ-C). LBG, also known as carob gum, is a polysaccharide obtained from the carob tree (*Ceratonia siliqua L.*). It has GRAS status for direct food use and has a huge industrial relevance due to its ability to form very viscous aqueous solutions at low concentrations, and it has emulsion stabilizing properties. The choice to use both LBG and κ-C to gel the aqueous phase was motivated because this synergy was found to improve apparent gel strength in many studies (Barak & Mudgil, 2014; Dea et al., 1972; Tako et al., 1998).

After this first screening, selected gels were combined with various bioactive compounds in different concentrations to determine the most suitable ones to be assessed in the following texture and microbiology analysis.

3.1 Materials

The vegetable oil used was refined sunflower oil (SO) from the brand Fula (Solvena, Algés, Portugal). Glycerol monostearate (GMS) was purchased from Alfa Aesar (Massachusetts, USA). Refined κ-carrageenan and clarified locust bean gum were from CEAMSA (Pontevedra, Spain). Herbalox® rosemary extract was from Kalsec® (Kalamazoo, USA). Rosemary essential oil was from Biover (Nazareth, Belgium). Gallic acid monohydrate was bought from Penta Manufactory Company (Livingston, USA). Turmeric was from Gold Coast Ingredients, Inc. (California, USA). Teavigo™ green tea extract (GT) was from was from DSM Nutritional Products (Heerlen, Netherlands). Tea polyphenol palmitate (TPP) was from Xian Tizan Tech & Industry Co., Ltd. (Shaanxi, China).

3.2 Preparation of the oleogel-in-hydrogel system

A total of five different formulations were tested, with varying concentrations of total LBG and κ-C in the hydrogel (HG) phase. Ratio between biopolymers was fixed at 1:1 (w/w) in all experiments, following other works that show that this proportion promotes a good synergy between those two biopolymers (Tako & Nakamura, 2014). The oleogel (OG) consisted of sunflower oil (SO) and 10 % GMS w/w, and it was the same for all groups of samples. The selected GMS concentration was based on other studies, that show that GMS can produce self-sustainable structured oils using concentrations between 5 and 25 % (Fasolin et al., 2018; Ferro et al., 2019; Uslu & Yilmaz, 2021).

For formulations 1, 2 and 3, the HG:OG ratios were 90:10, 80:20, 70:30, 60:40, and 50:50 (w/w). For formulations 4 and 5, that were done afterwards, the chosen HG:OG ratios were 90:10 and 80:20 (Table 3).

Table 3 – Different formulations tested for the hybrid gels

	Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
OG	SO + 10 % GMS				
HG	1:1 LBG/κ-C 0.5 %	1:1 LBG/κ-C 1 %	1:1 LBG/κ-C 2 %	1:1 LBG/κ-C 1.5 %	1:1 LBG/κ-C 1.75 %
HG:OG ratios	50:50 60:40 70:30 80:20 90:10			80:20 90:10	

Later on, bigels with higher amounts of GMS (15 % and 20 %) in the oleogel and 1 % and 1.5 % LBG/κ-C were produced to check whether this combination would result in stronger gels (Table 4).

Table 4 – Tested formulations with 15 % and 20 % GMS, using 90:10 HG:OG ratio

OG	SO + 15 % GMS	SO + 20 % GMS
HG	1 % LBG/κ-C and 1.5 % LBG/κ-C	1 % LBG/κ-C and 1.5 % LBG/κ-C

For the HG preparation, LBG and κ-C were carefully weighted using a precision scale. Separately, distilled water was measured and warmed up until 85 °C in a sealed bottle. Then, LBG was slowly poured into the warm water and constantly mixed with a magnetic stirrer at 350 rpm for 1 hour until complete LBG dissolution. After that, κ-C was added to the mixture and mixed at 350 rpm until dissolved. The resulting hydrogels were then weighted and put into five separate beakers. They were stored overnight at 4 ◦C.

For the OG preparation, sunflower oil was weighted and separated into five beakers containing the appropriate oil weight for each HG:OG ratio. Then, the beakers were heated up until the oil reached 85 ◦C, and 10 % GMS w/w was added to each one of the beakers. The mixtures were stirred at 350 rpm for 20 minutes. For the emulsification step, the heated OG was dispersed into the HG at 85 ◦C under mechanical stirring using a high shear homogenizer Hei-TORQUE Precision® from Heidolph, (Schwabach, Germany) at 1500 rpm, for 10 minutes (Figure 2). Afterwards, the resulting bigels were separated into 5 mL centrifuge tubes and distributed in Petri plates until characterization analyses.

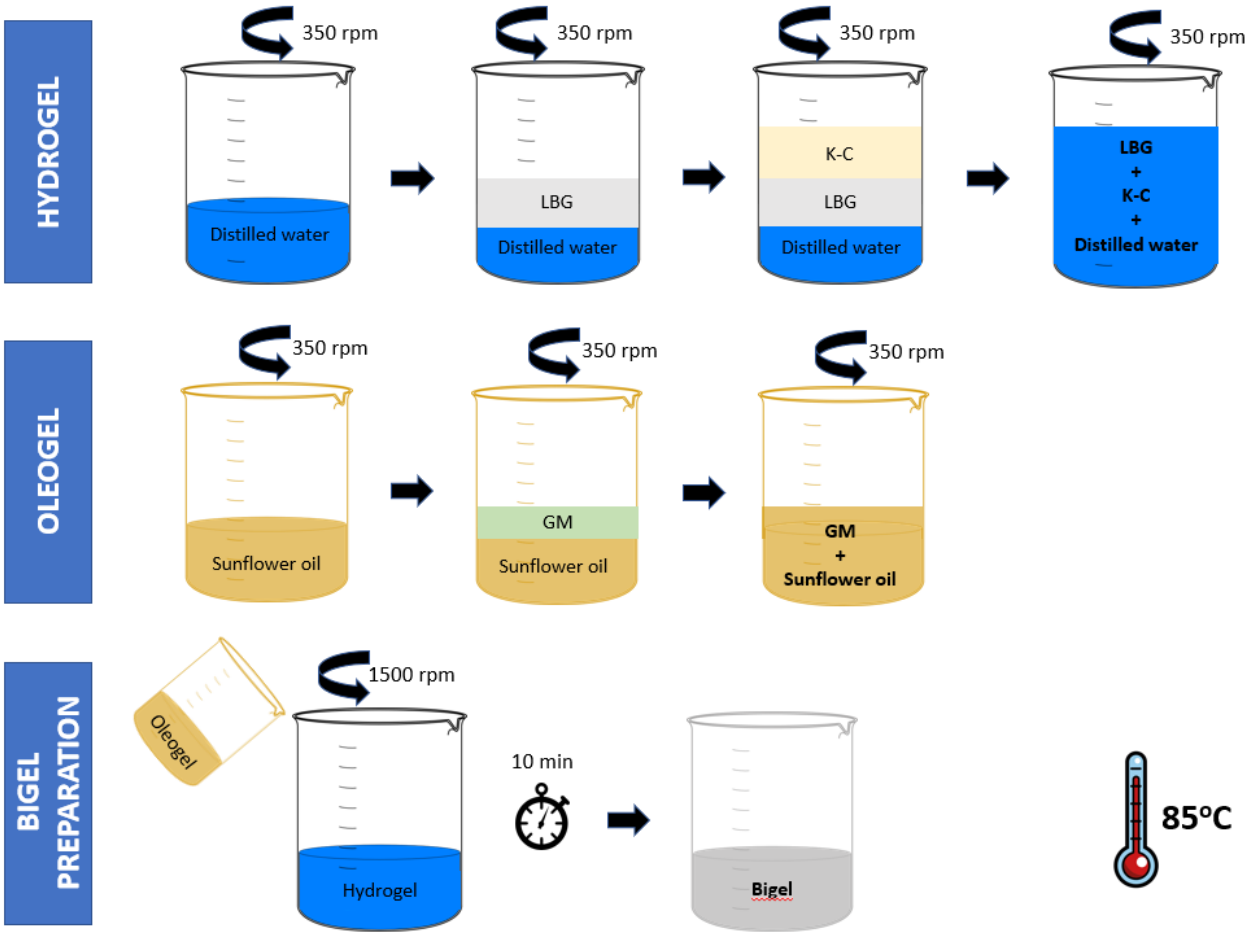

Figure 2 – Schematic representation of the methodology used for bigel preparation.

3.2.1 Preparation of the bigels loaded with bioactive compounds

The bioactive-loaded bigels were prepared according to the method described in the previous section. The chosen formulation for the incorporation of the bioactive compounds was the one with 1.5 % LBG/κ-C and 10 % GMS, which was tested for the HG:OG ratios of 90:10 and 80:20. Tested bioactive compounds were turmeric, gallic acid, tea polyphenol palmitate (TPP), green tea extract (GTE), rosemary extract (RE) and rosemary essential oil (REO).

Due to their different nature, RE, REO, TPP and turmeric were added to the oleogel, while GA and GTE were incorporated into the hydrogel. All compounds were added to the OG or HG and stirred for

approximately 2 minutes until completely dissolved. This addition was done right before the emulsification step to minimize the exposure to the high temperature.

The tested concentrations for each compound are shown in Table 5. Percentages are based on bigels' total weight (g of each compound/100 g bigel). Not all compounds were tested at the same concentrations, given complications in regard to their solubility or stability of the bigel. For example, bigels with 0,1 and 1 % green tea were tested first, but because of the damaging effects of this compound when 1 % was added, an intermediate concentration (0.5 %) was also evaluated. Following the same logic, gallic acid and turmeric were first incorporated into the bigels at 0.1 % concentration, but the former negatively affected its structure and the latter did not solubilize well. Hence, a lower concentration (0.01 %) was tested for both this compounds.

Table 5 – Tested concentrations of turmeric, GA, TPP, GTE, RE and REO in the bioactive-loaded bigels

	Concentration (g/100 g)
Turmeric	0.01 % and 0.1 %
Gallic acid	0.01 % and 0.1 %
Tea polyphenol palmitate	0.1 % and 1 %
Green tea extract	0.1 %, 0.5 % and 1 %
Rosemary extract	0.1 % and 1 %
Rosemary essential oil	0.1 % and 1 %

3.3 Gel characterization

3.3.1 Visual appearance

For the visual assessment, bigels were previously separated into 5 mL centrifuge tubes and Petri plates and stored at 4 °C for 24 hours. Then, tubes were inverted to evaluate the self-standing ability of each sample and to check whether there was phase separation. In addition to that, the samples stored in Petri dishes were analyzed and compared in regard of its appearance and gel strength. The visual assessment was performed in triplicate and repeated after 6 days.

3.3.2 Microscopy

A drop of each gel was collected immediately after the high-shear homogenization and placed on a glass slide, covered by a glass slip. After that, the samples were analyzed with a Wide-Field Upright Fluorescence Microscope – Nikon – Ni-E equipped with a polarizer and a Nikon DS-Fi2 camera (Nikon, Japan), for bright-field imaging. Pictures were taken with a magnification of 50x, 200x and 500x.

3.3.3 Droplet size measurements

Droplet size distribution in the bioactive-loaded bigels was calculated using Image J software. This estimate was performed by measuring the mean areas of 100 oleogel droplets in two micrographs for each analyzed sample, taken in different parts of the sample. Further, the average, minimum and maximum area values were determined.

3.3.4 Texture analysis

After the emulsification step, samples were put into circular molds with 13 cm of depth and 10 cm of diameter and stored at 4 °C for 24 hours. Texture profile analysis (TPA) was performed using Shimadzu Texture Analyzer (Kyoto, Japan), using Trapezium-V software. Bigel samples were compressed until 40 % deformation, using a pre-test speed of 1 mm s^{-1} and a test speed of 0.5 mm s^{-1}. The probe waited 2 s between the first and second compressions. Measurements were done in six replicates of each sample, and parameters such as hardness, springiness, adhesiveness, cohesiveness, gumminess, and chewiness were obtained. Figure 3 shows the ongoing texture analysis.

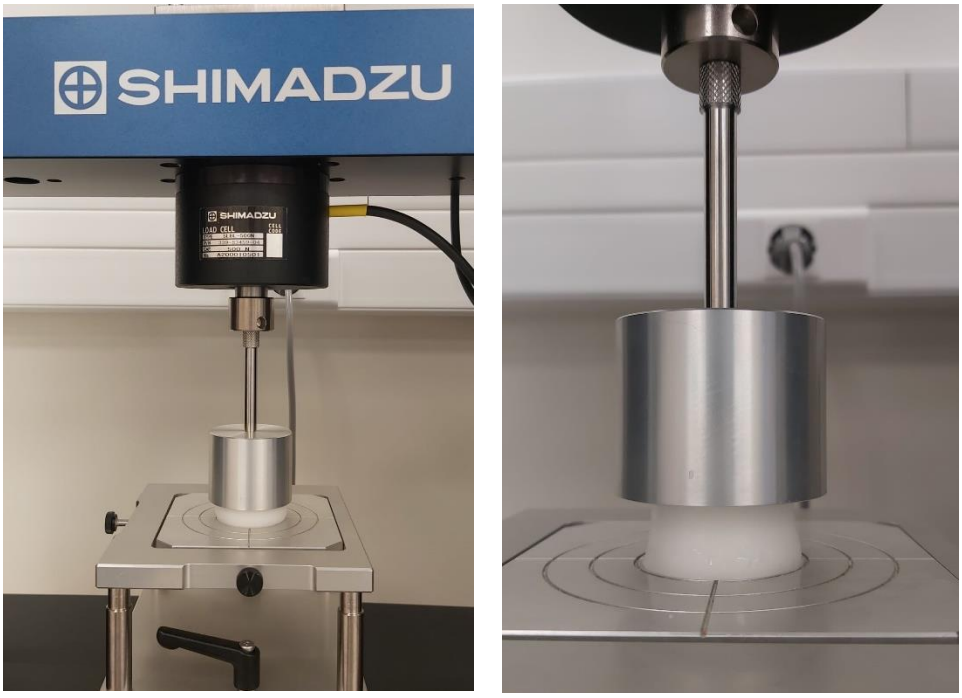

Figure 3 – Ongoing texture analysis on a bigel sample.

3.3.7 Statistical analysis

Statistical analysis of TPA and microbiology data was carried out using the OriginPro version 64-bit software (Northhampton, Massachusetts, USA). One-way ANOVA was employed to compare samples with 90:10 and 80:20 HG:OG ratios with each other and evaluate the effect of the addition of 1 % REO into those same HG:OG ratios. In the antimicrobial activity assays, one-way ANOVA was used to compare the effect of different REO concentrations over fungal growth. The pairwise differences between least-square means were examined using Tukey's test at a level of 5%.

3.3.8 Oil binding capacity (OBC)

This methodology was based on the one used by Martins et al. (2017) and aimed to evaluate the oil retention degree of the developed bigels. Thus, triplicates of 1 mL of each freshly prepared sample were placed into Eppendorf tubes and stored overnight at 4 °C. Before measurement, all tubes were centrifuged at 10g for 15 min at room temperature. After that, they were turned over onto a filter paper to recover the

released oil. This test was performed in triplicates and repeated on day 8 and day 15. OBC was calculated as follows (eqn 1):

$$OBC\ \% = \ 1 - \left(\frac{Wi - Wf}{Wi}\right)\ x\ 100 \qquad \text{Eqn 1}$$

3.4 Determination of the antioxidant and antimicrobial activities

3.4.1 Determination of the antioxidant activity

The oxidative stability of the samples stored at 4 ºC was measured on days 1, 8 and 15 after preparation (day 1 – 24-hour storage). Antioxidant activity of the compounds was analyzed in two bigels (90:10 and 80:20 HG:OG ratios) and also in sunflower oil, to understand if the added bioactive compounds would offer protection against oxidation in different matrices. The tested bioactive compounds were turmeric, rosemary extract, tea polyphenol palmitate and green tea extract, which were incorporated into the sunflower oil samples and bigels at the concentration 0.1 % (w/w). Rosemary essential oil was not evaluated in these tests, since the choice to use this compound was made in a later stage of this work. Controls without the addition of extracts were used for each bigel and for the sunflower oil.

Oxidation was determined by measuring the amount of 2-thiobarbituric acid-reactive substances (TBARS). For that, a food TBARS assay kit was used, bought from Oxford Biomedical Research (Rochester Hills, USA). The methodology followed precisely the guidelines provided with the kit.

3.4.2 Determination of the antimicrobial activity

To assess the antimicrobial activity of the compounds, fungal inhibition was evaluated primarily by a diffusion assay (testing the compounds solubilized in sunflower oil) and a fungal inhibition assay (testing the bigel with the compounds incorporated into the gel matrix). Antifungal activity was evaluated against four fungi: *A. westerdijkiae* (MUM 15.03) *and P. nordicum* (MUM 08.16), that were isolated from meat sausages and were obtained from Micoteca da Universidade do Minho (MUM); and *A. nomius* (12A18) *and P. solitum* (13 PPEN 2), that were isolated from the air of a meat industry and were obtained from Instituto Politécnico de Bragança (IPB). For *A. westerdijkiae*, *A. nomius* and *P. solitum*, the culture medium used was Malt Extract Agar (MEA); for *P. nordicum*, it was Yeast Extract Supplemented (YES) medium.

A 1 x 10^6 spores/mL solution of each species was prepared by first preparing and sterilizing the culture medium (MEA or YES) for 15 min at 121 °C. Then, culture media were distributed into 15 mL Falcon tubes, and each fungus was inoculated. Fungi grew for 5 days at 25 °C in the dark. After that, spore suspensions were prepared by adding 1 % peptone water (with tween 80) to the grown fungi and then agitating to suspend the spores. Solution was transferred to a clean tube and the spores were counting using a Neubaeur chamber. The solution was then diluted to a final concentration of 10^6 spores/mL.

For the diffusion assay 100 µL of spore solution (1 x 10^6 spores/mL) from each fungi were spread on Petri dishes using a sterile spreader and left to dry for 20 minutes. Then, a 6 mm diameter hole was aseptically punched in the agar, where 100 µL of each extract concentration was pipetted into the wells. Petri dishes with wells without the extracts (only with the carrier oil) and with distilled water plus acetic acid 10 % (antifungal) were used as negative and positive controls, respectively. All tests were performed in triplicate. The inhibition halo was measured daily. For the preliminary tests, each bioactive compound was solubilized in the oil for the diffusion assay in a concentration equivalent to the one used at the bigel, relatively to the oil weight in the oleogel (Table 6). This was calculated as follows:

$$\alpha = \frac{\beta}{\delta \cdot \gamma} \qquad \text{Eqn 2}$$

Where:

α is the [bioactive] in oil;

β is the[bioactive] in bigel;

δ is the [oleogel] in the bigel;

γ is the [oil] in the oleogel.

Therefore, for β = 0.1 % and bigel 90:10:

δ = 10 %

γ = 90 %

$\alpha = \frac{0.1\ \%}{10\ \% \cdot 90\ \%} \Longrightarrow \alpha = 1{,}11\ \%$

And for β = 0.1 % and bigel 80:20:

δ = 20 %

γ = 90 %

$$\alpha = \frac{0.1\,\%}{20\,\% \cdot 90\,\%} \Longrightarrow \alpha = 0.55\,\%$$

Table 6 – Used concentrations of each extract for the preliminary diffusion assay

	Diffusion assay – concentrations in sunflower oil (g/g)	
	90:10 ratio	80:20 ratio
Turmeric	1.11 %	0.55 %
Tea polyphenol palmitate		
Green tea extract		
Rosemary extract		
Rosemary essential oil		

Gallic acid was excluded from these tests because of its effects on the bigel, which will be discussed later. Later on, higher concentrations (11 % and 55 %) of turmeric, TPP and RE were also tested for the diffusion assay, to check whether those highly concentrated samples would have antifungal activity over the target fungi.

To assess the fungal inhibition activity of the loaded gels, culture media were prepared and evenly distributed through Petri dishes. Then, 100 µL of each fungus spore solution were spread on Petri dishes using a sterile spreader and left the dry for approximately 20 minutes. After that, the bigel was first cut into 1 cm^2 squares and then again, each square was cut transversally so both interior and exterior sides of the fat could be tested. Next, each square was placed in the center of the culture medium in separate Petri dishes, so that we had 6 petri dishes for each gel formulation (triplicates of the interior and exterior surfaces). Petri dishes with the structured fat produced without the extracts were used as negative controls.

Also, the antifungal potential of the extracts was tested using direct incorporation of the tested compounds into the culture medium. For that, each culture medium was prepared as usual and separated into glass bottles, one for each extract and concentration. The bioactives were incorporated while the agar was still in a liquid state but not too warm (50 ◦C) to avoid possible interference with the compounds' bioactivity. After that, 20 mL of culture medium were evenly distributed into Petri dishes and 10 µL of each fungi's spore solution were pipetted onto the center of the agar. Culture medium without any added compound was used as negative control. The evolution of the diameter of the colonies was measured daily, and experiments were done in triplicates. Tested concentrations are displayed at Table 7.

Table 7 – Concentrations of each extract incorporated into the culture media

	Concentration (g/100 mL)
Turmeric	0.1 % and 1 %
Tea polyphenol palmitate	0.1 % and 1 %
Green tea extract	0.1 %, 0.5 %, and 1 %
Rosemary extract	0.1 % and 1 %
Rosemary essential oil	0,05 %, 0.1 %, 0.5 % and 1 %

3.5 Mycotoxin analysis

The mycotoxin content was determined for the mycotoxigenic species *A. nomius* and *P. nordicum*. For that, the following protocol was used: after 7 days of incubation, the total content of plates was cut and placed into falcon tubes and extracted with 20 mL of an organic solvent solution of acetonitrile/methanol/acetic acid (78/10/2, v/v/v) by strong vortex agitation. Extracts were left overnight at room temperature in the dark, then filtered using 0.2 µm syringe filters (*nylon*) and analysed by HPLC and fluorescence detection with previously implemented methods (Soares et al. 2010).

Briefly, the HPLC system is comprised of a Varian Prostar 210 pump, a Varian Prostar 410 autosampler, a photochemical post-column derivatization reactor (PHRED unit, Aura Industries, USA) and a Jasco FP-920 fluorescence detector. The instrument and the chromatographic data were managed by a

Varian 850-MIB data system interface and a Galaxie chromatography data system, respectively. The chromatographic separation was performed on a C18 reversed-phase YMC-Pack ODS-AQ analytical column (250 x 4.6 mm I.D., 5 µm) that was fitted with a pre-column with the same stationary phase. The mycotoxins were eluted using a flow rate of 1.0 mL/min for 25- or 18-min isocratic run, for aflatoxins and OTA, respectively.

For aflatoxins (AFG2, AFG1, AFB2 and AFB1) the mobile phase was a mixture of water/acetonitrile/methanol (3:1:1, v/v/v) that was filtered and degassed with a 0.2 µm membrane filter (GHP, Gelman). The fluorescence detector was set to λ_{ex} 365 nm and λ_{em} 435 nm, and injection volume was of 30 µL. Calibration curves were constructed using a certified aflatoxins standard solution mixture at defined concentrations in the respective mobile phase.

For OTA, the mobile phase was a mixture of water/acetonitrile/acetic acid (99:99:2, v/v/v) that was also filtered and degassed with a 0.2 µm membrane filter (GHP, Gelman) and the fluorescence detector was set to λ_{ex} 333 nm and λ_{em} 460 nm, and an injection volume of 50 µL. Calibration curves were constructed using certified OTA and aflatoxins standard solutions at defined concentrations in the respective mobile phase. Mycotoxins were quantified by comparing the peak areas with the calibration curves.

4 RESULTS AND DISCUSSION

4.1 Influence of hydrogel-oleogel ratio and composition on bigel stability

The influence of the hydrogel:oleogel ratio was noticeable in all formulations tested, exhibiting a similar pattern. With higher proportions of oleogel, gel stability decreased, and a more disarranged distribution was observed, which is evident both visually and microscopically. Phase separation starts to be perceived visually from the ratio of 70:30 down, with samples 50:50 showing the highest instability and worst oil binding capacity. Figure 4 displays the differences in the appearance of 1 % LBG/κ-C samples developed with 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios. Samples have 10 % GMS unless stated otherwise.

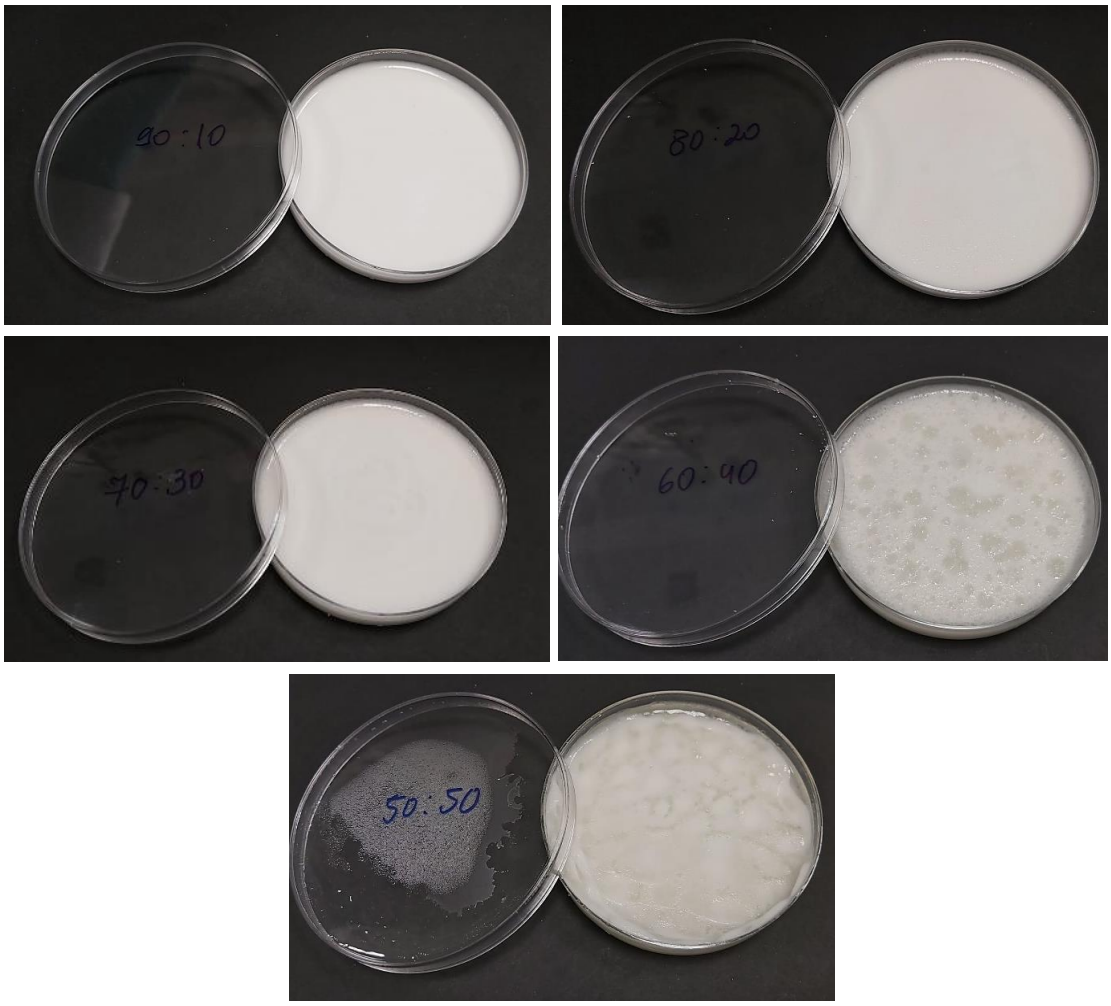

Figure 4 – Visual appearance of the gels with 1 % of LBG/κ-C. A, B, C, D and E correspond to samples with 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios, respectively.

Microscopically, it is possible to see dispersed oleogel particles inside the hydrogel matrix. The influence of hydrogel:oleogel ratio is demonstrated by an increase in oil droplet size and deformation when the oleogel proportion is augmented, with droplets losing its usual round shape and acquiring a misshapen elliptical conformation, with little uniformity in regard to shape and droplet size (Figure 5). The stability of an emulsion is dependent on a variety of factors including the presence or absence of the emulsifying agent, viscosity, and the age of the emulsion. Since for this bigel development no emulsifier was used, it is even more difficult to disperse higher amounts of oleogel without leading to coalescence, so it was expected that 50:50 ratio would be the hardest to stabilize. The dispersed oleogel droplets have their interfaces structured with crystallized GMS layers due to the amphiphilic nature of this molecule, with its hydrophobic tail turned to the inside of the oleogel droplets. Stabilization of this system is entirely due to the physical entrapment of the oleogel in the gelled aqueous phase (Patel & Dewettinck, 2016).

Further, gelator concentrations have an important role in gels' stability and appearance. Changing the gelator content (LBG/κ-C at 0.5 %, 1 %, 1.5 %, 1.75 % and 2 %) of the hydrogel, while maintaining a fixed amount at the oleogel (GMS at 10 %) resulted in bigels with distinct properties and gel strengths. The samples produced with 0.5 % gelator are the less homogeneous in terms of particle size and concentration, with separate oleogel droplet aggregates not evenly distributed along the aqueous phase. Droplets are larger, shapeless, and more interspaced. As the biopolymer concentration increases, bigels become more consistent, exhibiting a pattern with less dimension disparity between the oil globules. Additionally, stability seemed to have its peak using 1.5 % LBG/κ-C, where droplets are the smallest and most evenly distributed, which is an indicative of better stability of the gel (Dapčević Hadnađev et al., 2013; Martin et al., 2018; San Martin-González et al., 2009). Emulsion balance decreases from 1.75 % LBG/κ-C up (Figure 6), which may result from the increase in the viscosity of the continuous phase, that might result in a less effective emulsification.

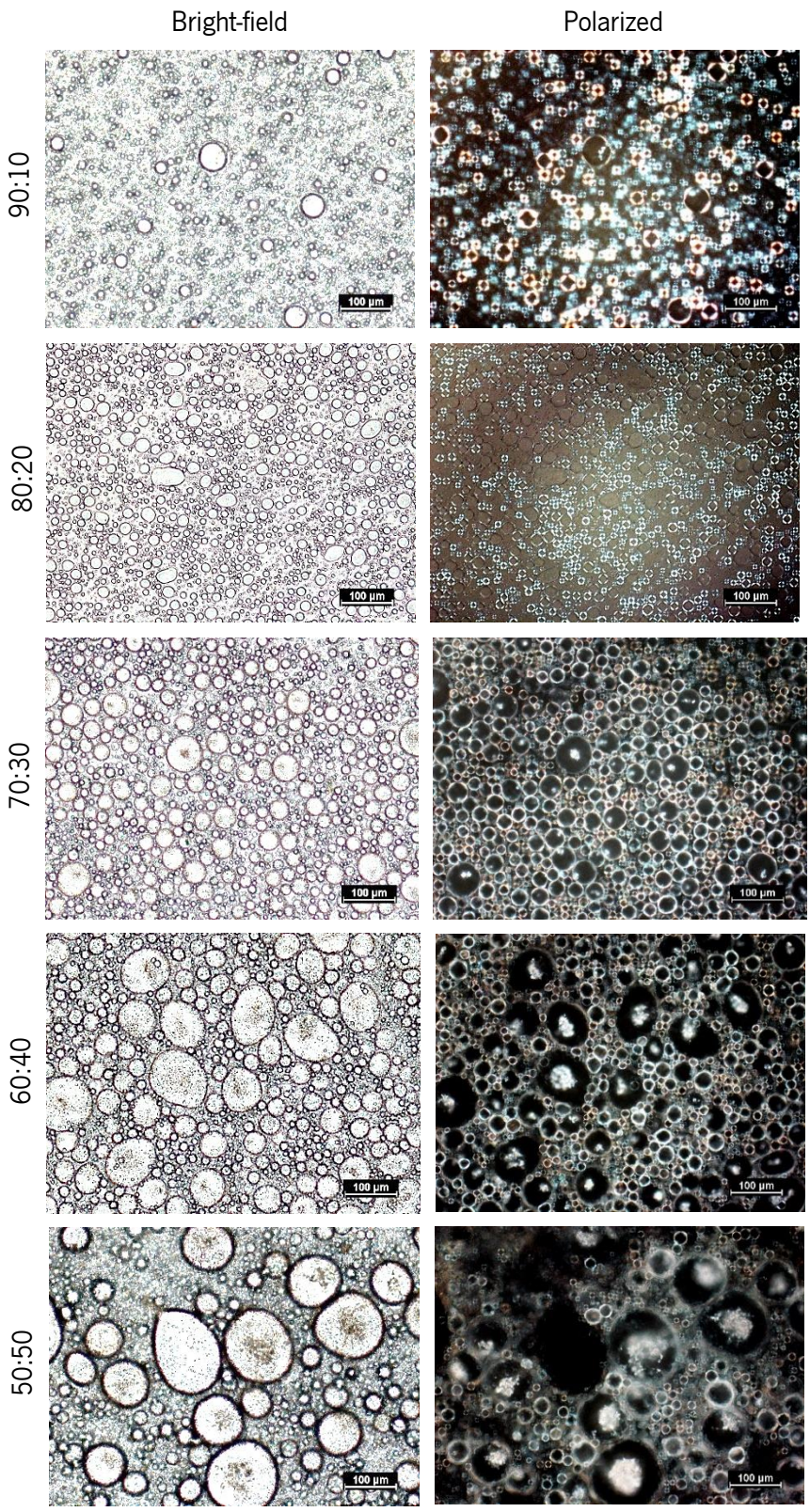

Figure 5 – Bright-field and polarized micrographs of the gels with 1 % of LBG/κ-C, taken with 200x magnification, for 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios.

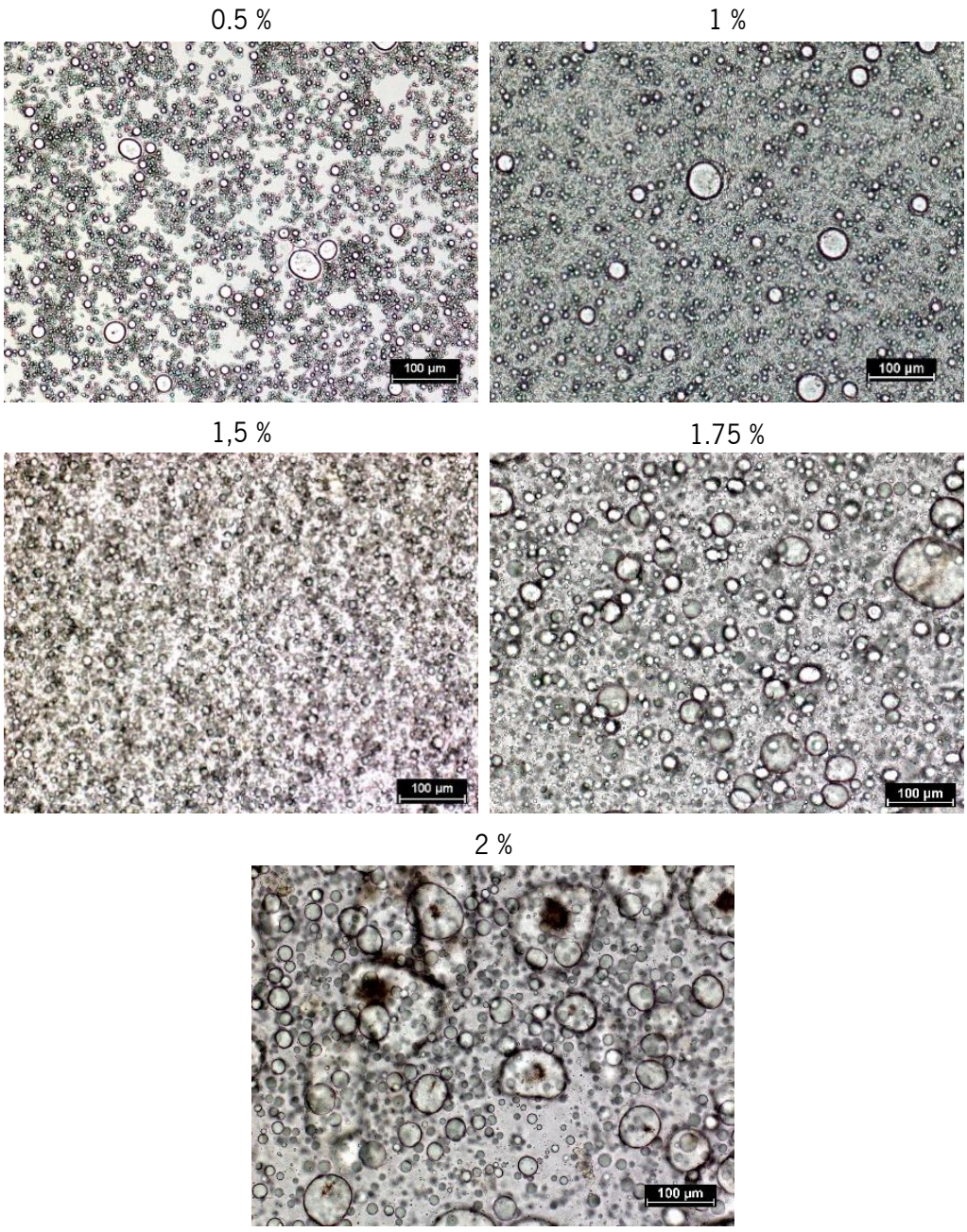

Figure 6 – Bright-field micrographs of the gels with 0.5 %, 1 %, 1,5 %, 1.75 % and 2 % of LBG/κ-C, taken with 200x magnification, for 90:10 HG:OG ratio.

The study on the increase of the oleogel strength showed that increasing the amount of GMS to 15 % and 20 % in the oleogel does not seem to result in stronger bigels; however, its effect on gel stability was different for samples with 1 % or 1.5 % LBG/κ-C. For the 1 % samples, droplets become smaller and more uniform with the increase in GMS, which points to an improvement in bigels' stability (Figure 7). On the other

hand, for the 1.5 % samples, their structures become slightly softer, and microscopy images show that the average droplet size increases with the GMS addition, which is an indicative of a worse gel stability (Figure 8). This corresponds to previous studies showing that above a certain GMS concentration, the excess GMS may interfere with the water/oil stability, leading to oil leakage (Samui et al., 2021).

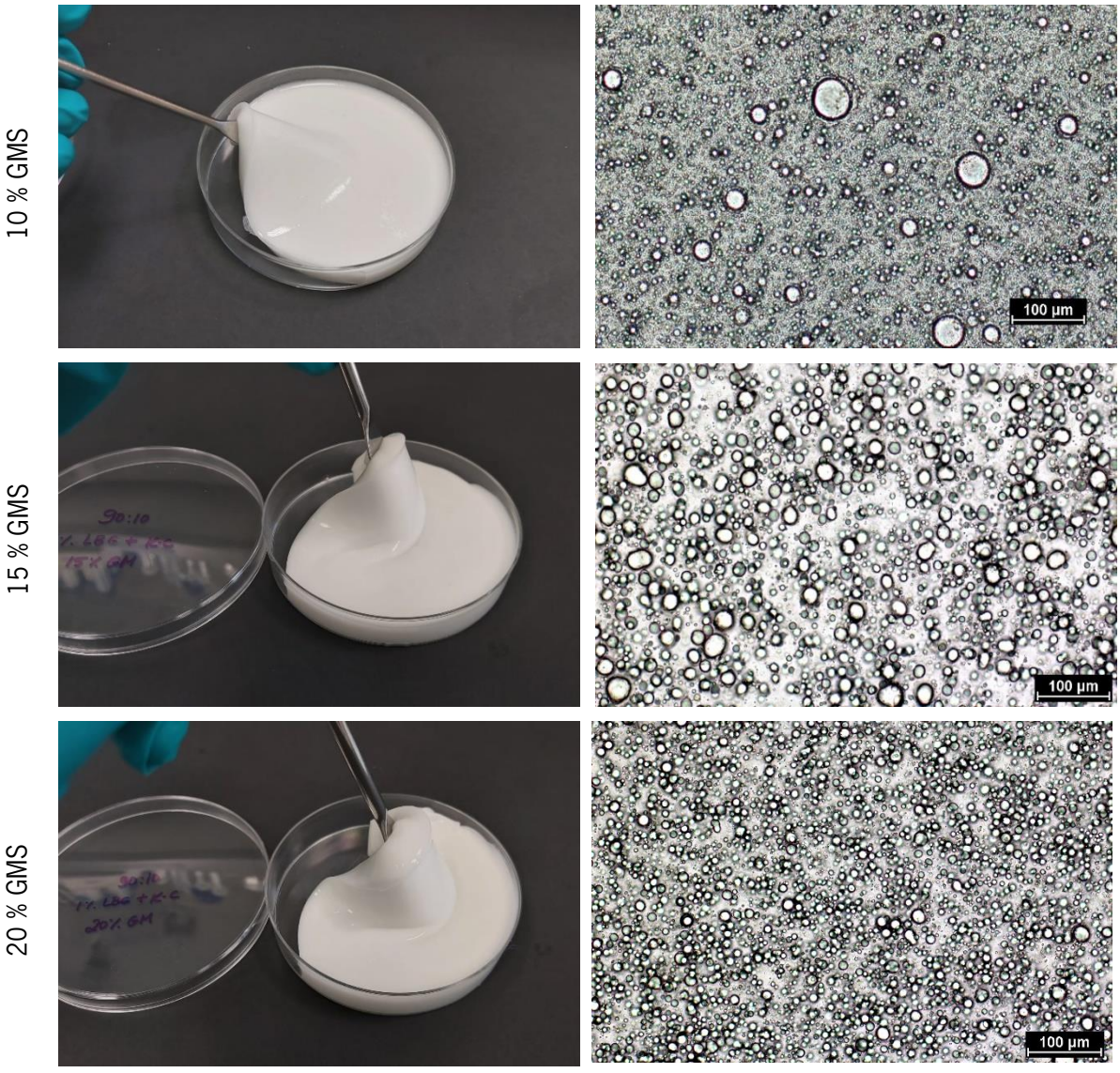

Figure 7 – Visual appearance of the gels (on the left) and bright-field micrographs (on the right) of the bigels with 90:10 HG:OG ratio, 1 % of LBG/κ-C and 10, 15 and 20 % of GMS.

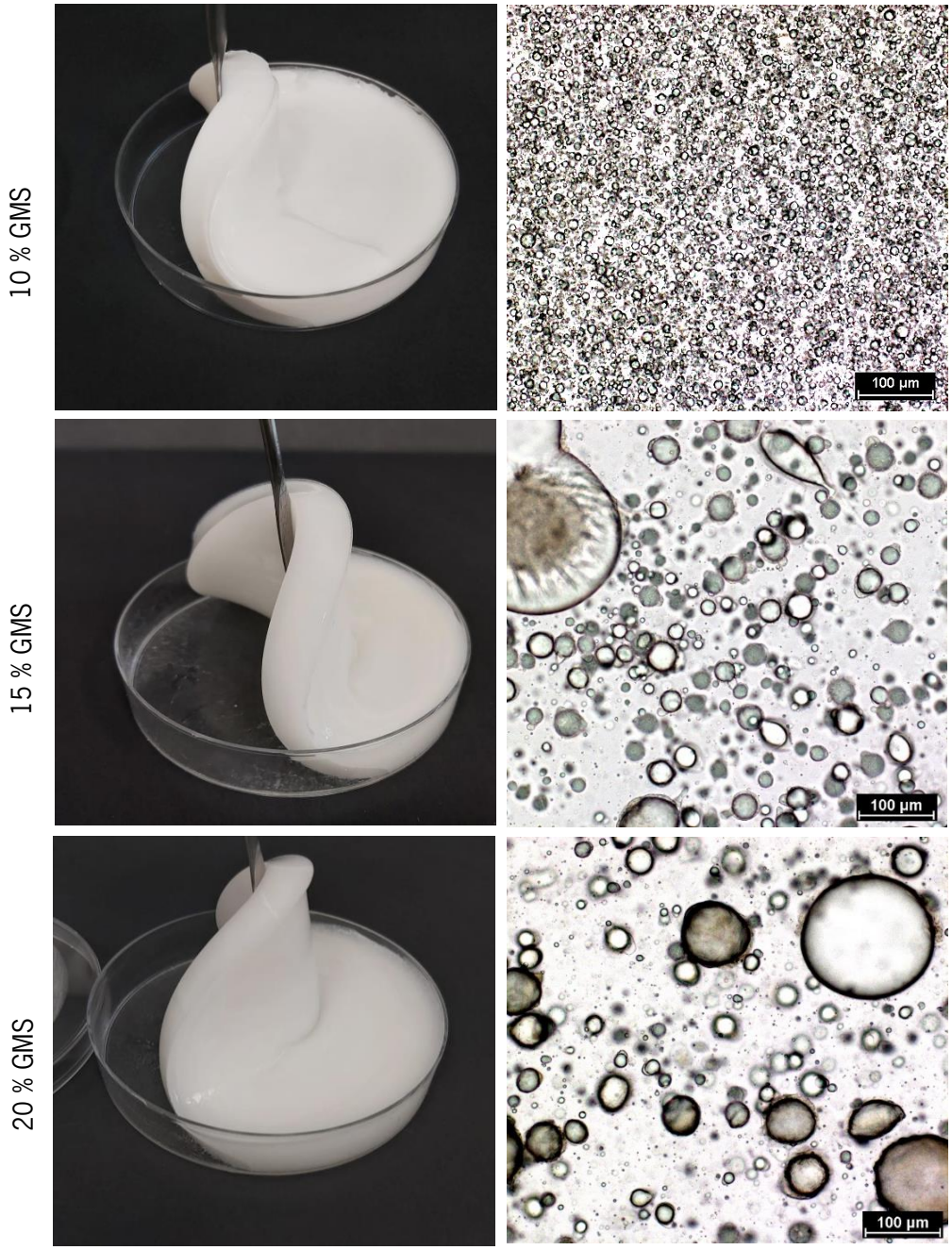

Figure 8 – Visual appearance of the gels (on the left) and bright-field micrographs (on the right) of the bigels with 90:10 HG:OG ratio, 1,5 % of LBG/κ-C and 10, 15 and 20 % of GMS.

Nonetheless, all gelator concentrations and HG:OG ratios resulted in self-standing gels upon inversion of the tubes, even after 15 days of storage in the cold (4 ◦C) (Figure 9).

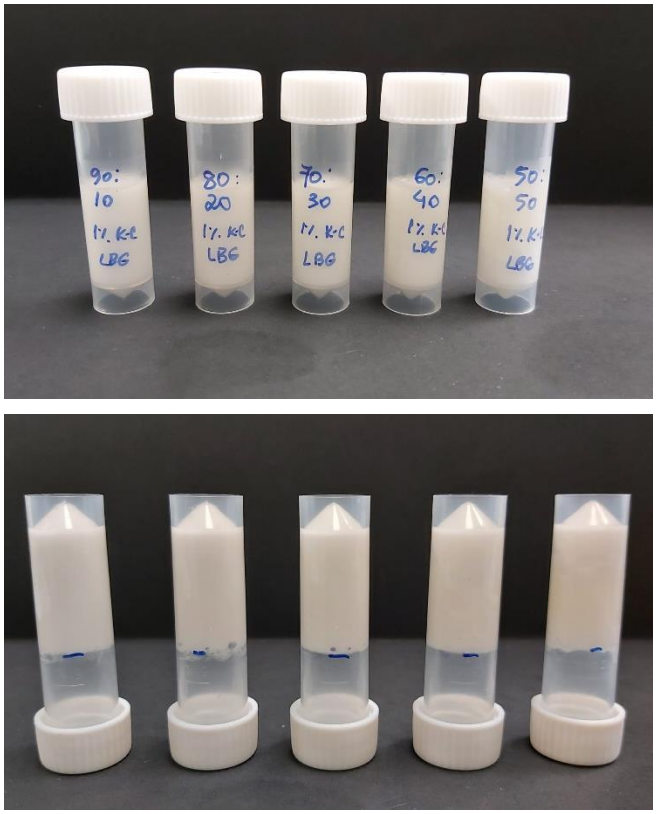

Figure 9 – Self-standing capacity of samples with 1 % of LBG/κ-C and 10 % GMS, upon inversion of the tubes.

Concerning all tested formulations, the ones with the most promising characteristics for incorporation into a meat product were the bigels with 1.5 % LBG/κ-C, 10 % GMS, and 90:10 and 80:20 ratios. Therefore, in the next steps these formulations were selected to be loaded with the antioxidant and antimicrobial compounds and to be evaluated in terms of textural parameters. Appendix A contains all micrographs for the different HG:OG ratios and various gelator concentrations tested. Appendix B includes a figure showing the differences in the visual appearance between samples with various HG:OG and gelator concentrations.

4.2 Influence of bioactive compounds on bigel stability and appearance

Comparing all tested compounds, the ones that least affected gels' microstructure were RE and TPP, followed by REO and turmeric. Gallic acid and GTE had deleterious effects over the bigels' stability. Gallic acid destroyed the gels' matrix completely and could not be tolerated even in very low concentrations (0.01 %), turning the gel into a liquid with no self-standing capacity (Figure 10). For this reason, it was excluded from

subsequent tests. Bright field micrographs confirm these bad emulsification results, showing very interspaced oil droplets with no homogeneity in size or form. In regard to GTE, it was tolerated in the concentration of 0.1 %, even though there were evident increased oil leakage levels for this gel. Above that concentration (0.5 % and 1 %), a disruption of bigels' original perceived texture was observed, as a much softer, almost liquid-like gel was formed. The reasons behind those deleterious effects caused by GA and GTE addition still need further clarification, but one possible explanation could be the acidifying properties of those compounds, particularly GA, that impacted emulsification the most. Also, it is interesting to note that GTE naturally contains a percentage of GA, which is a phenolic compound. Hence, GTE destabilizing issues could be linked to the presence of GA (Haslam & Cai, 1994).



Figure 10 – Visual appearance of the 90:10 HG:OG bigels with 0.1 % gallic acid upon inversion of the tubes.

On the other hand, micrographs show the RE and TPP gels are not significantly different from controls, with approximately the same particle size and distribution pattern (Figures 11 and 12). Interestingly, some studies showed that TPP, when combined with food-approved polymers, can form a three-dimensional gel-network that stabilizes oil-water interfaces in emulsions, thereby acting as a structurant (Luo et al., 2019; Pan et al., 2020). In REO and turmeric gels the overall particle size is only slightly bigger, and the addition of

these compounds does not seem to affect bigels' architecture considerably. However, the concentration of 0,1 % turmeric was difficult to fully solubilize during the oleogel development stage, leaving small aggregates that can be seen both macro and microscopically. Further, with the exception of GTE and turmeric, most of the bioactive compounds does not alter gels' color. Turmeric bigels' have a bright yellow pigment that may be undesirable for the final product, but GTE addition changes gels' color to a very subtle, pinkish tone (Figure 12).

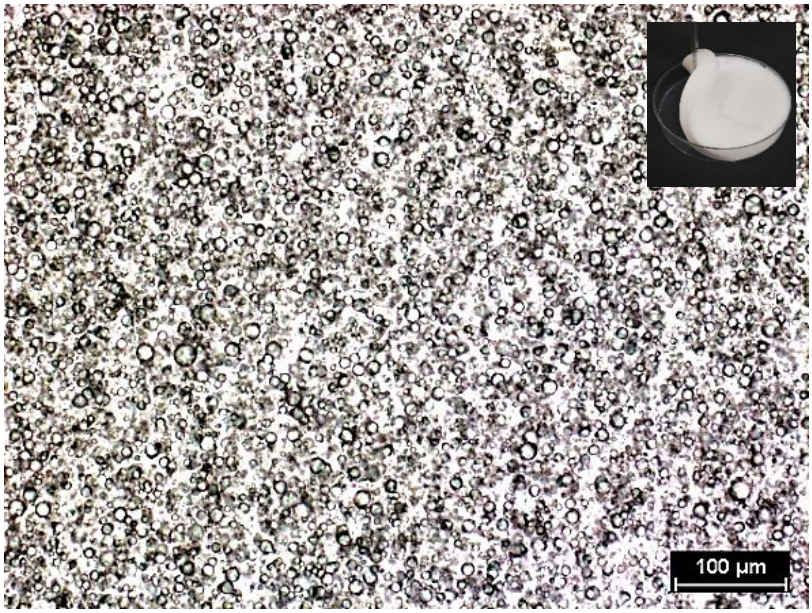

Figure 11 – Bright field micrographs for control bigel (no added extracts), with 90:10 HG:OG ratio, taken with 200x magnification.

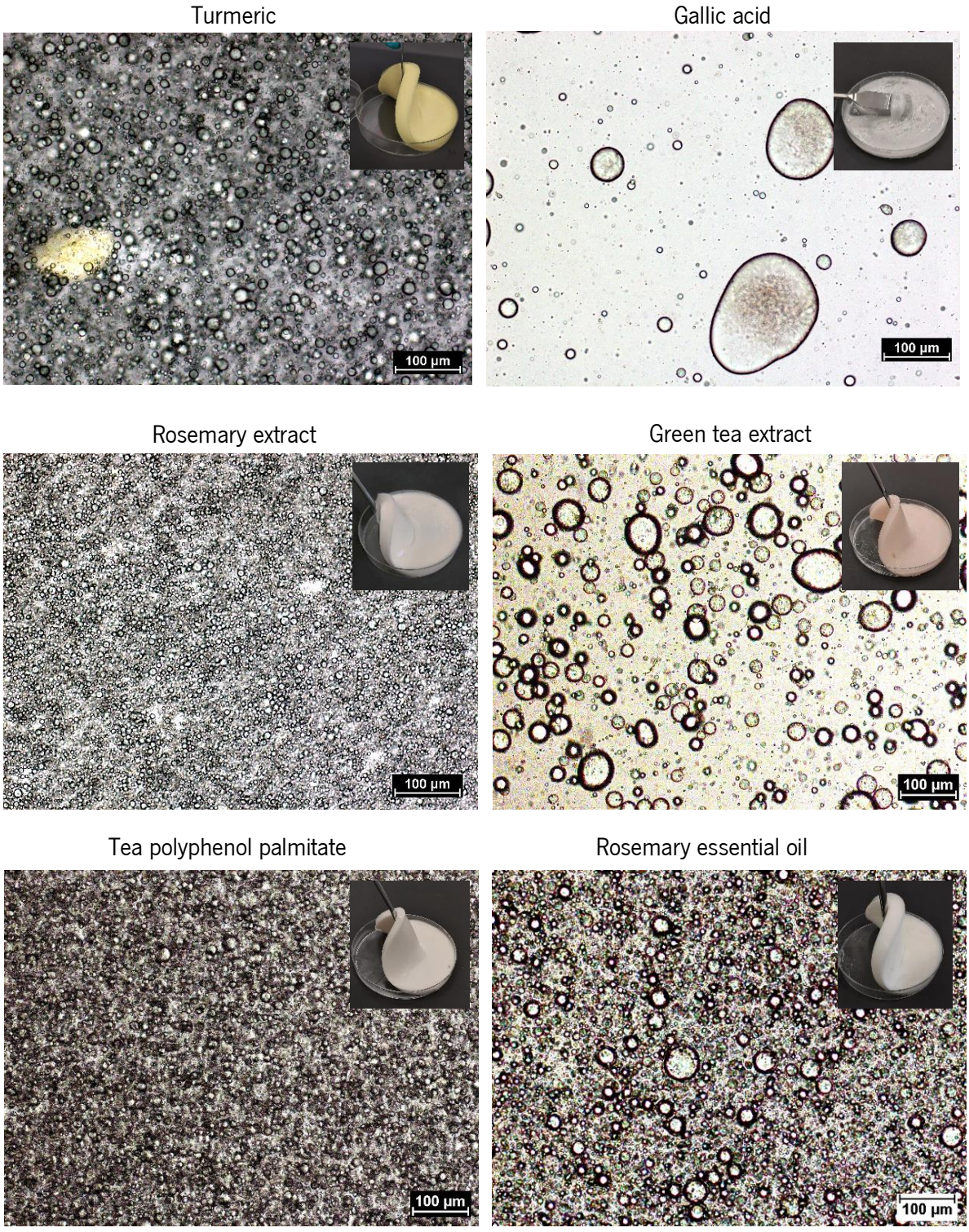

Figure 12 – Bright field micrographs for 90:10 samples with 0.1 % (w/w) of turmeric, gallic acid, rosemary extract, green tea, tea polyphenol palmitate and rosemary essential oil, taken with 200x magnification.

4.3 Droplet size measurements

Particle size measurements are represented in Figure 13 and Table 8.

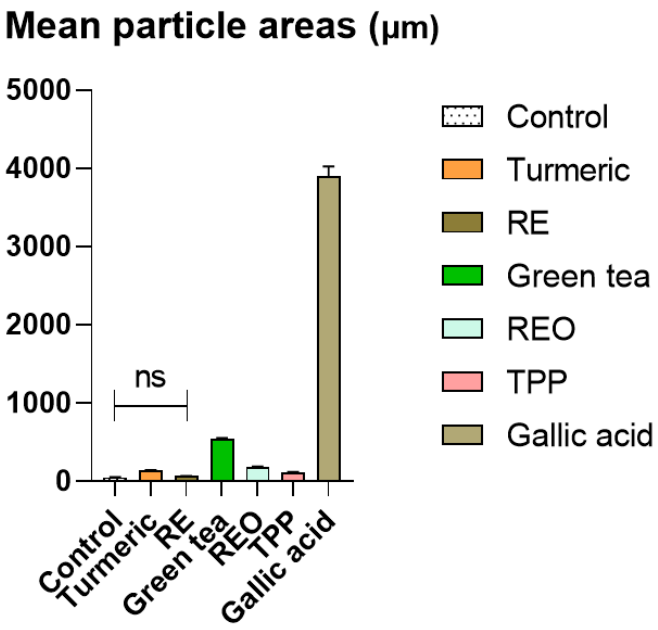

Figure 13 – Variation in particle size between 90:10 HG:OG ratio bigels. Samples have 0.1 % of each bioactive compound or none (control).

As shown above, mean oleogel droplets areas vary considerably between samples. As expected, gallic acid gels have the biggest mean particle size (3891.7 µm), in opposition to the control, that has the smallest mean particle area (42.4 µm). The second largest particle mean area belongs to green tea bigels, followed by REO, turmeric, TPP and RE. There is no statistical difference between the control and the samples with RE. Yet, in all formulations it is possible to find a wild range of particle sizes. As shown in Table 8, the biggest disparity between minimum and maximum droplet sizes are also found in gallic acid and green tea bigels, which are the ones where emulsification showed to be less effective.

Table 8 – Difference between minimum and maximum particle areas found in bigel samples. Values are in µm

	Control	Turmeric	RE	Gallic acid	GTE	REO	TPP
Min. area	4.5 ± 0.7	12.5 ± 3.5	4 ± 1.4	8 ± 2.8	29.2 ± 1.1	15.8 ± 1.7	13.9 ± 1.3
Max. area	115 ± 3.5	409.5 ± 7	424 ± 4.2	78179.5 ± 85.2	3280.3 ± 7.3	1559.7 ± 1.9	321.3 ± 0.8

4.4 Oil binding capacity

The OBC assessment expresses the ability of the gels to retain the oil phase into the bigel's structure, showing how well can the hydrogel matrix entrap the oleogel droplets. As expected, results show that bigels with higher HG:OG ratios were more successful in retaining the oil into the microstructure, since there's less oil and therefore it is easier to maintain oleogel droplets separate and avoid coalescence. Also, 0.5 % samples had the worst OBC, since those hydrogels are not strong enough to keep the oleogel imprisoned and the system emulsified. OBC values are compiled in Table 9.

Table 9 – OBC values for samples 0.5 %. 1 % and 2 %, without any added bioactive compound

HG:OG ratios	0.5 % LBG/κ-C	1 % LBG/κ-C	2 % LBG/κ-C
90:10	100 %	100 %	100 %
80:20	99.6 %	100 %	100 %
70:30	95.3 %	100 %	98.7 %
60:40	91.1 %	93.4 %	93.9 %
50:50	84.4 %	87.4 %	86.9 %

OBC was also assessed for bigels containing 1.5 % and 1.75 % LBG/κ-C (without bioactive compounds), and for the 1.5 % LBG/κ-C formulations with the incorporation of 0.1 % of turmeric, RE, TPP, GTE and REO, with 90:10 and 80:20 HG:OG ratios. All samples had OBC values equal to 100 %, which means that the addition of 0.1 % of those compounds did not negatively affect the capacity of the bigels to retain the oil.

4.5 Texture

The selected compound to be loaded into the bigels for the TPA was REO, since the other compounds either did not have antimicrobial or antioxidant activity, or negatively affected the bigel. TPA provided useful information on how the increase of the oleogel fraction and the addition of REO influenced the bigels' textural parameters of hardness, springiness, cohesiveness, chewiness, gumminess, and adhesiveness. Data analysis

show that the control bigels with 90:10 HG:OG ratio have higher values of hardness, springiness, chewiness, and gumminess in comparison to the bigels with 80:20 HG:OG ratio. This happens because the oleogel addition weakens the gel structure, promoting a more disaggregated structure that provides less resistance to the probe. On the other hand, values of adhesivity were lower, and no statistical difference in regard to cohesiveness was found when this formulation was compared to 80:20 samples. The adhesivity profile of a gel is an indication of the material's response and its consequent resistance to separation from the probe. Therefore, the 80:20 HG:OG control bigel is the stickiest (Martins, Silva, et al., 2019).

Moreover, results show that the incorporation of 1 % of REO seems to affect bigels with 90:10 and 80:20 HG:OG ratio differently. There is no statistical change in regard to hardness between 90:10 control and 90:10 with 1 % REO, but when this same amount of REO is added to the 80:20 formulation it becomes significantly softer as compared to control. Springiness, chewiness, and gumminess values decrease with the addition of 1 % REO at both tested HG:OG ratios, while adhesivity escalate. Cohesiveness values remains unchanged in all samples, which means that all tested bigels (with or without REO) have the same capacity to resist a second deformation.

Interestingly, bigels 80:20 control and 90:10 with 1 % REO have no statistical divergence on any of the tested textural parameters. Data can be visualized at Figure 14.

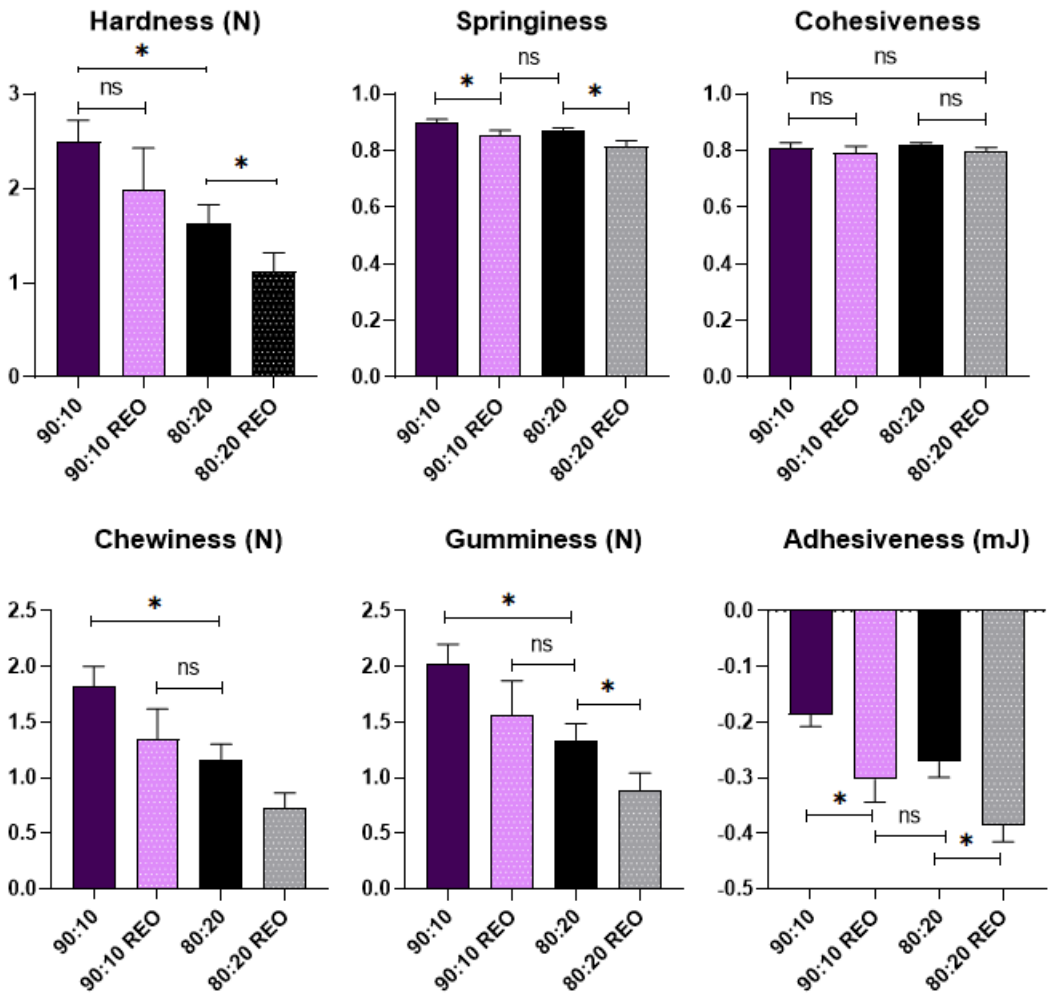

Figure 14 – TPA analysis results for samples 90:10 and 80:20, with and without 1 % REO.

4.6 Antioxidant activity

TBARS assay was performed to compare the production of oxidation products along storage. Tested samples were bigels with 90:10 and 80:20 HG:OG ratios and unheated sunflower oil, with and without natural antioxidants, intending to comprehend the effect of the gelator and the antioxidants on the bigels' oxidative profile. Results are compiled in Table 10, 11 and 12. Values are represented in [MDA] mg/L.

Table 10 – [MDA] mg/L in bigels with 90:10 HG:OG ratio

		Day 1	Day 8	Day 15
Bigel 90:10	Control (no added extracts)	0.25	-0.95	0.05
	Turmeric 0.1 %	-0.06	0.00	-0.15
	Rosemary extract 0.1 %	-0.50	0.90	1.22
	Tea polyphenol palmitate 0.1 %	-0.19	-0.07	-0.24
	Green tea extract 0.1 %	-0.28	-0.09	-0.14

Table 11 – [MDA] mg/L in bigels with 80:20 HG:OG ratio

		Day 1	Day 8	Day 15
Bigel 80:20	Control (no added extracts)	0.00	-0.02	-0.14
	Turmeric 0.1 %	0.01	0.08	-0.12
	Rosemary extract 0.1 %	0.00	0.15	-0.18
	Tea polyphenol palmitate 0.1 %	-0.01	-0.07	-0.14
	Green tea extract 0.1 %	-0.06	-0.03	-0.18

Table 12 – [MDA] mg/L in sunflower oil samples

		Day 1	Day 8	Day 15
Sunflower oil	Control (no added extracts)	-0.18	-0.07	-0.08
	Turmeric 0.1 %	-0.18	-0.12	-0.09
	Rosemary extract 0.1 %	-0.18	-0.08	-0.06
	Tea polyphenol palmitate 0.1 %	-0.16	-0.17	0.22
	Green tea extract 0.1 %	-0.16	-0.09	-0.07

As shown above, MDA concentration in all samples (with and without antioxidant compounds) have extremely low or even negative values, with no evident tendency. It was already expected that bigel oxidation levels would be low, since there is little amount of oil in their composition, and studies show that oil structuring in an oleogel or bigel form can increase oil resistance to oxidation, reducing product deterioration. (Samui et al., 2021). However, it was not expected that MDA values for pure oil would be in that range. This may be

explained by the fact that the sunflower oil used is refined and developed to resist high frying temperatures, with high amounts of a powerful antioxidant (0,06 % vitamin E) (Quiles et al., 2002). Also, all samples were kept in the cold and in the dark, added to the fact that the TBARS assay kit used does not require any heating of the samples. Therefore, stimuli for oil oxidation were too low, and the difference between the control and the samples with added antioxidants could not be perceived.

To check if these low values came from the type of oil used or from any complication with the methodology, a small experiment was done with olive oil at its plain state versus after it was boiled for 10 minutes. Results for the unheated olive oil, boiled olive oil and unheated sunflower oil were 0.030, 1.51 and -0.06 [MDA] mg/L, respectively. Olive oil MDA values are in accordance with other studies (Bergamo et al., 1998; Giannakopoulos et al., 2020).

Therefore, those results point out that the addition of an antioxidant in these bigel formulations developed with sunflower oil does not seem to be necessary as a mean to improve product's shelf life. However, the addition of bioactive compounds could still be relevant for the consumer by acting as a dietary source of antioxidants. Besides, to better perceive the antioxidants' potential over the oil and bigel samples, this experiment could be repeated over accelerated oxidation conditions and over a longer period of time. However, with the purpose of improving the microbial safety and shelf life of a meat product, these compounds could be useful as antimicrobials, since other works show they have potential antifungal and anti-mycotoxigenic activity.

4.7 Antimicrobial activity

In regard to the antifungal activity of the extracts, the only ones that inhibited the tested fungi were rosemary essential oil and green tea extract. Some samples had more evident spore formation around the wells with oil (Figure 15), which is confirmed by other studies that show that the presence of oil seems to enhance the sporulation of some fungi (Schlösser & Prange, 2019b).



Figure 15 – Spore formation around the well filled with sunflower oil with green tea extract.

To differentiate whether the lack of antifungal activity came from interference with the oil or as a result of the ineffectiveness of the compound itself, parallel experiments were performed by solubilizing the extracts into 1 % DMSO instead of sunflower oil. A control using 1 % DMSO without any extract was also developed, to discard that the inhibition could come from the DMSO and not from the added compounds.

In the diffusion assay, green tea did not show any inhibitory effect over fungal growth when it was solubilized in oil, even in high concentrations 55 % (w/w). When solubilized in DMSO, however, green tea did show fungal inhibition, but only in concentrations above 11 % (Figures 16 and 17). In the assays where green tea was dissolved into the culture medium, however, some inhibitory effects were noticeable with much lower concentrations (0.1 %, 0.5 % and 1 %).



Figure 16 – Inhibition halo when 55 % (g/g) of green tea extract is solubilized in oil (left) versus in DMSO (right).

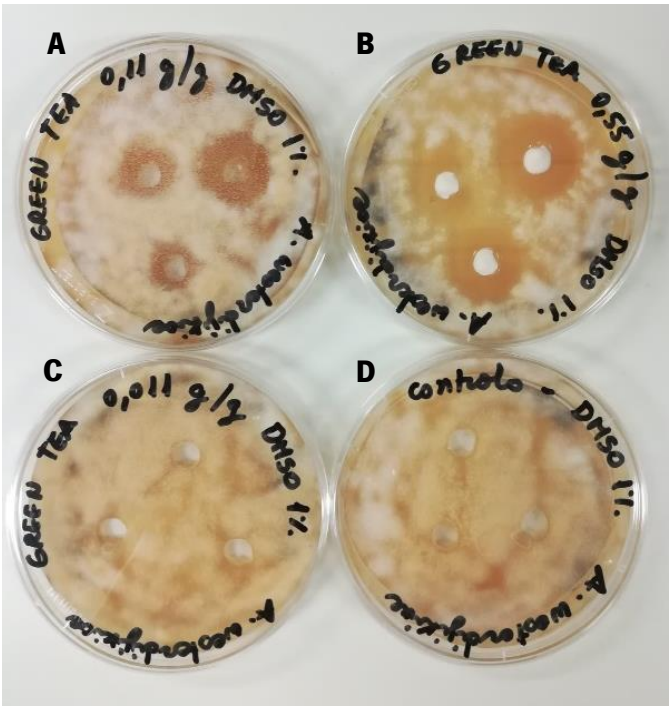

Figure 17 – Diffusion assay with GTE solubilized in DMSO in different concentrations: 11 % (A), 55 % (B), 1,1 % (C) and 0 % (D – control)

Rosemary essential oil, on the other hand, had antifungal activity over the four tested fungi species. In the diffusion assay, inhibition was not depicted as an inhibition halo, but as inhibition of growth and sporulation over the whole Petri dish (Figure 18). This happened because REOs antifungal activity comes from the existent volatile compounds, such as eucalyptol, α-pinene, camphor and β-pinene, and the oil alone was not enough to entrap them (Şerban et al., 2011).



Figure 18 – Diffusion assay for 11 %, 55 % and pure REO solubilized in sunflower oil.

In the assays where REO was solubilized in the culture medium, results showed an inhibitory effect on all fungi even using low concentrations (0,05 %, 0.1 %, 0.5 % and 1 % w/w). Diameter of the colonies was measured daily, from the third to the seventh day, and the results were represented in Figures 19 and 20.

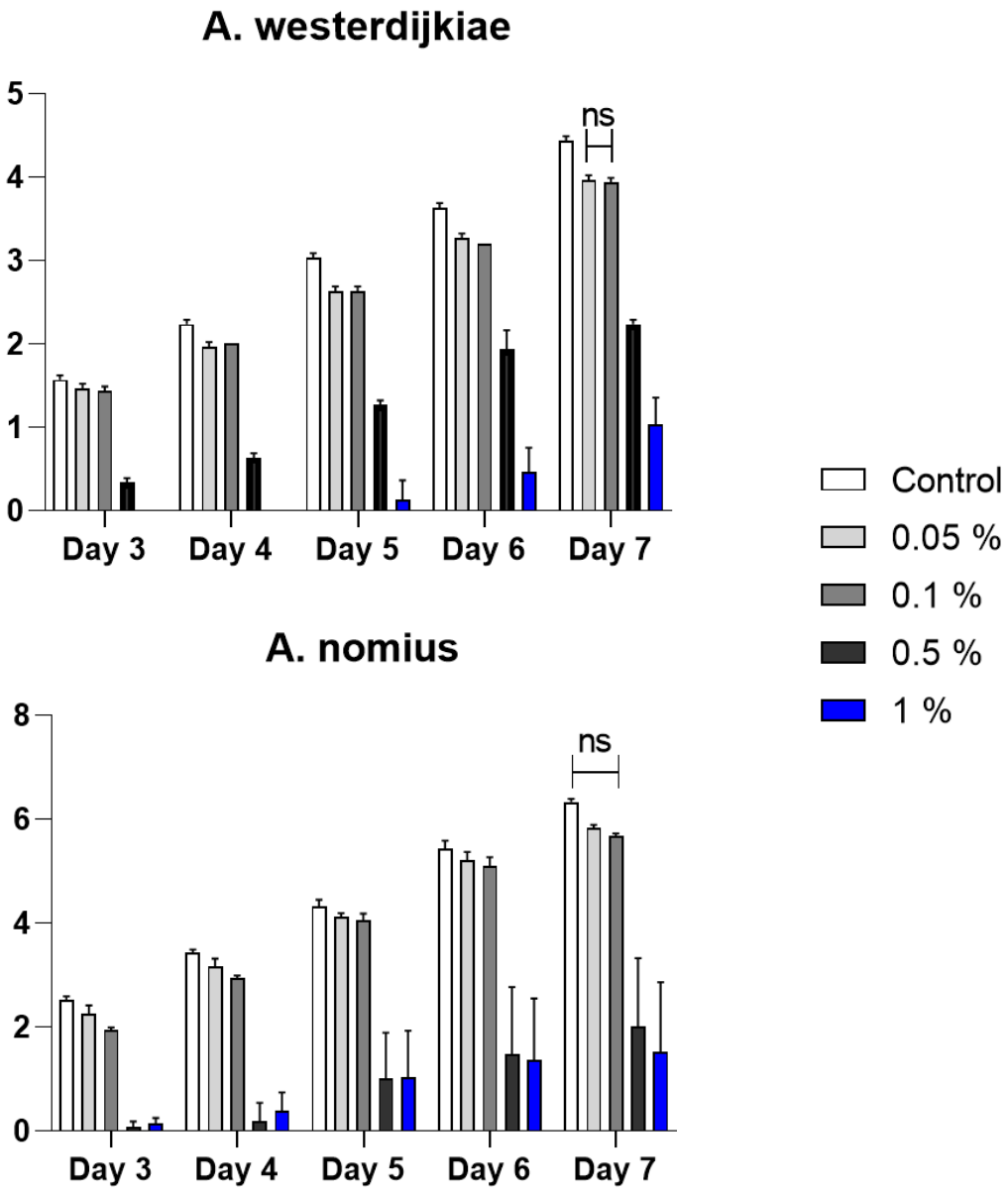

Figure 19 – Effect of REO added to the culture media at different concentrations (0 %, 0.05 %, 0.1 %, 0.5 % and 1 %) on colony diameters of *A. westerdijkiae* and *A. nomius,* from the third to the seventh day of growth.

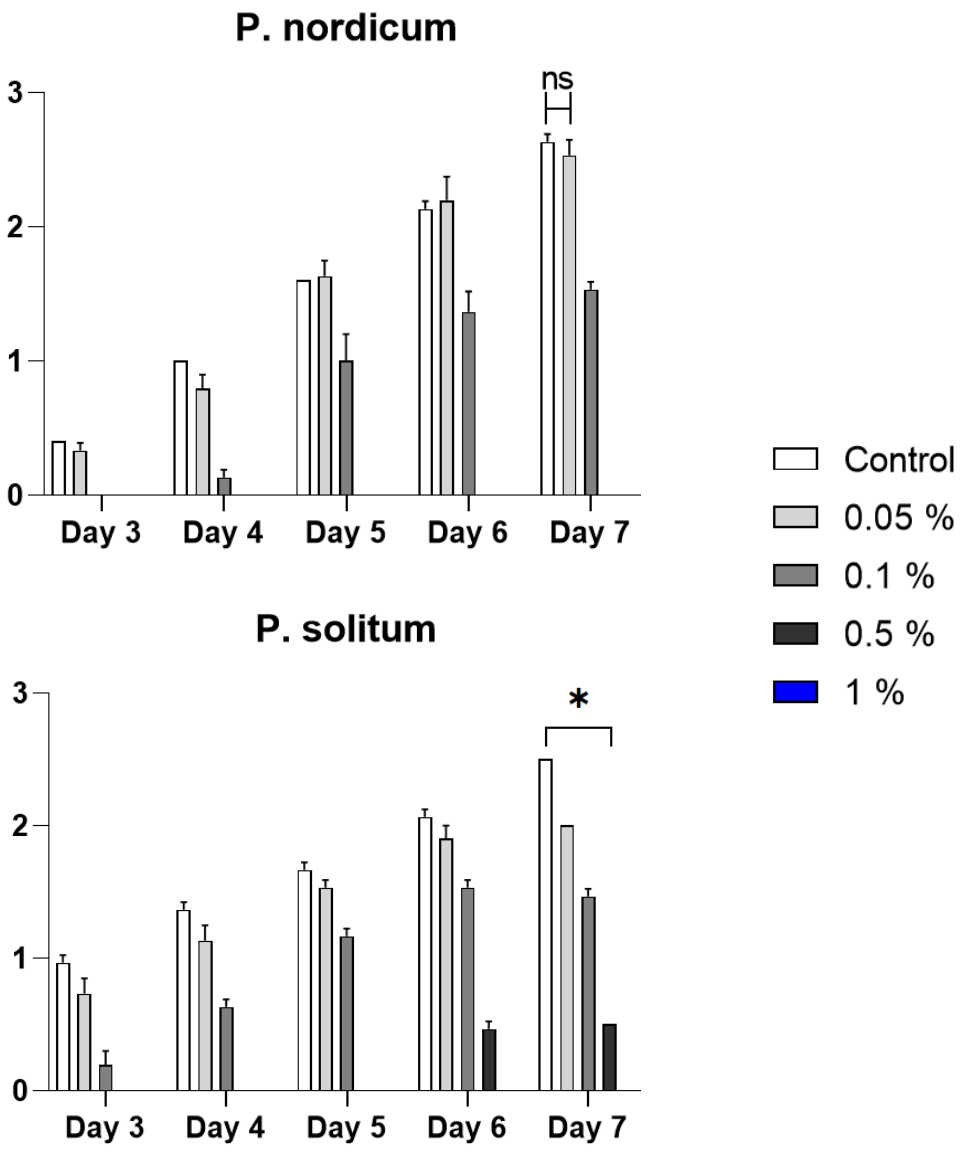

Figure 20 - Effect of REO added to the culture media at different concentrations (0 %, 0.05 %, 0.1 %, 0.5 % and 1 %) on colony diameters of *P. nordicum* and *P. solitum*, from the third to the seventh day of growth.

As seen in the figures above, all fungi had the highest degree of inhibition when 1 % REO was used. *P. solitum* and *P. nordicum* seemed to be the most sensitive species, not developing at all after the seventh day when this concentration was used. At day 7, there was no statistical difference between using 0.05 % or 0.1 % of REO in regard to the growth of *A. westerdijkiae*, and no difference between the control and the use of 0.1 % REO for *A. nomius*. However, it only seems to be worth it to use REO in concentrations above 0.5 %, where inhibition occurred more significantly. *P. nordicum* was 100 % inhibited at 0.5 % REO samples, so

higher concentrations would be unnecessary if the only aim was to inhibit fungal growth. *P. solitum* had significant differences between all tested concentrations, achieving 100 % inhibition only when 1 % REO was used.

The data demonstrates that REO could be used as a potential alternative to synthetic fungicides. Moreover, application of essential oils into meat formulations could be limited due to their intense aroma, especially in higher concentrations (0.5 % and 1 %). However, other technologies could be combined to improve both the sensory attributes and microbial stability, such as microencapsulation. Also, it is possible to combine lower levels of REO with preservation technologies such as low temperature and acidity, modified atmosphere packaging, high hydrostatic pressure or low-dose irradiation to achieve microbial safety while improving the sensorial attributes of the product (Jayasena & Jo, 2013).

4.7.1 Mycotoxin analysis

Data show that REO impacted mycotoxin production in both *A. nomius* and *P. nordicum*. For *A. nomius*, the addition of 0.05 % and 0.1 % REO did not significantly change the production of aflatoxin G2 (AFG2) and AFG1 in comparison to the control. However, those same REO concentrations significantly raised the production of aflatoxin B2 (AFB2) and AFB1 relatively to the control (Figure 20). This may be explained as a fungal response to stress when low doses of preservatives are employed, triggering the increase of the secondary metabolism of mycotoxins (da Cruz Cabral et al., 2013; Schlösser & Prange, 2019a). On the other hand, 0.5 % and 1 % REO reduced mycotoxin production of all aflatoxins when compared to the control. For AFB1, which is the most toxic aflatoxin, inhibition in relation to the control was 74 % when 1 % REO was used.

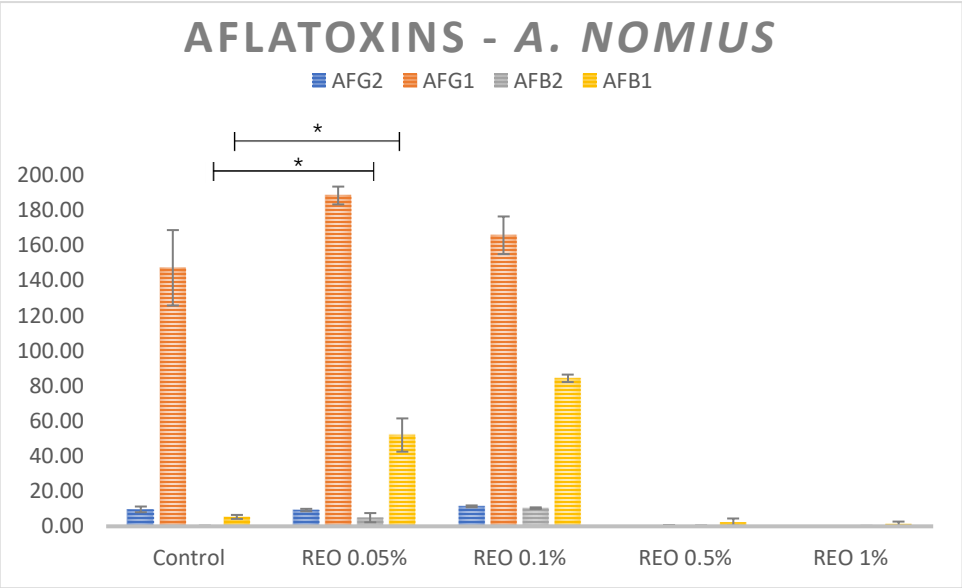

Figure 21 – Effect over the production of AFG2, AFG1, AFB2 and AFB1 by *A. nomius* as the result of the incorporation of 0 %, 0.05 %, 0.1 %, 0.5 %, and 1 % REO.

Production of OTA by *P. nordicum* is presented in Figure 21. Data demonstrates that over 0.1 % REO there was no production of OTA by this fungi. However, at this same concentration there is still fungal growth, which means inhibition of mycotoxin production cannot be correlated with growth inhibition. With 0.05 % REO there was no significant change in OTA levels relatively to the control.

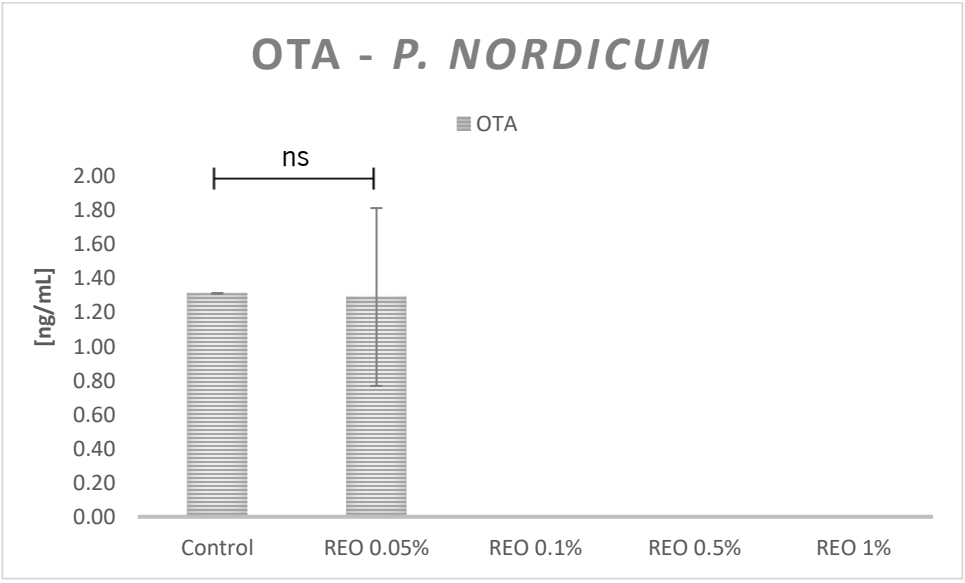

Figure 22 – Effect over the production of OTA by *P. nordicum* as the result of the incorporation of 0 %, 0.05 %, 0.1 %, 0.5 % and 1 % REO.

5 CONCLUSIONS

The main objectives of this dissertation were fulfilled, successfully characterizing, and developing bigels with and without the addition of antioxidants and antifungals. It was concluded that, to obtain stable formulations, a hydrogel with 1.5 % LBG/κ-C and an oleogel with 10 % GMS was the optimal combination. Also, it was confirmed that the hydrogel:oleogel ratio plays a big role in bigels' stability, and the most balanced ones are 90:10 and 80:20 ratios, with the highest capacities to retain the oil.

In addition, the antioxidant and antimicrobial potential of these novel formulations loaded with bioactive compounds was assessed. The addition of an antioxidant in bigels developed with sunflower oil did not seem to be necessary, since there were no significant oxidation levels in neither the control nor the loaded bigels. Yet, the antioxidant activity and potential applicability of the bioactive compounds into this type of structured system still needs further clarification.

In regard to the antimicrobial potential, only two bioactive compounds had antifungal activity against the tested fungi: green tea extract and rosemary essential oil. However, the former had disruptive effects over the bigel's structure and stability. The most promising results for application into meat formulations were of rosemary essential oil, which did not greatly disturb bigels quality and texture. Besides its capacity to inhibit fungal growth, rosemary essential oil also had anti-aflatoxigenic and anti-ochratoxigenic activity over the mycotoxin-producing fungi *A. nomius* and *P. nordicum*, respectively. Hence, rosemary essential oil could effectively be incorporated into bigels as a mean to improve a meat product's shelf life and microbial safety.

6 FUTURE PERSPECTIVES

In following works, it would be interesting to reassess the antioxidant activity of the tested compounds using a different methodology, possibly using an accelerated oxidation environment, a different oil medium, or a more sensitive methodology. In regard to the antifungal activity of rosemary essential oil, it would be interesting to determine its minimum inhibitory concentration ageainst *A. westerdijkiae, A. nomius, P. solitum* and *P. nordicum,* so that only the strictly necessary amount of REO would be added to the bigels. Also, to counteract its possible disadvantages in terms of odor, rosemary essential oil could be loaded into the bigels into its encapsulated form or combined with other preservation technologies in lower concentrations. Moreover, next steps would be the incorporation of the developed bigels into a meat product, evaluating its effect on product microstructure and texture, as well as further sensorial analysis of the final formulation.

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APPENDICES

APPENDIX A: Micrographs for bigels with 0.5 %, 1.5 %, 1.75 % and 2 % of LBG/κ-C.

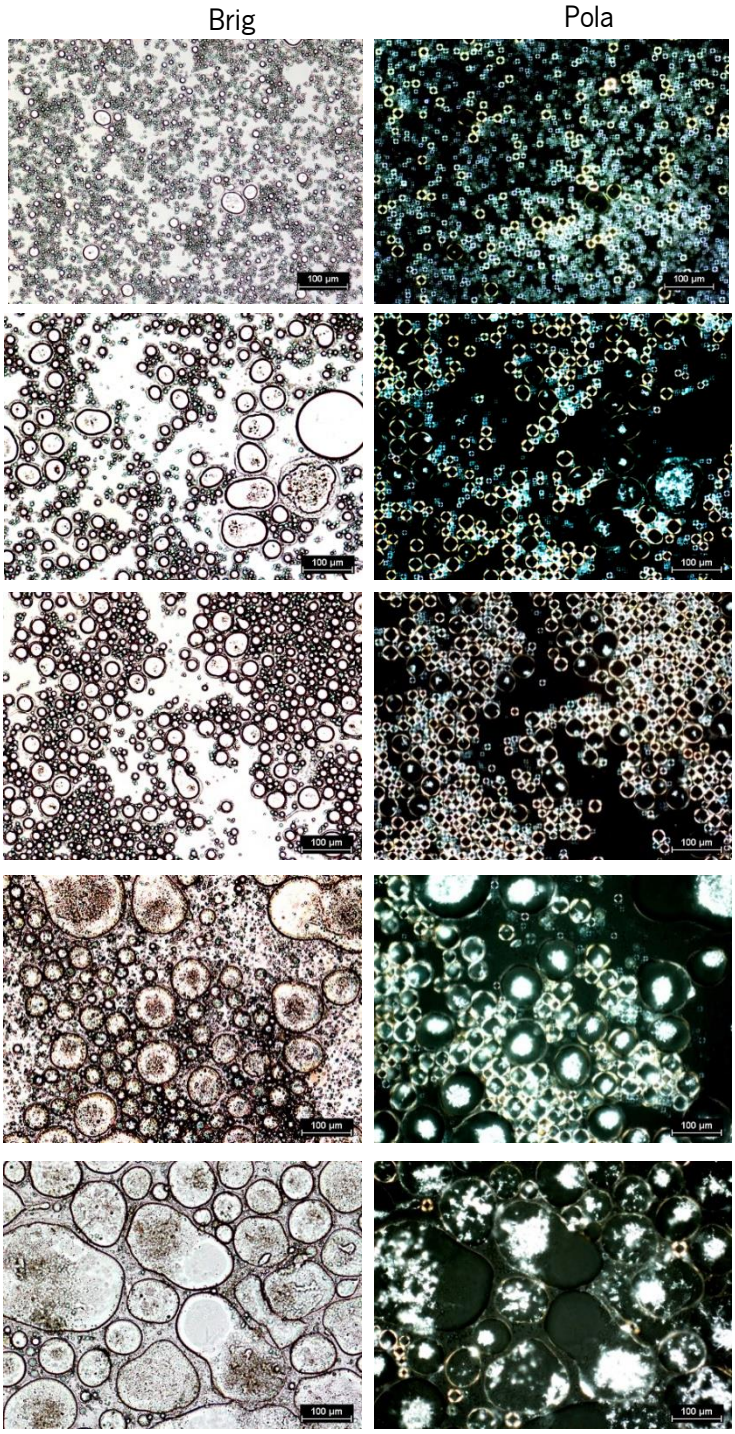

Figure 23 – Bright-field and polarized micrographs of the gels with 0.5 % LBG/κ-C, taken with 200x magnification, for 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios.

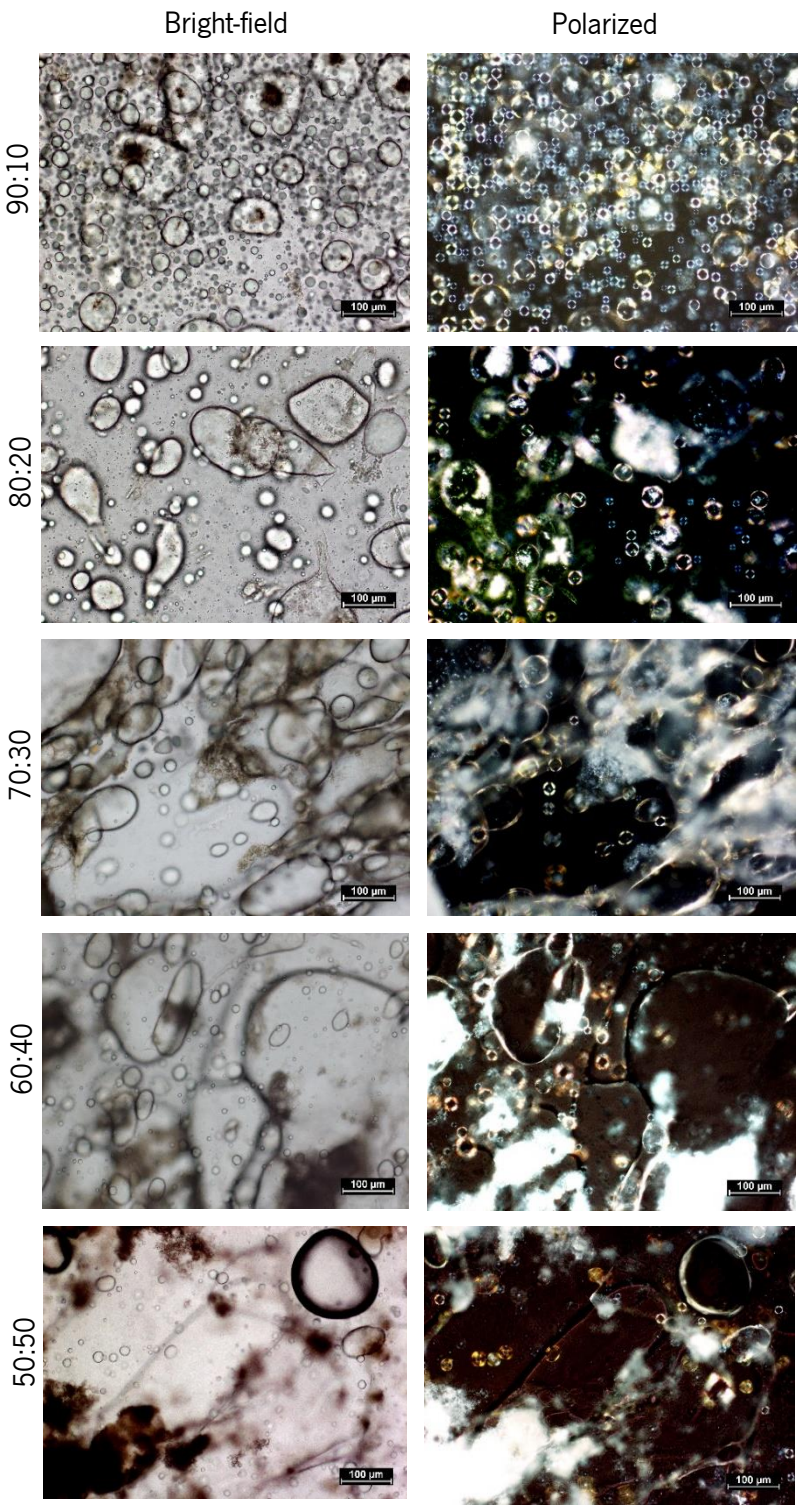

Figure 24 – Bright-field and polarized micrographs of the gels with 2 % LBG/κ-C, taken with 200x magnification, for 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios.

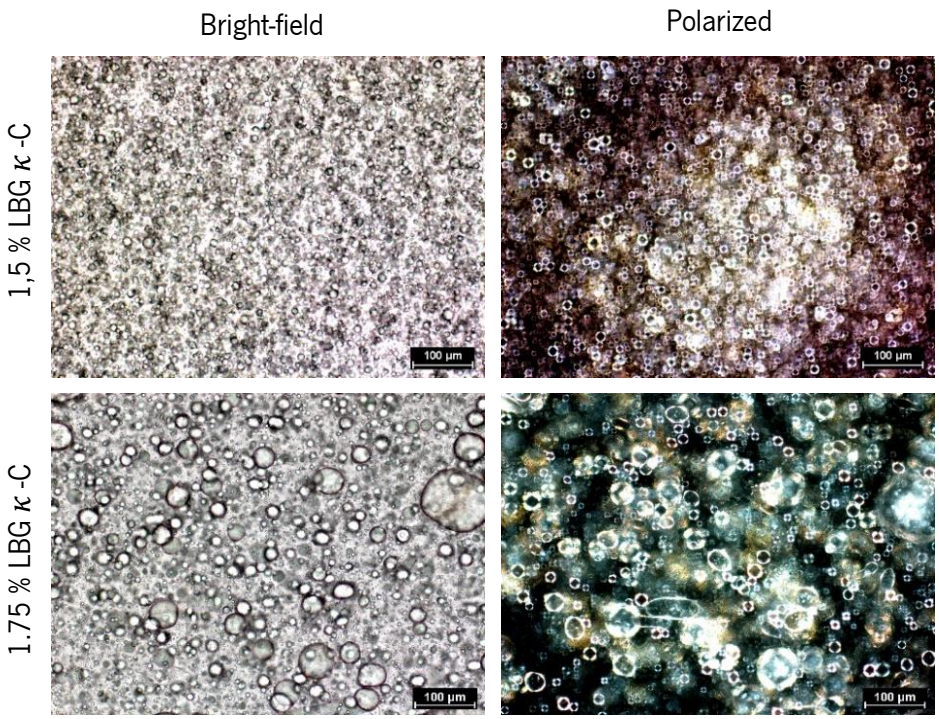

Figure 25 – Bright field and polarized micrographs of the gels with 1,5 % and 1.75 % LBG/κ-C, taken with 200x magnification, for 90:10 HG:OG ratio.

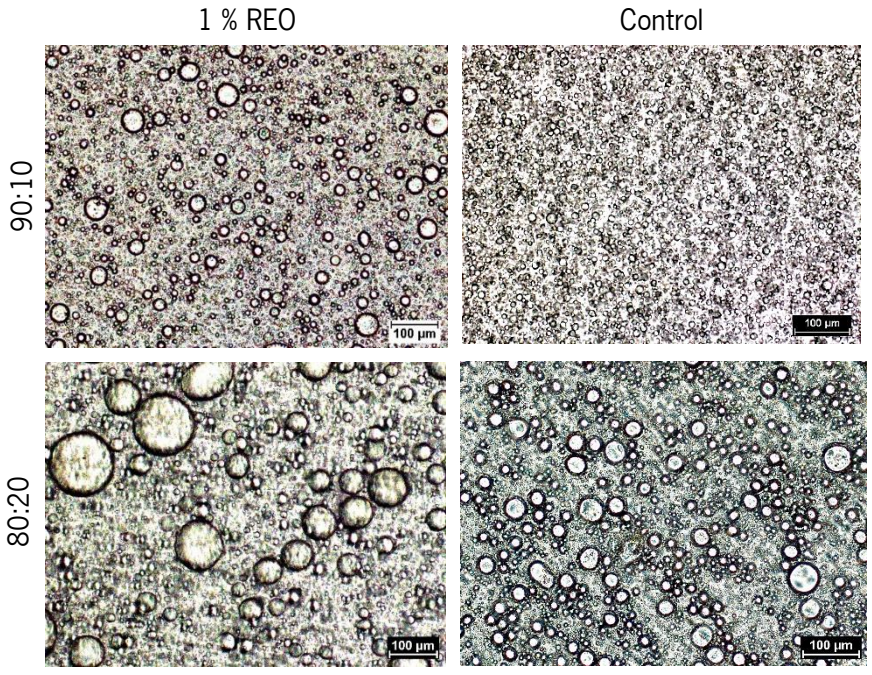

Figure 26 – Bright field micrographs for 90:10 and 80:20 samples with 1 % (w/w) of rosemary essential oil (REO) versus controls, taken with 200x magnification.

APPENDIX B: Differences in regard to the visual appearance of samples with various HG:OG ratios and gelator concentrations.

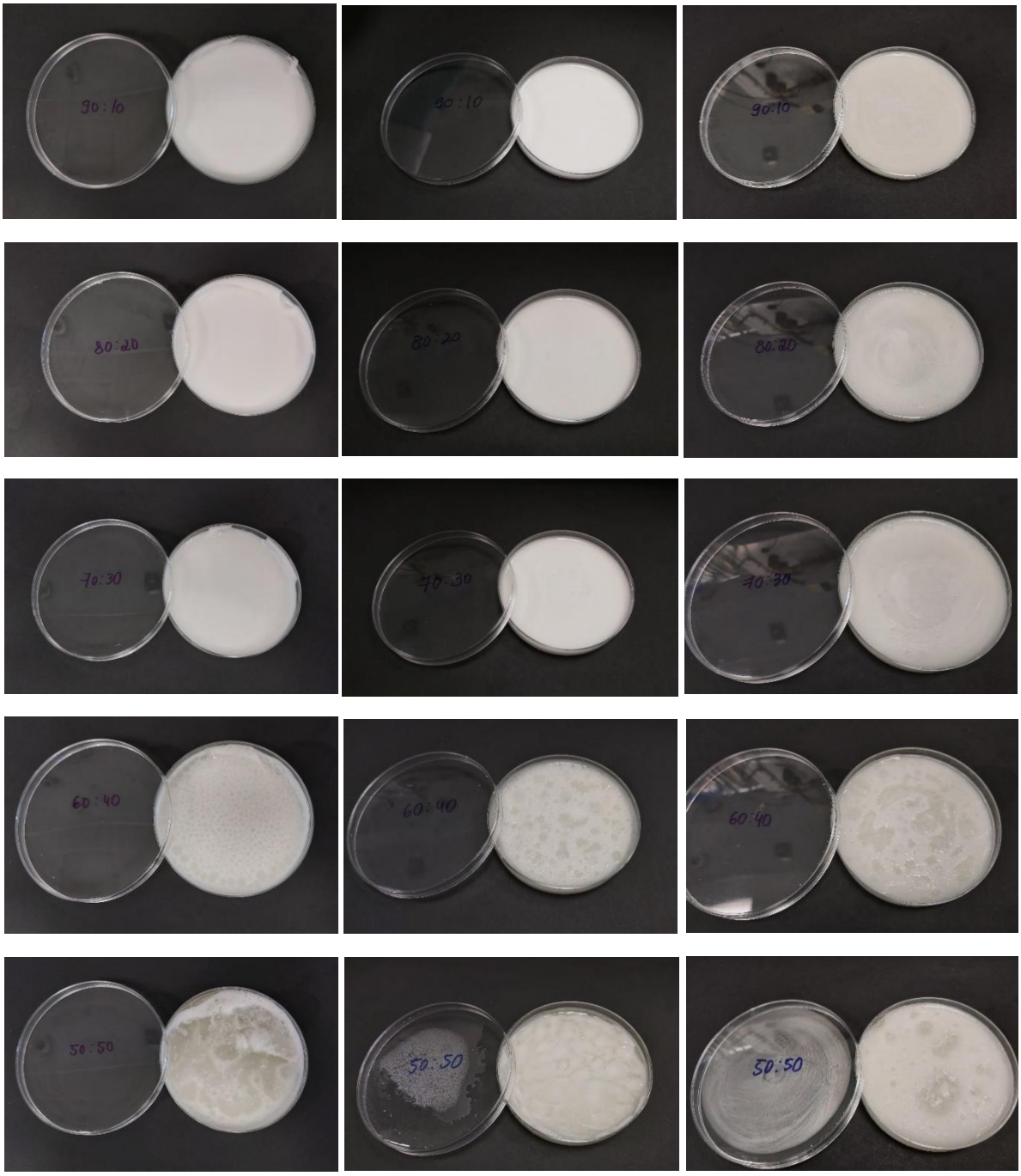

Figure 27 – Visual appearance of the gels with 0.5 %, 1 % and 2 % LBG/κ-C for 90:10, 80:20, 70:30, 60:40 and 50:50 HG:OG ratios