Investigating lipid oxidation in mayonnaise & Development of a new plant-based and gluten-free alternative to fish products

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Work carried out under the supervision of
**Giulia Battistel, Kraft Heinz Company**
**Daniela Marques, Irmãos Monteiro S.A.**

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Last but not the least, I owe everything to my parents and rest of the family. There are no words to express the love and gratitude I feel for you, thank you for inspiring me to be better.

## 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.

**Investigating lipid oxidation in mayonnaise and development of a new plant-based and gluten-free alternative to fish products**

**Abstract**: Nowadays, consumers are more aware of global issues and their wellbeing. Hence, food trends have been veering towards healthy, plant-based, and clean-label products. Thereupon, this study aimed to investigate lipid oxidation in mayonnaise to find an effective natural alternative to EDTA, as well as to develop a plant-based and gluten-free alternative to fish products that is healthy and has great organoleptic properties.

In a first step, mayonnaises with a rosemary and green tea extract (L), or a rosemary and spinach extract (X), were tracked over time. The samples containing extract L were not significantly impacted regarding their oxidation rates. Concerning extract X, vegan samples had a pH 3.0, so the natural antioxidant was mainly present at its less active form, still, it seems that the extract X is an effective alternative to EDTA because the lowest amount of high impact volatile compounds and the best sensory profile was associated with formulations without egg yolk. A factorial design was also carried out with 4 egg types and 4 oil types that led to 16 formulations of EDTA-free mayonnaise being monitored over time. The results suggested that in oil-in-water emulsions, like mayonnaise, the oxidative stability significantly increases when the level of unsaturation of the oil is higher. Mayonnaises with soybean oil exhibited the lowest high impact volatiles intensities, and the sensory profile of samples with whole egg or heat-stable egg yolk, and sunflower or soybean oils were the best. Thus, it seems that the "ideal recipe" to limit lipid oxidation would include whole egg and soybean oil.

In a second step, based on a market research, two prototypes of a plant-based and gluten-free *patanisca* were developed and sensory and nutritional analysis were performed. The data from a consumer sensory evaluation revealed a significant higher acceptance – sensory properties and intention of purchase – of the *patanisca* containing Ogonori algae, compared to one that only included vegetables. Focusing on the former sample's nutritional profile, the targeted quantity of nutrients such as saturated fats, carbohydrates, sugars and protein levels were achieved; however, the content in fats and salt was exceeded. In general, the formulation is healthier than what is currently available in the marketplace to consumers.

**Keywords:** Antioxidants, Lipid oxidation, New products, Nutritional analysis, Sensory analysis.

**Investigação da oxidação lipídica em maionese e desenvolvimento de um produto de peixe à base de plantas e sem glúten**

**Resumo**: Atualmente, os consumidores estão mais conscientes dos problemas globais e do seu bem-estar, logo as tendências alimentares têm convergido para produtos saudáveis, de base vegetal e de rótulo limpo. Portanto, este estudo tem como objetivos estudar a oxidação lipídica em maionese para encontrar uma alternativa natural ao EDTA, assim como desenvolver um produto de peixe à base de plantas e sem glúten que seja considerado saudável e com excelentes propriedades organoléticas.

Numa primeira etapa, maioneses com extrato de alecrim e chá verde (L), ou extrato de alecrim e espinafre (X), foram avaliadas ao longo do tempo. As amostras com extrato L não foram significativamente afetadas quanto à sua taxa de oxidação. Relativamente ao extrato X, nas amostras vegan com pH 3.0, o antioxidante natural apresentava-se na sua forma menos ativa, mesmo assim, parece que o extrato X é uma alternativa eficaz ao EDTA porque as formulações sem ovo apresentaram os menores níveis de voláteis de alto impacto e o melhor perfil sensorial. Adicionalmente, foi realizada uma análise fatorial com 4 tipos de ovo e 4 tipos de óleo, sendo obtidas 16 formulações que foram monitorizadas ao longo do tempo. Os resultados sugeriram que a maior estabilidade oxidativa da maionese é proporcional ao número de insaturações do óleo. A maionese com óleo de soja demonstrou as menores intensidades de voláteis de elevado impacto, e o melhor perfil sensorial foi atribuído às amostras com ovo inteiro ou gema de ovo estável ao calor, e óleo de girassol ou de soja. Sendo assim, parece que a "receita ideal" para limitar a oxidação lipídica incluiria ovo inteiro e óleo de soja.

Numa segunda etapa, com base num estudo de mercado, foram desenvolvidos dois protótipos de uma patanisca à base de vegetais e sem glúten, sendo estes analisados sensorial e nutricionalmente. Os resultados de uma análise sensorial por parte de consumidores revelaram que a patanisca com alga Ogonori apresentou uma aceitação, tanto em termos sensoriais como de intenção de compra, significativamente mais elevada do que uma patanisca que continha apenas vegetais. Quanto às propriedades nutricionais, os objetivos foram atingidos para nutrientes como lípidos saturados, hidratos de carbono, açúcares e proteína, mas o conteúdo em lípidos (totais) e sal foram excedidos. No entanto, a patanisca com algas foi considerada como mais saudável do que os produtos atualmente disponíveis no mercado.

**Palavras-chave:** Análise nutricional, Análise sensorial, Antioxidantes, Novos produtos, Oxidação lipídica.

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## List of abbreviations

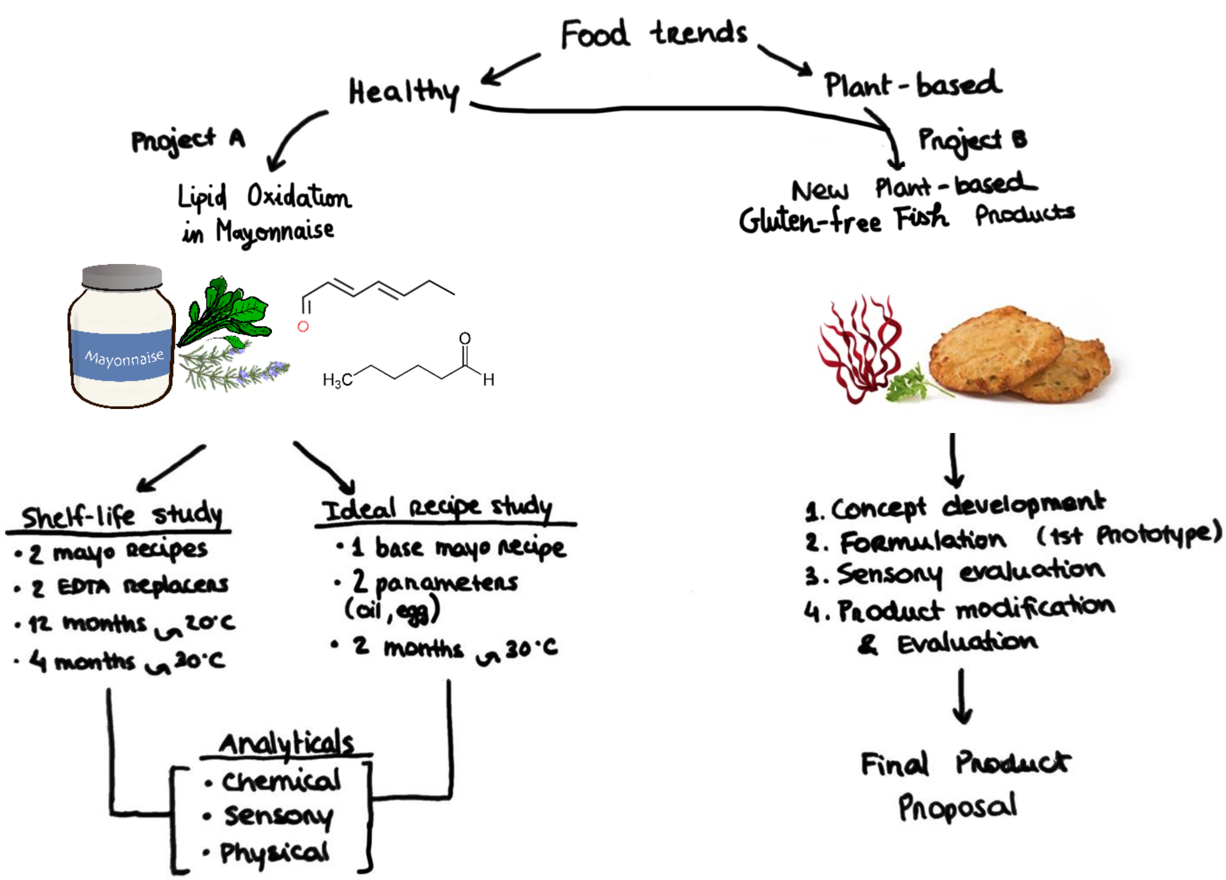
| | |
|---|---|
| ASL | Accelerated shelf life |
| ANOVA | Analysis of variance |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| CD | Celiac disease |
| Co | Cobalt |
| Cu | Copper |
| DOE | Design of experiments |
| E-nose | Electronic nose |
| EDTA | Ethylene diamine tetra-acetic acid |
| extract L | Rosemary and green tea extract |
| extract X | Rosemary and spinach extract |
| Fe | Iron |
| GC-FID | Gas chromatography coupled with flame ionization detector |
| GC-MS | Gas chromatography coupled with mass spectrometry |
| GFD | Gluten-free diet |
| HDL | High-density lipoproteins |
| HORS | High oleic rapeseed oil |
| HSEY | Heat stable egg yolk |
| HSG | Heinz seriously good full fat |
| LDL | Low-density lipoproteins |
| LEY | Liquid egg yolk |
| MUFA | Monounsaturated fatty acids |
| NPD | New product development |
| O/W | Oil-in-water |
| PB | Plant-based |
| PBD | Plant-based diet |
| PDCAAS | Protein digestibility-corrected amino acid score |

| | |
|---|---|
| PEY | Powdered egg yolk |
| pI | Isoelectric point |
| PUFA | Polyunsaturated fatty acids |
| PV | Peroxide value |
| RI | Reference intake |
| RS | Rapeseed oil |
| RSL | Real shelf life |
| SB | Soybean oil |
| SF | Sunflower oil |
| SFA | Saturated fatty acids |
| TEE | Total energy expenditure |
| UFA | Unsaturated fatty acids |
| W/O | Water-in-oil |
| WE | Whole egg/egg yolk mix |

## Dissertation workplan

The present dissertation has been organized in two parts, each dedicated to the work developed during two internships. In Part A it is discussed the project developed during 6 months at the Kraft Heinz Research and Development Center (Netherlands), related to the investigation of lipid oxidation in mayonnaise, with focus on finding natural alternatives to EDTA, but also to understand interactions between ingredients and its effect on oxidation rates. Regarding Part B, it concerns a 3 months internship at Irmãos Monteiro S.A. (Portugal), where the aim was the development of a new plant-based and gluten-free alternative to a fish product – *pataniscas.*

The following scheme depicts the overall dissertation workplan.



**Part A – Investigating Lipid Oxidation in Mayonnaise**

## 1. Introduction

Food provides nutrients essential for obtaining energy necessary for all body functions and to promote growth. In this vein, the food industry plays one of the most vital roles in society. Satisfaction of consumer need is one of the main goals of this industry, followed by being able to provide safe products and their nutritional information, and ultimately to maintain commercial viability. To meet these goals, product reformulation and development of new products is an essential part of the industry (Mettler, 1986).

The lifestyles and dietary patterns of consumers are deeply influenced by factors such as culture, politics, environment, demographics, and socioeconomics (Arenas-Jal et al., 2020). Since the beginning of the twenty-first century, the world has been seeing immense advances in mobile connectivity and other technologies, accordingly, in industrialized countries, consumers are much more aware of global issues such as climate change, food waste, animal abuse, among others (Arenas-Jal et al., 2020; Ayres & Williams, 2004). In addition, the understanding and concern with food components and their properties has risen (Arenas-Jal et al., 2020; Asioli et al., 2017). Thus, the food consumption is nowadays veering towards healthy, plant-based, and sustainable products (Arenas-Jal et al., 2020; Asioli et al., 2017; Portugal Foods, 2021).

Consumers are increasingly searching for clean label products (i.e., products perceived as healthier), which show absence or minimal presence of food additives, such as synthetic colours, preservatives, stabilizers, emulsifiers, and texturizers (Arenas-Jal et al., 2020; Asioli et al., 2017). In this vein, food companies have been investigating natural replacements or new technologies that allow products to maintain their quality and shelf life, as well as be perceived by consumers as something familiar, acceptable, and not artificial (Arenas-Jal et al., 2020).

Mayonnaise is a sauce with a creamy and smooth texture, that combined with a rich flavour, makes it one of the most widely used sauces in the world today (Li et al., 2014). In 2008, 26 % of the global sauce market value was comprised of the mayonnaise and salad dressing sector, the second largest. This product results from the emulsification process of vegetable oil with other components like eggs (whole or egg yolk), vinegar, mustard, among others (Morley, 2016; Raikos et al., 2016). Due to its low pH and low water content, mayonnaise is relatively resistant to microbial growth (Depree & Savage, 2001). However, its great fat percentage (traditionally, 70 % to 80 %) and the nature of the raw materials makes it highly susceptible to oxidation (Li et al., 2014; Raikos et al., 2016).

Within the food industry, oxidation of oil is very important as it leads to decreased shelf life due to rancidity. Oxidation products can also easily attack macromolecules, especially in the presence of metals, contributing to the decline of flavour, aroma, colour, and nutritional value of food (Li et al., 2014; Vieira et al., 2017). Moreover, the consumption of potentially toxic lipid oxidation products may contribute to inflammatory diseases, cancer, atherosclerosis, aging, etc (Vieira et al., 2017). Hence, the prevention of lipid oxidation is a necessity both for consumer health and product economic viability.

Heretofore, reduction and control of lipid oxidation has been attained by the addition of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ethylene diamine tetra-acetic acid (EDTA). The latter is an economical and effective antioxidant, commonly used as a food additive in mayonnaise (Li et al., 2014; Shahidi, 2015). Despite its great functionality, its synthetic nature does not align with the current consumers ideals, thus negatively influencing purchase decisions (Li et al., 2014). Thereupon, research is being conducted to find a natural alternative to EDTA that satisfies clean label requirements, without compromising shelf life.

### *1.1. Aim and Hypotheses*

This project mainly aims to find an efficient natural alternative to the ingredient EDTA, that is able not only to prevent oxidation of mayonnaise and other emulsified products, but also to guarantee comparable physical and sensory characteristics of the mayonnaise products. In this vein, the plan was carried out in two different workstreams: Natural antioxidants testing, and Ideal recipe design. The focus of the former is finding effective replacements of EDTA, therefore consisting in tracking the performance of two different natural antioxidants (extract L, a rosemary and green tea extract; and extract X, a rosemary and spinach extract) in preventing oxidation, over the course of 12 months, at different temperatures. In this study chemical, sensory and physical stability analysis were tracked over time. The second workstream aims to better understand how elements of the mayonnaise recipe, such as oil and egg, are influencing oxidative stability. Physicochemical and sensory analysis were collected over the course of 2 months. The outcomes of the design of experiment should allow to come up with the first suggestions of factor interactions and their impact on mayonnaise oxidation.

The current project set out to test the following hypotheses in mayonnaise and emulsified products:

- Both studied natural antioxidants, extracts L and X, have a radical scavenging or metal chelating activity, preventing oxidation without bringing any off flavours; hence lipid oxidation compounds have limited intensities comparable to samples with EDTA. Therefore, these ingredients can be used as an efficient replacement for EDTA in tested mayonnaise formulations.

- The mayonnaise with extract X version 1 has revealed significant off-notes (i.e., aromatic herbs) upon sensory analysis. Accordingly, extract X version 2, an improved version of the original formula, is going to have lower sensory impact in mayonnaise organoleptic properties.
- The active compound of the extract X has a pKa of 4, consequently, at $pH < 4$, the extract will be mainly in its protonated form, i.e., the less active form of the natural antioxidant. Therefore, the functionality of the extract X is greater in mayonnaise at pH 4.0, followed by pH 3.5, and pH 3.0, respectively.
- The usage of different types of emulsifiers (i.e., egg) impacts the density and composition of the oil droplet interphase, therefore the physical and oxidative stability of mayonnaise is affected. Heat-stable egg yolk increases the density of the emulsion interphase, so mayonnaise using this type of egg should show higher resistance to oxidation. On the other hand, whole egg due to the presence of egg white proteins that impact the emulsion physical stability, ought to show higher levels of oxidation.
- The type of raw material (i.e., seeds) used to obtain vegetable oils impacts their composition in fatty acids. Generally, a greater level of unsaturation correlates to lower oxidative stability, therefore soybean oil should perform worse and sunflower oil ought to perform better comparing to other types of oils.

## 2. Literature Review

### *2.1. Food Emulsions*

#### 2.1.1. Emulsions: Definition, Formation and Characterisation

Emulsions are colloidal systems that result from the mixture of two immiscible liquids, where one is dispersed as spherical droplets (dispersed phase) in the other liquid (continuous phase), both separated by an interfacial region. In food industry, both types of emulsions coexist (Figure 2.1), water-in-oil (W/O) emulsions (i.e. water droplets exist in an oil phase), such as butter and margarine; and oil-in-water (O/W) emulsions (e.g. mayonnaise, salad dressings, milk) which consist of oil globules surrounded by water (Liu et al., 2022; Sharma & Shad, 1985).

Pertaining to O/W emulsions, these are formed by applying energy to the system (e.g., high shear, high pressure), leading to the development of small oil droplets (> 0.1 μm). The contact between immiscible liquids is thermodynamically unstable, and the emulsification process greatly increases the interfacial area, consequently in these systems problems arise related to physical and oxidative stability, respectively (Lam & Nickerson, 2013; Sharma & Shad, 1985).

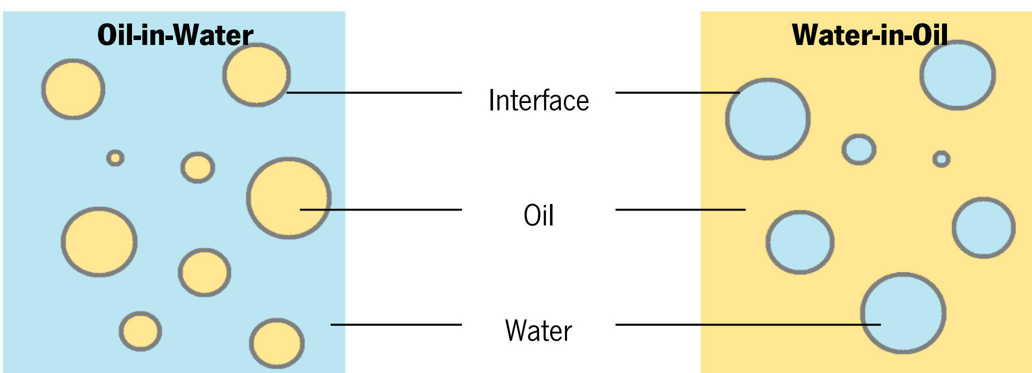



*Figure 2.1. Types of emulsions. Oil-in-water emulsion with oil globules dispersed in an aqueous phase (left) and a water-in-oil emulsion with water droplets dispersed in an oil phase (right). Both cases show the two phases separated by an interface. Adapted from* Horn (2012).

Physical instability includes different processes, for instance oil droplets have the tendency to aggregate (i.e., Coalescence or Flocculation) or there is a gravitational separation (i.e., Creaming or Sedimentation) (Figure 2.2). Food emulsions are required to remain stable for several months, therefore emulsifiers (e.g., egg lecithin, faba bean protein) are used so that they can adsorb oil droplets surface and interfacial tension is lowered, forming viscoelastic films to keep physical stability over time (Lam & Nickerson, 2013).

Regarding oxidation, the phenomenon intensifies because emulsification process implies greater superficial area, hence higher contact between unsaturated fatty acids in the oil phase and oxygen and/or pro-oxidants (e.g., metal ions) in the water phase (Liu et al., 2022; Sharma & Shad, 1985). Therefore, compounds with antioxidative activity are applied to food emulsions, so that oxidation is reduced and controlled over time.

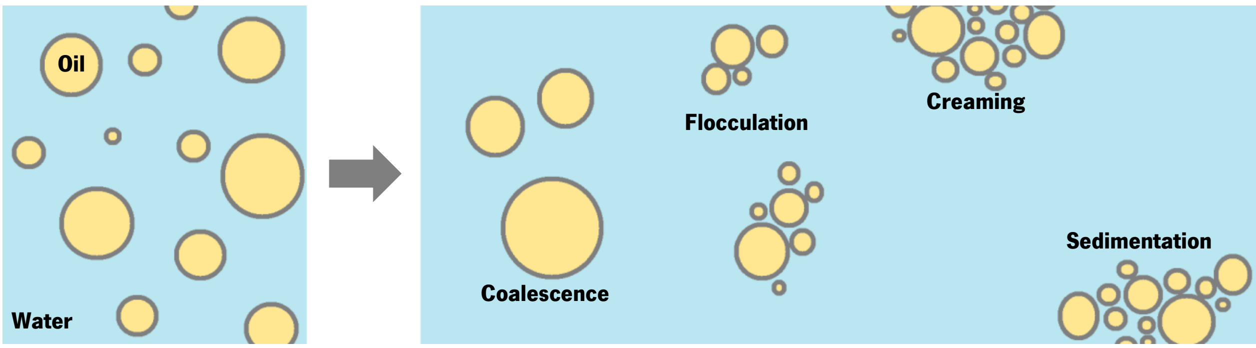



*Figure 2.2. Physical instability in oil-in-water emulsions. Coalescence: Collision and fusion of oil droplets. Flocculation: Collision of oil droplets without forming a bigger droplet. Creaming: Oil globules with lower density than surrounding liquid accumulate at the top of the emulsion. Sedimentation: Oil globules show higher density than surrounding phase accumulate at the bottom of the emulsion. Adapted from* Horn (2012).

### 2.1.1.1. Mayonnaise: A Global Food Emulsion

Mayonnaise is a globally appreciated condiment sauce used to improve the flavour and taste of other foods (Ghorbani Gorji et al., 2016; Khalid et al., 2021). In 1989, on the authority of the Codex Alimentarius, the European standard of identity established 78.5 % and 6 % as the minimum total fat content and "technically pure" egg yolk content, respectively, for mayonnaise (Codex Coordinating Committee for Europe, 1989). Nowadays, the previous information is not listed in the Codex standards, so in 2015, Culinaria Europe established a code of practice for mayonnaise defining the standard as containing a minimum of 70 % and 5 % minimum of total fat and egg yolk content, respectively (Culinaria Europe, 2015).

Traditional full fat mayonnaise (70-80 % oil) is still the most common type of mayonnaise, nonetheless consumer preferences have been changing, so there is now more mayonnaise-like spreads available in the market, such as light (i.e., low fat) and plant-based (i.e., with no animal derived products, including egg). The former shows only 20-30 % fat content and was developed because of perceived unhealthiness of high oil content products. The latter targets people who are allergic to eggs or want to avoid animal fat and cholesterol (Featherstone, 2016; Morley, 2016).

Mayonnaise is a complex product where every ingredient has a function. To acquire great sensory profile, this food emulsion is a blend of different ingredients such as oil, egg, mustard, sugar, salt, etc.; but as an O/W emulsion, mayonnaise consists of three main components: oil (dispersed phase), vinegar solution (continuous phase) and egg yolk, the emulsifier at the interface (Ghorbani Gorji et al., 2016; Khalid et al., 2021). The proteins in egg yolk adsorb onto the surface of oil droplets making the emulsion more stable. The common high proportion of oil gives these emulsions their great structural viscosity, hence mayonnaise is a creamy product that usually has a thick and smooth texture, its opaque colour varies between pale yellow and white, and is low in pH (Ghorbani Gorji et al., 2016; Khalid et al., 2021).

## *2.2. Lipid Oxidation in Emulsions*

Lipid oxidation can occur in three manners: autoxidation, enzyme-catalysed oxidation, and photo-oxidation (Domínguez et al., 2019). During storage of emulsions, autoxidation and photosensitized oxidation may arise, however, in food emulsion systems such as mayonnaise the former is the most important process (Choe & Min, 2006; Ghorbani Gorji et al., 2016).

Generally, the rate of lipid oxidation is higher in O/W emulsions than in bulk oil, denoting the different characteristics and mechanism of oxidation in the former system compared to the latter. O/W emulsions include three phases, so the oxidation process is more complex since it may initiate in all of them. Therefore, despite the basic lipid oxidation reactions being the same for both bulk oil and O/W emulsions, the factors influencing emulsions' oxidation are significantly different (Ghorbani Gorji et al., 2016; Jacobsen, 2016).

### 2.2.1. Lipid Autoxidation

Autoxidation of lipids normally includes three steps (Figure 2.3): Initiation (i.e., formation of free radicals), Propagation (i.e., number of reactive compounds multiplies) and Termination (i.e., reactive compounds deteriorate or react with each other originating non-reactive compounds) (Choe & Min, 2006; Domínguez et al., 2019). In the initiation step, a source of energy (temperature or light) and/or a catalytic compound (e.g., free radical, transition metal ion), act on unsaturated fatty acids (RH) extracting an atom of hydrogen, with that forming an alkyl radical (R•). These are extremely reactive products but in the presence of nearby double-bonds, alkyl radicals tend to be stabilized by rearrangement to form conjugated dienes or trienes (Figure 2.4) (Choe & Min, 2006; Domínguez et al., 2019).

| | |
|---|---|
| Initiation | $RH \rightarrow R\bullet + H\bullet$ |
| Propagation | $R\bullet + O_2 \rightarrow ROO\bullet$ |
| | $ROO\bullet + RH \rightarrow ROOH + R\bullet$ |
| Termination | $ROO\bullet + R\bullet \rightarrow ROOR$ |
| | $R\bullet + R\bullet \rightarrow RR$ |

*Figure 2.3. Lipid Autoxidation steps. R: lipid alkyl. Adapted from Choe & Min (2006).*

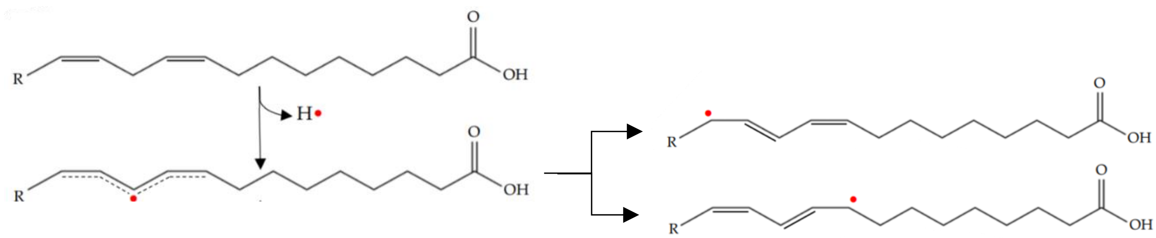



*Figure 2.4. Mechanism of Initiation step of Lipid Autoxidation. Generation of alkyl radical, followed by double-bond rearrangement with production of conjugated dienes. Adapted from* Domínguez et al. (2019).

Before hydroperoxides accumulate, free radicals are leisurely formed, additionally, food emulsions generally contain antioxidants that easily neutralize free radicals. Subsequently, during the initiation stage, the accumulation of lipid oxidation products is normally slow – lag phase (Domínguez et al., 2019).

At normal oxygen pressure, lipid alkyl radicals rapidly react with atmospheric oxygen to generate a lipid peroxy radical (ROO•) (Figure 2.5), onsetting the propagation step of autoxidation. This stage is then characterized by successively reactions between lipid peroxy radical and other lipid molecules to generate new alkyl radicals and hydroperoxides (ROOH) (Choe & Min, 2006; Domínguez et al., 2019).

Lipid hydroperoxides are the primary oxidation products; these molecules are both taste- and odourless, and, in the absence of metals, relatively stable at room temperature. Despite initial product deterioration, primary oxidation does not contribute negatively to sensory patterns. However, reactions mediated by metal ions or other hydroperoxides, can further decompose original hydroperoxides into radicals, then allowing the formation of an array of compounds (Table 2.1), such as aldehydes, ketones, acids, esters, alcohols and hydrocarbons, known as secondary oxidation products, which are the ones responsible for off-flavour and off-odour of oxidized oil (Choe & Min, 2006; Domínguez et al., 2019; Ghorbani Gorji et al., 2016).

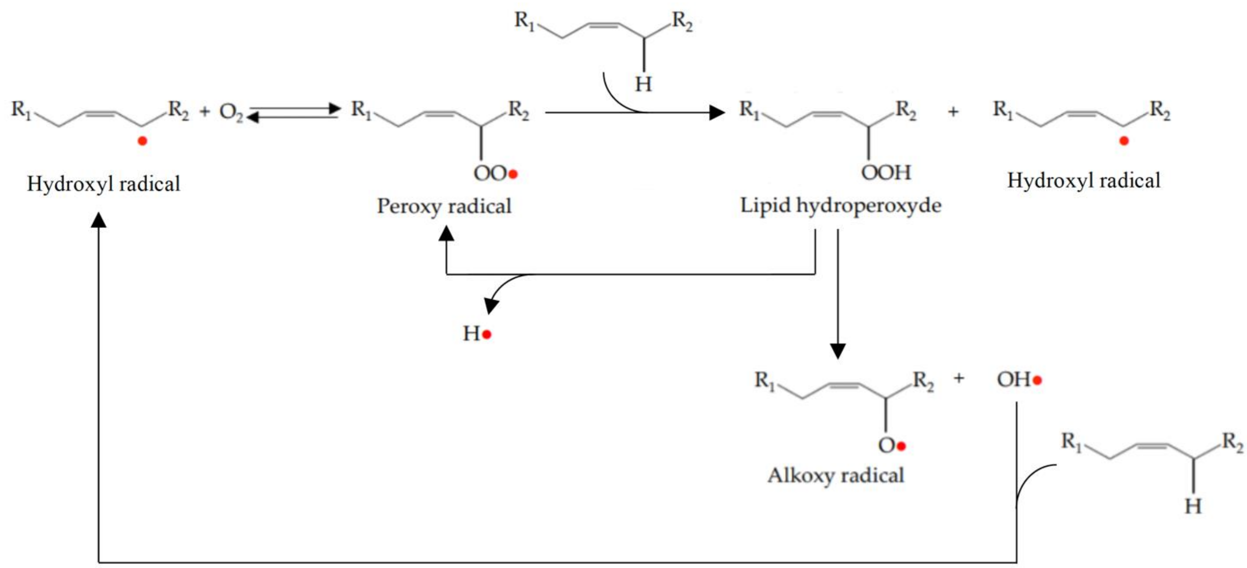



*Figure 2.5. Mechanism of Propagation step of Lipid Autoxidation. Formation of peroxy radical and necessary reaction to produce lipid hydroperoxides, followed by their decomposition in new hydroxyl, peroxy and alkoxy radicals. Adapted from* Domínguez et al. (2019).

Lastly, the termination step comprises reactions between radicals from the propagation step to form non-radical species, represented as followed (adapted from Domínguez et al. (2019)):

$$\mathrm{R} \bullet + \mathrm{R} \bullet \rightarrow \mathrm{RR}$$

$$\mathrm{R} \bullet + \mathrm{ROO} \bullet \rightarrow \mathrm{ROOR}$$

$$\mathrm{RO} \bullet + \mathrm{RO} \bullet \rightarrow \mathrm{ROOR}$$

$$\mathrm{RO} \bullet + \mathrm{R} \bullet \rightarrow \mathrm{ROR}$$

$$\mathrm{ROO} \bullet + \mathrm{ROO} \bullet \rightarrow \mathrm{ROOR} + O_2$$

$$2\ \mathrm{RO} \bullet + 2\ \mathrm{ROO} \bullet \rightarrow 2\ \mathrm{ROOR} + O_2$$

*Table 2.1. Examples of Secondary oxidation products produced in Lipid Autoxidation. Adapted from* Domínguez et al. (2019)

| Class | Oleic acid | Linoleic acid | Linolenic acid |
|---|---|---|---|
| Alcohols | Hexanol<br>Heptanol<br>Octanol<br>Nonanol<br>1-Nonenol | Butanol<br>Pentanol | 2-Pentenol<br>1,3,6-Nonatrienol |
| Aldehydes | Hexanal<br>Heptanal<br>Octanal<br>Nonanal<br>Decanal<br>2-Decenal<br>2-Undecanal<br>Formaldehyde | Butanal<br>Pentanal<br>Hexanal<br>3-Nonenal<br>2,4-Decadienal<br>Formaldehyde | Propanal<br>2-Pentenal<br>3-Hexenal<br>2,4-Heptadienal<br>3,6-Nonadienal<br>2,4,7-Decatrienal<br>Formaldehyde |
| Carboxylic acids | Hexanoic acid<br>Heptanoic acid<br>Octanoic acid<br>8-Nonenoic acid<br>9-Decenoic acid | Octanoic acid<br>9-Undecenoic acid | Octanoic acid<br>9-Decenoic acid<br>9-Undecenoic acid<br>9,11-Dodecadienoic acid |
| Hydrocarbons | Hexane<br>Heptane<br>Octane<br>Nonane<br>1-Nonene<br>1-Decene | Butane<br>Pentane<br>1,3-Nonadiene | Ethane<br>Butene<br>2-Pentene<br>3-Hexene<br>1,3,6-Nonatriene |

#### 2.2.1.1. Transition Metal Ions

Derived from processing, packaging or even naturally (free-state and/or protein-linked), foods always contain metal ions (primarily, Iron (Fe), Copper (Cu) and Cobalt (Co)); in the case of oils, the refining process can reduce metal concentrations but never eliminate them completely (Belitz et al., 2009).

Metal ions actively promote lipid oxidation, acting as catalysts by two pathways. To a lesser extent, these ions can directly react with an unsaturated fatty acid to produce an alkyl radical (Figure 2.6. (1)); this process occurs at a leaden rate, thus not significantly impacting initiation step of autoxidation (Belitz et al., 2009). In addition, metal ions can lead to lipid hydroperoxides decomposition into new radicals (Figure 2.6. (2) & (3)), triggering significant autoxidation. Based on reactions (2) and (3), Figure 2.6, it is evidenced the possibility of renovation of the oxidation state of the metal ions, consequently proving that metal ions only need to be present in small amounts to promote oxidation. The impact of metal ions in the rate of linoleic acid hydroperoxides decomposition was previously studied according to type and oxidative state of metal ions as well as medium pH (Table 2.2). Besides metal ions showing higher activity with the decrease of pH from 7.0

to 5.5, it was also observed a great increase in the deterioration rate of hydroperoxides for lower oxidation state ions ($Fe^{2+} > Fe^{3+}$) (Belitz et al., 2009; E. Frankel, 2005).

$$M^{(n+1)+} + RH \longrightarrow M^{n+} + R\bullet + H^{+} \quad (1)$$
$$M^{n+} + ROOH \longrightarrow M^{(n+1)+} + RO\bullet + OH^{-} \quad (2)$$
$$M^{(n+1)+} + ROOH \longrightarrow M^{n+} + ROO\bullet + H^{+} \quad (3)$$

*Figure 2.6. Lipid Autoxidation catalysed by metal ions. (1) Direct lipid oxidation to generate alkyl radical; (2) & (3) Hydroperoxide decomposition to generate new free radical. M: Transition metal ions; RH: Unsaturated fatty acid; R•: Lipid alkyl radical; ROOH: Lipid hydroperoxides; RO•: Lipid alkoxyl radical; ROO•: Lipid peroxy radical. Adapted from* Belitz et al. (2009).

*Table 2.2. Linoleic acid hydroperoxides decomposition by metal ions at 23°C. Adapted from* Belitz et al. (2009)

| Metal ion | Relative reaction rate | |
|---|---|---|
| | pH 7.0 | pH 5.5 |
| $\boldsymbol{Fe^{3+}}$ | 1 | 100 |
| $\boldsymbol{Fe^{2+}}$ | 14 | 1000 |
| $\boldsymbol{Cu^{2+}}$ | 0.2 | 1.5 |

#### 2.2.1.2. Volatile Secondary Oxidation Products

Autoxidation is a complex process giving rise to a myriad of end products, among them volatile secondary oxidation products are one of the most important mixture of compounds to the sensory quality of products. In contrast with hydroperoxides, most volatiles interact with olfactory receptors contributing to the change in flavour of an oxidized product.

Table 2.3 summarizes some of the volatiles derived from three different edible oils. Here it is possible to understand how dependent the sensory profiling is from the product matrix since different oils when oxidized will generate diverse volatiles mixtures, thus dissimilar sensory profiles. In addition, the volatiles show a wide range of odour thresholds (i.e., lowest concentration at which a panel is able to recognize an odorant), meaning that some compounds may be present in a product but if in too low concentrations will not be detected, and vice versa (Doty, 2019). In conclusion, understanding how volatile secondary oxidation products evolve over time is of great importance for maintaining food products' acceptability.

*Table 2.3. Volatile Secondary Oxidation Products from Lipid Autoxidation of three edible oils; nd, non-detectable. Adapted from* Xu et al. (2017)

| Key compounds | Odour threshold (ng/L) | Relative Odour Activity Value | | | Sensory description |
|---|---|---|---|---|---|
| | | Rapeseed oil | Peanut oil | Soybean oil | |
| Pentanal | 38 | 1.53 | 1.13 | 11.54 | Pungent, almond, malt |
| Hexanal | 51 | 10.17 | 100 | 11.39 | Grass, green, rancid, tallowy, fat |
| Heptanal | 46 | 0.84 | 0.75 | 3.34 | Rancid, fatty, citrus |
| Octanal | 9.3 | 40.37 | 26.73 | 41.24 | Fatty, soapy, green, oily, fresh |
| Nonanal | 12 | 100 | 85.48 | 100 | Citrus, green, fatty, oily, soapy, tallowy, fruity |
| Decanal | 4.3 | 17.1 | nd | 19.11 | Floral, orange peel, soapy, tallowy, green, fresh |
| 2-Butenal | 350 | 0.11 | nd | nd | Pungent |
| (E)-2-pentenal | 1500 | 0.13 | nd | nd | Strawberry, fruity, tomato, pungent, apple |
| (E)-2-heptenal | 88 | 2.46 | 1.07 | 3.61 | Fatty, soapy, almond, oily |
| (E)-2-octenal | 20 | nd | 2.14 | 4.66 | Green, nutty, fatty, oily |
| E-2-decenal | 3.2 | 2.42 | 2.68 | 10.27 | Tallowy, orange-like |
| (E,E)-2,4-heptadienal | 38 | 1.02 | nd | nd | Fatty |
| Benzaldehyde | 186 | 1.91 | 3.82 | 1.80 | Almond, burnt sugar |
| 1-Pentanol | 3590 | nd | 0.016 | 0.11 | Balsamic |
| 1-Hexanol | 360 | nd | nd | nd | Green, floral |
| 1-Octanol | 73 | 0.53 | 0.43 | 0.52 | Chemical, metallic, burnt |
| Acetic acid | 1384 | nd | 0.12 | nd | Sour |
| Hexanoic acid | 3000 | 0.02 | 0.02 | 0.06 | Sour, fatty, sweaty, cheese |
| Nonanoic acid | 12 | 9.35 | nd | 10.96 | Green, fatty |
| Dodecane | 5300 | 0.064 | 0.14 | 0.026 | Gasoline-like |
| D-Limonene | 718 | 0.38 | 0.57 | 0.2 | Lemon, orange |
| 2-Pentylfuran | 181 | nd | 1.07 | 2.75 | Butter, green beans |

#### 2.2.1.3. Antioxidants

The presence of antioxidants can reduce and control the oxidation process by interfering at certain stages of the autoxidation process. Based on their action mechanism, antioxidants are classified as primary or secondary antioxidants. It is worth noting that certain substances act through more than one mechanism (McClements & Decker, 2000). Primary antioxidants also known as radical scavengers (e.g., BHA, BHT) are those capable of accepting free radicals. These antioxidants can react with alkyl and peroxy radicals converting them to more stable, radical, or nonradical products (Figure 2.7. (1) – (3)). The antioxidant radicals are usually stabilized by an aromatic resonance system, thus much less reactive and less effective at promoting oxidation. In addition, radical scavengers can terminate lipid oxidation by directly reacting with peroxy radicals, alkoxyl radicals and other antioxidants (Figure 2.7. (4) – (6)) (McClements & Decker, 2000).

$$ROO\bullet + AH \longrightarrow ROOH + A\bullet \quad (1)$$

$$RO\bullet + AH \longrightarrow ROH + A\bullet \quad (2)$$

$$R\bullet + AH \longrightarrow RH + A\bullet \quad (3)$$

$$ROO\bullet + A\bullet \longrightarrow ROOA \quad (4)$$

$$RO\bullet + A\bullet \longrightarrow ROA \quad (5)$$

$$A\bullet + A\bullet \longrightarrow AA \quad (6)$$

*Figure 2.7. Activity of an antioxidant as a radical scavenger. AH: antioxidant. Adapted from McClements & Decker (2000).*

Secondary Antioxidants hinder lipid oxidation process by chelating metals ions, regenerating primary antioxidants, oxygen scavenging, and deactivation of reactive species. Concerning O/W emulsions, presence of transition metals in the water phase is a major factor in the promotion of the propagation of lipid oxidation. Hence, the most relevant secondary antioxidants in retarding lipid oxidation are the metal chelating ones. Their mechanism of action varies from formation of insoluble metal complexes, prevention of metal redox recycling, occupation of metal coordination sites, and steric interference between metals and lipid substrates, resulting in the reduction of metal-catalysed reactions near droplet surface (McClements & Decker, 2000).

### 2.2.2. Factors influencing Lipid Oxidation in Mayonnaise

Rancidity is one of the major problems in high fat content products as, for example, mayonnaise. By forming an emulsion, the surface area of the oil exposed to air, water, among other parameters, increases significantly, thus the interfacial oxidation is of serious issue (Jacobsen, 2016; Khalid et al., 2021).

Studies of lipid oxidation suggest that many factors play a role regarding oxidative instability of O/W emulsions. Lipid oxidation may be induced by transition metals, egg, and is also affected by pH, among other factors (Ghorbani Gorji et al., 2016; Horn, 2012; Jacobsen, 2016). In Figure 2.8, the most important factors influencing lipid oxidation in these systems are summarized.

#### 2.2.2.1. Vegetable oil

Current nutritional recommendations highlight the importance of polyunsaturated fatty acids (PUFA), such as omega-3 (e.g., α-linolenic acid) and omega-6 (e.g., linoleic acid) PUFA, consumption for the health of human beings.

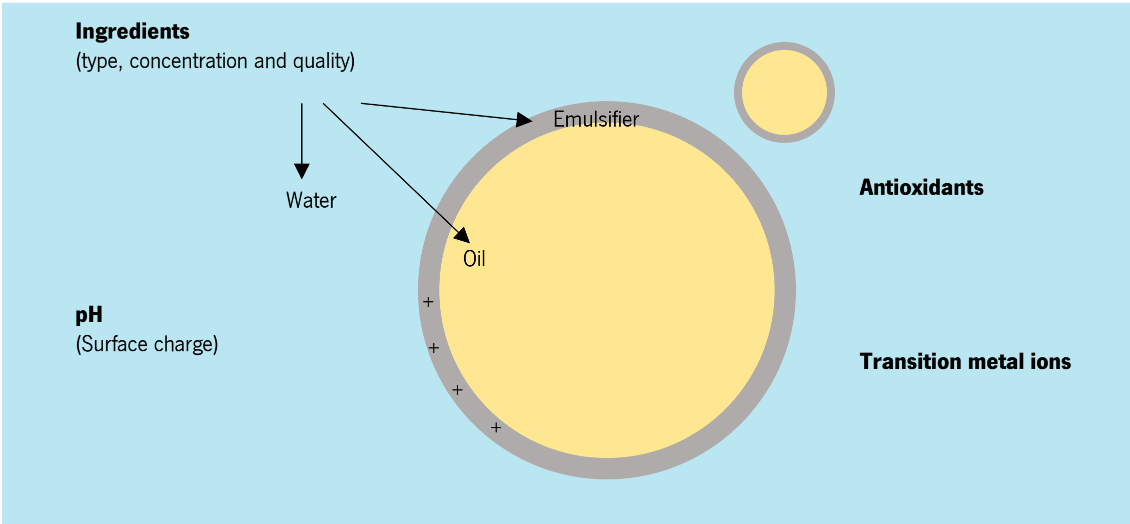



*Figure 2.8. Important factors influencing Lipid Oxidation in O/W emulsions. Adapted from* Horn (2012).

Fatty acids are susceptible to oxidation, and the rate at which the process happens greatly depends on the degree of unsaturation. For example, it has been reported that in the series of 18-carbon-atom fatty acids stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), the relative rate of oxidation is in the ratio of 1:100:1200:2500. Regarding unsaturated lipids, oleic acid is more stable than linoleic acid, thus, a high level of oleic acid might be desirable in the aspect of stability (Belitz et al., 2009; DeMan et al., 2018). Due to the high content of PUFA, the nutritional profile of vegetable oils, as well as the products they are used in, is highly valued. Nevertheless, as food ingredients, PUFA may be problematic due to the heighten vulnerability of the final product to oxidative deterioration (Jacobsen, 2016; Raikos et al., 2016). Thus, the type and quality of the oil used in emulsions influences lipid oxidation (Horn, 2012; Jacobsen, 2016).

Presently mayonnaise production involves the usage of three main vegetable oils: sunflower, rapeseed and soybean. These oils are characterised by their affordability and sensory properties, as well as their positive influence on the final product's texture (Morley, 2016; Raikos et al., 2016). According to Table 2.4, rapeseed oil (RS) is the one with lowest saturated fatty acids (SFA) (≈5 %) and the richest in unsaturated fatty acids (UFA), with around 70 % of monounsaturated fatty acids (MUFA) and 23 % of PUFA. When in bulk oils, MUFA are known to be more resistant to oxidation, but this oil shows the highest amount of $\alpha$-linolenic acid which is very unstable (Chew, 2020; Woodfield & Harwood, 2017). Compared to the previous oil, soybean oil (SB) and sunflower oil (SF) have much more linoleic acid (54-61 % >>> 14 %), moreover soybean oil has comparable levels of $\alpha$-linolenic acid. Therefore, it seems that SB oxidizes more quickly than RS and SF, leading to higher generation of volatile compounds, such as hexanal, which would negatively impact the final product's sensory characteristics. Between rapeseed and sunflower, due to elevated concentration of α-linolenic acid in rapeseed oil, that presents one of the higher susceptibilities to oxidation, as it has three conjugated double bonds, then sunflower oil should have the highest oxidative stability (Belitz et al., 2009; Romanić, 2020; Woodfield & Harwood, 2017). To increase oxidative stability and the possible applications of the previous oils, some hybrid seeds have been created, and changes in the composition of fatty acids took place. High oleic acid oils are examples of hybrid oils, where the goal was to reduce levels of PUFA and their replacement by oleic acid, monounsaturated C18:1. In this way, emerging oils with higher smoking temperature, i.e., great heat stability, and more resistant to oxidation (Sharafi et al., 2015).

*Table 2.4. Fatty acid composition (expressed as percentages) of three oils: sunflower, rapeseed and soybean (Romanić, 2020; Woodfield & Harwood, 2017)*

| | **Sunflower oil** | **Rapeseed oil** | **Soybean oil** |
|---|---|---|---|
| **Total saturated acids** | 12.1 | 5.0 | 15 |
| ***Palmitic acid, 16:0*** | 6.3 | 4.0 | 11 |
| ***Stearic acid, 18:0*** | 4.6 | 1.0 | 4 |
| **Total monounsaturated acids** | 26.8 | 69.9 | 23 |
| ***Oleic acid, 18:1*** | 26.7 | 14.8 | 23 |
| **Total polyunsaturated acids** | 61.1 | 23.2 | 62 |
| ***Linoleic acid, 18:2 (n-6)*** | 61.1 | 14.1 | 54 |
| ***α-Linolenic acid, 18:3 (n-3)*** | - | 9.1 | 8 |
| **Total unsaturated acids** | 87.9 | 93.1 | 85 |

Legend: Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the figure afterward indicating the number of double bonds; n-6 and n-3 correspond to omega-6 and omega-3 fatty acids, respectively.

Waraho et al. (2011) investigated soybean O/W emulsions, finding that free fatty acids, a minor component of oils, were strong pro-oxidants that when present in 0.1% accelerated oxidation by 1.0 %. More importantly, lipid oxidation was significantly lower for emulsions with linolenic acid than those with linoleic acid and oleic acid; besides, oleic acid's emulsions showed the lowest oxidative stability. In sum, it was discovered that the emulsion oxidative stability was correlated with the level of unsaturation of the free fatty acids, possibly because the free fatty acids oxidise and promote oxidation of triacylglycerols, as well as due to the higher mobility of MUFA – with linear geometry – compared to PUFA, leading to a superior negative charge of oil droplets, that draws the metal ions (i.e., pro-oxidants) to the interface.

In conclusion, considering the bulk oil's behaviour, the average oil's composition, and how high oleic rapeseed oil (HORS) is designed to have a lower ratio of PUFA/MUFA than RS, accordingly the expected oxidative stability were as follows: SF > HORS > RS > SB. However, free fatty acids impact O/W emulsions oxidation rate; for example, soybean oil that has higher level of PUFA, and in more detail, a greater concentration of omega-3 than sunflower oil, and more omega 6-than rapeseed oil, consequently, should also have higher levels of the correspondent free fatty acids, thus, based on Waraho et al. (2011), if soybean oil is included in mayonnaise, it might lead to a better oxidation profile than expected. In opposition, rapeseed oil, with 3 times less PUFA than sunflower oil , might show greater oxidation rates in emulsions than expected.

Current food trends veered towards healthy products with low fat content, for example, light mayonnaise with only ≈25 % lipids was developed as an alternative to the ≈75 % fat in full fat mayonnaise (Featherstone, 2016; Morley, 2016). Oils are highly prone to oxidation, therefore, regarding oil concentration in emulsions, the expected behaviour would be the increase of oxidative stability with the decrease of fat content. However, studies have been revealing contradicting findings. For instance, Sørensen, Nielsen, Hyldig, et al (2010) investigated fish-oil enriched light mayonnaise finding that lipid oxidation in 40 % oil mayonnaise was comparable to full fat mayonnaise (80 %), but higher in 63 % oil mayonnaise. In contrast, the study of Sørensen, Nielsen, & Jacobse (2010) revealed that mayonnaise with 63 % oil had higher oxidative stability than a 24 % emulsion. In conclusion, oil concentration might influence the rate of lipid oxidation, but further investigations need to occur.

Regarding the oil's quality, a low-quality oil (i.e., oil containing high levels of primary or secondary oxidation products) will oxidize faster compared to one with good quality (Featherstone, 2016). Generally, the peroxide value (PV) of fresh vegetable oils is less than 1.0 mEq $O_2$/kg oil, with a higher PV related to increased reactive oxygen species and secondary oxidation products (Woodfield & Harwood, 2017). Therefore, certain product specifications can be adopted, for example, requirement of negative result to rancidity test, free fatty acid content below than 0.05 % (expressed as oleic acid), and PV close to 1.0 mEq $O_2$/kg oil; in addition, bland and neutral odour, flavour, and taste should also be expected (Featherstone, 2016; Woodfield & Harwood, 2017).

#### 2.2.2.2. Egg

Typical mayonnaise uses egg yolk as an emulsifier, contributing to the characteristic colour and flavour of the product, as well as to the physical stability of the emulsion. Egg yolk contains phospholipids (e.g., lecithin) and proteins (e.g., livetin, lipovitellin and lipovitellinin) that adsorb to the surface of oil droplets, imparting the emulsification ability of the egg yolk (Belitz et al., 2009; Khalid et al., 2021; Morley, 2016).

Egg yolk is an O/W emulsion composed of approximately 50 % water, 32 % lipids, 16 % proteins and 2 % carbohydrates, vitamins and minerals (Belitz et al., 2009). This ingredient can be fractioned into plasma and granules, that differ in their composition. Plasma (75-81 % of the yolk dry matter) consists of 85 % low-density lipoproteins (LDL) and 15 % livetins. Granules (19-25 % of the yolk dry matter) correspond to 70 % high-density lipoproteins (HDL), 16 % phosvitin and 12 % LDL. Anton & Gandemer (1997), Dyer-Hurdon & Nnanna (1993) and le Denmat et al. (2000) studied the stability of emulsions using full egg yolk, egg yolk plasma and granules, discovering that emulsions made with plasma had properties closer to emulsions prepared with complete egg yolk. Therefore, concluding that egg yolk emulsifiers must belong to plasma instead of granules.

The type, concentration and quality of the egg (emulsifier) influences the oxidative stability of the product. Firstly, because the ability of the emulsifier components to interact with the oil droplet impacts the shielding effect of the lipids from pro-oxidants in the water phase, and secondly due to the presence of metal ions in the egg (Castellani et al., 2004; Horn, 2012).

Whole egg and egg yolk pose the ideal media to the microorganisms' growth, as a result in industry the preservation of these egg products is done by pasteurization, addition of salt and/or sugar, or spray drying (Anton et al., 2018). Consequently, mayonnaise can be made using various types of eggs, such as liquid egg yolk, heat-stable egg yolk, powdered egg yolk, whole egg, among others (Belitz et al., 2009).

Liquid egg yolk is the reference emulsifier in sauce industry, and it is usually 10 % salted, which increases 10 times the yolk viscosity compared to the natural version. The increase in viscosity leads to a more stable emulsion (Anton et al., 2018).

Heat stable egg yolk consists of egg yolk enzymatically modified with phospholipase A2, that hydrolyses the acyl group in the second position of triglycerides, leading to the conversion of phospholipids to lysophospholipids (Daimer & Kulozik, 2009; Gazolu-Rusanova et al., 2020). Gazolu-Rusanova et al. (2020) reported that the enzymatic modification led this type of egg yolk to not gel under severe heat treatments, also results showed a significant increase in protein solubility (especially, granules' proteins), a lower interfacial tension of the oil/water interface, a higher and faster protein adsorption on the interface compared to untreated egg yolk (Figure 2.9).

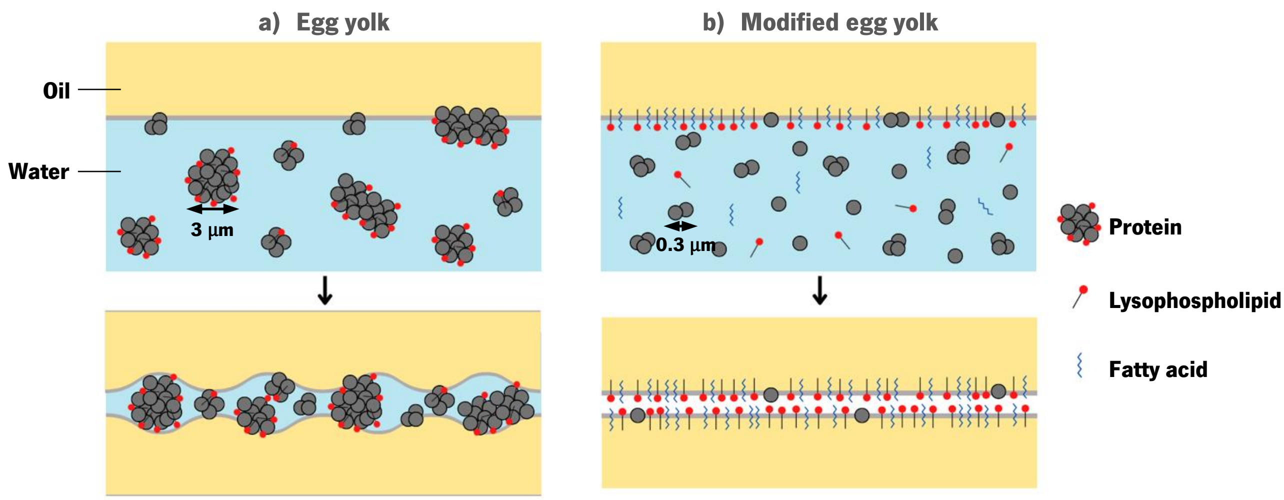



*Figure 2.9. Oil/water interface in emulsions using (A) egg yolk and (B) modified egg yolk. Adapted from Gazolu-Rusanova et al. (2020).*

A previous study was done by Kraft Heinz regarding the effect of different types of egg in the oxidation of mayonnaise after 4 months at 30°C; results showed that heat stable egg yolk was able to form a more stable emulsion with a lower perceived off taste compared to the liquid egg yolk and powdered egg yolk, being concluded that a denser interfacial film layer, can result in a higher oxidative stability. Powdered egg yolk results demonstrated a behaviour similar to the liquid egg yolk sample (i.e., reference) in terms of physical stability, and sensory profile, therefore oxidative stability seemed comparable.

Ariizumi et al. (2017) investigated the impact of whole egg on the stability of mayonnaise concluding that the quantity of protein and the type of proteins adsorbed to the interface of oil droplets diminished the physical stability of mayonnaise. Besides egg white proteins, such as ovoalbumin and ovotransferrin, were found to be responsible for flocculation of the emulsion. Therefore, it is possible that the lower physical stability of oil droplets might lead to increased oxidation rates.

Phosvitin is a protein with around 50 % of the amino acids corresponding to phosphorylated serine, thus forming a unique structure capable of chelating metals, for example, one phosvitin molecule anchors 113 ions of manganese (Castellani et al., 2004). A crucial fact is that phosvitin can also chelate ferric ions with great affinity (Kf = $10^{18}$), and egg yolk is very rich in iron (734 µM), therefore phosvitin shows antioxidant properties in the egg (Castellani et al., 2004; Hegenauer et al., 2002). At pH 6.5 and ionic strength 0.15M, Castellani et al. (2004) reported that phosvitin performs the best, binding one iron every two phosphoserines. Besides it was shown that the iron binding capacity was affected by changes in external factors, such as pH, ionic strength, temperature, etc. For example, by lowering the pH the affinity to iron was reduced, moreover, at pH lower than 3.5, phosvitin did not significantly complex with iron. The same behaviour was demonstrated by Jacobsen et al. (2001), where during mayonnaise storage the pH decrease from 6.0 to 4.2 led to increase of PV, indicating higher oxidation levels. Therefore, it was proposed that at pH close to neutral, iron established bridges between LDL, lipovitellin, and phosvitin, and was located at the O/W interface of the emulsion, so it would not act as a pro-oxidant. However, low pH values should promote breakage of the iron bridges, thus iron ions become more accessible as oxidation initiators (Jacobsen et al., 2001).

As previously stated, transition metal ions are strong pro-oxidants, thus only needing to be present in trace amounts to promote oxidation. Mayonnaise can be made using various types of eggs, however, all of them have in common the presence of phosvitin, meaning that depending on the concentration and quality of the ingredient that is used to obtain the food emulsion, higher or lower levels of iron ions can be introduced in the final product, therefore increasing the potential to product deterioration (Morley, 2016).

#### 2.2.2.3. Antioxidants

With lipid oxidation being one of the major problems in high fat content products, antioxidants are used as additives to retard deterioration. But the effectiveness with which these compounds act depends on their type, concentration and quality (Horn, 2012; McClements & Decker, 2000).

As priorly explained antioxidants can be of two categories: Primary antioxidants (i.e., radical scavengers) or Secondary antioxidants (i.e., Metal chelators, Primary antioxidants regenerators, Oxygen scavengers, and Reactive species deactivators) (McClements & Decker, 2000).

Regarding radical scavengers (e.g., α-tocopherol, BHA, BHT), variations in their chemical properties and physical location on the emulsion impacts their effectiveness. This is explained because hydroperoxides are relatively polar, thus likely accumulating at the interface. Therefore, pro-oxidants like metal ions originating in the aqueous phase will interact with them at that location, and lipid oxidation will occur more rapidly at the oil/water interface. Antioxidants with lower polarity or high surface activity are mainly present in oil droplets interior and/or at the oil/water interface, so they have greater effectiveness in O/W emulsions (McClements & Decker, 2000). It is worth noting that certain conditions lead to lower resonance stabilization of radical scavengers, so they become pro-oxidants by acting as radical carriers generating new radicals (E. Frankel, 2005).

Regarding secondary antioxidants (e.g., EDTA, citric acid), in emulsions the most important antioxidants act as metal chelating agents. Transition metals catalytic activity increases dramatically with higher proximity to droplet surfaces. Consequently, metal chelators can decrease the ions availability near oil surface, leading to lipid oxidation prevention (McClements & Decker, 2000). Of great importance is that most of these agents' activity is concentration dependent, meaning that at relatively low concentrations they may have pro-oxidant effects instead of antioxidant activity. For example, Frankel et al., 2002 studied a fish oil emulsion supplemented with 100 and 200μM of $Fe^{2+}$, and when using EDTA in equimolar quantity to iron, EDTA promoted oxidation – $Fe^{3+}$ chelation is preferable to $Fe^{2+}$, which is a more active lipid oxidation catalyst –, in opposition, excess molar concentrations effectively inhibited oxidation. The same behaviour was also observed for ascorbic acid (Frankel et al., 2002).

#### 2.2.2.3.1. EDTA and Natural Antioxidants

Ethylene diamine tetra-acetic acid, EDTA, is a water-soluble and hydrophilic synthetic food additive (E385) that acts as a metal chelator. EDTA contains five membered rings that enhance its stability and allows the generation of stable complexes with metals ions (Shahidi, 2015). This agent is suggested to impact micronutrients (e.g., iron, zinc, copper) absorption in the human body (Autoridade de Segurança Alimentar e Económica (ASAE), n.d.). In spite of its high effectiveness at improving the oxidative stability of food emulsions (e.g., mayonnaise), due to its possible impacts on human health as well as its synthetic nature, studies on natural antioxidant alternatives are nowadays of topical importance (Frankel, 2005).

Citric acid is a natural chelating agent, that can be used as an antioxidant however it tends to be less effective and has limited use due to its flavour, solubility, and/or requirement for acidic pH (McClements & Decker, 2000).

Currently the search for natural antioxidants resides in plants and spices constituents due to their numerous compounds and activities. For example, it is known that a number of natural essential oils obtained from herbs are resistant to autoxidation due to phenolic compounds (e.g., rosemary extract, tea catechin, tannins) improving food deterioration when incorporated (Maqsood & Benjakul, 2010). Until now several studies have been carried out in mayonnaise to find effective natural antioxidants such as gallic, ascorbic, and phytic acids, rosemary, grape seed and purple corn husk extracts, tocopherol, lactoferrin, lycopene, ginger powder, among others (Altunkaya et al., 2013; Jacobsen et al., 1999, 2000, 2001, 2003; Kaur et al., 2011; Kishk & Elsheshetawy, 2013; Lagunes-Galvez et al., 2002; Li et al., 2014; Nielsen et al., 2004). Some extracts showed no significant antioxidant activity, acted as pro-oxidants or inhibited lipid oxidation. The latter was the case of purple corn husk extracts, however the extract application in mayonnaise resulted in the change of colour of the product, which is a concern to consumer acceptability (Li et al., 2014).

In conclusion, there are still many other vegetables and plants, with valuable compounds that have not been explored yet in mayonnaise but that remain as new possibilities to study.

#### 2.2.2.4. Other ingredients

In addition to the ingredients previously presented as impacting lipid oxidation, some others can also be of relevance, as water, salt, sugar, lemon juice, vinegar and thickeners.

Mayonnaise is a O/W emulsion, so water is required to produce the final product. Regular water usually carries transition metal ions, so like egg yolk, increased amounts of this ingredient may potentiate product deterioration (Featherstone, 2016).

Salt is used in mayonnaise, as a flavour enhancer, but also to promote emulsion stability. Sugar plays a role in preservation, flavour and texture of the product. However, they also might influence autoxidation (Ghorbani Gorji et al., 2016; Morley, 2016). When investigating three types of salt (NaCl, mineral salt (65 % NaCl, 25 % KCl and 10 % $MgSO_4.6H_2O$) and Morton Lite salt (50 % NaCl, 50 % KCl)) in the oxidation of mayonnaise, it was reported that NaCl and mineral salt increased the oxidation of mayonnaise (Ghorbani Gorji et al., 2016). Besides, Yamauchi et al. (1982) studied the impact of sugar on autoxidation of methyl linoleate and safflower oil-sugar-cellulose aqueous emulsion, and the results pointed to the inhibition of autoxidation at low humidity but reducing sugars accelerated deterioration at high humidity. In contrast, Thomson et al. (2000) investigation showed that the formation of radicals in mayonnaise was not induced by NaCl or sugar.

Vinegar and lemon juice may be used in mayonnaise to slightly contribute to the flavour of the final product, but mainly to lower pH below 4 to reach higher microbial stability (Thomson et al., 2000). However, both can impact lipid oxidation by acting as pro-oxidants (E. N. Frankel et al., 2002; Ghorbani Gorji et al., 2016; Thomson et al., 2000). Lemon juice, probably due to ascorbic acid, in mayonnaise's water phase can promote radical generation. Ascorbic acid in low concentrations can form an iron/ascorbate complex, that deteriorates hydroperoxides at the oil-water interface (Frankel et al., 2002; Ghorbani Gorji et al., 2016). Vinegar typically supplied at 12 % acetic acid solution, reduces the pH and subsequently increase the release of iron from egg yolk (Thomson et al., 2000).

Mustard is used in mayonnaise, and its pungent flavour adds to the characteristic sensory profile of the emulsion. Previous studies revealed that mustard can improve emulsion stability aside from acting as an emulsifier in mayonnaise (Harrison & Cunningham, 1985). Lagunes-Galvez et al. (2002) investigated the effect of mustard paste absence in sunflower oil mayonnaise over 10 months, concluding that the presence of mustard contributed to lower amounts of conjugated dienes (i.e., oxidation compounds) and slower oxidative deterioration of the product. Another study followed the changes in the physicochemical properties of mayonnaise with different concentrations of mustard (powder and paste), determining that high concentrations of mustard paste (0.75 %-1.50 %) improved viscosity and emulsion stability; more importantly, reduced peroxide value and rancidity (Milani et al., 2013). In conclusion, mustard seeds contain natural antioxidants, such as tocopherol, flavones, flavonols and ascorbic acid, that might intervene in the protection of the oil from lipid oxidation in emulsions (Lagunes-Galvez et al., 2002; Milani et al., 2013).

In emulsions where fat reductions happen, with the final product showing 70 % or lower levels of lipids, water-soluble gelling agents (e.g., modified starch, xanthan gum) are needed. Oil reductions lead to a decrease in oil droplets' density, so emulsion becomes less stable due to the weaking of the interactions between droplets (Depree & Savage, 2001). Consequently, gelling agents' usage guarantees that the viscosity of the water phase is improved, and that the required texture and droplet stabilization against creaming is achieved (Morley, 2016). Moreover, studies have revealed that these agents are able to retard lipid oxidation in O/W emulsions. Shimada et al. (2002) studied xanthan on the autoxidation of soybean oil emulsion discovering that this gelling agent strongly inhibited oil peroxidation due to the chelating of metal ions at negatively charged pyruvate sites.

#### 2.2.2.5. pH

Mayonnaise is an O/W emulsion with low pH (3.0 to 4.0) that contributes to the product preservation but also to its physical stability. Lipid oxidation is affected by changes in pH because of proteins and their isoelectric point (pI), due to changes in the physical location of volatile secondary oxidation compounds, as well as if emulsions contain ionizable antioxidants (Depree & Savage, 2001; Ghorbani Gorji et al., 2016; Jacobsen et al., 2001; McClements & Decker, 2000; Takai et al., 2003).

In proteins, a pH below their pI makes the protein positively charged, whereas at a pH above the pI the proteins become negatively charged. Proteins are mainly located at the droplet surface of emulsions, hence changes in the pH impact the oil globules' surface charge, turning it positive or negatively charged (Ghorbani

Gorji et al., 2016). Typical mayonnaise should show its highest viscoelasticity and stability when the pH is close to the egg yolk proteins' pI. In those conditions, the charge of proteins is minimized so there is no limitations on other proteins adsorbing to droplets surface and no droplet repulsion (Depree & Savage, 2001). Nevertheless, pH decreases from 6.0 to 4.2 have been shown to lead to a strong pro-oxidant activity because it promotes breakage of the iron bridges with egg yolk proteins, thus making iron ions more accessible as oxidation initiators (Jacobsen et al., 2001).

Concerning volatile oxidation compounds, their distribution in an emulsion is pH dependent. It was shown that under acidic conditions (pH 4) volatiles easily migrated from liquid phase to gas phase, so interactions between protein emulsifiers and carbonyl compounds (propanal) are probably weak at pH 4. At low pH, mayonnaise had lower flavour stability but higher oxidative stability (Takai et al., 2003).

Lastly, pH affects the charge (and polarity) of antioxidants with ionizable groups thus influencing their effectiveness in preventing lipid oxidation by leading to changes in their physical location. In case the antioxidant has high polarity, then both the ionized and the nonionized forms will be predominantly water-soluble, so neither antioxidant partitioning, nor lipid oxidation are significantly affected. On the other hand, if aside from the ionizable group the antioxidant has low polarity, then the nonionized form would be partitioned between the oil and its interface. However, ionization would lead the antioxidant to be predominantly water-soluble, mainly moving to the water phase, consequently affecting oxidative stability of the emulsion (McClements & Decker, 2000).

## 3. Materials and Methods

### *3.1. Materials*

In general, mayonnaise was produced using different types of oil and egg, as well as sugar, salt, mustard and vinegar. To attain emulsion stability, the ingredients list included starches, such as cook up, cold swelling and modified starches.

Regarding the Natural antioxidants testing workstream, samples consisted of mayonnaise [Heinz Seriously Good full fat (HSG) and Vegan] to which no antioxidant was added, as well as mayonnaise with EDTA, rosemary and green tea extract (extract L) or rosemary and spinach extract (extract X); and trisodium citrate, if pH adjustment was necessary.

Pertaining to the Ideal Recipe Design workstream, HSG standard mayonnaise served as a base for the study, with some changes being made in the type of ingredients used. For instance, it was used different vegetable oils (i.e., sunflower, rapeseed, high oleic rapeseed, soybean) and emulsifiers [i.e., liquid egg yolk (LEY), heat stable egg yolk (HSEY), powdered egg yolk (PEY), whole egg/egg yolk mix (WE)].

### *3.2. Production of mayonnaises*

Mayonnaise samples were produced in the pilot plant in 15 kg batches using Fryma Koruma MaxxD Lab (capacity: 20 kg), the emulsifying unit. The overview of mayonnaise's production is illustrated in Figure 3.1.

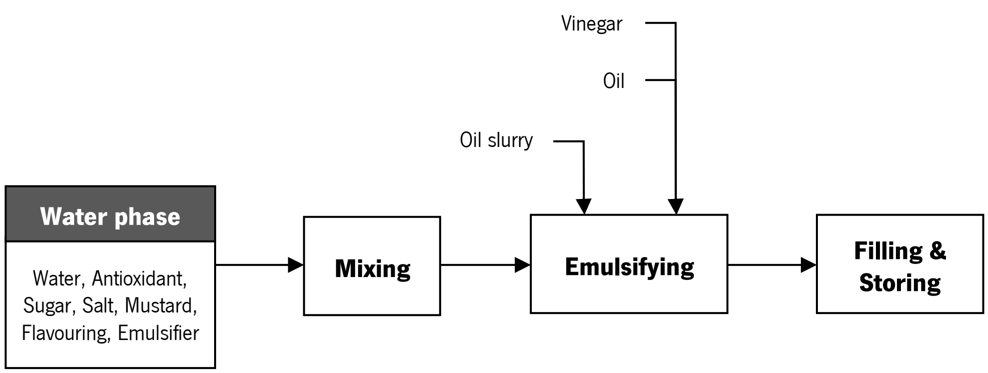



*Figure 3.1. Mayonnaise production steps*

First, the water phase ingredients (i.e., sugar, salt, mustard, natural flavour, EDTA or natural antioxidant) were mixed in water, followed by the addition and mixture of the emulsifier (i.e., LEY, HSEY, PEY, WE, Modified starch). Meanwhile, the oil slurry was prepared by mixing part of the oil with cold swelling starches to prevent lumping, in a proportion of 1:3 (starch/oil). The water phase mix was added to the vacuum mixer and the oil slurry was gradually added while mixing continued. Then, the vinegar was added, followed right after by the rest of the oil. The emulsification time was 30 seconds after all ingredients were added to the emulsifying vessel. The different mayonnaise formulations are shown in Appendix A – Table A.1 to Table A.3, Table A.5 and Table A.6.

Mayonnaise samples were manually filled into glass jars (220 mL) ensuring similar quantities in each jar, then jars were tightly closed straight after filling and firstly stored at 4°C until all trials were available. The samples were then transferred to the assigned storage conditions at 4°C, 20°C (Real Shelf Life, RSL) and 30°C (Accelerated Shelf Life, ASL). At Kraft Heinz, it is assumed that lower temperatures are able to keep the product fresh for longer so the 4°C can be used as references, besides mayonnaise stored for 1 month at 30°C is comparable to mayonnaise stored for 3 months at 20°C.

### *3.3. Analytical Procedures*

Emulsions' sensory properties, texture and safety are of great importance in mayonnaise, therefore quality analysis such as peroxide value (PV), pH, and viscosity were tested at every sampling timepoint. Furthermore, physicochemical analysis [i.e., particle size, globule size and electronic nose (E-nose) for volatile identification] were also done at Kraft Heinz facilities. Gas Chromatography Coupled with Mass Spectrometry (GC-MS) was performed by an external party.

#### 3.3.1. Peroxide Value

The determination of the peroxide value of the oils was adapted from the official method AOAC 965.33. for Oils and Fats, due to the titration with the Mettler Toledo (USA) T50 automatic titrator, with electrode DMi 147 SC.

The analysis was started by weighing at least 3.00 grams of oil sample in a titrator vessel, followed by the dissolution in 20.0 mL of acetic acid/chloroform solution (3:2 v/v). Next, around 1.0 mL of saturated potassium iodide solution was added to the previous mixture, and to allow the formation of iodine, the solution rested for exactly 5 min. Subsequently, it was added 50 mL of distilled water, followed by iodine titration with 0.01 M sodium thiosulfate solution. The determinations were made in duplicates, with differences not greater than 0.20 mEq $O_2$/kg. The PV, expressed in milliequivalent of peroxides per kg of sample, was given by the following formula:

$$\frac{(S - B) \times N \times 1000}{m}$$

where:

S – sample titration volume, in mL
B – blank titration volume, in mL
N – normality of sodium thiosulfate solution
M – mass of sample portion, in g

### 3.3.2. pH & Viscosity

The pH and viscosity measurements used room temperature (25 °C) mayonnaise samples. The former was determined using a pH meter (i.e., Metrohm 913, Switzerland); the analysis was executed in duplicates, with results differences not greater than 0.02. Regarding viscosity evaluation, a rotational viscometer (HA DV2T Brookfield Viscometer) was used with spindle number 5, setting the rotation speed and time for 30 rpm and 30 s. The results were expressed in centipoise (cP), with duplicate values not showing differences greater than 533 cP.

### 3.3.3. Particle Size

The particle size of mayonnaise's oil droplets was assessed with a particle sizer (Ambivalue EyeTech – ACM-101 Magnetic Stirring Cell, Netherlands) in duplicates. The procedure started with two successive dilutions. First, at least 15 g of room temperature sample was mixed with the same amount of water using a magnetic stirrer on 500 rpm for 5 min. Afterwards, 200 μL of diluted sample was mixed in 100 g of water using a magnetic stirrer on 500 rpm for 3 min. Finally, after the previous solution was transferred to a cuvette with a stirrer until the marked line, the oil droplets size was measured using EyeTech software. Results were reported as surface mean diameters (D[3,2]) in micrometre (μm).

### 3.3.4. Globule Size

Mayonnaise's oil globules size and their size distribution was determined using Bresser Euromex Scope BH-2 series (Netherlands) set to 40x magnification. This analysis exhibited the emulsion stability, which can be divided in 5 levels: level I – excellent stability; level II – good stability; level III – acceptable; level IV and V – unacceptable. Visual references are shown in Figure 3.2.

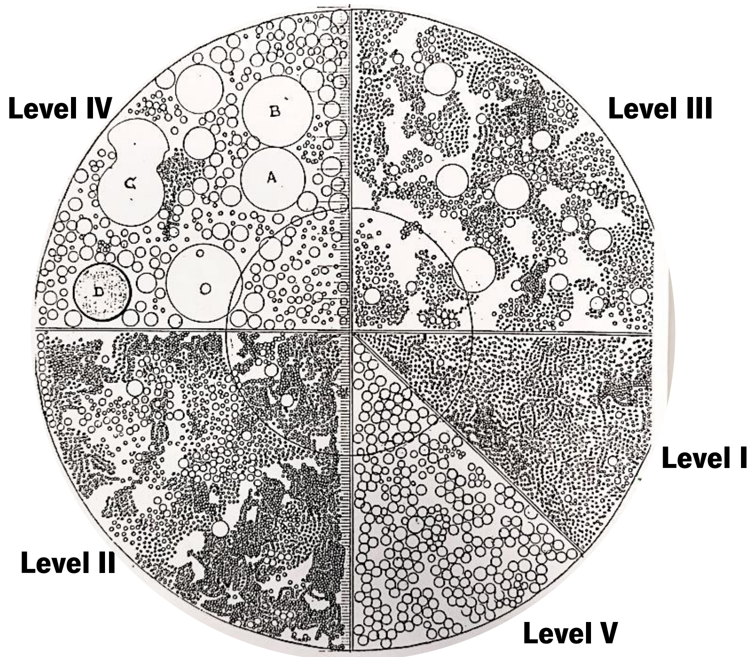



*Figure 3.2. Emulsion stability levels: Level I – excellent stability; level II – good stability; level III – acceptable; level IV – unacceptable and V – unacceptable. Adapted from The Kraft Heinz Company (2022).*

#### 3.3.5. Volatiles Identification (E-nose)

An E-nose (Heracles II Alpha MOS, France), consisting of a gas chromatography coupled with flame ionization detector (GC-FID), was used to identify the volatile compounds in the headspace of mayonnaise. The equipment is made up of an autosampler attached to a GC tailored with two columns, a non-polar and a slightly polar column.

First, to a 20 mL vial, it was added 2 g of room temperature mayonnaise and 2 mL of saturated salt solution. The vial was sealed and agitated for 10 s, then samples went into incubation (70°C, 1700 s) to stimulate volatiles release to the headspace. Subsequently, 5 mL of the vial headspace was aspirated, and the volatiles were collected on an adsorbent trap. The compounds were injected into columns, where the separation took place based on the volatility (molecular weight) and the interactions of the analytes with the stationary phase. Analytes were detected by FID and converted into chromatograms. Each sample had 3 replicates. The results were represented as peak intensities.

### *3.4. Sensory Analysis – Bipolar Difference from Control*

The sensory analysis of mayonnaise samples was performed by an expert tasting panel using the bipolar difference from control test. The expert tasters were 9 to 11 Kraft Heinz employees that work within the mayonnaise team and had been trained with references and on the descriptors of fresh and oxidised mayonnaise.

Starting with the samples' preparation, mayonnaise jars were retrieved from storage and then around 20 g of product was transferred to transparent plastic-tasting cups, which were coded accordingly: reference, code 00X (X = number from 1 to 9); samples, three-digit random number. Before the tasting session, for each taster, a tray was prepared with the reference and sample, plastic spoons, as well as water and plain crackers to cleanse the palate in between samples. The product in the cups had to reach ambient temperature.

For the tasting session, in a tasting room, panelists would individually have access to a tray and the tasting form was available online following the structure presented in Appendix B. The tasting instructions were to first taste the reference (i.e., mayonnaise with EDTA) followed by the sample, and subsequently state the degree of difference of the sample to the reference considering the specified attributes. The degree of difference was in the range of -8 to 8 (with 0 = same as reference, 2 = slight difference, 4 = slight to moderate difference, 6 = moderate difference and 8 = large difference). The negative scores meant that the sample was lower in intensity than the reference, positive scores indicated a higher intensity than the reference. The taste attributes in evaluation varied depending on the workstream. In the case of the natural antioxidants testing, the attributes were thickness, and taste of painty, sour, egg, sulphuric egg, mustard, fatty/oily, buttery, rancid oil, green/grass, aromatic herbs, metal and off taste. The ideal recipe design attributes focused on rancid oil, metal and egg tastes. In Table 3.1 is shown the mayonnaise reference against which sample was scored.

*Table 3.1. Sensory analysis of mayonnaise samples and respective references*

| | | **Reference** | **Samples** |
|---|---|---|---|
| **Natural Antioxidants Testing** | *Extract L* | HSG EDTA | HSG no EDTA, HSG L |
| | | Vegan EDTA | Vegan no EDTA, Vegan L |
| | *Extract X* | HSG EDTA | HSG no EDTA, HSG X1 pH 3.5, HSG X2 pH 3.5 |
| | | Vegan EDTA | Vegan no EDTA, Vegan X2 pH 3.0 |
| **Ideal Recipe Design** | | Sunflower EDTA | DOE 1, DOE 5, DOE 9, DOE 13 |
| | | Rapeseed EDTA | DOE 2, DOE 3, DOE 6, DOE 7<br>DOE 10, DOE 11, DOE 14, DOE 15 |
| | | Soybean EDTA | DOE 4, DOE 8, DOE 12, DOE 16 |

### *3.5. Experimental Design*

This project was divided into two main parts: (1) Natural antioxidants testing, and (2) Ideal Recipe Design. For every study, mayonnaise references were prepared: positive – HSG and Vegan mayonnaise with EDTA – , and negative – HSG and Vegan mayonnaise without EDTA.

#### 3.5.1. Natural Antioxidants Testing

Regarding natural antioxidants testing workstream, the aim was to find a natural EDTA replacement that when applied to mayonnaise would be performing better than the no EDTA samples. In this vein, two extracts were studied in full fat mayonnaise (± 70 % oil) concerning oxidation prevention: (1) extract L, a rosemary and green tea extract; and (2) extract X, a rosemary and spinach extract. The doses of natural extracts were decided based on the supplier's recommendations.

##### 3.5.1.1. Rosemary & Green Tea Extract (L) Study

To understand the influence of the rosemary and green tea extract on the oxidation of mayonnaise, the ingredient was applied at the same concentration on both mayonnaise recipes, and samples were assessed during shelf life.

Mayonnaise was produced always using rapeseed oil, the standard oil for Kraft Heinz, and the manufacturing process variables were kept constant. The oil quality was measured via peroxide value (PV) determination before mayonnaise's production.

Lastly, the jars were stored at three different temperatures (4°C, 20°C, and 30°C), and were tested for 12 months or equivalent. The current study samples are identified in Table 3.2, and Table 3.3 resumes the experimental plan.

*Table 3.2. Extract L study sample identification*

| **Sample name** | **Recipe** |
|---|---|
| HSG EDTA | HSG standard recipe with EDTA |
| HSG no EDTA | HSG standard recipe without EDTA |
| HSG L | HSG standard recipe with extract L |
| Vegan EDTA | Vegan mayonnaise recipe with EDTA |
| Vegan no EDTA | Vegan mayonnaise recipe without EDTA |
| Vegan L | Vegan mayonnaise recipe with extract L |

*Table 3.3. Chemical and Sensory analysis on extract L study*

<table>
<tr><th rowspan="3">Analysis</th><th colspan="17">Storage time and temperature</th></tr>
<tr><th rowspan="2">Fresh</th><th colspan="2">1 month</th><th colspan="2">2 months</th><th colspan="3">3 months</th><th colspan="2">4 months</th><th colspan="2">6 months</th><th colspan="2">9 months</th><th colspan="2">12 months</th></tr>
<tr><th>4°C</th><th>30°C</th><th>4°C</th><th>30°C</th><th>4°C</th><th>20°C</th><th>30°C</th><th>4°C</th><th>30°C</th><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th></tr>
<tr><td>Volatiles identification</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Sensory analysis</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
</table>

#### 3.5.1.2. Rosemary & Spinach Extract (X) Study

To gauge the impact of the rosemary and spinach extract on mayonnaise's oxidation, the ingredient was integrated at the same concentration on HSG and Vegan mayonnaise recipes, and samples were evaluated during shelf life.

The first factor being monitored was the extract X version used in HSG mayonnaise. Previous Kraft Heinz studies revealed significant off-notes (i.e., aromatic herbs) in mayonnaise with the rosemary and spinach extract. Accordingly, the original extract X, herein referred to as extract X1, was improved by the supplier in order to possibly have lower sensory impact (i.e., extract X2).

The second factor being followed in this study was the functionality of the ingredient at different pH (i.e., 3.0, 3.5 and 4.0) within Kraft Heinz mayonnaise pH range. Considering that prior studies revealed distinct quantities of oxidation related compounds in extract X mayonnaise when at different pH.

All mayonnaise samples were produced using rapeseed oil and the manufacturing process was kept constant. The PV was determined before mayonnaise's production. The various trials were saved at various storage conditions (4°C, 20°C, and 30°C) and tested for 12 months or equivalent. In Table 3.4 mayonnaise samples are identified, and the experimental plan is described in Table 3.5. Samples with pH 4.0 were only followed in terms of analytical data, has the higher pH poses an increased microbiologic risk.

*Table 3.4. Extract X study sample identification*

| **Sample name** | **Recipe** |
|---|---|
| HSG EDTA | HSG standard recipe with EDTA |
| HSG no EDTA | HSG standard recipe without EDTA |
| HSG X1 pH 3.5 | HSG standard recipe with extract X1 |
| HSG X2 pH 3.5 | HSG standard recipe with extract X2 |
| HSG X2 pH 4.0 | HSG standard recipe with extract X2 and trisodium citrate (0.26 %) |
| Vegan EDTA | Vegan mayonnaise recipe with EDTA |
| Vegan no EDTA | Vegan mayonnaise recipe without EDTA |
| Vegan X2 pH 3.0 | Vegan mayonnaise recipe with extract X2 |

*Table 3.5. Physical, chemical, and sensory analysis on extract X study*

<table>
<tr><th rowspan="3">Analysis</th><th colspan="16">Storage time and temperature</th></tr>
<tr><th rowspan="2">Fresh</th><th colspan="2">1 month</th><th colspan="2">2 months</th><th colspan="3">3 months</th><th colspan="2">4 months</th><th colspan="2">6 months</th><th colspan="2">9 months</th><th colspan="2">12 months</th></tr>
<tr><th>4°C</th><th>30°C</th><th>4°C</th><th>30°C</th><th>4°C</th><th>20°C</th><th>30°C</th><th>4°C</th><th>30°C</th><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th><th>4°C</th><th>20°C</th></tr>
<tr><td>pH</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Viscosity</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Particle size</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Globule size</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Volatiles identification</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Volatiles quantification</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
<tr><td>Sensory analysis</td><td>✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="3">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td><td colspan="2">✓</td></tr>
</table>

### 3.5.2. Ideal Recipe Design

With the purpose of understanding how mayonnaise ingredients are influencing the oxidative stability of the product, the Design of Experiments (DOE) method was carried out. Based on the multiple ingredients of mayonnaise and their possible impact in oxidation, the experiment would take great effort to test, so as a first study, 2 independent factors were selected: egg type and oil type.

The selected egg types were the standard liquid egg yolk, the heat stable egg yolk, powdered egg yolk and whole egg/egg yolk mix. The latter has egg white therefore the composition of the emulsifier is different; thus, the emulsion and oxidative stability of mayonnaise may be affected. HSEY and PEY are highly accessible processed versions of egg yolk.

Oils' fatty acid composition varies according to their source, therefore might impact the oxidative stability of mayonnaise, the types and abundance of volatiles compounds formed during shelf life, and ultimately influence the sensory profile. Rapeseed oil, sunflower oil, and soybean oil were chosen due to their high accessibility and similar cost. High oleic rapeseed oil was selected as it is the version of Kraft Heinz standard oil with higher content in oleic acid (i.e., MUFA) and lower PUFA content, the latter being the most susceptible to oxidation.

The design matrix for the experiment is given Table 3.6. The matrix shows the experiments that were conducted based on a 16 full factorial design, i.e., 2 factors with 4 levels each. The statistical software Minitab was used to generate DOE.

*Table 3.6. Design matrix*

| Egg type | Oil type | | | |
|---|---|---|---|---|
| | *Sunflower* | *Rapeseed* | *High Oleic Rapeseed* | *Soybean* |
| *Liquid Egg Yolk* | DOE 1 | DOE 2 | DOE 3 | DOE 4 |
| *Heat Stable Egg Yolk* | DOE 5 | DOE 6 | DOE 7 | DOE 8 |
| *Powdered Egg Yolk* | DOE 9 | DOE 10 | DOE 11 | DOE 12 |
| *Whole Egg/ Egg Yolk mix* | DOE 13 | DOE 14 | DOE 15 | DOE 16 |

The developed formulations are described in Appendix A – Table A.5 and Table A.6. The ingredient dosage was adjusted to maintain the final sugar and salt content of the several mayonnaises. Furthermore, to switch between egg types it was always taken into consideration the actual amount of egg yolk in the recipe. In addition to the recipes shown, HSG standard mayonnaise with EDTA (i.e., references) were also produced, with following labels: Sunflower EDTA, Rapeseed EDTA and Soybean EDTA. All mayonnaise samples were manufactured following the standard HSG mayonnaise procedure. The PV, an oil quality parameter, was determined before mayonnaise's production. The various trials were saved at 4°C and 30°C, then physical, chemical, and sensory results were collected over the course of 2 months (Table 3.7).

*Table 3.7. Physical, chemical, and sensory analyses on ideal recipe design study*

| Analysis | Temperature and Storage period | | | | | | |
|---|---|---|---|---|---|---|---|
| | Fresh | 2 weeks | | 1 month | | 2 months | |
| | | 4°C | 30°C | 4°C | 30°C | 4°C | 30°C |
| pH | ✓ | ✓ | | ✓ | | ✓ | |
| Viscosity | ✓ | ✓ | | ✓ | | ✓ | |
| Particle size | ✓ | ✓ | | ✓ | | ✓ | |
| Globule size | ✓ | ✓ | | ✓ | | ✓ | |
| Volatiles identification | ✓ | ✓ | | ✓ | | ✓ | |
| Sensory analysis | ✓ | ✓ | | ✓ | | ✓ | |

### *3.6. Data Analysis*

In the case of the natural antioxidant testing workstream, all analytical data presented were expressed as mean and standard deviation. For the sensory analysis, the Grubbs test was used to detect outliers, afterward the scores for each attribute were averaged and rounded to the nearest whole number. A significant difference was reported for sensory attributes when the absolute difference given by the average score of the reference and sample was equal or greater than 2. The comments on the off taste and/or other parameters were analysed and summarised, only being considered as significant if mentioned by more than 50 % of the tasters.

Concerning the ideal recipe design workstream, data analysis included determination of the coefficient of determination ($R^2$) and analysis of variance (ANOVA). The regression equation used for the experimental design was: $y = b_0 + b_1X_1 + b_2X_2 + \cdots + b_kX_k$, where $y$ is the response function with respect to factors $X_1, X_2, \ldots, X_k$, $b_0$ is the constant and $b_1, b_2, \ldots, b_k$ are the regression coefficients.

# 4. Results and Discussion

Regarding emulsified products such as mayonnaise, a lot of factors play a role in the oxidation and the acceptability of the product. A natural antioxidant as effective as EDTA is yet to be found, hence EDTA removal remains a challenge that must be tackled from different angles.

The present project involved two workstreams: Natural antioxidants testing, and Ideal Recipe design. The former focused on finding an efficient natural alternative to EDTA, and the latter aimed to understand how elements of the mayonnaise recipe are influencing oxidative stability.

E-nose analyses on fresh and aged mayonnaise were carried out to identify the volatiles naturally present in the product, the ones developed during storage and associated with lipid oxidation, and ultimately, to know how these evolve over storage time. In general, more than 30 different volatile compounds were detected in each mayonnaise sample. Amongst detected compounds were, for example, acetic acid, ethyl acetate, ethyl butyrate commonly found in vinegar, and one of the mayonnaise ingredients (Aurand et al., 1966). Moreover, groups of volatiles as alcohols, alkanals, ketones, 2-alkenals, 2,4-alkadienals, esters, alkanes were also identified, all of which have been associated with the lipid oxidation process (Ghorbani Gorji et al., 2019). Odour detection threshold is the lowest concentration of an odorant that can be reliably detected, consequently the lower the odour threshold, the lesser the quantity of odorant needs to be present to be detected. In sum, lower odour threshold compounds have higher impact on the sensory profile of a determined product, hence based on the general odour threshold value of volatile compounds generated during lipid autoxidation (Table 4.1), only aldehydes and vinyl ketones were assessed in the present work.

*Table 4.1. Odour threshold value (in ppm) per group of compounds* (E. N. Frankel, 1985)

| **Group of Compounds** | **Odour threshold value (ppm)** |
|---|---|
| Hydrocarbons | 90-2150 |
| Substituted furans | 2-27 |
| Vinyl alcohols | 0.5-3 |
| 1-Alkenes | 0.02-9 |
| 2-Alkenals | 0.04-2.5 |
| Alkanals | 0.04-1.0 |
| (E,E)-2,4-alkadienals | 0.04-0.3 |
| Isolated alkadienals | 0.002-0.3 |
| Isolated cis-alkenals | 0.003-0.1 |
| (E,Z)-2,4-alkadienals | 0.002-0.006 |
| Vinyl ketones | 0.00002-0.007 |

### *4.1. Natural antioxidants testing*

In attempt to find an efficient natural alternative to EDTA, the rapeseed oil mayonnaise samples were evaluated concerning their oxidative and emulsion stability, as well as their sensory properties. The results presented were based on the data obtained on EDTA and EDTA free mayonnaises. Concerning volatiles identification and/or quantification, from the selected volatiles categories, greater relevance was given to 2,4-heptadienal. The latter compound being one of the preferred oxidation markers for rapeseed oil mayonnaise, based on previous Kraft Heinz studies on mayonnaise.

#### 4.1.1. Rosemary & Green Tea Extract (L) Study

To understand the impact of the rosemary and green tea extract on the oxidation and shelf life of mayonnaise, the results presented were based on the data acquired from mayonnaise tested until 3 and 4 months of storage at 20°C and 30°C, respectively. Some figures, tables, and images are shown in Appendix C due to the resemblance of drawn conclusions.

##### 4.1.1.1. Volatiles Profile

Rancidity is one of the main problems in mayonnaise, as lipid oxidation is a complex process arising to a myriad of end products, among them volatile compounds that alter the sensory properties of an oxidized product. EDTA is a highly effective metal chelator, meaning that the abundance of volatile compounds associated with lipid oxidation is expected to be much greater in EDTA free mayonnaise than in the samples containing the antioxidant. Besides, samples with extract L acting as a natural antioxidant, are expected to show lower abundance of lipid oxidation volatiles than EDTA free samples.

Fresh and aged mayonnaises were analysed to determine the naturally occurring volatiles, as well as the ones produced during shelf life and linked to rancidity in mayonnaise. As can be seen in Figure 4.1, at t = 0 months, the 2,4-heptadienal intensities were close to null and similar despite the presence of EDTA or extract L. During accelerated shelf life (ASL, 30°C), mayonnaise with EDTA and EDTA-free presented the expected behaviour over time. The "HSG EDTA" and "Vegan EDTA" samples showed low abundance of high impact oxidation compounds, only slightly increasing over time but in general remaining under a relative intensity of 1000. For the mayonnaises free of EDTA, higher intensities of 2,4-heptadienal were achieved: "HSG no EDTA" reached an intensity of 12330 after 4 months of ASL; and "Vegan no EDTA" showed an intensity of 8767 after 3 months at 30°C. During real shelf life (RSL, 20°C) (Figure 4.2), the same volatile profile was detected. Concerning mayonnaise with the rosemary and green tea extract, both in HSG and Vegan samples, the volatile profile for ASL and RSL was comparable to the respective recipes without EDTA. Hence, the observed intensities were much higher than the ones associated with samples containing EDTA, for example, 3-months old HSG mayonnaise with extract L at 20°C revealed a 2,4-heptadienal intensity of around 24 times higher than the HSG EDTA mayonnaise.

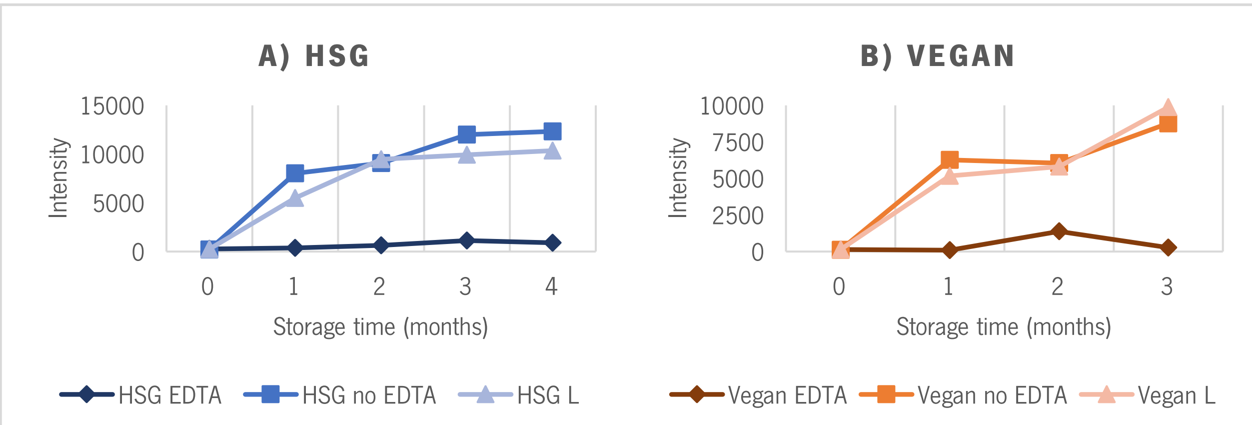



*Figure 4.1. Intensity of (E,E)-2,4-heptadienal in a) HSG and b) Vegan mayonnaises stored at 30°C during 4 and 3 months of storage, respectively.*

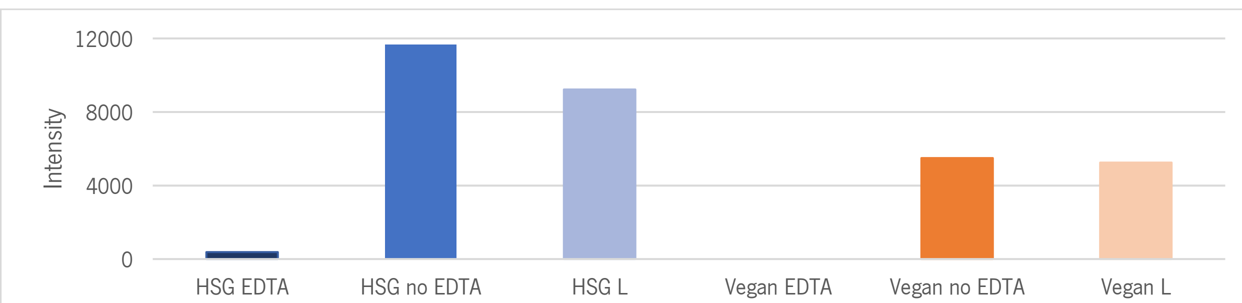



*Figure 4.2. Intensity of (E,E)-2,4-heptadienal in HSG and Vegan mayonnaises stored at 20°C for 3 months.*

Several studies have evaluated the volatile profile of food products rich in lipids, such as fish oil, mayonnaise and milk, where fishy, rancid and metallic off flavours have been perceived (Hartvigsen et al., 2000; Hsleh et al., 1989; Jacobsen, 1999; Jacobsen, Hartvigsen, Lund, Thomsen, et al., 2000; Karahadian & Lindsay, 1989; Venkateshwarlu et al., 2004a, 2004b). The authors found potent volatile compounds (i.e., 1-penten-3-one, (Z)-4-heptenal, 1-octen-3-one, 1,5-octadien-3-one, (E,E)-2,4-heptadienal, and (E,Z)-2,6-nonadienal) in these products, however, in general, the individual odorants were not characterised by a fishy or metallic odour. High levels of 2,4-heptadienal in mayonnaise has been positively correlated with fishy, metallic and rancid off flavours (Jacobsen, 1999; Jacobsen, Hartvigsen, Lund, Thomsen, et al., 2000). In a study by Venkateshwarlu et al. (2004b), a selection of volatile compounds, such as (E,Z)-2,6-nonadienal, 1-penten-3-one, (Z)-4-heptenal, and (E,E)-2,4-heptadienal, which have been reported to result from the oxidation of omega-3 fatty acids, were added to milk, finding that, in opposition to samples with individual compounds, the combination of all or specific volatiles resulted in the perception of fishy and metallic off flavours.

In this vein, the volatiles analysis of "HSG L" and "Vegan L" suggests a sensory profile characterised by high scores in the "rancid oil" and "metal" taste attributes, as the one found for the corresponding no EDTA mayonnaises.

#### 4.1.1.2. Sensory Profile

Mayonnaise is a complex product with multiple ingredients, the major part impacting the flavour of the final product. Some ingredients in addition to imparting flavour may act as masking agents, possibly leading, for example, to lower perception of oxidation compounds (Kumar & Tanwar, 2011). Consequently, the volatile profile of a mayonnaise might not be entirely related to its sensory profile.

The scores for the oxidation linked sensory attributes can be seen in Table 4.2, Table 4.3 and Appendix C – Table C.1 and Table C.2 –, with the significant differences highlighted in red. In general, the evaluated sensory attributes for fresh mayonnaises were scored 0, and oxidation started to be perceived after equivalent time, i.e., 1 month at 30°C and 3 months at 20°C. The HSG mayonnaises with extract L were perceived significantly worse on the "rancid oil" and "metal" taste, and "off taste" compared to the no EDTA mayonnaise. In the case of the Vegan samples, the natural extract mayonnaise showed a sensory profile comparable to the one of mayonnaise without EDTA.

*Table 4.2. Sensory scores of HSG mayonnaise samples after 3 months storage at 20°C, and 2- and 4-months storage at 30°C*

| **Storage** | | **Sample** | **Taste** | | | | | **Off taste** |
|---|---|---|---|---|---|---|---|---|
| **T (°C)** | **t (months)** | | **Painty** | **Fatty/oily** | **Rancid oil** | **Green/Grass** | **Metal** | |
| 20 | 3 | **HSG no EDTA** | 1 | 1 | 3 | 0 | 0 | 2 |
| | | **HSG L** | 1 | 1 | 3 | 0 | **2** | 3 |
| 30 | 2 | **HSG no EDTA** | 1 | 3 | 3 | 0 | 0 | 2 |
| | | **HSG L** | 2 | 2 | **5** | 0 | **4** | **4** |
| | 4 | **HSG no EDTA** | 1 | 1 | 3 | 0 | 0 | 2 |
| | | **HSG L** | 1 | 1 | 3 | 0 | **2** | 1 |

*Table 4.3. Sensory scores of Vegan mayonnaise samples after 3 months storage at 20°C, and 2- and 4-months storage at 30°C*

| Storage | | Sample | Taste | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|
| **T (°C)** | **t (months)** | | **Painty** | **Fatty/oily** | **Rancid oil** | **Green/Grass** | **Metal** | |
| 20 | 3 | **Vegan no EDTA** | 3 | 1 | 2 | 0 | 0 | 1 |
| | | **Vegan L** | **1** | 0 | 3 | 0 | 0 | 2 |
| 30 | 2 | **Vegan no EDTA** | 4 | 2 | 2 | 4 | 2 | 3 |
| | | **Vegan L** | 4 | 2 | 2 | 4 | 3 | 3 |
| | 4 | **Vegan no EDTA** | 2 | 1 | 3 | 1 | 0 | 1 |
| | | **Vegan L** | 3 | 2 | 3 | 1 | 1 | 2 |

The worst sensory profile of "HSG L" mayonnaise, in comparison to "Vegan L", might be owed to the presence of egg yolk, as egg yolk contributes to higher iron (i.e., pro-oxidant) content in the HSG formulations (Morley, 2016). Moreover, possible variances in the physicochemical profile of analysed samples might have impacted flavour perception. For instance, some authors revealed that sample viscosity was not significantly impacting the quantity of released volatiles (Cook et al., 2003; Hollowood et al., 2002; Lethuaut et al., 2004; Štern et al., 2001; Weel et al., 2002). However, there is evidence that flavour perception decreased when food viscosity was greater (Baines & Morris, 1987; Moskowitz & Arabie, 1970; Pangborn et al., 1973; Vaisey et al., 1969).

In summary, the high intensity volatiles associated with oxidation (e.g., 2,4-heptadienal) were significantly perceived in sensory tasting, and samples containing the natural extract had similar or worse performance than no EDTA samples, thus the rosemary and green tea extract (extract L) did not perform effectively in the prevention and control of lipid oxidation of tested mayonnaise matrixes.

#### 4.1.2. Rosemary & Spinach Extract (X) Study

To understand the functionality of the rosemary and spinach extract, as a natural antioxidant, in HSG and Vegan mayonnaise samples, as well as to evaluate its performance at different pH, the results presented were based on the data acquired from mayonnaise tested until 9 and 4 months of storage at 20°C and 30°C, respectively. Some figures, tables, and images are in Appendix C due to the resemblance of drawn conclusions.

##### 4.1.2.1. Physicochemical Profile

The pH and viscosity variability over time give an indication of the emulsion stability.

Figure 4.3 shows the average pH and the respective standard deviations of mayonnaises during storage at 20°C. Analysis revealed that samples had pH values around the expected: pH 3.5 for all HSG samples, except "HSG X2 pH 4.0", and pH 3.0 for Vegan mayonnaises. All recipes showed high pH stability during shelf-life, with the highest relative standard deviation (RSD) being only 2.4 %. The pH results during ASL (Appendix C – Figure C.1) showed analogous behaviour.

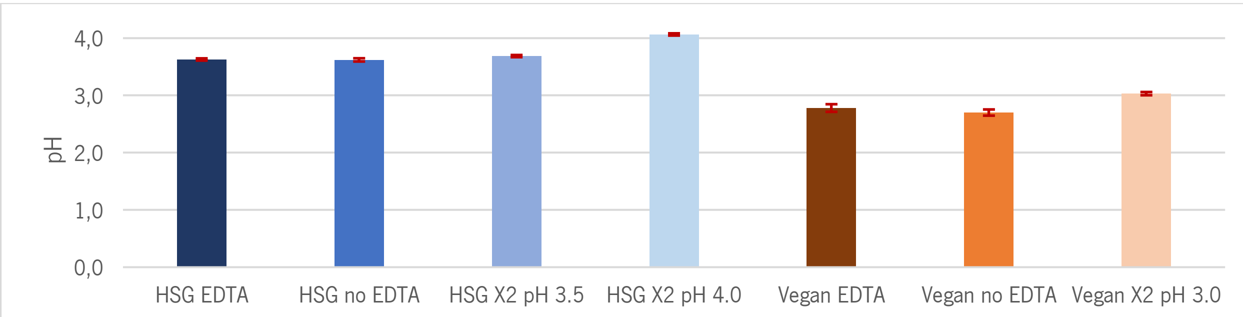



*Figure 4.3. Average pH of HSG and Vegan mayonnaises after 9 months and 4 months of storage at 20°C and 30°C, respectively, with error bars showing the standard deviations.*

At t = 0 months, generally samples had viscosities within the range of 11000 – 12000 cP (Figure 4.4). All mayonnaises started has the most viscous, then as storage time increased, the viscosity of mayonnaise decreased. Thus, the average particle size of the mayonnaises was expected to become higher, i.e., larger droplets are produced due to coalescence (Depree & Savage, 2001). The previous data are to be analysed in chapter 4.1.2.1.1 Particle and Globule size.

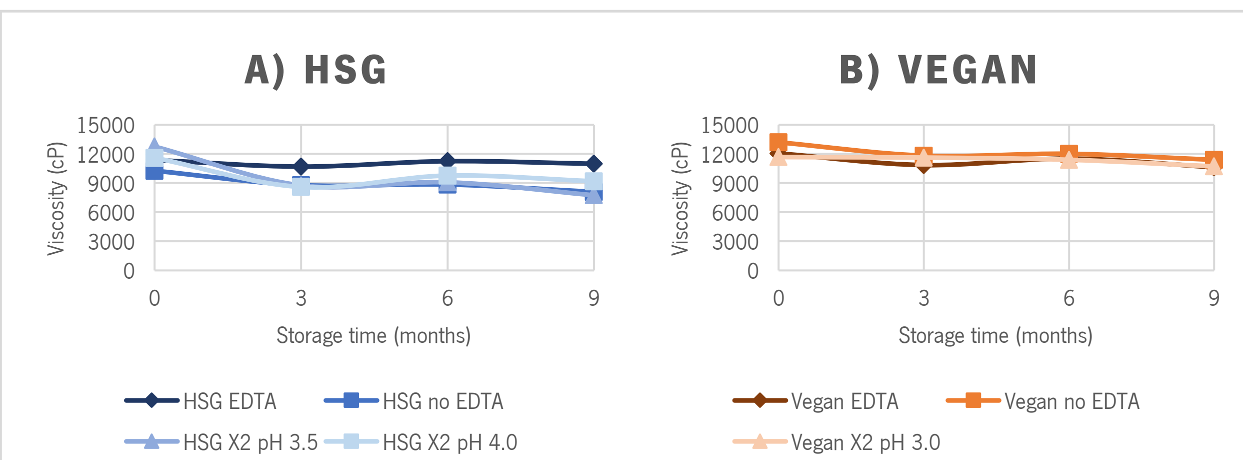



*Figure 4.4. Viscosity of a) HSG and b) Vegan mayonnaises until 9 months of storage at 20°C.*

In terms of viscosity, the sample “HSG EDTA” and all vegan mayonnaises exhibited a more stable behaviour over time, showing RSD lower than 6 %. In contrast, “HSG no EDTA” and the HSG samples containing the extract X had more substantial changes in viscosity. For example, the viscosity reduction until t = 9 months, seen in “HSG X2 pH 3.5” was approximately two times higher than “HSG X2 pH 4.0” and 11 times higher than “HSG EDTA”. At 30°C storage conditions (Appendix C – Figure C.2), the viscosity variations were aligned with RSL results, thus, the viscosity of mayonnaise samples became lower, the variations being more noticeable in the case of “HSG no EDTA” and “HSG X2” samples.

The effect of viscosity variation in sensory properties, namely flavour, has been investigated. Various studies revealed that sample viscosity was not significantly impacting the quantity of released volatiles (Cook et al., 2003; Hollowood et al., 2002; Lethuaut et al., 2004; Štern et al., 2001; Weel et al., 2002). Conversely, there is evidence that flavour perception decreased when food viscosity was greater (Baines & Morris, 1987; Moskowitz & Arabie, 1970; Pangborn et al., 1973; Vaisey et al., 1969).

In conclusion, mayonnaise with extract X2, had higher emulsion stability when at pH 3.0, followed by at pH 4.0 and was least stable when at pH 3.5. The conflicting findings regarding viscosity’s impact on sensory profile do not show a clear path as to what to expect from studied mayonnaises, however, it was hypothesised that the lower emulsion stability of HSG samples with the rosemary and spinach extract, mainly at pH 3.5, lead to higher perception of flavour compounds, for example, lipid oxidation volatiles.

#### 4.1.2.1.1. Particle and Globule size

Particle size measurements and globule structure help to determine the impact of oxidation on the physical structure of mayonnaises during shelf life.

Initial droplet size (Figure 4.5) for HSG samples was on average 7.3 ± 0.6 µm, excluding “HSG X2 pH 4.0” which showed a mean diameter around 1.5 times higher. In the case of Vegan samples, the average particle size was 16.8 ± 0.9 µm, almost double than HSG sample.

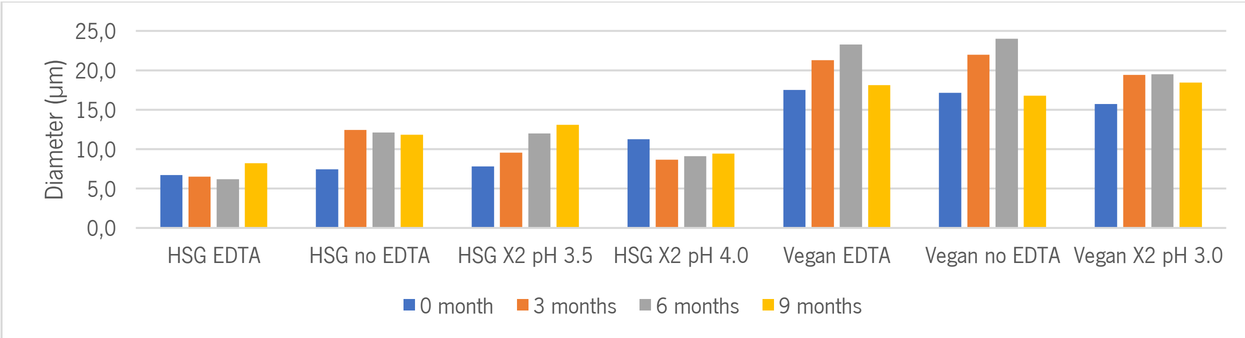



*Figure 4.5. Particle size (in µm) of HSG and Vegan mayonnaise for 9 months of storage at 20°C.*

The effect of droplet size on oxidative stability of O/W emulsions has been studied. In some reports, when decreasing the emulsions' droplets size, the rate of lipid oxidation increased, due to higher surface area of lipids exposed to the water phase, the carrier of pro-oxidants (Berton-Carabin et al., 2014; Gohtani et al., 1999; Jacobsen, Hartvigsen, Lund, Thomsen, et al., 2000; Uluata et al., 2016). However, Hu et al. (2003) and Osborn & Akoh (2004) reported that particle size did not significantly impact lipid oxidation of emulsions. Furthermore, a kinetic study using fish and olive O/W emulsions concluded that droplet size had insignificant effect on oxidative stability and on the distribution and concentration of antioxidants, proposing that oxidative stability varied according to the interfacial concentration of reactants, i.e., lipids and pro-oxidants, and not on surface area (Costa et al., 2020). The studies have shown incongruous results, nevertheless, changes in oxidation rate to some extent can be due to alterations in particle size.

The droplet size of mayonnaise is known to increase over storage time, hence this being the expected behaviour for the tested samples (Ariizumi et al., 2017). Particle size behaviour during storage suffered some variations, that might denote different oxidation rates. As storage time increased, the mean diameter of "HSG EDTA", "HSG no EDTA", "HSG X2 pH 4.0" and "Vegan X2 pH 3.0" was fairly constant, which might indicate a slower oxidation process. Conversely, for the "Vegan EDTA", "Vegan no EDTA" and the sample with extract X at pH 3.5, the mean diameter progressively increased with growing storage time, thus higher oxidation levels might be expected. In addition, HSG mayonnaise, that has egg yolk as an ingredient, should have higher concentration of metal ions (i.e., pro-oxidant). Hence, it can be hypothesised that the Vegan sample with the rosemary and spinach extract at pH 3.0 can present the best oxidative stability, followed by "HSG X2 pH 4.0" mayonnaises, and lastly mayonnaise with extract X2 at pH 3.5 should have the worst performance, showing higher concentration of lipid oxidation compounds. When at ASL, for "HSG EDTA", as well as for all vegan mayonnaises, the particle size changes over time were low, being observed a maximum RSD of 8%; the former variations were much lower than decrease in droplet size showed by "HSG no EDTA", “HSG X2 pH 3.5” and “HSG X2 pH 4.0”, where the minimum RSD was 14 % – associated with the mayonnaise with extract X2 at higher pH.

As formerly stated, in general, mayonnaises’ viscosity diminished as storage time increased, and according to Depree & Savage (2001) larger droplets are produced due to coalescence, consequently, the mean diameter of the droplets was expected to become higher (negative correlation). In the case of “HSG no EDTA” and “HSG X2 pH 3.5”, the previous behaviour was observed, and a satisfactory correlation was established ($R^2$ = 75 %). However, the HSG mayonnaise with extract X2 at pH 4.0 presented an inverse behaviour, showing a strong positive correlation ($R^2$ = 91 %), in addition, all vegan samples and “HSG EDTA”, showed no substantial correlation between viscosity and particle size ($R^2$ < 6 %).

At both storage temperatures, comparable results were obtained between particle size measurements and microscope observations (Appendix C – Table C.3 to Table C.6). All fresh mayonnaises had excellent or good emulsion stability, and over time irrespective to samples in test, oil droplets augmented indicating slightly lower emulsion stability and the occurrence of oxidation. Yet, coalescence rate seemed less evident in vegan mayonnaises, for example, in sample containing the natural extract at pH 3.0, followed by “HSG X2 pH 4.0”. The sample “HSG X2 pH 3.5” showed similar profile to EDTA-free HSG mayonnaise, with microscope images showing sizable differences in terms of globule size.

#### 4.1.2.2. Volatiles Profile

Fresh and aged mayonnaises were analysed to determine the volatiles linked to oxidation that were produced during storage and their evolution over time. The high impact volatile compound 2,4-heptadienal was quantified (Figure 4.6 and Appendix C – Figure C.4) during shelf-life for HSG and Vegan mayonnaises.

The initial 2,4-heptadienal concentration for all samples was below detection limit. During RSL, mayonnaises with EDTA and EDTA-free showed the expected behaviour over time. The concentrations of 2,4-heptadienal in “HSG no EDTA” and “Vegan no EDTA” increased immensely reaching a maximum of 6491 ppb and 3489 ppb, respectively, at 9 months. In comparison, volatiles growth in EDTA mayonnaises was considered of no relevance, since its greatest value (i.e., 140 ppb in “HSG EDTA”) was 46 and 24 times, respectively, lower than the previous stated maximums.

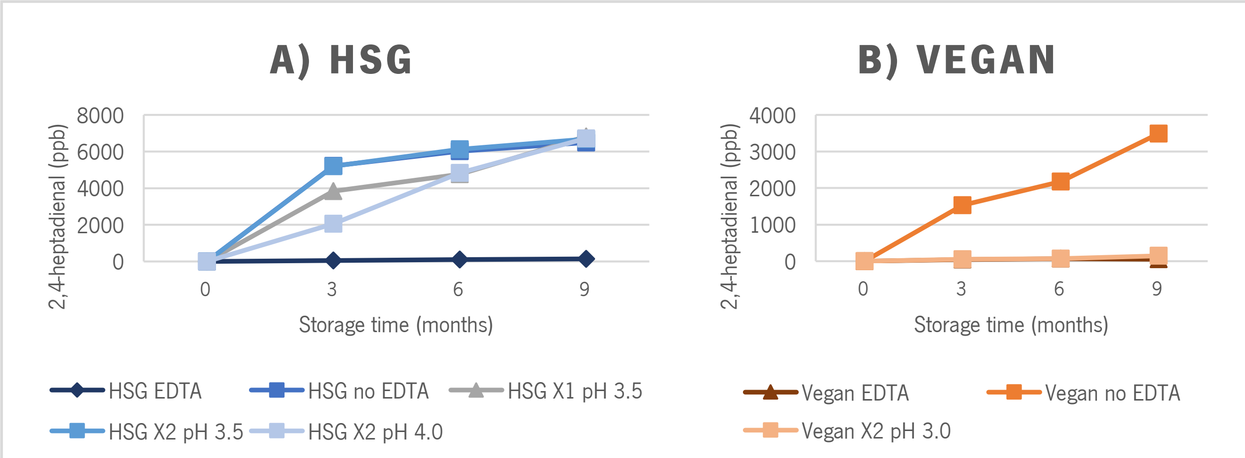



*Figure 4.6. Quantification of (E,E)-2,4-heptadienal (in ppb) concerning a) HSG and b) Vegan mayonnaises stored at 20°C for 9 months.*

Until 6 months of storage, contrary to anticipated, the performance of extract X1 in HSG mayonnaise was slightly better than the extract X2 (4754 ppb < 6120 ppb); besides, by the end of evaluated checkpoints similar 2,4-heptadienal concentrations were measured in both extract versions, as well as analogous volatile profile to "HSG no EDTA" sample. Hence, in the initial steps of storage, sensory evaluation of "HSG X1 pH 3.5" is suggested to be better perceived than "HSG X2 pH 3.5", although by the end of shelf life it is expected to have similar scores among the previous samples and "HSG no EDTA".

When pH changes in formulations were factored in, results were aligned with predictions based on physicochemical profile variations. The highest oxidative stability was associated with "Vegan X2 pH 3.0", succeeded by "HSG X2 pH 4.0" and the lowest linked to "HSG X2 pH 3.5". Thus, the lower content of 2,4-heptadieanal in mayonnaises, i.e., the higher functionality of the extract X, was linked to the lowest tested pH values (pH 3.0). The active compound of the rosemary and spinach extract has a pKa of 4, so when at lower pH, the extract will be mainly in its protonated form, the less active form of the antioxidant. Unpredictably, Figure 4.6 showed that by the end of RSL "Vegan X2 pH 3.0" sample exhibited 46 times lower content of 2,4-heptadienal than "HSG X2 pH 4.0" mayonnaise, despite the pH being around 3.0. The result may be explained by the absence of egg in vegan samples and its associated lower content in iron. Transition metal ions (e.g., iron) are one of the main pro-oxidants leading to lipid oxidation, therefore, the lower concentration of these compounds in vegan mayonnaise might explain its higher oxidative stability.

In sum, it was estimated that both the "HSG X2 pH 3.5" and "HSG no EDTA" samples were perceived similarly and the version 1 of the extract to be perceived comparably or slightly better than the previous mayonnaises. Moreover, "Vegan X2 pH 3.0" sample showed oxidation concentrations analogous to "Vegan

EDTA" mayonnaise, thus being expected that the vegan sample with natural extract was perceived considerably better than no EDTA sample, on oxidation related attributes.

#### 4.1.2.3. Sensory Profile

The scores for the oxidation linked sensory attributes, as well as those possibly associated with natural compounds originating from plants, can be seen in Table 4.4,

Table 4.5 and Appendix C (Table C.7 and Table C.8), with the significant differences highlighted in red.

Mostly sensory profile of EDTA-free fresh mayonnaises was similar to EDTA references, and oxidation started to be pointedly perceived in 3-months stored samples. In the beginning of RSL, HSG samples with extract X1 presented a sensory profile comparable to "HSG EDTA" and were perceived significantly better than the version 2 of the natural extract on attributes such as "painty", "sulphuric egg" and "fatty/oily" taste. However, with increased storage time, the samples containing the original extract were perceived equally or significantly worse than the extract X2, both being comparable to EDTA free sample on oxidation attributes such as "painty" and "rancid oil".

For the vegan samples, as analytical results suggested samples containing the rosemary and spinach extract had similar sensory profile as "Vegan EDTA" and performed significantly better than no EDTA mayonnaise on the attributes "painty", "rancid oil" and "off taste".

*Table 4.4. Sensory scores of HSG mayonnaise samples after 3- and 9-months storage at 20°C*

| Storage | | Sample | Taste | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|
| T (°C) | t (months) | | Painty | Sulphuric egg | Fatty/oily | Rancid oil | Aromatic herbs | |
| 20 | 3 | **HSG no EDTA** | 2 | 2 | 2 | 2 | 0 | 2 |
| | | **HSG X1 pH 3.5** | **0** | **0** | **0** | 1 | 0 | 2 |
| | | **HSG X2 pH 3.5** | 2 | 2 | 2 | 2 | 0 | 1 |
| | 9 | **HSG no EDTA** | 2 | 2 | 3 | 3 | 0 | 2 |
| | | **HSG X1 pH 3.5** | 2 | 1 | **1** | 3 | 0 | **4** |
| | | **HSG X2 pH 3.5** | 1 | **0** | **1** | 2 | 0 | **0** |

*Table 4.5. Sensory scores of HSG mayonnaise samples after 3- and 9-months storage at 20°C*

| Storage | | Sample | Taste | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|
| T (°C) | t (months) | | Painty | Fatty/oily | Rancid oil | Green / Grass | Metal | |
| 20 | 3 | **Vegan no EDTA** | 4 | 2 | 2 | 4 | 0 | 2 |
| | | **Vegan X2 pH 3.0** | **2** | **0** | **0** | **0** | 0 | **0** |
| | 9 | **Vegan no EDTA** | 3 | 1 | 3 | 0 | 1 | 4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Vegan X2 pH 3.0** | **0** | 0 | **0** | 1 | 0 | **0** |

Lastly, considering the functionality of the rosemary and spinach extract with pH variations, sensory results supported previous projections, with extract X2 at pH 3.0 showing greatest oxidative stability than when at higher pH (3.5).

In sum, sensory evaluation results were consistent with the analytical data; by the end of storage, the high intensity volatiles (e.g., 2,4-heptadienal) were significantly perceived in extract X samples, with the version 2 performing slightly better. For Vegan mayonnaise, the natural extract samples had a great performance, effectively preventing and controlling lipid oxidation. Hence, to confirm extract X performance, the investigation should proceed until the data from 12 months of storage is recovered.

### *4.2. Ideal Recipe design*

With the challenge of finding how mayonnaise ingredients, such as oil and egg, are influencing its oxidative stability, and possibly establish optimal ingredient composition to prevent oxidation, HSG standard formulation was adapted so that in a full factorial design with 16 trials it would be possible to study the impact of changing the oil and emulsifier in the oxidative and emulsion stability, as well as the sensory properties of the samples.

Regarding E-nose data, the numerous volatiles compounds, such as alcohols, alkanals, ketones, 2-alkenals, 2,4-alkadienals, esters, alkanes, arising from oxidation have distinct odour thresholds; low threshold compounds have a higher impact on the sensory profile of a determined product.

The current study involved the usage of different oils that accordingly led to different flavour profiles. Since oils differ in their MUFA and PUFA composition, as previously investigated by Warner et al. (1989), the formed lipid oxidation compounds as well as their quantities are variant, for example, soybean oil in comparison to sunflower oil and low erucic acid rapeseed oil showed significantly lower production of compounds imparting flavour to the samples. Besides, a Kraft Heinz study concluded that the multiple oils have different oxidation markers compounds, as 2,4-heptadienal (rapeseed), 2,3-pentanedione (soybean), and 2,3-dimethylpyrazine and 2,4-decadienal (sunflower). Rapeseed mayonnaise had a blander taste, while soybean and sunflower mayonnaises tended to have more dairy and nuttier off taste. The off tastes linked to each oil could impact and mask the rancid taste (i.e., old oil and metallic).

In this vein, since multiple compounds had to be pondered, based on the general odour thresholds (Table 4.1), only aldehydes and vinyl ketones were assessed in the present work. The volatiles intensities were added, so the response results correspond to the total intensities of high impact volatiles.

### 4.2.1. Storage time and Changes on Mayonnaises Profile

#### 4.2.1.1. Physicochemical Profile

Figure 4.7 shows pH variability in mayonnaises over 1 month at 30°C. The pH results indicated that, at t = 0 months, EDTA references and DOE 1-8, that included LEY or HSEY, had an average pH value of 3.6, the closest to the expected pH of 3.5. And in mayonnaises with PEY (DOE 9-12, pH 3.7) and WE (DOE 13-16, pH 3.8) this property was slightly augmented. When storage time increased, generally mayonnaises' pH values barely increased (up to 3 %), and the same pattern remained, i.e. (LEY ≃ HSEY) < PEY < WE.

The viscosity data (Figure 4.8) showed that fresh mayonnaises started with different viscosities despite going through the same process. The samples' ageing led mayonnaises to become less viscous – minimum 9 % decline observed in sample "DOE 13" –, except for HSEY samples which after 1 month of ASL had similar viscosities to when fresh.

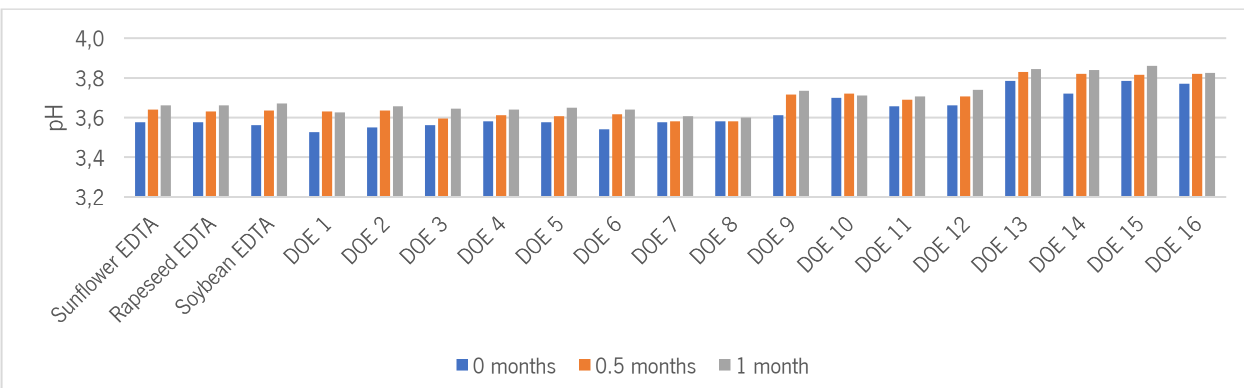



*Figure 4.7. pH of mayonnaises from the design of experiment for 1 month of storage at 30°C.*

Data comparison founded on egg type, revealed a clear behaviour of mayonnaises viscosities; regardless of the oil type, mayonnaises using LEY (i.e., DOE 1-4) were the less viscous (13851 cP ± 13 %), followed by the samples with PEY (i.e., DOE 9-12) that had a viscosity of 17488 cP ± 11 %, lastly, formulations that included HSEY (DOE 5-8) and WE (DOE 13-16) exhibited the highest viscosities (20171 cP ± 9 % and 19867 cP ± 11 %, respectively). When comparing samples' viscosity based on the oil type, unlike the aforementioned factor, the pattern was only somewhat evident for samples containing SB (i.e., DOE 4, 8, 12 and 16) that generally showed the highest viscosities (18659 cP ± 17 %). Overall, during storage time the viscosity suggested a greater dependence on the egg type versus the oil type used in formulations, and the viscosity followed the pattern: LEY < PEY < (HSEY ≃ WE).

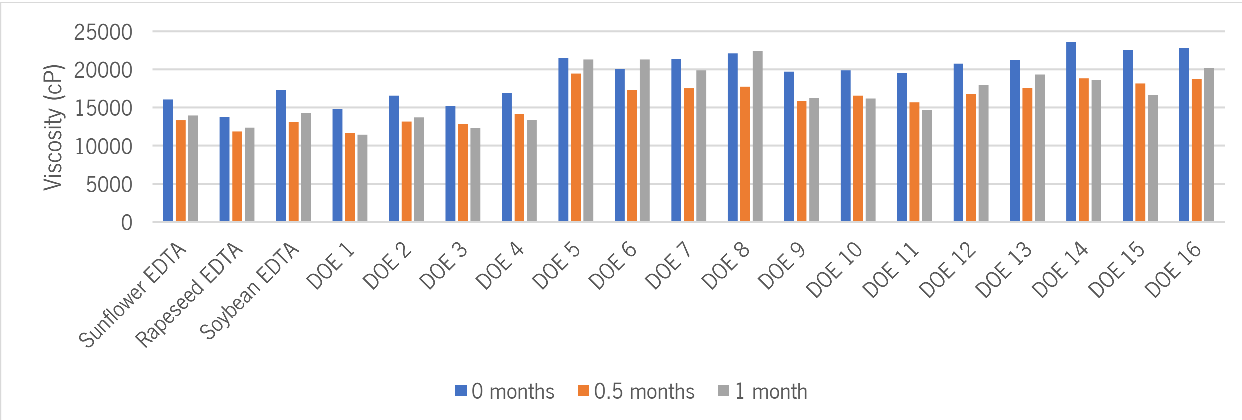



*Figure 4.8. Viscosity (in cP) of mayonnaises from the design of experiment for 1 month of storage at 30°C.*

Mayonnaises' particle size (Figure 4.9) identically to viscosity results were variable among samples.

Results appraisal by egg type present in samples ensued the discovery that, unrelatedly to the oil type, mayonnaises using LEY (i.e., DOE 1-4) had the smallest diameter (6.7 µm ± 14.9 %), followed by the samples with PEY (i.e., DOE 9-12) that had an average particle size of 13.5 µm ± 19.7 %, finally, formulations that included HSEY (DOE 5-8) and WE (DOE 13-16) exhibited the largest oil droplets (15.4 µm ± 15.4 % and 15.9 µm ± 13.5 %, respectively). The comparison of samples' particle size built upon oil type, in contrast with the afore factor, did not unveil an apparent association (min. RSD = 33.3%).

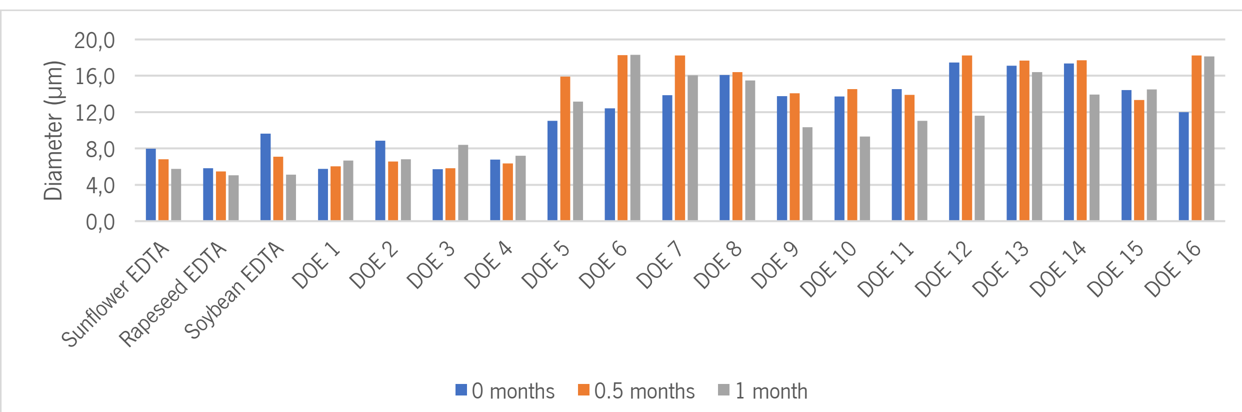



*Figure 4.9. Particle size (in µm) of mayonnaises from the design of experiment for 1 month of storage at 30°C.*

#### 4.2.1.2. Volatiles Profile

The high impact volatile compounds related to oxidation were tracked over time (Figure 4.10). Overall fresh mayonnaises had extremely low intensities of oxidation compounds and based on the factor "egg type" a trend was observed: mayonnaises with PEY (i.e., DOE 9-12) showed at least doubled intensities of oxidation compounds compared to HSEY samples, i.e., DOE 5-8, the trials with the second highest volatiles' intensity. Then, the samples showing the minimum intensities were the ones including WE (i.e., DOE 13-16), proceeded by mayonnaises with LEY (i.e., DOE 1-4). During storage time, as expected relative volatiles intensities increased, and results comparison based on egg type after 1 month of ASL remained approximately the same. Furthermore, the analysis of data built on oil type led to new findings, with the intensities for the oxidation compounds showing the subsequent behaviour in terms of oxidative stability: SB > HORS > (RS ≃ SF).

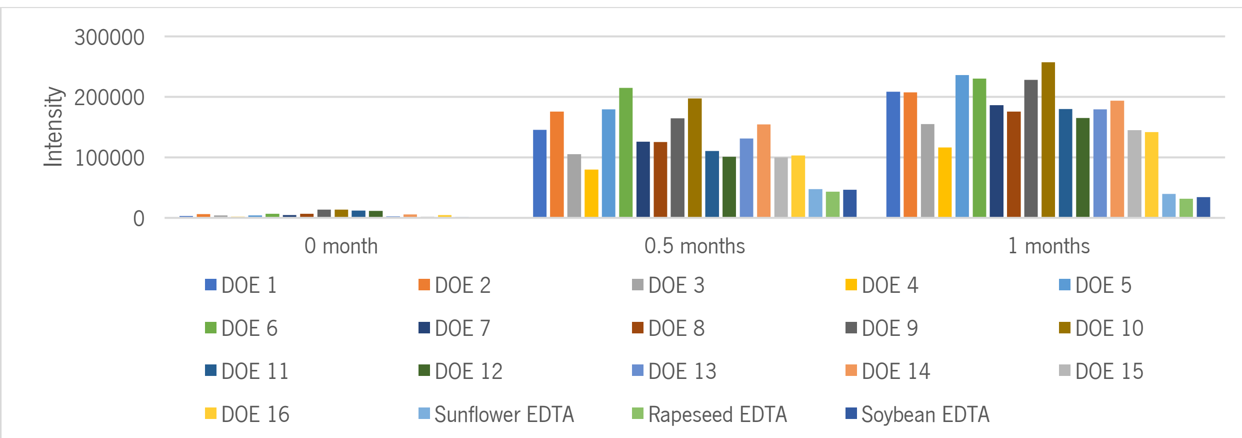



*Figure 4.10. Global intensity of selected oxidation compounds in DOE mayonnaises when fresh and after 0.5- and 1-month storage at 30°C.*

#### 4.2.1.3. Sensory Profile

Lastly, for the sensory profile (Appendix C – Table C.9), the results showed that in general fresh mayonnaises did not differ significantly from respective EDTA references. The exceptions being the "DOE 2", and all samples having HORS or PEY (i.e., DOE 3, 7, 9-12 and 15) for the "rancid oil taste", in addition, mayonnaises with HORS (i.e., DOE 7, 11 and 15) and "DOE 12" were significantly perceived as having a metallic taste. As noted in the previous section, at t = 0 months, mayonnaises with PEY showed the uppermost intensities of oxidation compounds, which can explain the significant perception of "rancid oil taste". Regarding formulations including HORS, the measured PV for the present study (Appendix A – Table A.4) highlight the lower quality of HORS when compared to the other oils, which might be leading to the worse sensory profile for the samples containing this oil. During storage time, as expected sensory scores increased, however, in opposition to former variables, with the sensory profile results no clear trend was detected for both factors, egg and oil type.

### 4.2.2. ANOVA and Design of Experiments

As samples with the same type of egg or oil exhibited similar patterns over the time, the ANOVA was applied to ascertain the significant factors and levels that impact the physicochemical and sensory profile of mayonnaises. The ANOVA method hypothesises that data follows a normal distribution, hence based on residuals analysis, the confidence of the ANOVA results was assessed. The normality plots of the residuals at 95 % confidence level for each studied response were shown in Appendix C – Figure C.5 and Figure C.6 –, all the plots reveal that residuals closely followed the reference line, assuring the normality of the data and subsequently the veracity of the ANOVA information.

#### 4.2.2.1. Effects on Physicochemical Profile

The pH, viscosity and particle size data were analysed and Table 4.6 presents the regression coefficients and respective probability values (p-value), as well as the equation for the regression models for each studied response considering only the statistically significant factors at a 95 % confidence level ($\alpha$ = 0.05).

*Table 4.6. ANOVA for the model of the physicochemical properties (responses): pH, viscosity and particle size*

| **Term** | **pH** | | **Viscosity** | | **Diameter** | |
|---|---|---|---|---|---|---|
| | **Coefficient** | **p-value** | **Coefficient** | **p-value** | **Coefficient** | **p-value** |
| Constant | 3.70750 | 0.000* | 17224 | 0.000* | 12.332 | 0.000* |
| Egg type | | | | | | |
| *LEY* | -0.06625 | 0.000* | -4518 | 0.000* | -5.067 | 0.000* |
| *HSEY* | -0.08375 | 0.000* | 4011 | 0.000* | 3.419 | 0.001* |
| *PEY* | 0.01500 | 0.111 | -964 | 0.015* | -1.758 | 0.036* |
| *WE* | 0.13500 | 0.000* | 1472 | 0.001* | 3.406 | 0.001* |
| Oil type | | | | | | |
| *SF* | 0.00625 | 0.481 | -148 | 0.654 | -0.698 | 0.355 |
| *RS* | 0.00375 | 0.669 | 231 | 0.489 | -0.243 | 0.742 |
| *HORS* | -0.00375 | 0.669 | -1337 | 0.002* | 0.162 | 0.826 |
| *SB* | -0.00625 | 0.481 | 1254 | 0.003* | 0.779 | 0.304 |
| $R^2$ | 97 % | | 97 % | | 90 % | |
| Model equation | y = 3.70750 - 0.06625 x LEY - 0.08375 x HSEY + 0.13500 x WE | | y = 17224 - 4518 x LEY + 4011 x HSEY - 964 x PEY + 1472 x WE - 1337 x HORS + 1254 x SB | | y = 12.332 - 5.067 x LEY + 3.419 x HSEY - 1.758 x HSEY + 3.406 x WE | |

* Significant at the 95 % confidence level. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.

The pH values were significantly dependent on the presence of egg types such as LEY, HSEY and WE. For the droplets' diameter and the viscosity, all egg types significantly impacted these properties; besides, the usage of HORS and SB were associated with significant changes in the latter property. As the $R^2$ was high (> 90 %), the linear regression models were statistically significant and reliable to forecast the physicochemical properties.

The main effect plots (Figure 4.11) represent the results of the regression analysis. The use of LEY or HSEY led to analogous pH values but a smaller pH than the overall average; the exchange of these ingredients for WE brought a significant increase of about 6 % of the pH values. A previous study by Kraft Heinz regarding the influence of different egg types, such as LEY, HSEY and PEY, in the mayonnaise revealed analogous pH for all the samples, not sustaining the pattern found in the present study.

Focusing on the viscosity variability (Figure 4.11. b)), in opposition to HSEY and WE, the usage of LEY and PEY was associated with less viscous samples, with the overall behaviour being as follows: LEY < PEY < WE < HSEY. Besides, the incorporation of HORS or SB in the formulations significantly decreased or increased the response, respectively. As viscosity of mayonnaise decreases, the average particle size is expected to become higher, i.e., larger droplets are produced due to coalescence (Depree & Savage, 2001). Unexpectedly, the variability of the oil's droplet size (Figure 4.11. c)) was positively correlated with the viscosity, thus a similar pattern was observed for droplet's diameter: LEY < PEY < (WE $\simeq$ HSEY).

Some studies have been done on the impact of egg yolk in O/W emulsions properties. A former Kraft Heinz investigation on the influence of LEY, HSEY and PEY, in the mayonnaise revealed that samples containing HSEY were slightly less viscous than samples with LEY and PEY, the previous samples showing similar viscosity. Lastly, the smallest to the largest particle size were linked to mayonnaises as follows: PEY, LEY and HSEY. Daimer & Kulozik (2009) compared the properties of emulsions with HSEY and LEY, showing that at pH 4, the consistency index was remarkably lower for samples with modified egg yolk (i.e., HSEY), which suggested that these samples would be less viscous than LEY emulsions; yet, in contrast to the prior Kraft Heinz study, HSEY samples had significantly smaller oil droplets. Ariizumi et al. (2017) investigated the impact of whole egg on the stability of mayonnaise discovering the lower physical stability of this mayonnaise, therefore being possible that compared to LEY emulsions, a lower viscosity is observed. Concerning the impact of the types of oil used in emulsions properties, an investigation has been done with sunflower oil and soybean oil emulsions discovering that differences in viscosity were not significant (Gu et al., 2009). For high oleic rapeseed oil and rapeseed oil, no studies were found on its effects on emulsions properties. The current study and the aforementioned investigations findings did not wholly align.

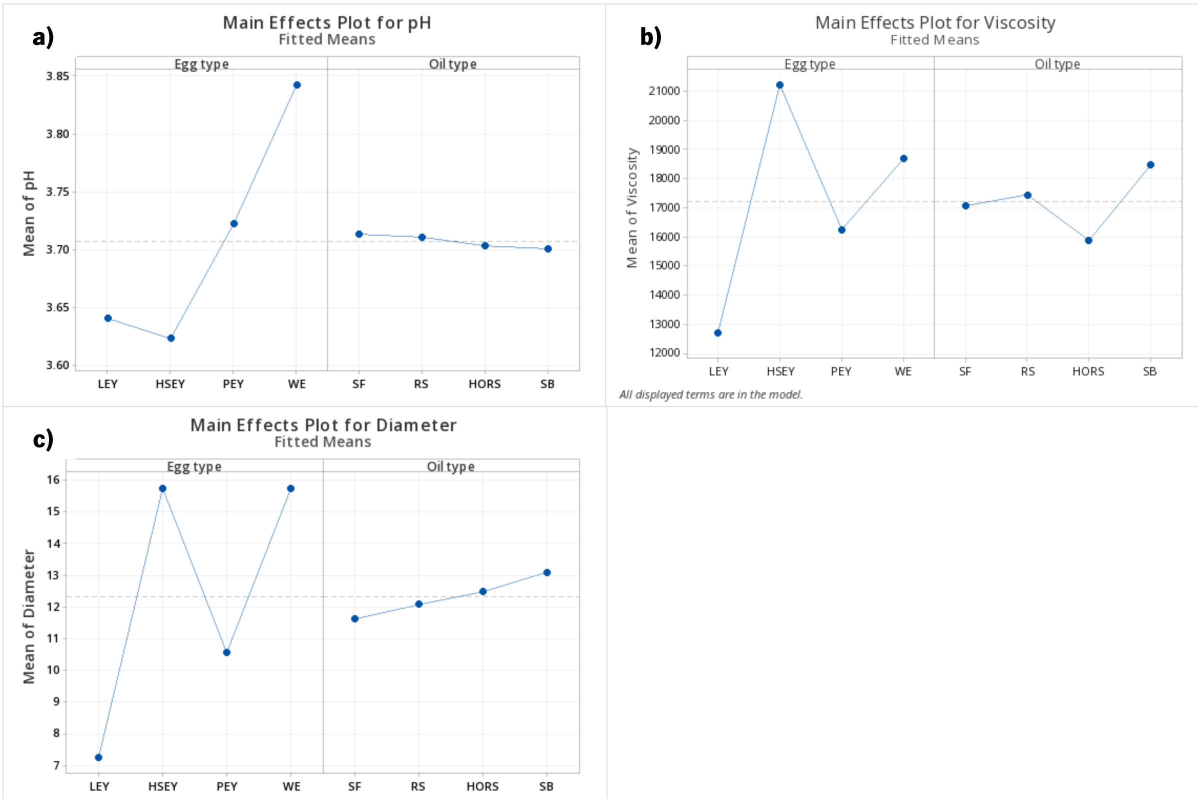



*Figure 4.11. Main effects plot for measured physicochemical properties (responses): a) pH, b) viscosity and c) diameter. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.*

As viscosity changes might interfere with oxidation rate and its perception, then considering that sample with LEY had the lowest viscosity, those samples might be associated with higher perception of lipid oxidation volatiles (Baines & Morris, 1987; Cook et al., 2003; Hollowood et al., 2002; Lethuaut et al., 2004; Moskowitz & Arabie, 1970; Pangborn et al., 1973; Štern et al., 2001; Vaisey et al., 1969; Weel et al., 2002). In turn, mayonnaises with HSEY might show the best sensory profile, i.e., low scores on oxidation related attributes. Similarly, to other responses the droplet size impact on O/W emulsions' oxidative stability has been investigated, leading to believe that changes in particle size can somewhat influence oxidation rate (Berton-Carabin et al., 2014; Costa et al., 2020; Gohtani et al., 1999; Hu et al., 2003; Jacobsen, Hartvigsen, Lund, Thomsen, et al., 2000; Osborn & Akoh, 2004; Uluata et al., 2016). Seeing as particle size was characterised by the following sequence: LEY < PEY < (WE ≃ HSEY), in juxtaposition to viscosity predictions, mayonnaises including HSEY and WE, may show the worst oxidative stability, on the contrary, samples with LEY can be linked to lower oxidation rates, further than PEY-mayonnaises.

In summary, further analysis are needed to define the impact of the ingredients in study, egg and oil type, on the properties of mayonnaise.

#### 4.2.2.2. Effects on Volatiles Profile

About the E-nose analysis, the total intensities of the high impact volatiles compounds were accounted, and ANOVA results were presented in Table 4.7. The volatiles intensities were significantly dependent on both egg type and oil type, on all the studied levels. The $R^2$ was high (> 90 %), the volatile profile of mayonnaises could be affected by the egg type in use, as well as the oil type included in the formulations.

*Table 4.7. ANOVA for the model of the volatiles intensities (response)*

| **Term** | **Volatiles intensities** | |
|---|---|---|
| | **Coefficient** | **p-value** |
| Constant | 187859 | 0.000* |
| Egg type | | |
| *LEY* | -16141 | 0.013* |
| *HSEY* | 19233 | 0.005* |
| *PEY* | 19771 | 0.004* |
| *WE* | -22863 | 0.002* |
| Oil type | | |
| *SF* | 25103 | 0.001* |
| *RS* | 34321 | 0.000* |
| *HORS* | -21270 | 0.003* |
| *SB* | -38154 | 0.000* |
| $R^2$ | 94 % | |
| Model equation | y = 187859 - 16141 x LEY + 19233 x HSEY + 19771 x PEY - 22863 x WE + 25103 x SF + 34321 x RS - 212270 x HORS - 38154 x SB | |

* Significant at the 95 % confidence level. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.

Figure 4.12 shows the overall mean of the volatiles' intensities, as well as the magnitude and direction of the effects that each factor has on the response. Compared to LEY, most common egg type, the inclusion of WE in mayonnaises led to a slight decrease (3 %) in the identified volatiles, in contrast, when HSEY or PEY were used in samples, the volatile profile was aggravated by around 24 %. In terms of the effects of the type of oils, the observed pattern was as follows: SB < HORS < SF < RS.

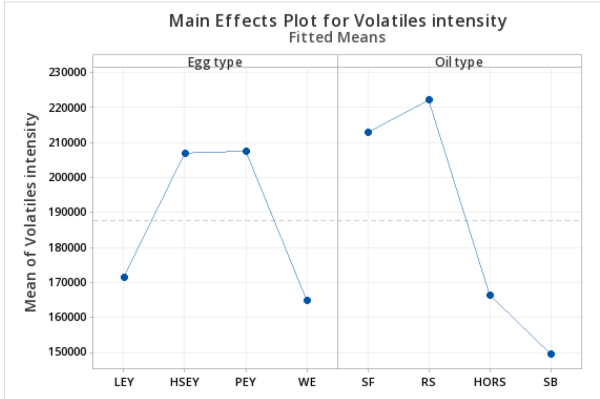



*Figure 4.12. Main effects plot for volatiles intensity. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.*

Daimer & Kulozik (2009) compared HSEY and LEY emulsions' properties, discovering that samples with the former had higher interfacial protein concentration, which might constraint oxidation by limiting lipids exposure to pro-oxidants. In a similar way, Kraft Heinz has studied the effect of egg in mayonnaise oxidation finding that HSEY-mayonnaises had higher emulsion and oxidative stability than samples with PEY or LEY, which were analogous. Ariizumi et al. (2017) investigated the impact of whole egg on mayonnaise finding the physical stability to be diminished, therefore increased oxidation rates were expected. Hence, the trend for samples' oxidative stability was expected to be as follows: HSEY > (PEY = LEY) > WE. Conversely, the current volatile profile showed diverging conclusions, as samples containing WE had the highest oxidative stability (i.e., lowest global volatiles intensities) and formulations with HSEY and PEY had the least oxidative stability.

The degree of unsaturation of fatty acids is an important factor to define the rate at which oxidation occurs; per each additional double bond, the oxidation rate can increase, approximately, 10 times. Thus, a lower level of unsaturation is desirable in the aspect of bulk oil stability (Belitz et al., 2009; DeMan et al., 2018). Pondering only on the bulk oil's behaviour, the expected oxidative stability were as follows: SF > HORS > RS > SB (Romanić, 2020; Woodfield & Harwood, 2017). However, the present findings did not entirely correlate with the anticipated pattern, as mayonnaises with RS had one of the highest volatiles intensities and similar intensities to SF samples, consequently implying analogous oxidative stabilities. Besides, the SB-samples which were estimated to present the overall highest volatiles intensities, i.e., lowest oxidative stability, instead showed the best resistance to oxidation. Waraho et al. (2011) investigated oxidation in O/W emulsions, discovering that oxidative stability was improved with higher levels of unsaturation. Then, accounting the average oil's composition (Table 2.4), it is possible that higher degree of tri-unsaturated fatty acids in soybean oil compared to sunflower oil, led to higher oxidation resistance of the former. The evaluation of the PV (Appendix A – Table A.4) of the fresh oils revealed the much inferior quality of HORS (PV = 3.29 mEq $O_2$/kg) compared to RS (PV = 1.60 mEq $O_2$/kg) and the other oils, so lipid oxidation compounds intensities during storage time might have been augmented, but not enough to lead the HORS-samples to perform worse than the mayonnaises with rapeseed oil.

In conclusion, the findings associated with egg type should impact the sensory profile of mayonnaises in a way that HSEY and PEY samples are expected to have significant oxidation being perceived. Regarding the factor "oil type", the sensory profile of samples containing RS and SF are expected to perform the worst, having much higher sensory scores for oxidation attributes than formulations with SB and HORS. Ultimately, the volatile profile suggests that a formulation with WE and SB would lead to the best sensory profile, hence a mayonnaise with the optimal combination of ingredients to prevent lipid oxidation.

#### 4.2.2.3. Effects on Sensory Profile

Regarding the sensory analysis, the scores for each oxidation linked attribute were investigated and an analysis of variance was applied (Table 4.8). The $R^2$ was acceptable (> 65 %) for the "rancid oil" and "metal" tastes, thus the models were statistically significant and fairly reliable to predict the sensory properties. The sensory profile of mayonnaises were significantly affected by some of the egg types in use, such as LEY and HSEY, as well as some of the oil types included in the formulations, for example, RS and HORS. At a 90 % confidence level, WE, SF and SB also significantly impacted the sensory profile of mayonnaise.

Based on the main effects plot for the significant sensory attributes (Figure 4.13), the "rancid oil" taste was the highest when using LEY, the standard egg for mayonnaise, and when it was replaced by WE or HSEY, it significantly improved the sensory profile, reducing the oxidation perception in around 70 %. Moreover, compared to the other oils the usage of RS significantly increased the "rancid oil" taste. When it refers to the "metal" taste, samples including HORS had significantly higher rancidity being perceived, in disparity with SF or SB-mayonnaises, which showed 4 times lower scores in this attribute.

*Table 4.8. ANOVA for the model of the sensory properties (responses)*

| Term | Rancid oil taste | | Metal taste | | Egg taste | |
|---|---|---|---|---|---|---|
| | **Coefficient** | **p-value** | **Coefficient** | **p-value** | **Coefficient** | **p-value** |
| Constant | 1.688 | 0.000* | 1.063 | 0.000* | 0.750 | 0.004* |
| Egg type | | | | | | |
| *LEY* | 1.563 | 0.002* | 0.437 | 0.143 | -0.250 | 0.479 |
| *HSEY* | -0.938 | 0.027* | -0.063 | 0.824 | -0.750 | 0.054** |
| *PEY* | 0.062 | 0.864 | -0.063 | 0.824 | 0.500 | 0.174 |
| *WE* | -0.688 | 0.085** | -0.312 | 0.281 | 0.500 | 0.174 |
| Oil type | | | | | | |
| *SF* | -0.438 | 0.250 | -0.563 | 0.069** | 0.250 | 0.479 |
| *RS* | 1.062 | 0.015* | 0.187 | 0.509 | -0.250 | 0.479 |
| *HORS* | -0.187 | 0.611 | 0.938 | 0.007* | -0.250 | 0.479 |
| *SB* | -0.437 | 0.250 | -0.562 | 0.069** | 0.250 | 0.479 |
| $R^2$ | 78 % | | 67 % | | 50 % | |
| Model equation | y = 1.688 + 1.563 x LEY - 0.938 x HSEY + 1.062 x RS | | y = 1.063 + 0.938 x HORS | | – | |

* Significant at the 95 % confidence level. ** Significant at the 90 % confidence level. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.

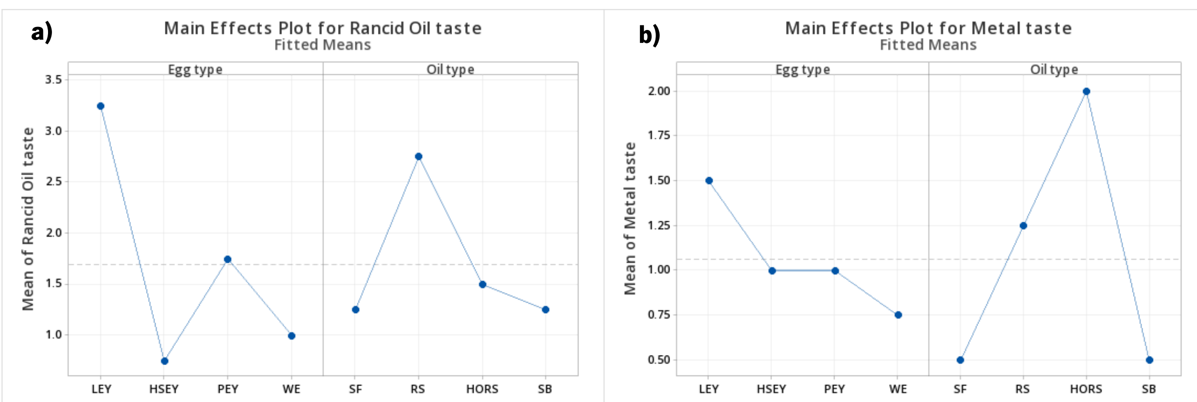



*Figure 4.13. Main effects plot for sensory properties: a) rancid oil taste, and b) metal taste. Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.*

LEY-mayonnaises had the lowest viscosity, thus higher perception of lipid oxidation volatiles was expected; additionally, mayonnaises with HSEY had been hypothesised to show the best sensory profile, so overall these predictions aligned with sensory profile results. Yet, the particle size projections were not observed.

Mayonnaise has multiple ingredients, that contribute to the particular flavour of the final product. However, there are ingredients with masking flavour effects that lead, for example, to lower perception of oxidation compounds (Kumar & Tanwar, 2011). Consequently, the volatile profile of a mayonnaise might not be entirely related to its sensory profile. Concerning the volatiles profile forecasts, unlike expected neither

HSEY nor PEY samples were correlated to the highest oxidation perception, however, the usage of WE in fact led to decreased scores in both “rancid oil” and “metal” taste attributes. Regarding the “oil type”, samples containing RS as hypothesised performed the worst in the “rancid oil” taste. Finally, the optimal ingredient combination to prevent oxidation, i.e., mayonnaise with WE and SB also aligned with the sensory profile data.

### 4.2.3. Optimisation test

The results of the factorial design enabled to ascertain the most reliable parameters affecting mayonnaise. To determine the formulations that lead to the highest oxidative stability, i.e., lower levels of oxidation and its perception, an optimisation test was carried out consisting of minimizing the parameters volatiles intensity, and sensory attributes such as “rancid oil” and “metal” tastes.

Table 4.9 shows the optimisation results, being determined that the possible best solution to reduce oxidation rate and its perception is formulating mayonnaise with WE in combination with SB. In opposition, the possible worst recipe to prevent oxidation, appears to be associated with samples that include the standard egg, i.e., LEY, and one of the most common oils, i.e., RS.

*Table 4.9. Optimisation test results showing the formulations solutions from best to worst*

| Solution | Egg type | Oil type | Metal taste Fit | Rancid Oil taste Fit | Volatiles intensity Fit | Desirability (%) |
|---|---|---|---|---|---|---|
| 1 | WE | SB | 0.1875 | 0.5625 | 126843 | 86 |
| 2 | HSEY | SB | 0.4375 | 0.3125 | 168939 | 75 |
| 3 | WE | SF | 0.1875 | 0.5625 | 190099 | 69 |
| 4 | PEY | SB | 0.4375 | 1.3125 | 169476 | 69 |
| 5 | WE | HORS | 1.6875 | 0.8125 | 143727 | 63 |
| 6 | LEY | SB | 0.9375 | 2.8125 | 133565 | 60 |
| 7 | WE | RS | 0.9375 | 2.0625 | 199318 | 52 |
| 8 | HSEY | HORS | 1.9375 | 0.5625 | 185823 | 51 |
| 9 | HSEY | SF | 0.4375 | 0.3125 | 232195 | 49 |
| 10 | LEY | SF | 0.9375 | 2.8125 | 196821 | 48 |
| 11 | PEY | HORS | 1.9375 | 1.5625 | 186360 | 47 |
| 12 | PEY | SF | 0.4375 | 1.3125 | 232733 | 45 |
| 13 | LEY | HORS | 2.4375 | 3.0625 | 150448 | 36 |
| 14 | HSEY | RS | 1.1875 | 1.8125 | 241414 | 33 |
| 15 | PEY | RS | 1.1875 | 2.8125 | 241951 | 29 |
| 16 | LEY | RS | 1.6875 | 4.3125 | 206039 | 26 |

Legend: LEY = Liquid egg yolk, HSEY = Heat stable egg yolk, PEY = Powdered egg yolk, WE = Whole egg/egg yolk mix, SF = Sunflower oil, RS = Rapeseed oil, HORS = High oleic rapeseed oil, SB = Soybean oil.

Summarizing, it can be established as a result of the design of experiments, that egg and oil play a role in the oxidation rate of mayonnaise; the investigated types of ingredients were capable to significantly impact oxidative stability of mayonnaise samples, i.e., maximising or minimising volatile compounds production, as well as influencing the sensory perception of oxidation in mayonnaise. However, the inferences should be confirmed by proceeding the study and analysis of the data from the 2 months storage of samples.

## 5. Conclusion

The current research aimed to find an efficient solution to replace EDTA as an ingredient in mayonnaise, assuring analogous product performance during shelf-life. Accordingly, two different workstreams were carried out, viz.: the natural antioxidant testing, and the ideal recipe design.

In the workstream involving the natural antioxidants testing, generally, with increased storage time mayonnaise had diminished viscosity and greater droplet size, as well as higher concentrations of volatile compounds (e.g., 2,4-heptadienal), and a deteriorated sensory profile. The extract L (i.e., rosemary and green tea extract) when incorporated in HSG and Vegan mayonnaises, after 3 and 4 months of storage at 20°C and 30°C, respectively, did not enhance the volatile and sensory profile of mayonnaises, compared to EDTA free samples; hence, suggesting that lipid oxidation rate was not significantly affected. The study with the rosemary and spinach extract gave insight in the impact of both versions of the extract in the properties of mayonnaise, as in terms of sensory profile, the extract X version 2 had slightly lower oxidation perception than the original extract. Moreover, data revealed how the pH influences the functionality of the extract X; at pH 4.0 in the presence of egg yolk, the 2,4-heptadienal concentration was lower than at pH 3.5, due to the less active form of the natural antioxidant being at pH < pKa values. Interestingly, Vegan samples with a pH 3.0 exhibited overall the smallest amount of high impact volatile compounds, alike EDTA samples. Thus, one can infer the relevance of the contribution of pro-oxidants (i.e., iron) originated from egg yolk, in the lipid oxidation rate observed in O/W emulsions. Nonetheless, input from the 12 months storage time are still to be evaluated, so conclusions might suffer some changes. In summary, at the present time results point only to the ability of the rosemary and spinach extract having a significant radical scavenging or metal chelating activity, with the Vegan mayonnaise being the greatest matrix to implement the extract, in order to have an effective alternative to EDTA in the prevention and control of lipid oxidation.

The ideal recipe design study allowed to gather information about factor impact on mayonnaise oxidation. The use of different types of egg and of oil significantly impacted the viscosity, volatiles intensities and the "rancid oil" taste of mayonnaise. Besides, the pH and particle size were significantly influenced by the type of egg, and finally, the "metal" taste was affected by the oil type in the formulations. In terms of physicochemical profile, pH varied only slightly according to the egg type in the product. Besides, the viscosity and particle size presented similar patterns, with LEY and HSEY being responsible for, respectively, the significantly inferior and superior values of these properties. Concerning the volatiles profile, formulations with HSEY, WE, SB and SF had unexpected effects. Despite the increased interphase density of HSEY mayonnaise, and the lower level of unsaturation in SF, the data showed great amounts of high impact volatile compounds. Inversely, an enhanced volatile profile was associated with WE, that possibly confers lower physical stability to O/W emulsions, and with SB, the bulk oil with the lowest oxidative stability. Hence, results are in line with a previous investigation, whose findings revealed that in case of O/W emulsions the oxidative stability is positively correlated with the levels of unsaturation  Waraho et al., 2011. Lastly, the sensory profile improved around 4 times when common ingredients, such as LEY and RS, were replaced by WE or HSEY, and SF or SB. Ultimately, an optimisation test was carried out signalling that the "ideal recipe" to curtail lipid oxidation, i.e., minimal volatiles intensities and scores for the "rancid oil" and "metal" attributes, would include WE and SB. However, the findings should be confirmed after the 2 months storage data analysis is accomplished.

This study shed some light into the future directions, especially concerning the factors affecting lipid oxidation in mayonnaise, and how to control it. Additional factorial designs should be done to determine other factors affecting lipid oxidation in mayonnaise, and a final design should be carried out to establish a final optimal formulation to control lipid oxidation. For further studies, it is proposed to use oils with analogous and the lowest peroxide values, of more relevance when different oils are in analysis, in this way allowing for a more accurate comparison of the oxidation rate. In addition, composition analysis of both oils and emulsifiers might be of great importance to understand, for example, the definite level of lipids unsaturation, or the concentration of pro-oxidants (e.g., iron), and its impact on lipid oxidation development. Moreover, it is advised to measure the surface charge (zeta-potential) of oil droplets, especially since interphase charge might vary due to new ingredients or because of the composition and properties of mayonnaise, further influencing oxidation of lipids. Furthermore, concerning natural antioxidant tests, it is recommended the use of a consumer evaluation to determine the acceptability of mayonnaises containing alternatives to EDTA; besides, regarding the factorial designs, the use of an official trained panel is suggested, so more precise and exact scores are obtained. Finally, the scale up of mayonnaise production can also bring better insight on lipid oxidation in consumer available products, since factory equipment produces emulsions with better physical properties (i.e., higher viscosity, smaller particle size, and better globule structure) than the pilot plant equipment.

**Part B - Development of a New Plant-Based and Gluten-Free Alternative to Fish Products**

## 6. Introduction

Food provides nutrients essential for obtaining energy necessary for all body functions and to promote growth. In this vein, the food industry plays one of the most vital roles in society. Satisfaction of consumer need is one of the main goals of this industry, followed by being able to provide safe products and their nutritional information, and ultimately to maintain commercial viability. To meet these goals, product reformulation and development of new products is an essential part of the industry (Mettler, 1986).

The lifestyles and dietary patterns of consumers are deeply influenced by factors such as culture, politics, environment, demographics, and socioeconomics (Arenas-Jal et al., 2020). Since the beginning of the twenty-first century, the world has been seeing immense advances in technology, accordingly, in industrialized countries, consumers are much more aware of global issues such as climate change, food waste, animal abuse, among others (Arenas-Jal et al., 2020; Ayres & Williams, 2004). Also, the understanding and concern with food components and their properties has risen (Arenas-Jal et al., 2020; Asioli et al., 2017). Moreover, the interest in locally produced and traditional products have been expanding among consumers, as these products are perceived as more genuine, tastier, healthful, and contribute to the local economy (Chambers et al., 2007; Pieniak et al., 2009; Vlontzos et al., 2018). Thus, the food consumption is presently veering towards traditional, healthier, plant-based (PB) and sustainable products (Arenas-Jal et al., 2020; Asioli et al., 2017; Portugal Foods, 2021).

For a few years now the drive towards PB products has been increasing. Currently, the global PB protein market (e.g. meat, fish, dairy, egg) amounts to 11.3 billion US$ and is expected to surpass 22.5 billion US$ by 2032, exhibiting a compound annual growth rate (CAGR) of 7.2%, (Future Market Insights, 2022). Plant-based alternatives to seafood and fish compose only around 1% of the alternative meat market; nonetheless, in 2021, the former products outpaced the “meat” sales. The projection for the global PB fish market is around 28% CAGR from 2021 to 2031, thus being a category with great interest for innovation (Emma Ignaszewski, 2022; Fact.MR, 2021).

Additionally, diseases caused by gluten ingestion, as celiac disease, have been rising in prevalence, possibly due to advances in science and technology, which allows the patients to be more effectively diagnosed (Thompson et al., 2005; Vici et al., 2016). Hitherto, the global gluten-free market amounts to 6.4 billion US$ and is estimated to grow at a CAGR of 9.8% from 2022 to 2030 (Grand View Research, 2022).

More and more consumers are in the search for healthy gluten-free foods and beverages that will not prompt intestinal damages, therefore pushing companies to innovate and supply gluten-free formulations with great characteristics.

Nowadays, PB meat and fish alternatives are able to reach non-vegetarians, vegetarians and vegans (Choudhury et al., 2020; Toribio-Mateas et al., 2021). Consumers are more than ever trying to be conscious of their food habits and the ingredients present in what they eat. For example, PB products are usually perceived as intrinsically healthy, and in some parameters they are, on the contrary, gluten-free foods' formulations have higher sugar and fat contents and low fibre and protein amounts (Kupper, 2005; Öhlund et al., 2010; van Hees et al., 2015; Zuccotti et al., 2013). Consequently, optimizing the nutritional profile of these types of products to meet consumer expectations and to contribute to healthy diets can be of great interest (Boukid et al., 2022; Curtain & Grafenauer, 2019; Harnack et al., 2021).

In this regard, the conception of new plant-based fish products, which do not contain gluten, and that are great from a nutritional and an organoleptic perspective might be the future of the plant-based products market. Thus, research is being conducted to develop a *patanisca* based on raw materials of vegetal origin.

### *6.1. Aim*

Current food trends and market research have driven the current project to aim to develop a new plant-based and gluten-free alternative to fish products; additionally, showing a healthier nutritional profile and great organoleptic characteristics. In this vein, the following steps are proposed: (1) Concept development; (2) Formulation; (3) Sensory and Nutritional evaluation; and (4) Product modification and evaluation. The planned workflow intends to provide a way of designing and testing a concept for a plant-based fish product, so that the first prototypes are produced, and then analysed by an intern sensory panel. In case of failed attempt, information should be gathered, so that other prototypes may be developed and evaluated. In contrast, if prototype meets the standards, slight modifications may be needed, otherwise the final product may be proposed for future shelf-life studies and other analysis.

# 7. Literature review

## *7.1. Food & the 21st century*

In the past century, the food and agricultural industries significantly contributed to the reduction of world hunger and malnutrition, by generating an abundant supply of inexpensive, tasty, convenient and safe foods. However, modern practices have also been deemed harmful, thus why both people and the planet are dealing with some major health and environmental problems (Kazir & Livney, 2021; McClements & Grossmann, 2021a, 2021b). First, global population is growing remarkably, with projections pointing to around 9.7 thousand million in 2050 and 10.4 thousand million by 2100 (*World Population Prospects 2022: Summary of Results*, 2022). Besides, due to increasing economic power of society, animal-based products became an integral part of human diets, and, compared to growing crops intended for direct consumption, rearing livestock for food as well as marine farming often leads to significantly greater usage of natural resources. Consequently, there is a pressure to produce higher quantities of great quality food, that negatively impacts the environment and leads to the loss of biodiversity, escalates greenhouse gas emissions (i.e., pollution), global warming and other related effects (Kazir & Livney, 2021; McClements & Grossmann, 2021a, 2021b). Second, it has been observed a rise in chronic diseases, cancer and metabolic syndrome, all related to unhealthy lifestyles, i.e., unbalanced nutrition and insufficient physical activity (Kazir & Livney, 2021; McClements & Grossmann, 2021a). For instance, because of amplified consumption of meat not only in developed countries but also in developing countries. Meat is a good source of protein, providing essential amino acids and vitamins such as vitamin B, vitamin A, zinc, and iron. Nevertheless, its richness in saturated fat and cholesterol, has led meat to being linked with chronic diseases such as obesity, type 2 diabetes, cardiovascular disease, and different types of cancer (Boada et al., 2016). In this vein, as a result of the health, environmental and also ethical concerns, the shift from animal- to PB diets is rapidly becoming more typical, thus the food industry has been increasingly trying to fulfil the consumer demands. It is worth noting that moving towards PB diets has different degrees of impact on the environment, depending on the geographic location and climate, foods are subjected to; the proteins' nutritional value; and the types of plant and animal products that are compared. For example, rocky hills, in contrast with livestock production, are one of the places where it is not feasible to crop farming (McClements & Grossmann, 2021a).

### 7.1.1. Plant-based diet

The term "plant-based diet" is commonly attributed to dietary patterns that focus on the consumption of PB foods, with little to no intake of products of animal origin (Silva et al., 2015). All these diets share its consumption of fruits, vegetables, fungi, cereals, nuts, seeds, seaweed and microalgae, and the exclusion of all direct animal products, such as seafood, meat, insects, meat broth, gelatine, lard and tallow. The main differentiating factors being the inclusion of eggs and/or dairy. In this way, the PB diet can range from lacto-ovo vegetarian (includes dairy and eggs), lactovegetarian (includes dairy), ovovegetarian (includes eggs) and vegan (i.e., strict vegetarian) (Boukid et al., 2022; Kent et al., 2022; Silva et al., 2015).

Overall, inappropriate choices in every lifestyle can cause problems concerning health (Dwyer, 1999). Still, a more plant-based lifestyle, characterised by a higher intake of fresh or minimally processed foods, e.g., vegetables, fruits, whole grains and nuts, has been linked to lower prevalence of obesity (Appleby et al., 1998; Ledoux et al., 2011; Orlich & Fraser, 2014; Singh & Lindsted, 1998), diabetes (Carter et al., 2010; Orlich & Fraser, 2014; Snowdon & Phillips, 1985), cancer (Catsburg et al., 2015; Fraser, 1999; Key et al., 2014; Orlich et al., 2015; Snowdon & Phillips, 1985; Thorogood et al., 1994; Turner-McGrievy et al., 2015), as well as increased longevity (Fraser, 1999; Orlich et al., 2013; Orlich & Fraser, 2014). In spite of the raw materials' origin, if highly processed products are the base of the day-to-day meals, then the nutritional benefits are lost because of the frequent high levels of saturated lipids, added sugars and salt (Herpich et al., 2022; McClements & Grossmann, 2021a).

The movement towards PB foods has given more space for the food industry to increase the effort put in the exploration of the versatile non-animal raw materials and create innovative plant-based products. The segment of plant-based foods has been steadily growing, with most investigations focused on designing products that mimic the texture, structure and organoleptic characteristics of animal-based options, such as meat, seafood, eggs and dairy. Along these lines, the available PB meat and fish alternatives are able to reach a wide range of lifestyles, for example, flexitarians (includes dairy, eggs, meat and seafood scarcely), pescatarian (includes dairy, eggs and fish), vegetarians and vegans, as well as people who consume "Kosher", as there is no animal killing involved in the process of getting the products (Boukid et al., 2022; Kent et al., 2022; Silva et al., 2015).

Currently, most meat and fish replacers are constituted by vegetal ingredients, e.g., pea, wheat, soybeans (Table 7.1), that are processed to produce extracts and/or derived ingredients (Choudhury et al., 2020; Toribio-Mateas et al., 2021). Ideally, non-animal analogues and their counterparts would be nutritionally equivalent, in this sense the health benefits associated with, for example, fish consumption, which is rich in omega-3 PUFA (e.g. eicosapentaenoic and docosahexaenoic acids), would also be seen in PB seafood products (Emery et al., 2016). Harnack et al. (2021) focused on the measurement of energy, macronutrients, fatty acids, vitamins and minerals on PB ground beef alternative products. Despite some limitations on the brand selection, the investigation concluded that regrettably most of the products contained less protein, zinc, and vitamin B12 than ground beef, on the other hand most had lower levels of saturated fats, and greater dietary fibre content. Protein quality was not assessed, but plant protein commonly has lower biologic value than the one from animal sources (Harnack et al., 2021). Another study, on seafood alternatives nutritional comparison to the conventional products, revealed that the analysed nutritional profiles were tremendously variable. On the one hand, a selection of products were comparable in terms of lipids, SFA, carbohydrates and protein contents, also showing lower salt content. On the other hand, a portion of products revealed higher calories, lipids and salt contents, as well as lower protein contents (Boukid et al., 2022).

*Table 7.1. Plant-based seafood alternatives on the market (Kazir & Livney, 2021)*

| **Product type** | **Main Ingredients** |
|---|---|
| Caviar | Seaweeds |
| Fish burgers, fish cakes, crab cakes and tuna chunks | Peas, chickpeas, lentils, soy, fava beans and navy beans |
| Fish filet and crab cakes | Soy, wheat and potato |
| Fish fingers, tuna pate, fish cakes and smoked salmon | Soy, potato, konjac, wheat |
| Shrimp | Seaweed |

### 7.1.2. Gluten-free diet

Disorders linked to gluten encompass ailments like celiac disease (CD), gluten allergy, non-celiac gluten sensitivity, among others. Celiac disease is an autoimmune disorder resulting from genetic and/or environmental factors, characterised by small intestinal enteropathy (Catassi et al., 2022; Koehler et al., 2014a; Richardson et al., 2022). It is triggered by the ingestion of gluten, and it is one of the most common chronic diseases, with an estimated prevalence between 0.5% and 2% worldwide and a growing incidence; however, since symptomatic CD is only a minority, many patients remain undiagnosed due to atypical, minimal or non-existent complaints (Catassi et al., 2022; Koehler et al., 2014a).

Gluten is a term used to designate the storage proteins of wheat, barley and rye. These proteins are named prolamins and contain a high proportion of proline and glutamine residues (Catassi et al., 2022;

Koehler et al., 2014c). In part because of the unusual repetitive amino acid sequences, gastric and pancreatic enzymes are unable to completely degrade these proteins. In celiac individuals, the gluten peptides are going to bind to enterocytes receptors, eventually crossing the intestinal epithelium. Then, after deamination the negatively charged peptides are endocytosed by antigen-presenting cells (e.g., macrophages and dendritic cells), leading to activation of gluten-specific CD4 T cells with subsequent initiation of an inflammatory response (Dieckman et al., 2022; Gandini et al., 2021; Richardson et al., 2022). Ultimately, the release of cytokines and other mediators of inflammation causes injuries in the small intestine mucosa, which results in partial or total villous atrophy, crypt elongation and hypercellularity, and it is linked to a deficient absorption of essential nutrients and vitamins, such as folate and iron (Koehler et al., 2014a; Richardson et al., 2022).

Presently, a gluten-free diet (GFD) is the only effective treatment for celiac disease, nonetheless investigations are being carried out in order to find other viable routes (C. P. Kelly et al., 2021; Koehler et al., 2014d; Lähdeaho et al., 2019; Schuppan et al., 2021). CD patients may consume a wide range of common foods such as meat, fish, milk products, vegetables, and fruits, excluding from their meals any foods containing or contaminated with gluten, for example, wheat, oats, rye, barley, puddings, sauces and soups (Koehler et al., 2014c; Polo et al., 2020).

The GFD has been growing in its popularity, thus gluten-free products are now ubiquitous, and commonly replace ordinary wheat-based foods like bread, pasta, cakes, with gluten-free options made of grains (e.g., rice, corn) or pseudocereals (e.g., quinoa, buckwheat and amaranth). A strict GFD means that the daily intake of gluten should be less than 20 mg, in comparison an average person ingests about 20000 mg of gluten (Koehler et al., 2014b). Gluten proteins lack in some essential amino acids, consequently having a low biological value (Hoffman & Falvo, 2004). Thus, replacing gluten by other proteins might not be unfavourable. However, despite divergent results among authors, usually CD patients following a GFD have a tendency towards an unbalanced intake of macronutrients, i.e., carbohydrates, lipids and protein, as well as reduced intake of certain essential micronutrients, such as B6, B9 and B12 vitamins, calcium and iron (Koehler et al., 2014b; Polo et al., 2020). In general, a GFD leads to a higher intake of sugars, contrastingly, dietary fibre daily consumption is rather low, possibly due to gluten-free recipes frequently using gums, starches and/or refined flours, to achieve similar textures to original food products (Thompson et al., 2005; Vici et al., 2016). Additionally, protein and fat intake is lower and higher, respectively, in CD individuals compared to the normal populations (Kupper, 2005; Öhlund et al., 2010; van Hees et al., 2015; Zuccotti et al., 2013). These findings have led the gluten-free industry to try to develop nutritionally richer products by introducing naturally rich raw materials, like legumes and pseudocereals, as well as adding cellulose, psyllium husk, bamboo fibre, beetroot fibre, among others (Koehler et al., 2014b). Nevertheless, the consumer is not always pleased with the final flavour and texture of these gluten-free foods. Thus, highlighting the existing food industry challenge of supplying nutritionally balanced gluten-free formulations with great organoleptic characteristics.

### 7.1.3. Healthy diet

The determinants of health go beyond nutrition, encompassing factors such as lifestyle, genetics and environment (Patwardhan et al., 2015; World Health Organization, 2017). Notwithstanding, nutrition and lifestyle are easily adjustable elements of the day-to-day, meaning that leading an unbalanced diet, the lack of physical activity, the high levels of stress, among other factors contributes to the heighten prevalence of many health conditions (e.g., obesity, diabetes, heart disease); contrastingly, healthy and balanced dietary patterns and regular exercise minimize the risk of the same diseases (Appleby et al., 1998; Carter et al., 2010; Dwyer, 1999; Ledoux et al., 2011; Orlich & Fraser, 2014; Patwardhan et al., 2015; Schneiderman et al., 2005; Singh & Lindsted, 1998; Snowdon & Phillips, 1985). In sum, food plays a crucial role in the maintenance and improvement of health.

A healthy diet implies an adjustment of the dietary patterns to the individuals, their environment and stage of life, in order to provide all essential quantities of nutrients, as well as of energy and fluids. The principles governing this diet are adequacy, balance and moderation, hence breaking one of the pillars possibly conducts to malnutrition (i.e., undernutrition, overweight and obesity). In view of this, nutritional guidelines have been created globally so the population is educated with respect to a proper diet (Dwyer, 1999; Kauffmann, 2016; Skerrett & Willett, 2010). In Portugal, the current dietary recommendations are in line with the advice of the World Health Organization (WHO) as well as with the European Union (EU) legislation (Candeias et al., 2005; European Parliament & Council of the European Union, 2011; World Health Organization, 2020). The total energy expenditure (TEE) can be defined as the overall energy expended daily by an individual considering its basal metabolism and physical activity, among other factors. Under regular circumstances the TEE will be fully provided by consumption of food (Kreymann et al., 2009). Considering an average healthy adult, the daily energy intake should round the 2000 kcal, distributed over 5 meals. Table 7.2 shows the nutritional recommendations according to the TEE, and Table 7.3 presents the reference intake (RI) values for energy and the main macronutrients (Candeias et al., 2005; European Parliament & Council of the European Union, 2011; World Health Organization, 2020). Moreover, it is noteworthy that, albeit the total energy intake, the minimum amount of fibre ingestion per day is 25 g.

*Table 7.2. Total energy expenditure (TEE) distribution per macronutrients and per recommended daily meals* (Candeias et al., 2005)

| | **Percentage of TEE** |
|---|---|
| **Macronutrients** | |
| Carbohydrates | 55 – 75 |
| Fats | 15 – 30 |
| Proteins | 10 – 15 |
| **Meal** | |
| Breakfast | 15 |
| Morning snack | 5 |
| Lunch | 35 |
| Afternoon snack | 15 |
| Dinner | 30 |

*Table 7.3. Reference intake values for energy and selected macronutrients of an average adult (Candeias et al., 2005; European Parliament & Council of the European Union, 2011)*

| | **Reference intake** |
|---|---|
| Energy | 8400 kJ / 2000 kcal |
| Fat | 70 g |
| Saturates | 20 g |
| Carbohydrate | 260 g |
| Sugars | 90 g |
| Protein | 50 g |
| Salt | 6 g |

## 8. New product development

The society and its surroundings (e.g., products, technologies, legislations) change, which means that the products' acceptance by consumers fluctuates over time impacting its life cycle; however, now more than ever the variations of consumers' requirements occur at a great speed, shortening the life cycle of the product (Azanedo et al., 2020; Świąder & Marczewska, 2021). After being launched, the sales volume is low as consumers are starting to get to know the product. Then, starts a period of a high number of sales due to repurchasing and new buyers, followed by a slight decline in sales until a period of stagnation arrives. Lastly, the sales volume drops because of new products being introduced in the marketplace (Fuller, 2011). Therefore, the development of new products as, for example, those using alternative protein sources, as well as better formulations, or new/improved processes, that stem from the competitiveness amid businesses, are the key element for companies to answer the necessities of the consumer and to prosper (Fuller, 2011; Świąder & Marczewska, 2021).

New product development (NPD) is a complex and potentially risky process, linked to the concept of innovation, that allows an idea to become a product at the marketplace. The success of a new product relies on several factors (Table 8.1), since the product has to meet the consumers' expectations, has to reach the appropriate number of buyers at a competitive price and, finally, has to be profitable to sell. Thus, meaning that NPD requires capital investment and encompasses many counterparts such as marketing, finance, manufacturing and design (Azanedo et al., 2020; Gao & Bernard, 2018; Świąder & Marczewska, 2021). To potentiate the product's chances, the NPD process consists of many stages, from bibliographic research that evaluates the idea or concept and its potential; furthermore, it demands thorough planning of the product development since its first prototype until the final product (Fuller, 2011; Świąder & Marczewska, 2021).

*Table 8.1. Common reasons for the success of new products. Adapted from* Fuller (2011)

| **Successful new product** | |
|---|---|
| - Good management of NPD process | - Good market analysis |
| - Innovation drives the product | - Clear marketing strategy |
| - Coworking of business and technology functions | - Clear benefit to consumer |

Consumers understanding and concern with food ingredients and their functions has been emerging, accordingly nowadays their preferences are directed to products that are healthier and plant-based (Arenas-Jal et al., 2020; Asioli et al., 2017; Portugal Foods, 2021). Yet, PB fish alternatives make only a small part of the marketplace, and several PB products are lacking in their nutritional profile to be considered healthy and balanced (Boukid et al., 2022; Curtain & Grafenauer, 2019; Harnack et al., 2021). Furthermore, with the better education practices regarding celiac disease and the improvement of its diagnostic tests, more and more individuals are being diagnosed every day. Regrettably, gluten-free products are available but just in a limited range and at a higher price; additionally, consumers are many times unsatisfied with the quality (especially sensory) of the current products compared to the traditional (i.e., with gluten) counterparts (A. L. Kelly et al., 2008).

In Portugal, cuisine changes slightly from region to region, however a strong food culture is present everywhere and many influences can be perceived in the traditional dishes. Prior to Portugal's foundation in 1143, for centuries other cultures had been exposing "Portuguese" people to new foods. The Roman occupation left lasting imprints such as wine and olive oil production, and introduced wheat, onions and garlic to agricultural activities. Later on, the Moors brought rice, almonds, figs, lemons and oranges (Dias, 2022; Ministry of Foreign Affairs, n.d.). During the 15th and 16th centuries, Portugal had a principal role in the Age of Discoveries; many explorers went on long maritime expeditions, with that discovering and colonizing parts of Africa, the Middle East, Asia, the Americas, among others. Therefore, coming back with several nuts, spices, exotic fruits, vegetables and grains, that until today Portuguese are very fond of (Nicolle, 2012).

Portuguese cuisine uses various seafood species, such as sea bass, clam, sardine and cod. In 2016, Portuguese seafood consumption (56.8 kg/capita/year) was more than double the European average (24.9 kg/capita/year); thus, consumers have been acknowledged has one of the highest seafood consumers in Europe (Szalaj et al., 2021). This is the result of Portugal's history, as the proximity to the ocean and the marine institution have always been present, thus leading seafood products to become a part of tradition, and the seafood industry to have a vital role that is still maintained (Almeida et al., 2015; Szalaj et al., 2021).

*Pataniscas* are a famous traditional savoury fritter prepared using a batter that includes cod, wheat flour, onion, egg, parsley, salt, pepper and milk (or the water used to cook the cod). In recent years, some innovations have happened, for example, formulations of *pataniscas* with other seafood (e.g., tuna, hake, shrimp) and vegetables, but also *pataniscas* that are prepared in the oven instead of being fried. Nevertheless, the marketplace has yet to see a *patanisca* that is free of gluten and/or plant-based (i.e., vegan) and has a good nutritional profile.

As per the nutritional recommendations for an average adult's lunch (i.e., 35% of the TEE), this meal should apport 700 kcal (Table 7.2 and Table 7.3), and the energy contribution for the meal concerning fat, carbohydrate and protein can be, respectively, 30%, 55% and 15%. Hence, generally a balanced lunch would be composed, approximately, of 23 g, 96 g and 26 g of fat, carbohydrate and protein, correspondingly (Candeias et al., 2005; European Parliament & Council of the European Union, 2011; World Health Organization, 2020). A portion of *pataniscas* can be served as a starter/snack or as a main course, subsequently it should be a source of protein. Following the Portuguese Nutrition Association guidelines, an example of a healthy lunch is composed of a vegetable soup, a turkey breast (90 g) with spinach and rice, followed by a small apple; in sum, the meat is the principal source of protein, apporting around 20 g of protein (Cordeiro & Bento, 2011; Instituto Nacional de Saúde Doutor Ricardo Jorge, n.d.). Therefore, it is a goal to reach the former value with the new product development, as this can be one of the hardest nutrients to consume in adequate amounts when following a plant-based and/or gluten-free diet. Besides, due to the health effects of nutrients such as fibre, fats, saturated fats, sugars and salt, then the aimed quantity of fibre is a minimum of 17.5 % – half of the target amount for a lunch that represents 35 % of the TEE – of the RI value, as well as a maximum of 17.5 % of the RI values for composition in fats, saturated fats, sugars and salt (Candeias et al., 2005; European Parliament & Council of the European Union, 2011; World Health Organization, 2020).

In conclusion, the goal is to develop a *patanisca* which is plant-based and gluten-free, that follows the nutritional objectives described in Table 8.2.

*Table 8.2. Recommended nutritional macronutrients target for a portion of prepared new plant-based and gluten-free pataniscas*

| | **Quantity (g)** |
|---|---|
| Fat | < 11.9 |
| Saturates | < 3.4 |
| Carbohydrate | < 44.2 |
| Sugars | < 15.3 |
| Fibre | > 4.2 |
| Protein | > 20 |
| Salt | < 1.0 |

### *8.1. Plant-based & Gluten-free Ingredients*

As to develop a particular food product, it is important to pinpoint an adequate mixture of ingredients.

Traditional *pataniscas* include cod, wheat flour, onion, egg, parsley, salt, pepper and milk (or the water used to cook the cod). For instance, cod is the main source of protein, texture and flavour, wheat flour and egg are sources of carbohydrates, protein and/or fats, and act as a thickening and structural agents, finally, the remaining ingredients are incorporated to give the distinctive flavour to the *patanisca*. Also, due to the frying process of the batter, *pataniscas* have a specific appearance, texture and taste, aside from absorbing considerable amounts of oil that impacts the nutritional profile of the final product. In this vein, the search for appropriate plant-based and gluten-free ingredients can be based on the original ingredients' function.

### 8.1.1. Cod substitute

In a traditional *patanisca,* the presence of cod leads to the fritter having some areas of fish-like texture, besides contributing to the flavour and protein content of the final product.

Proteins are of particular significance as they take part in many biological functions, such as structural (collagen), transport (haemoglobin), digestion (enzymes), among others (Belitz et al., 2009; Nelson et al., 2008). Proteins consist of several amino acids interlinked by peptide bonds to form chains; amino acids can be classified as essential (i.e., not synthesised by the organism; must be obtained through diet) or as non-essential (i.e., synthesised by the organism), and the presence or absence of the former determines the quality of the protein. When a protein is missing one or more essential amino acids, even if the remaining are present in great amounts, then it is considered of low quality, i.e., a low biological value (Belitz et al., 2009; McClements & Grossmann, 2021a, 2022; Nelson et al., 2008). Moreover, the proportion of the protein that is accessible to the organism – bioavailability –, and the ability of the organism to absorb the proteins into the bloodstream – digestibility – are also of great importance to the organism's wellbeing, as a protein with high biological value but low digestibility and bioavailability would signify an undervalued proportion of amino acids arriving at the cells (McClements & Grossmann, 2021a, 2022).

In this vein, the protein digestibility-corrected amino acid score (PDCAAS) (Table 8.3) was developed to access protein's quality by comparing the essential amino acids composition of a protein to a reference protein that meets nutritional requirements (Hertzler et al., 2020; Silva et al., 2015). Overall, animal proteins score around 1.00 – the maximum score –, thus being highlighted has complete proteins. Soy protein is one of the most whole plant protein (PDCAAS = 98%), so in combination with its low cost, it is readily available in the marketplace; however, soy is also known for the characteristic "bean" (undesirable) flavour (Hertzler et al., 2020; McClements & Grossmann, 2022). Unfortunately, plant proteins usually are of inferior quality, but the combination of different protein sources can lead to adequate amino acid intake (Hertzler et al., 2020; Marsh et al., 2013).

*Table 8.3. Protein quality of milk and selected plant-based protein sources, along with information about flavour acceptability and cost (Hertzler et al., 2020; McClements & Grossmann, 2022)*

| | PDCAAS | Limiting Amino acid(s) | Flavour | Cost |
|---|---|---|---|---|
| *Milk* | 1.00 | - | Neutral | Low |
| *Soy* | 0.98 | Methionine, Cysteine | Undesirable | Low |
| *Pea* | 0.84 | Methionine, Cysteine, Tryptophan | Acceptable | Low |
| *Chickpea* | 0.76 | Leucine, Lysine, Methionine, Cysteine, Threonine, Tryptophan, Valine | Acceptable | High |
| *Fava bean* | 0.64 | Lysine, Methionine, Cysteine, Threonine, Tryptophan, Valine | Acceptable | High |
| *Wheat* | 0.49 | Isoleucine, Leucine, Lysine, Phenylalanine, Tyrosine, Threonine, Valine | Acceptable | Very low |

In the food industry, plant-based proteins exist in multiple forms, for example, concentrates or isolates, that differ in their content of protein; with isolates (> 90 %) being much richer in protein than the concentrates (60 – 90 %). In addition, plant protein can be in the form of a texturized vegetable protein (50-70 % protein), which is designed to replicate the texture and structure of burgers, fish fillet, among other animal products (McClements & Grossmann, 2022). Consequently, the development of the new *patanisca* is recommended to include a texturized protein that will allow the consumer to have enhanced mouthfeel perception of the product.

Based in Table 8.3, wheat's protein – gluten – does not contain many essential amino acids, moreover it is the trigger of celiac disease, and other gluten linked disorders, therefore it would not be suitable as an alternative to cod in the development of a new *patanisca* that aims to reach consumers following a GFD. Chickpea and fava bean proteins incur in increased production costs and show lower quality when compared to soy and pea protein, still as textured proteins their inclusion in *pataniscas'* formulations might contribute to better product performance. Soy and pea protein's show the highest protein quality and lowest cost, thus, despite the undesirable flavour of soy protein, the previous proteins might possibly the best ingredients for a plant-based and gluten-free alternative to fish.

The distinctive seafood flavour is explained by two main motives. First, from seafood derives a complex mixture of volatiles compounds that are responsible for its characteristic fish aroma. These molecules can originate from different sources: a) the autoxidation of polyunsaturated fatty acids (PUFA) forms "fishy" volatiles, like alcohols (e.g., 3,5-octadien-2-ol), ketones (3,5-octadien-2-one) and aldehydes (e.g., 4-heptenal, hexanal); b) sulfuric compounds; and c) nitrogen-containing compounds. Second, seafood is known for a characteristic taste designated as umami, that it is associated to the presence of non-volatile compounds, such as glutamate, aspartate and salt, as well as 5'-ribonucleotides (i.e., inosine, guanosine and/or adenosine) monophosphate (Coleman et al., 2022; Kazir & Livney, 2021).

In the recent years, algae (i.e., micro and macroalgae – seaweeds) have become common in many meals, like sushi, soups, pasta, snacks, among others. They are a healthy source of omega-3 and omega-6 fatty acids, proteins and carbohydrates (e.g., agar). Many seaweeds have been investigated and similarly to seafood, it has been discovered that they contain "fishy" volatiles, such as alcohols, carbonyls and sulfur compounds, therefore explaining the latest and more notable uses of seaweeds as a natural source of marine flavour in plant-based seafood products (ALGAplus, n.d.; Belattmania et al., 2021; Coleman et al., 2022; Francavilla et al., 2013; Garicano Vilar et al., 2020; Kazir & Livney, 2021).

Ogonori algae (i.e., *Gracilaria gracilis* ) is a seaweed that grows in Portugal, where it can be found at Ria de Aveiro lagoon. In the past, it was used as a component of "moliço", i.e., a mixture of seaweed and seagrasses used as a natural soil fertilizer, however it is an edible algae that can be integrated in food products (Abreu et al., 2011; ALGAplus, n.d.). As a local raw material from Aveiro, and due to its marine flavour and nutritional properties, these algae can be integrated in the new plant-based *pataniscas.*

### 8.1.2. Wheat and Egg substitutes

In traditional *pataniscas*, wheat flour and egg are sources of carbohydrates, protein and/or fats, and act as a thickening and structural agents; for example, starch act as a thickening agents when incorporated in the batter; gluten and egg proteins are responsible for the viscoelasticity and cohesivity, and during the baking process the product gains a porous, spongy texture unlike many other ingredients are able to provide. Egg is not plant-based and in spite of wheat flour being a plant-based ingredient, it is not appropriate for the gluten-free marketplace.

Carbohydrates are one of the most widely spread compounds on earth, and exhibit many molecular, physicochemical, functional and biological properties. For instance, since glucose can be readily used by cells and converted in energy, or it can be stored in muscles and liver has glycogen for future use, carbohydrates are deemed the main source of energy for the organism (Belitz et al., 2009; Nelson et al., 2008). Carbohydrates consist of one or more monosaccharide units linked by glycosidic bonds and depending on their chemical structures they are classified as monosaccharides ($n = 1$), oligosaccharides ($n = 2$ to 10), or polysaccharides ($n > 20$). Because their molecular structure is diverse, these molecules behave differently within the digestive system. Most monosaccharides (e.g., glucose, fructose) and oligosaccharides (e.g., saccharose) are categorized as sugars, due to their ability to interact with specific mouth receptors that induce the perception of sweetness (Belitz et al., 2009; McClements & Grossmann, 2022; Nelson et al., 2008).

Moreover, commonly carbohydrates occur as polysaccharides (e.g., cellulose, glycogen, starch, guar gum), and are present in many food products because of their thickening, gelling, emulsifying, stabilizing and structural properties; when arriving to the human gut, they can be fermentable or non-fermentable, as well as digestible or indigestible (i.e., fibers) hence impacting nutrition and health (McClements & Grossmann, 2021b, 2022). In conclusion, the carbohydrate ingredients included in a formulation define the attained quality and nutritional properties of the final product.

Currently, rice, corn or legumes (e.g., chickpea, lentil, soybean), are being used as substitutes of wheat flour and eggs. These raw materials might have better nutritional characteristics – lower carbohydrates and higher protein amounts – but possibly induce changes in flavour (e.g., "bean" off taste). Furthermore, the aforementioned flours lead to denser doughs, so to achieve great organoleptic properties the replacements do not occur in a ratio of 1:1 (Hertzler et al., 2020; Hopkin et al., 2022; M. Kaur et al., 2017; Kılıç Keskin et al., 2022; McClements & Grossmann, 2022; Sung & Chai, 2017).

In recent years, flaxseed (*Linum usitatissimum* L.) has become more popular as investigations revealed its exceptional nutritional profile: high content of oil (38 %), of which more than 50 % is alpha-linolenic acid, dietary fiber (28 – 35 %) and protein (21 – 30 %) and highlighted its inclusion in food products as a source of omega-3 fatty acids and fiber (Kajla et al., 2015; M. Kaur et al., 2017; Sung & Chai, 2017). Flaxseed is high in mucilage which can increase the water holding properties of the dough, and impact its rheological properties (Korus et al., 2015). Interestingly, Sung & Chai (2017) studied the effect of flaxseed flour on gluten-free cake properties, discovering that the supplementation of rice flour with up to 40 % flaxseed flour led to darker cakes, but augmented batter viscosity and cake volume, and reduced cake hardness compared to when using 100 % rice flour; moreover, 40 % flaxseed supplementation led to the better sensory properties in terms of aroma, texture, flavour and overall acceptability than control cake (Sung & Chai, 2017).

#### 8.1.3. Vegetable oil

The traditional process of preparing *pataniscas* consists of frying the batter that has been shaped into flat disks with a variable thickness, as such the final product develops organoleptic properties (e.g., taste, appearance, texture) that are characteristic of fried products; and, regrettably, it absorbs a considerable amount of fats (up to 45 %), that adversely influences nutritional characteristics of the product (Rimac-Brnčić et al., 2004). Many factors affect the quantity of absorbed oil: a) frying temperature and its duration; b) food characteristics – composition, shape, surface area, porosity; c) oil quality; and others (Rimac-Brnčić et al., 2004).

The new plant-based and gluten-free *patanisca* it is proposed to be a frozen product (i.e., not pre-fried). Thus, in the case of commercialization, consumers would have to fry the product at home. Since among consumers food characteristics would be fairly constant, and assuming that preparation instructions would be followed, the frying temperature and its duration would be more or less uniform, then oil quality is possibly the factor with higher impact on the nutritional characteristics of the *pataniscas*.

Fats are extremely important components of cell structures, and the major source of energy coming from food (9 kcal/g). Lipids are present in nature in different forms, such as triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, among others. Fatty acids are the basic component of many fats; therefore, the latter physicochemical, nutritional and functional properties depends on fatty acids composition (Belitz et al., 2009; McClements & Grossmann, 2022).

Current nutritional recommendations highlight the importance of polyunsaturated fatty acids (PUFA), such as omega-3 (e.g., α-linolenic acid) and omega-6 (e.g., linoleic acid) PUFA, consumption for the health of human beings. Consequently, since vegetable oils have high levels of monounsaturated fatty acids (MUFA) and PUFA, they are greatly valued (Belitz et al., 2009; DeMan et al., 2018). The most used vegetable oils are produced from olive, sunflower, rapeseed and soybean (Morley, 2016; Raikos et al., 2016). Table 8.4 shows the fatty acid composition of plant oils; rapeseed oil is the richest in unsaturated fatty acids (UFA), with around 70 % of MUFA and 23 % of PUFA and with the lowest level of saturated fatty acids (SFA) (5 %). Compared to the previous oil, soybean oil and sunflower oil have much more linoleic acid (54-61 % >>> 14 %), moreover soybean oil has comparable levels of α-linolenic acid. Lastly, olive oil is the richest oil in MUFA and poorest in PUFA (McClements & Grossmann, 2022; Romanić, 2020; Woodfield & Harwood, 2017). In sum, vegetable oils vary a lot in their composition, accordingly the preparation of a frozen product like a *patanisca* will determine a big part of their nutrition.

*Table 8.4. Fatty acid composition (expressed as percentages) of four oils: olive, sunflower, rapeseed and soybean. Adapted from (McClements & Grossmann, 2022; Romanić, 2020; Woodfield & Harwood, 2017)*

| | **Olive oil** | **Sunflower oil** | **Rapeseed oil** | **Soybean oil** |
|---|---|---|---|---|
| **Total saturated acids** | 12 | 12.1 | 5.0 | 15 |
| ***Palmitic acid, 16:0*** | 10 | 6.3 | 4.0 | 11 |
| ***Stearic acid, 18:0*** | 2 | 4.6 | 1.0 | 4 |
| **Total monounsaturated acids** | 78 | 26.8 | 69.9 | 23 |
| ***Oleic acid, 18:1*** | 78 | 26.7 | 14.8 | 23 |
| **Total polyunsaturated acids** | 8 | 61.1 | 23.2 | 62 |
| ***Linoleic acid, 18:2 (n-6)*** | 7 | 61.1 | 14.1 | 54 |
| ***$\alpha$-Linolenic acid, 18:3 (n-3)*** | 1 | - | 9.1 | 8 |
| **Total unsaturated acids** | 86 | 87.9 | 93.1 | 85 |

Legend: Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the figure afterward indicating the number of double bonds; n-6 and n-3 correspond to omega-6 and omega-3 fatty acids, respectively.

## 9. Materials and Methods

As the goal was to produce nutritionally balanced plant-based *pataniscas* without gluten, as first steps dietary guidelines were established, and ingredients commonly seen in the traditional product were analysed. Then, non-animal and gluten-free alternatives to conventional products were selected, to be analysed and new formulations to be developed.

### *9.1. Nutritional simulator*

With data retrieved from the Food Composition Table from "Instituto Nacional de Saúde Doutor Ricardo Jorge" (Instituto Nacional de Saúde Doutor Ricardo Jorge, n.d.), a nutritional simulator was developed, first, to help idealize sample's recipes to produce at the production site; second, to evaluate the nutritional composition (i.e., energy, fats, saturated fats, carbohydrates, sugars, fibre, proteins and salt) of the developed formulations.

### *9.2. Production of plant-based pataniscas*

Initial formulations were produced based on the output of the nutritional simulator, which were then improved and analysed, finally originating two approved prototypes. *"Patanisca A"* and *"Patanisca B"* formulations shared the same ingredients type and dosage, aside from the differentiating factors, those being: the type of proteins included in the formulation; and the usage of algae – Ogonori algae (i.e., *Gracilaria gracilis*). To gauge the impact of using proteins with different texture, as seen in Table 9.1 and Table 9.2, *Patanisca A* used only textured pea protein, and *Patanisca B* included pea and fava bean textured proteins in a ratio of 50:50, so that the amount of textured protein were equal to the other formulation. Lastly, in the former product's formulation was incorporated an algae, that should impact the flavour, and possibly the mouthfeel of the final product.

*Table 9.1. Ingredient declaration for Patanisca A*

| **Ingredients:** |
|---|
| Water, textured pea protein, corn starch, soy protein isolate, onion, carrot, red bell pepper, parsley, vinegar, flaxseed flour, baking powder, salt, garlic powder, black pepper powder |

*Table 9.2. Ingredient declaration for Patanisca B*

| **Ingredients:** |
|---|
| Water, textured pea protein, textured fava bean protein, corn starch, soy protein isolate, onion, carrot, red bell pepper, parsley, vinegar, flaxseed flour, baking powder, Ogonori algae, salt, garlic powder, black pepper powder |

The procedure to prepare both versions of the gluten-free plant-based product is as shown in the following steps, with exception of the algae hydration step for the "Patanisca A":

1) Weighing of ingredients
2) Preparation of textured proteins
    a. Hydration of pea and fava bean proteins, ratio of 1 to 4, for 60 min at room temperature (Figure 9.1)
    b. Size reduction until around 1 cm in bowl cutters (CUTMIX 90 L K+G Wetter, Germany)

*Figure 9.1. Hydration of textured proteins, i.e. pea and faba bean*

3) Hydration of flax seed flour and algae, ratio of 1 to 4, for 15 min (Figure 9.2)

*Figure 9.2. Hydration of flax seed flour and algae Onogori (i.e. Gracilaria gracilis)*

4) Mixing and size reduction (until ± 0.5 cm) of frozen onion, carrot and red bell pepper in bowl cutters (CUTMIX 90 L K+G Wetter, Germany) (Figure 9.3)

*Figure 9.3. Vegetables mixture after size reduction.*

5) Preparation of dough by adding prior preparations and remaining ingredients (i.e. corn starch, soy protein isolate, frozen parsley, vinegar, baking powder, salt, garlic powder, black pepper powder) to a mixing bowl and combine until homogeneous
6) Formatting of the *pataniscas* (Figure 9.4)
    a. Transfer of dough (± 50 g) to plastic covered trays with the help of a measuring spoon
    b. Shaping of dough into rounded shape with around 10 cm diameter and 0.7 cm height

*Figure 9.4. Formatting of dough to obtain "pataniscas". On the left, after dough transferring to tray, and on the right, after being shaped into disks.*

7) Ultra freezing of product in a tunnel freezer for 1 h
8) Packaging and storage at freezing conditions (- 18 °C)

## *9.3. Sensory Analysis*

The sensory analysis of *pataniscas* samples was performed by an untrained panel using the acceptance test and the paired preference test. The panel was composed of 31 employees – 45 % men and 55 % women – with ages between 18 and 66 years and had been previously informed about the products in test.

Concerning the preparation of samples, the *pataniscas* (A and B) were retrieved from storage and then the product was fried in sunflower oil at 180 °C for 3 min, after it was transferred to absorbent paper to drain for around 20 min. The samples were coded with a three-digit random number. Before the tasting session, for each taster, a tray was prepared with both products (± 25 g of representative sample), as well as water and plain crackers to cleanse the palate in between samples. The samples were presented simultaneously at the same temperature, and the order of sample's presentation was balanced, i.e., half of the participants received the *pataniscas* in the order A-B, and the other half received them in the order B-A (Civille & Carr, 2015).

During the tasting session, panelists would have access to a tray and the tasting form was presented in paper form following the structure shown in Appendix D. The instructions were to evaluate the acceptance of certain attributes (i.e., overall acceptance, appearance, odour, texture, taste and intention of purchase) of the products in test using a 9-point hedonic scale, where higher scores meant that the attribute in test for the corresponding samples was more liked by the panel.

### *9.4. Nutritional Analysis*

The nutritional analysis were performed by an accredited laboratory, ALS Life Sciences Portugal, S.A.. Table 9.3 shows the parameter in analysis, the applied method and respective reference. The data was used to organize the nutrition declaration according to Regulation (EU) n° 1169/2011 (European Parliament & Council of the European Union, 2011).

Due to cost restraints, nutritional analysis were carried out only on the sample with higher acceptance from the consumer side. Additionally, to evaluate the impact of the frying process in the nutritional properties of the *patanisca*, both a frozen and a fried – following the same procedure as when preparing samples for consumer tasting – product were analysed.

*Table 9.3. Nutritional analysis methods and references*

| **Parameter** | **Method** | **Reference** |
|---|---|---|
| *Moisture* | Thermogravimetry | NP 875, NP 2282, NP 1614-1 |
| *Total Ash* | Gravimetry | NP 872, NP 1615, NP 3913:1991 |
| *Fat* | Nuclear magnetic resonance<br>- Fat content determined by comparison with calibration curve of a certified olive oil standard | Internal methodology |
| *Fatty acids profile* | Gas chromatography–flame ionization detection (GC-FID) | ISO 12966-2 and internal methodology |
| *Sugars* | High performance ion-exchange chromatography | Internal methodology |
| *Carbohydrates* | Calculation, by diference, based on the content in moisture, ash, protein, fat and dietary fibre, i.e. Carbohydrates (%) =100 – [Moisture (%) + Ash (%) + Protein (%) + Fat (%) + Fibre (%) | Regulation (EU) n° 1169/2011 |
| *Dietary fibre* | Enzymatic-Gravimetry | AOAC 985.29 |
| *Protein* | Combustion (i.e. Dumas method) | ISO 16634, AOAC 992.15, Regulation (EU) n° 1169/2011 |
| *Sodium* | Flame atomic absorption spectrophotometry | EN 14084, ISO 6869, EN 14082 |
| *Salt* | Calculation based on sodium content | Regulation (EU) n° 1169/2011 |
| *Energy value* | Calculation based on nutrients content | Regulation (EU) n° 1169/2011 |

### *9.5. Data Analysis*

In the case of the sensory analysis, it was followed the procedures described by Civille & Carr, 2015. In sum, regarding the acceptance test, the statistical method used to determine significant differences among samples was the paired *t*-test, and the mean difference was considered significant at a 95 % confidence level ($\alpha$ = 0.05). The comments on each sample were analysed and summarised, only being considered as significant if mentioned by more than 50 % of the panelists.

In terms of the nutritional analysis, all data was presented as provided by the accredited laboratory.

## 10. Results and Discussion

The marketplace offers plant-based and gluten-free food products that are overall lacking in their nutritional profile, and sometimes even concerning their sensory attributes. Accordingly, the development of a new plant-based fish product, which is free of gluten, and that contributes to better health and presents acceptable organoleptic properties is of great interest. The current project focused on developing a healthy *patanisca* using only raw materials from vegetal origin and that did not include gluten. Sensory analyses were carried out to evaluate consumers acceptability regarding appearance, odour, texture, taste, among others. Additionally, nutritional analysis were used to determine the final nutrients' composition, as well as to compare it to a *patanisca de legumes* available in the marketplace.

### *10.1. Sensory Analysis*

#### 10.1.1. Acceptance test

For the acceptance test, the sensory scores of the studied attributes are shown in Figure 10.1 and Figure 10.2. Based on the overall results, the average and standard deviation per attribute per sample were determined and summarized in Table 10.1. The sensory data was treated statistically based on a paired *t*-test (Table 10.2) in order to assess significant differences between samples, and potentially improve the formulations according to sensory evidence (Civille & Carr, 2015).

*Table 10.1. Sensory scores' average and standard deviation for the acceptance test of Patanisca A and Patanisca B.*

| | **Patanisca A** | **Patanisca B** |
|---|---|---|
| *Overall acceptance* | 7.4 ± 1.1 | 8.0 ± 0.9 |
| *Appearance* | 7.6 ± 1.2 | 7.9 ± 0.8 |
| *Odour* | 7.3 ± 1.1 | 7.6 ± 1.0 |
| *Texture* | 7.2 ± 1.5 | 8.1 ± 0.8 |
| *Taste* | 7.4 ± 1.2 | 7.9 ± 0.9 |
| *Intention of purchase* | 3.9 ± 1.3 | 4.4 ± 0.9 |

Overall, on average sample B was considered moderately pleasant for all attributes in comparison to sample A being only considered pleasant. In addition, Figure 10.1 showed that the distribution of scores in regard to *Patanisca A* was somewhat more erratic compared to *Patanisca B;* in more detail, the panelists evaluated the former sample from slightly unpleasant (4) to extremely pleasant (9), while the latter product was mainly classified from pleasant (7) to extremely pleasant (9).

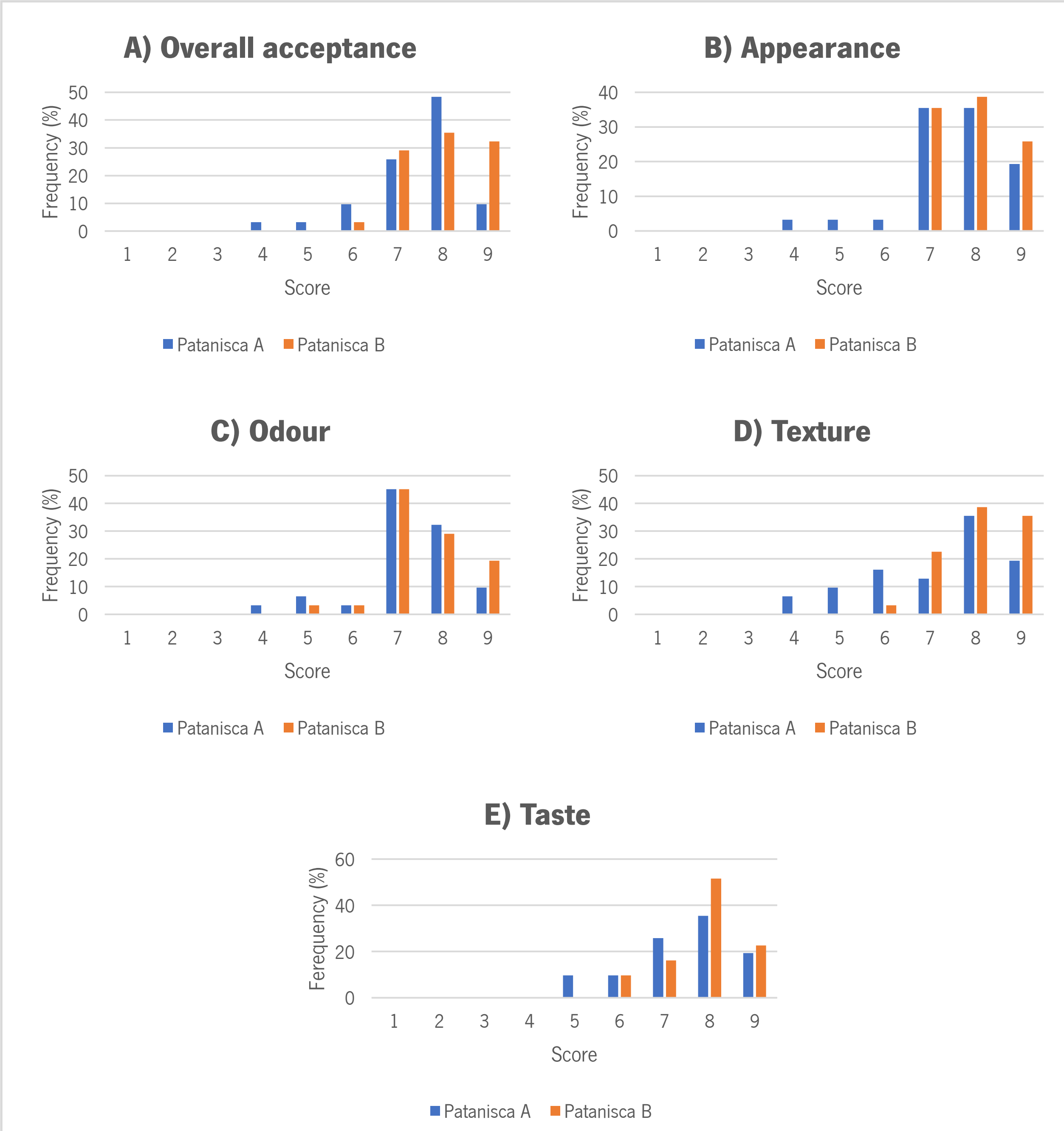



*Figure 10.1. Sensory scores of an acceptance test of pataniscas A and B, concerning A) Overall acceptance, B) Appearance, C) Odour, D) Texture and E) Taste. Legend: 1 – Extremely unpleasant; 2 – Moderately unpleasant; 3 – Unpleasant; 4 – Slightly unpleasant; 5 – Neutral; 6 – Slightly pleasant; 7 – Pleasant; 8 – Moderately pleasant; 9 – Extremely pleasant.*

The evaluation of the intention of purchase (Figure 10.2) revealed that 8 out of 31 (26 %) tasters probably would not buy *Patanisca A* compared to only 2 (6 %) for *Patanisca B*, moreover more consumers (87 % > 68 %) would probably or definitely buy sample B, concluding that the tendency to purchase the *Patanisca B* was higher.

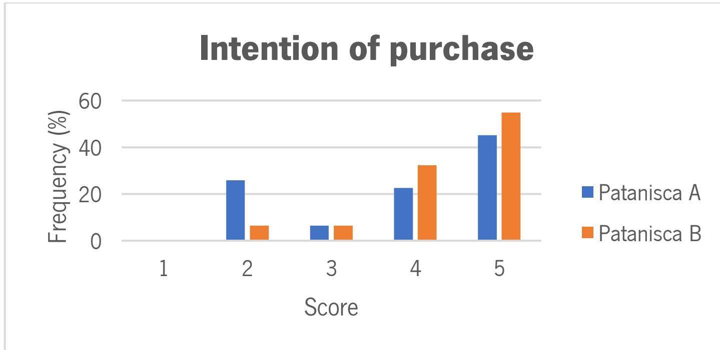



*Figure 10.2. Sensory scores of an acceptance test of pataniscas A and B, regarding the intention of purchase. Legend: 1 – Definitely would not buy; 2 – Probably would not buy; 3 – Maybe/Maybe not; 4 – Probably would buy; 5 – Definitely would buy.*

The attributes with higher difference among samples were "overall acceptance", "texture" and "taste", the latter two being two of the most central sensory properties in a product. Contrastingly to sample A, the formulation B contained the Ogonori algae which is a source of marine flavour, therefore it seems that consumers were significantly more interested in a plant-based product that conveyed a more traditional familiar taste. Furthermore, the applied seaweed is one of the main sources of food-grade agar, consequently it is possible that its presence in the dough is positively affecting not only the flavour but also the texture of the final product (ALGAplus, n.d.; Belattmania et al., 2021).

Ultimately, it can be hypothesised that consumers had a significantly higher level of acceptance of *Patanisca B* than sample A. Nonetheless, the relative standard deviation (RSD) for *Patanisca B* was ranging from 10 % to 20 %, and *Patanisca A* was linked to a RSD range of 15 % to 33 %; in sum, both samples showed fairly high (> 5 %) standard deviations, denoting the need for a superior number of panelists. Furthermore, panelists were employers of Irmãos Monteiro S.A., despite having different roles at the company, their judgement might be prejudiced (Fuller, 2011).

Concerning the statistical analysis, in the present case for each attribute the goal was to determine if *Patanisca B* was perceived has significantly better than *Patanisca A,* which was translated as the average ratings for sample B being significantly greater than the average ratings for sample A. The null hypothesis in this case is $H_0$: $\delta \leq 0$ versus the alternative hypothesis $H_1$: $\delta > 0$, and the null hypothesis is rejected if the upper-$\alpha$ critical value of the *t*-distribution with (n – 1) degrees of freedom is surpassed by the calculated *t*-value. The confidence level was set at 95 % ($\alpha$ = 0.05), and tasting included 31 tasters, hence reference *t*-value was $t_{0.05,30}$= 1.697 (Civille & Carr, 2015). Based on data from Table 10.2, the calculated *t*-value for all attributes exceeded the reference value, so the null hypothesis was rejected, and differences between average ratings were significant. In conclusion, as hypothesised *Patanisca B* had higher acceptance rate than *Patanisca A* in regard to all studied attributes, even in terms of "intention of purchase", suggesting that sample B could be a viable product for the marketplace.

*Table 10.2. Paired t-statistic for the acceptance test of Patanisca A and Patanisca B.*

| Term | Overall acceptance | Appearance | Odour | Texture | Taste | Intention of purchase |
|---|---|---|---|---|---|---|
| $\bar{\delta}$ | 0.548 | 0.355 | 0.323 | 0.871 | 0.419 | 0.484 |
| s | 0.850 | 1.050 | 0.871 | 1.176 | 0.958 | 0.926 |
| Calculated $t$ - value | 3.592 | 1.881 | 2.061 | 4.124 | 2.436 | 2.908 |

Legend: $\bar{\delta}$= average of the differences; s = standard deviation of the differences

## *10.2. Nutritional Analysis*

The nutritional analysis of *Patanisca B* (Table 10.3 and Table 10.4) both fried and frozen, was carried out, aiming to understand the final composition of the new plant-based and gluten-free product, and to ascertain if nutritional recommendations (Table 8.2) were achieved.

*Table 10.3. Nutritional declaration of a fried Patanisca B*

| Nutritional declaration | per 100 g | per portion (150 g) | %RI* | RI* |
|---|---|---|---|---|
| **Energy** | 915 kJ<br>218 kcal | 1373 kJ<br>327 kcal | 16 | 8400 kJ<br>2000 kcal |
| **Fat** | 10.1 g | 15.2 g | 22 | 70 g |
| **of which** | | | | |
| **saturates** | 1.2 g | 1.8 g | 9 | 20 g |
| **mono-unsaturates** | 2.7 g | 4.0 g | | |
| **polyunsaturates** | 6.2 g | 9.3 g | | |
| **Carbohydrate** | 18.1 g | 27.2 g | 10 | 260 g |
| **of which sugars** | 1.6 g | 2.5 g | 3 | 90 g |
| **Fibre** | 1.7 g | 2.6 g | | |
| **Protein** | 12.9 g | 19.4 g | 39 | 50 g |
| **Salt** | 0.93 g | 1.4 g | 23 | 6 g |

First, as the goal was to develop a new alternative to a fish product that would be considered healthier while being both plant-based and gluten-free, thus following the proposed nutrients level summarized in Table 8.2. Table 10.3 shows that considering a portion of 150 g (i.e., approximately 3 small *pataniscas*), the level of saturated fats, carbohydrates, sugars and protein were 1.8 g, 27.2 g, 2.5 g and 19.4 g, respectively, which was in alignment with the targeted values. Contrastingly, in terms of fats (15.2 g > 11.9 g) and salt (1.4 g >

1.0 g) the portion was exceeding the recommended nutritional values; also, the fibre content was regrettably smaller than expected.

*Table 10.4. Nutritional declaration of frozen Patanisca B*

| Nutritional declaration | per 100 g | per portion (150 g) | %RI* | RI* |
|---|---|---|---|---|
| **Energy** | 455 kJ<br>108 kcal | 693 kJ<br>162 kcal | 8 | 8400 kJ<br>2000 kcal |
| **Fat** | 1.1 g | 1.7 g | 2 | 70 g |
| **of which** | | | | |
| **saturates** | 0.20 g | 0.30 g | 2 | 20 g |
| **mono-unsaturates** | 0.25 g | 0.40 g | | |
| **polyunsaturates** | 0.64 g | 1.0 g | | |
| **Carbohydrate** | 14.6 g | 21.9 g | 8 | 260 g |
| **of which sugars** | 0.59 g | 0.9 g | 1 | 90 g |
| **Fibre** | 1.6 g | 2.4 g | | |
| **Protein** | 9.0 g | 13.5 g | 27 | 50 g |
| **Salt** | 0.74 g | 1.1 g | 18 | 6 g |

* Reference intake of an average adult (8400 kJ/ 2000 kcal)

Focusing on the comparison of the composition of the products before (Table 10.4) and after (Table 10.3) the frying process, it is possible to verify the occurrence of water losses and consequent increase in the levels of carbohydrates, protein and salt; as well as the absorption of vegetable oil, that led the product to be a greater source of energy and of fats. In this case, the oil used to fry the product was a sunflower oil that is naturally richer in PUFA, followed by MUFA, and poorer in SFA (Romanić, 2020; Woodfield & Harwood, 2017); moreover, in the formulation flaxseed flour and Ogonori algae were included, both being sources of omega-3 and omega-6 fatty acids (Abreu et al., 2011; ALGAplus, n.d.; Kajla et al., 2015; M. Kaur et al., 2017; Sung & Chai, 2017). Understandably, the fats' composition pattern found in *Patanisca B* is as the one described for the oil, with only some influences of the flaxseed and algae.

In sum, the reduction of the salt's content of the *pataniscas* would be a possible next step, so that it would be as recommended. Concerning the fats, it seems that due to great oil absorption while frying, the lipid profile of this new product is much more dependable on the oil used to prepare it, than the ingredients used in its formulation. A possible solution to guarantee a more stable nutritional profile, could be to enhance the lipid profile of the frozen product so it would present the appropriate amount of fat and a correct balance between SFA, MUFA and PUFA. However, the preparation method for the *patanisca* would possibly have to change from frying to cooking it in the oven or in a pan, which might negatively influence the texture and taste consumers are demanding when consuming a traditional product that it is fried.

Table 10.5 summarizes the nutritional composition per 100 g of *Patanisca B* (i.e., *patanisca* with algae) and a *patanisca* with vegetables available in the marketplace that it is plant-based, therefore not containing cod, yet that contains egg. In comparison with the *patanisca* with vegetables, the new *patanisca* with algae contained around 2 times lower percentages of fat, including saturated fats that can negatively impact health; besides, it had a higher protein content (12.9 g > 5 g) and similar levels of salt and sugars.

In conclusion, the plant-based and gluten-free formulation – *Patanisca* with algae – can be considered healthier than what is currently available for consumers.

*Table 10.5. Nutritional declaration comparison between the new plant-based and gluten-free patanisca, and a marketplace patanisca with vegetables*

| **Nutritional declaration** | **per 100 g *Patanisca* with algae** | **per 100 g *Patanisca* with vegetables** |
|---|---|---|
| **Energy** | 915 kJ<br>218 kcal | 1561 kJ<br>375 kcal |
| **Fat** | 10.1 g | 26.7 g |
| **of which** | | |
| **saturates** | 1.2 g | 3.2 g |
| **mono-unsaturates** | 2.7 g | |
| **polyunsaturates** | 6.2 g | |
| **Carbohydrate** | 18.1 g | 28.7 g |
| **of which sugars** | 1.6 g | 1.8 g |
| **Fibre** | 1.7 g | |
| **Protein** | 12.9 g | 5 g |
| **Salt** | 0.93 g | 0.9 g |

* Reference intake of an average adult (8400 kJ/ 2000 kcal)

## 11. Conclusion

The current research aimed to develop a new plant-based and gluten-free alternative to fish products, showing a healthier nutritional profile and great organoleptic characteristics. Accordingly, after market research, some formulations of *pataniscas* were developed and sensory and nutritional analysis were carried out.

After initial testing, two prototypes were proposed for sensory analysis. Based on the acceptance rating of consumers, in comparison to a *patanisca* with vegetables and two textured proteins, a *patanisca* with Ogonori algae was perceived has having significantly better acceptance for all studied attributes, including the intention of purchase. Thus, results were promising suggesting that the latter product might be of consumers' interest.

The nutritional analysis of the *patanisca* with algae revealed that, on the one hand, the target for some nutrients (i.e., saturated fats, carbohydrates, sugars and protein) levels was achieved; on the other hand, content in fats and salt was exceeded. In sum, there is still some nutritional properties that should be improved; for example, the increase of the fiber amount, that contributes to better health and can lead to nutritional allegations on the product. Nonetheless, in general, the formulations is healthier than what is currently available in the marketplace to consumers.

This study shed some light into the future directions, especially concerning the improvement of the product that included algae by introducing some changes, both nutritionally and process wise, and its sensory evaluation by a higher number of consumers. Furthermore, the development of a sustainable packaging that aligns with the product vision and target consumers is needed, as well as the assessment of the costs of production.

## References


Abreu, M. H., Pereira, R., Sousa-Pinto, I., & Yarish, C. (2011). Ecophysiological studies of the non-indigenous species *Gracilaria vermiculophylla* (Rhodophyta) and its abundance patterns in Ria de Aveiro lagoon, Portugal. *European Journal of Phycology*, *46*(4), 453–464. https://doi.org/10.1080/09670262.2011.633174

ALGAplus. (n.d.). *Cabelo-de-velha ou Ogonori desidratada*. Retrieved 23 May 2022, from https://algaplus.lojasonlinectt.pt/product/cabelo-de-velha-desidratada-100g

Almeida, C., Karadzic, V., & Vaz, S. (2015). The seafood market in Portugal: Driving forces and consequences. *Marine Policy*, *61*, 87–94. https://doi.org/10.1016/j.marpol.2015.07.012

Altunkaya, A., Hedegaard, R. v., Harholt, J., Brimer, L., Gökmen, V., & Skibsted, L. H. (2013). Oxidative stability and chemical safety of mayonnaise enriched with grape seed extract. *Food & Function*, *4*(11), 1647–1653. https://doi.org/10.1039/C3FO60204D

Anton, M., & Gandemer, G. (1997). Composition, Solubility and Emulsifying Properties of Granules and Plasma of Egg Yolk. *Journal of Food Science*, *62*(3), 484–487. https://doi.org/10.1111/J.1365-2621.1997.TB04411.X

Anton, M., Nau, F., Lechevalier, V., & Meynier, A. (2018). Presentation of the Ovoproduct Matrix. In O. Gonçalves & J. Legrand (Eds.), *Alteration of Ovoproducts: From Metabolomics to Online Control* (pp. 1–50). ISTE Press - Elsevier. https://doi.org/10.1016/B978-1-78548-271-7.50001-8

Appleby, P. N., Thorogood, M., Mann, J. I., & Key, T. J. (1998). Low body mass index in non-meat eaters: the possible roles of animal fat, dietary fibre and alcohol. *International Journal of Obesity*, *22*(5), 454–460. https://doi.org/10.1038/sj.ijo.0800607

Arenas-Jal, M., Suñé-Negre, J. M., Pérez-Lozano, P., & García-Montoya, E. (2020). Trends in the food and sports nutrition industry: A review. *Critical Reviews in Food Science and Nutrition*, *60*(14), 2405–2421. https://doi.org/10.1080/10408398.2019.1643287

Ariizumi, M., Kubo, M., Handa, A., Hayakawa, T., Matsumiya, K., & Matsumura, Y. (2017). Influence of processing factors on the stability of model mayonnaise with whole egg during long-term storage. *Bioscience, Biotechnology, and Biochemistry*, *81*(4), 803–811. https://doi.org/10.1080/09168451.2017.1281725

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International*, *99*, 58–71. https://doi.org/10.1016/j.foodres.2017.07.022

Aurand, L. W., Singleton, J. A., Bell, T. A., & Etchells, J. L. (1966). Volatile Components in the Vapors of Natural and Distilled Vinegars. *Journal of Food Science*, *31*(2), 172–177. https://doi.org/10.1111/J.1365-2621.1966.TB00474.X

Autoridade de Segurança Alimentar e Económica (ASAE). (n.d.). *Outros Aditivos*. Retrieved 4 January 2022, from https://www.asae.gov.pt/seguranca-alimentar/aditivos-alimentares/outros-aditivos.aspx

Ayres, R. U., & Williams, E. (2004). The digital economy: Where do we stand? *Technological Forecasting and Social Change*, *71*(4), 315–339. https://doi.org/10.1016/J.TECHFORE.2003.11.001

Azanedo, L., Garcia-Garcia, G., Stone, J., & Rahimifard, S. (2020). *An Overview of Current Challenges in New Food Product Development*. *12*, 3364. https://doi.org/10.3390/su12083364

Baines, Z. v, & Morris, E. R. (1987). Flavour/taste perception in thickened systems: the effect of guar gum above and below c*. *Food Hydrocolloids*, *1*(3), 197–205. https://doi.org/https://doi.org/10.1016/S0268-005X(87)80003-6

Belattmania, Z., Bhaby, S., Nadri, A., Khaya, K., Bentiss, F., Jama, C., Reani, A., Vasconcelos, V., & Sabour, B. (2021). Gracilaria gracilis (Gracilariales, Rhodophyta) from Dakhla (Southern Moroccan Atlantic Coast) as Source of Agar: Content, Chemical Characteristics, and Gelling Properties. *Marine Drugs*, *19*(12), 672. https://doi.org/10.3390/md19120672

Belitz, H. D., Grosch, W., & Schieberle, P. (2009). Food chemistry. In *Food Chemistry*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-69934-7

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer. *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 945–977. https://doi.org/https://doi.org/10.1111/1541-4337.12097

Boada, L. D., Henríquez-Hernández, L. A., & Luzardo, O. P. (2016). The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. *Food and Chemical Toxicology*, *92*, 236–244. https://doi.org/10.1016/J.FCT.2016.04.008

Boukid, F., Baune, M.-C., Gagaoua, M., & Castellari, M. (2022). Seafood alternatives: assessing the nutritional profile of products sold in the global market. *European Food Research and Technology*, *248*(7), 1777–1786. https://doi.org/10.1007/s00217-022-04004-z

Candeias, V., Nunes, E., Morais, C., Cabral, M., & Silva, P. (2005). *Princípios para uma Alimentação Saudável*. Direção Geral da Saúde.

Carter, P., Gray, L. J., Troughton, J., Khunti, K., & Davies, M. J. (2010). Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ*, *341*(aug18 4), c4229–c4229. https://doi.org/10.1136/bmj.c4229

Castellani, O., Guérin-Dubiard, C., David-Briand, E., & Anton, M. (2004). Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. *Food Chemistry*, *85*(4), 569–577. https://doi.org/10.1016/J.FOODCHEM.2003.08.002

Catassi, C., Verdu, E. F., Bai, J. C., & Lionetti, E. (2022). Coeliac disease. *The Lancet*, *399*(10344), 2413–2426. https://doi.org/10.1016/S0140-6736(22)00794-2

Catsburg, C., Kim, R. S., Kirsh, V. A., Soskolne, C. L., Kreiger, N., & Rohan, T. E. (2015). Dietary patterns and breast cancer risk: a study in 2 cohorts. *The American Journal of Clinical Nutrition*, *101*(4), 817–823. https://doi.org/10.3945/ajcn.114.097659

Chambers, S., Lobb, A., Butler, L., Harvey, K., & Bruce Traill, W. (2007). Local, national and imported foods: A qualitative study. *Appetite*, *49*(1), 208–213. https://doi.org/10.1016/j.appet.2007.02.003

Chew, S. C. (2020). Cold pressed rapeseed (Brassica napus) oil. In M. F. Ramadan (Ed.), *Cold Pressed Oils* (pp. 65–80). Academic Press. https://doi.org/10.1016/B978-0-12-818188-1.00007-4

Choe, E., & Min, D. B. (2006). Mechanisms and Factors for Edible Oil Oxidation. *Comprehensive Reviews in Food Science and Food Safety*, *5*(4), 169–186. https://doi.org/https://doi.org/10.1111/j.1541-4337.2006.00009.x

Choudhury, D., Singh, S., Seah, J. S. H., Yeo, D. C. L., & Tan, L. P. (2020). Commercialization of Plant-Based Meat Alternatives. *Trends in Plant Science*, *25*(11), 1055–1058. https://doi.org/10.1016/J.TPLANTS.2020.08.006

Civille, G. V., & Carr, B. T. (2015). *Sensory Evaluation Techniques* (5th ed.). CRC Press. https://doi.org/10.1201/b19493

Codex Coordinating Committee for Europe. (1989). Codex Stan 168-1989: Codex Standard for Mayonnaise. In *Codex Alimentarius* (Vol. 11). FAO/WHO Joint Publications.

Coleman, B., van Poucke, C., Dewitte, B., Ruttens, A., Moerdijk-Poortvliet, T., Latsos, C., de Reu, K., Blommaert, L., Duquenne, B., Timmermans, K., van Houcke, J., Muylaert, K., & Robbens, J. (2022).

Potential of microalgae as flavoring agents for plant-based seafood alternatives. *Future Foods*, *5*, 100139. https://doi.org/10.1016/J.FUFO.2022.100139

Cook, D. J., Linforth, R. S. T., & Taylor, A. J. (2003). Effects of Hydrocolloid Thickeners on the Perception of Savory Flavors. *Journal of Agricultural and Food Chemistry*, *51*(10), 3067–3072. https://doi.org/10.1021/jf0211581

Cordeiro, T., & Bento, A. (2011). *Alimentação Adequada: Faça mais pela sua Saúde!* (A. Bento & T. Cordeiro, Eds.). Associação Portuguesa de Nutrição.

Costa, M., Freiría-Gándara, J., Losada-Barreiro, S., Paiva-Martins, F., & Bravo-Díaz, C. (2020). Effects of droplet size on the interfacial concentrations of antioxidants in fish and olive oil-in-water emulsions and nanoemulsions and on their oxidative stability. *Journal of Colloid and Interface Science*, *562*, 352–362. https://doi.org/10.1016/J.JCIS.2019.12.011

Culinaria Europe. (2015). *Code of Practice for Mayonnaise*. https://www.culinaria-europe.eu/index-culinaria-europe.html/sectors/mayonnaise

Curtain, F., & Grafenauer, S. (2019). Plant-Based Meat Substitutes in the Flexitarian Age: An Audit of Products on Supermarket Shelves. *Nutrients*, *11*(11), 2603. https://doi.org/10.3390/NU11112603

Daimer, K., & Kulozik, U. (2009). Oil-in-water emulsion properties of egg yolk: Effect of enzymatic modification by phospholipase A2. *Food Hydrocolloids*, *23*(5), 1366–1373. https://doi.org/10.1016/J.FOODHYD.2008.10.002

DeMan, J. M., Finley, J. W., Hurst, W. J., & Lee, C. Y. (2018). *Principles of Food Chemistry* (4th ed.). Springer Internacional. https://doi.org/10.1007/978-3-319-63607-8

Depree, J. A., & Savage, G. P. (2001). Physical and flavour stability of mayonnaise. *Trends in Food Science & Technology*, *12*(5–6), 157–163. https://doi.org/10.1016/S0924-2244(01)00079-6

Dias, J. (2022). The use of cheese from Alentejo in Portuguese gastronomy: A travel through history. *International Journal of Gastronomy and Food Science*, *29*, 100579. https://doi.org/10.1016/J.IJGFS.2022.100579

Dieckman, T., Koning, F., & Bouma, G. (2022). Celiac disease: New therapies on the horizon. *Current Opinion in Pharmacology*, *66*, 102268. https://doi.org/10.1016/J.COPH.2022.102268

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants (Basel, Switzerland)*, *8*(10), 429. https://doi.org/10.3390/antiox8100429

Doty, R. L. (2019). Psychophysical testing of smell and taste function. *Handbook of Clinical Neurology*, *164*, 229–246. https://doi.org/10.1016/B978-0-444-63855-7.00015-0

Dwyer, J. (1999). Convergence of plant-rich and plant-only diets. *The American Journal of Clinical Nutrition*, *70*(3 Suppl), 620s–622s. https://doi.org/10.1093/ajcn/70.3.620s

Dyer-Hurdon, J. N., & Nnanna, I. A. (1993). Cholesterol Content and Functionality of Plasma and Granules Fractionated from Egg Yolk. *Journal of Food Science*, *58*(6), 1277–1281. https://doi.org/10.1111/J.1365-2621.1993.TB06165.X

Emery, J. A., Norambuena, F., Trushenski, J., & Turchini, G. M. (2016). Uncoupling EPA and DHA in Fish Nutrition: Dietary Demand is Limited in Atlantic Salmon and Effectively Met by DHA Alone. *Lipids*, *51*(4), 399–412. https://doi.org/10.1007/S11745-016-4136-Y/FIGURES/1

Emma Ignaszewski. (2022). *2021 U.S. Retail Market Insights: Plant-based foods*. https://gfi.org/wp-content/uploads/2022/03/2021-U.S.-retail-market-insights_Plant-based-foods-GFI.pdf

European Parliament, & Council of the European Union. (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union*, *L 304*, 18–63.

Fact.MR. (2021). *Plant-based Fish Market: Analysis Report 2021 to 2031*. https://www.factmr.com/report/plant-based-fish-market

Featherstone, S. (Ed.). (2016). Mayonnaise and salad dressing products. In *A Complete Course in Canning and Related Processes* (pp. 369–384). Elsevier. https://doi.org/10.1016/B978-0-85709-679-1.00011-8

Francavilla, M., Franchi, M., Monteleone, M., & Caroppo, C. (2013). The Red Seaweed Gracilaria gracilis as a Multi Products Source. *Marine Drugs*, *11*(10), 3754–3776. https://doi.org/10.3390/md11103754

Frankel, E. (2005). Lipid Oxidation. In *Lipid Oxidation: Second Edition* (2nd ed.). Oily Press. https://doi.org/10.1533/9780857097927

Frankel, E. N. (1985). Chemistry of Autoxidation: Mechanism, products and flavor significance. *Flavor Chemistry of Fats and Oils*, 1–37.

Frankel, E. N., Satué-Gracia, T., Meyer, A. S., & German, J. B. (2002). Oxidative Stability of Fish and Algae Oils Containing Long-Chain Polyunsaturated Fatty Acids in Bulk and in Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry*, *50*(7), 2094–2099. https://doi.org/10.1021/jf0111458

Fraser, G. E. (1999). Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. *The American Journal of Clinical Nutrition*, *70*(3), 532s–538s. https://doi.org/10.1093/ajcn/70.3.532s

Fuller, G. W. (2011). *New Food Product Development: From Concept to Marketplace* (3rd ed.). CRC Press. https://doi.org/10.1201/b10521

Future Market Insights. (2022). *Plant-Based Protein Market*. https://www.futuremarketinsights.com/reports/plant-based-protein-market

Gandini, A., Gededzha, M. P., de Maayer, T., Barrow, P., & Mayne, E. (2021). Diagnosing coeliac disease: A literature review. *Human Immunology*, *82*(12), 930–936. https://doi.org/10.1016/J.HUMIMM.2021.07.015

Gao, J., & Bernard, A. (2018). An overview of knowledge sharing in new product development. *The International Journal of Advanced Manufacturing Technology*, *94*(5–8), 1545–1550. https://doi.org/10.1007/s00170-017-0140-5

Garicano Vilar, E., O'Sullivan, M. G., Kerry, J. P., & Kilcawley, K. N. (2020). Volatile compounds of six species of edible seaweed: A review. *Algal Research*, *45*, 101740. https://doi.org/10.1016/J.ALGAL.2019.101740

Gazolu-Rusanova, D., Mustan, F., Vinarov, Z., Tcholakova, S., Denkov, N., Stoyanov, S., & de Folter, J. W. J. (2020). Role of lysophospholipids on the interfacial and liquid film properties of enzymatically modified egg yolk solutions. *Food Hydrocolloids*, *99*, 105319. https://doi.org/10.1016/J.FOODHYD.2019.105319

Ghorbani Gorji, S., Calingacion, M., Smyth, H. E., & Fitzgerald, M. (2019). Effect of natural antioxidants on lipid oxidation in mayonnaise compared with BHA, the industry standard. *Metabolomics*, *15*(8), 106. https://doi.org/10.1007/s11306-019-1568-4

Ghorbani Gorji, S., Smyth, H. E., Sharma, M., & Fitzgerald, M. (2016). Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. *Trends in Food Science & Technology*, *56*, 88–102. https://doi.org/10.1016/J.TIFS.2016.08.002

Gohtani, S., Sirendi, M., Yamamoto, N., Kajikawa, K., & Yamano, Y. (1999). Effect of droplet size on oxidation of docosahexaenoic in emulsion system. *Journal of Dispersion Science and Technology*, *20*(5), 1319–1325. https://doi.org/10.1080/01932699908943855

Grand View Research. (2022). *Gluten-Free Products Market Size*. https://www.grandviewresearch.com/industry-analysis/gluten-free-products-market

Gu, X., Campbell, L. J., & Euston, S. R. (2009). Effects of different oils on the properties of soy protein isolate emulsions and gels. *Food Research International*, *42*(8), 925–932. https://doi.org/10.1016/J.FOODRES.2009.04.015

Harnack, L., Mork, S., Valluri, S., Weber, C., Schmitz, K., Stevenson, J., & Pettit, J. (2021). Nutrient Composition of a Selection of Plant-Based Ground Beef Alternative Products Available in the United States. *Journal of the Academy of Nutrition and Dietetics*, *121*(12), 2401-2408.e12. https://doi.org/10.1016/J.JAND.2021.05.002

Harrison, L. J., & Cunningham, F. E. (1985). Factors Influencing the Quality of Mayonnaise: A review. *Journal of Food Quality*, *8*(1), 1–20. https://doi.org/10.1111/J.1745-4557.1985.TB00828.X

Hartvigsen, K., Lund, P., Hansen, L. F., & Hølmer, G. (2000). *Dynamic Headspace Gas Chromatography/Mass Spectrometry Characterization of Volatiles Produced in Fish Oil Enriched Mayonnaise during Storage*. https://doi.org/10.1021/jf991385b

Hegenauer, J., Saltman, P., & Nace, G. (2002). Iron(III)-phosphoprotein chelates: stoichiometric equilibrium constant for interaction of iron(III) and phosphorylserine residues of phosvitin and casein. *Biochemistry*, *18*(18), 3865–3879. https://doi.org/10.1021/BI00585A006

Herpich, C., Müller-Werdan, U., & Norman, K. (2022). Role of plant-based diets in promoting health and longevity. *Maturitas*, *165*, 47–51. https://doi.org/10.1016/j.maturitas.2022.07.003

Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M., & Allgeier, C. (2020). Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. *Nutrients*, *12*(12), 3704. https://doi.org/10.3390/nu12123704

Hoffman, J. R., & Falvo, M. J. (2004). Protein - Which is the best? *Journal of Sports Science and Medicine*, *3*. http://www.jssm.org

Hollowood, T. A., Linforth, R. S. T., & Taylor, A. J. (2002). The Effect of Viscosity on the Perception of Flavour. *Chemical Senses*, *27*(7), 583–591. https://doi.org/10.1093/chemse/27.7.583

Hopkin, L., Broadbent, H., & Ahlborn, G. J. (2022). Influence of almond and coconut flours on Ketogenic, Gluten-Free cupcakes. *Food Chemistry: X*, *13*, 100182. https://doi.org/10.1016/J.FOCHX.2021.100182

Horn, A. F. (2012). Factors Influencing the Effect of Milkbased Emulsifiers on Lipid Oxidation in Omega-3 Emulsions. In *Citation*. APA.

Hsleh, T. C. Y., Williams, S. S., Vejaphan, W., & Meyers, S. P. (1989). Characterization of volatile components of menhaden fish (brevoortia tyrannus) oil. *Journal of the American Oil Chemists' Society*, *66*(1), 114. https://doi.org/10.1007/BF02661797

Hu, M., McClements, D. J., & Decker, E. A. (2003). Lipid Oxidation in Corn Oil-in-Water Emulsions Stabilized by Casein, Whey Protein Isolate, and Soy Protein Isolate. *Journal of Agricultural and Food Chemistry*, *51*(6), 1696–1700. https://doi.org/10.1021/jf020952j

Instituto Nacional de Saúde Doutor Ricardo Jorge. (n.d.). *PortFIR - Tabela de Composição de Alimentos*. Retrieved 19 May 2022, from http://portfir.insa.pt/foodcomp/introduction

Jacobsen, C. (1999). Sensory impact of lipid oxidation in complex food systems. *Lipid / Fett*, *101*(12), 484–492. https://doi.org/https://doi.org/10.1002/(SICI)1521-4133(199912)101:12<484::AID-LIPI484>3.0.CO;2-H

Jacobsen, C. (2016). Oxidative Stability and Shelf Life of Food Emulsions. In M. Hu & C. Jacobsen (Eds.), *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats* (pp. 287–312). AOCS Press. https://doi.org/10.1016/B978-1-63067-056-6.00008-2

Jacobsen, C., Adler-Nissen, J., & Meyer, A. S. (1999). Effect of Ascorbic Acid on Iron Release from the Emulsifier Interface and on the Oxidative Flavor Deterioration in Fish Oil Enriched Mayonnaise. *Journal of Agricultural and Food Chemistry*, *47*(12), 4917–4926. https://doi.org/10.1021/JF990241U

Jacobsen, C., Hartvigsen, K., Lund, P., Adler-Nissen, J., Hømler, G., & Meyer, A. S. (2000). Oxidation in fish-oil-enriched mayonnaise. *European Food Research and Technology 2000 210:4*, *210*(4), 242–257. https://doi.org/10.1007/S002179900070

Jacobsen, C., Hartvigsen, K., Lund, P., Thomsen, M., Skibsted, L., Adler-Nissen, J., Hølmer, G., & Meyer, A. S. (2000). Oxidation in fish oil-enriched mayonnaise 3. Assessment of the influence of the emulsion structure on oxidation by discriminant partial least squares regression analysis. *European Food Research and Technology*, *211*(2), 86–98. https://doi.org/10.1007/s002179900132

Jacobsen, C., Timm, M., & Meyer, A. (2001). Oxidation in Fish Oil Enriched Mayonnaise:  Ascorbic Acid and Low pH Increase Oxidative Deterioration. *Journal of Agricultural and Food Chemistry*, *49*(8), 3947–3956. https://doi.org/10.1021/JF001253E

Jacobsen, C., Xu, X., Nielsen, N. S., & Timm-Heinrich, M. (2003). Oxidative stability of mayonnaise containing structured lipids produced from sunflower oil and caprylic acid. *European Journal of Lipid Science and Technology*, *105*(8), 449–458. https://doi.org/10.1002/EJLT.200300796

Kajla, P., Sharma, A., & Sood, D. R. (2015). Flaxseed—a potential functional food source. *Journal of Food Science and Technology*, *52*(4), 1857–1871. https://doi.org/10.1007/S13197-014-1293-Y/TABLES/4

Karahadian, C., & Lindsay, R. C. (1989). Evaluation of compounds contributing characterizing fishy flavors in fish oils. *Journal of the American Oil Chemists' Society*, *66*(7), 953–960. https://doi.org/10.1007/BF02682616

Kauffmann, D. (2016). *Nutrition in protracted crises*. Food and Agriculture Organization of the United Nations. https://www.fao.org/documents/card/en/c/9bd90d6c-196e-4802-a69e-50f969eac6b8/

Kaur, D., Wani, A. A., Singh, P., & Sogi, D. S. (2011). Shelf Life Enhancement of Butter, Ice-Cream, and Mayonnaise by Addition of Lycopene. *International Journal of Food Properties*, *14*, 1217–1231. https://doi.org/10.1080/10942911003637335

Kaur, M., Singh, V., & Kaur, R. (2017). Effect of partial replacement of wheat flour with varying levels of flaxseed flour on physicochemical, antioxidant and sensory characteristics of cookies. *Bioactive Carbohydrates and Dietary Fibre*, *9*, 14–20. https://doi.org/10.1016/J.BCDF.2016.12.002

Kazir, M., & Livney, Y. D. (2021). Plant-Based Seafood Analogs. *Molecules*, *26*(6), 1559. https://doi.org/10.3390/molecules26061559

Kelly, A. L., Moore, M. M., & Arendt, E. K. (2008). New product development: the case of gluten-free food products. In E. K. Arendt & F. Dal Bello (Eds.), *Gluten-Free Cereal Products and Beverages* (1st ed., pp. 413–431). Elsevier. https://doi.org/10.1016/B978-012373739-7.50020-4

Kelly, C. P., Murray, J. A., Leffler, D. A., Getts, D. R., Bledsoe, A. C., Smithson, G., First, M. R., Morris, A., Boyne, M., Elhofy, A., Wu, T. T., Podojil, J. R., Miller, S. D., Fogel, R., Freitag, T. L., Gerber, M., Haynes, P. K., Koren, M., Matson, M., ... Turner, M. (2021). TAK-101 Nanoparticles Induce Gluten-Specific Tolerance in Celiac Disease: A Randomized, Double-Blind, Placebo-Controlled Study. *Gastroenterology*, *161*(1), 66-80.e8. https://doi.org/10.1053/j.gastro.2021.03.014

Kent, G., Kehoe, L., McNulty, B. A., Nugent, A. P., Flynn, A., & Walton, J. (2022). A standardised methodological approach for characterising the plant-based component of population or individual diets.

*Journal of Food Composition and Analysis*, *114*, 104727. https://doi.org/https://doi.org/10.1016/j.jfca.2022.104727

Key, T. J., Appleby, P. N., Crowe, F. L., Bradbury, K. E., Schmidt, J. A., & Travis, R. C. (2014). Cancer in British vegetarians: updated analyses of 4998 incident cancers in a cohort of 32,491 meat eaters, 8612 fish eaters, 18,298 vegetarians, and 2246 vegans. *The American Journal of Clinical Nutrition*, *100*(suppl_1), 378S-385S. https://doi.org/10.3945/ajcn.113.071266

Khalid, M. U., Shabbir, M. A., Mustafa, S., Hina, S., Quddoos, M. Y., Mahmood, S., Maryam, Y., Faisal, F., & Rafique, A. (2021). Effect of Apple peel as an antioxidant on the quality characteristics and oxidative stability of mayonnaise. *Applied Food Research*, *1*(2), 100023. https://doi.org/10.1016/J.AFRES.2021.100023

Kishk, Y. F. M., & Elsheshetawy, H. E. (2013). Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics. *Annals of Agricultural Sciences*, *58*(2), 213–220. https://doi.org/10.1016/J.AOAS.2013.07.016

Kılıç Keskin, H., Bilgiçli, N., & Yaver, E. (2022). Development of gluten-free tarhana formulations: Part II. Utilization of legume composite flours and baker's yeast. *Food Bioscience*, *47*, 101692. https://doi.org/10.1016/J.FBIO.2022.101692

Koehler, P., Wieser, H., & Konitzer, K. (2014a). Celiac Disease—A Complex Disorder. In P. Koehler, H. Wieser, & K. Konitzer (Eds.), *Celiac Disease and Gluten* (1st ed., pp. 1–96). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-420220-7.00001-8

Koehler, P., Wieser, H., & Konitzer, K. (2014b). Gluten-Free Products. In P. Koehler, H. Wieser, & K. Konitzer (Eds.), *Celiac Disease and Gluten* (1st ed., pp. 173–223). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-420220-7.00004-3

Koehler, P., Wieser, H., & Konitzer, K. (2014c). Gluten—The Precipitating Factor. In P. Koehler, H. Wieser, & K. Konitzer (Eds.), *Celiac Disease and Gluten* (1st ed., pp. 97–148). Academic Press. https://doi.org/10.1016/B978-0-12-420220-7.00002-X

Koehler, P., Wieser, H., & Konitzer, K. (2014d). Treatment of Celiac Disease. In P. Koehler, H. Wieser, & K. Konitzer (Eds.), *Celiac Disease and Gluten* (1st ed., pp. 149–171). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-420220-7.00003-1

Korus, J., Witczak, T., Ziobro, R., & Juszczak, L. (2015). Linseed (Linum usitatissimum L.) mucilage as a novel structure forming agent in gluten-free bread. *LWT - Food Science and Technology*, *62*(1), 257–264. https://doi.org/10.1016/J.LWT.2015.01.040

Kreymann, G., Adolph, M., Mueller, M. J., & Working group for developing the guidelines for parenteral nutrition of The German Association for Nutritional Medicine. (2009). Energy expenditure and energy intake-Guidelines on Parenteral Nutrition, Chapter 3. *German Medical Science*, *7*(25). https://doi.org/10.3205/000084

Kumar, D., & Tanwar, V. K. (2011). Effects of incorporation of ground mustard on quality attributes of chicken nuggets. *Journal of Food Science and Technology*, *48*(6), 759–762. https://doi.org/10.1007/S13197-010-0149-3

Kupper, C. (2005). Dietary guidelines and implementation for celiac disease. *Gastroenterology*, *128*(4 SUPPL. 1), S121–S127. https://doi.org/10.1053/j.gastro.2005.02.024

Lagunes-Galvez, L., Cuvelier, M.-E., Ordonnaud, C., & Berset, C. (2002). Oxidative stability of some mayonnaise formulations during storage and daylight irradiation. *Journal of Food Lipids*, *9*(3), 211–224. https://doi.org/10.1111/j.1745-4522.2002.tb00220.x

Lähdeaho, M. L., Scheinin, M., Vuotikka, P., Taavela, J., Popp, A., Laukkarinen, J., Koffert, J., Koivurova, O. P., Pesu, M., Kivelä, L., Lovró, Z., Keisala, J., Isola, J., Parnes, J. R., Leon, F., & Mäki, M. (2019). Safety and efficacy of AMG 714 in adults with coeliac disease exposed to gluten challenge: a phase 2a, randomised, double-blind, placebo-controlled study. *The Lancet Gastroenterology & Hepatology*, *4*(12), 948–959. https://doi.org/10.1016/S2468-1253(19)30264-X

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure–function approach. *Food Chemistry*, *141*(2), 975–984. https://doi.org/10.1016/J.FOODCHEM.2013.04.038

le Denmat, M., Anton, M., & Beaumal, V. (2000). Characterisation of emulsion properties and of interface composition in O/W emulsions prepared with hen egg yolk, plasma and granules. In *Food Hydrocolloids* (Vol. 14, Issue 6, pp. 539–549). https://doi.org/10.1016/S0268-005X(00)00034-5

Ledoux, T. A., Hingle, M. D., & Baranowski, T. (2011). Relationship of fruit and vegetable intake with adiposity: a systematic review. *Obesity Reviews*, *12*(5), e143–e150. https://doi.org/https://doi.org/10.1111/j.1467-789X.2010.00786.x

Lethuaut, L., Weel, K. G. C., Boelrijk, A. E. M., & Brossard, C. D. (2004). Flavor Perception and Aroma Release from Model Dairy Desserts. *Journal of Agricultural and Food Chemistry*, *52*(11), 3478–3485. https://doi.org/10.1021/jf035488c

Li, C. Y., Kim, H. W., Li, H., Lee, D. C., & Rhee, H. I. (2014). Antioxidative effect of purple corn extracts during storage of mayonnaise. *Food Chemistry*, *152*, 592–596. https://doi.org/10.1016/J.FOODCHEM.2013.11.152

Li, C.-Y., Kim, H.-W., Li, H., Lee, D.-C., & Rhee, H.-I. (2013). *Antioxidative effect of purple corn extracts during storage of mayonnaise q*. https://doi.org/10.1016/j.foodchem.2013.11.152

Liu, C., Pei, R., & Heinonen, M. (2022). Faba bean protein: A promising plant-based emulsifier for improving physical and oxidative stabilities of oil-in-water emulsions. *Food Chemistry*, *369*, 130879. https://doi.org/10.1016/J.FOODCHEM.2021.130879

Maqsood, S., & Benjakul, S. (2010). Comparative studies of four different phenolic compounds on in vitro antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. *Food Chemistry*, *119*(1), 123–132. https://doi.org/10.1016/J.FOODCHEM.2009.06.004

Marsh, K. A., Munn, E. A., & Baines, S. K. (2013). Protein and vegetarian diets. *Medical Journal of Australia*, *199*(4), S7–S10. https://doi.org/10.5694/MJA11.11492

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., & Grossmann, L. (2021a). The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 4049–4100. https://doi.org/10.1111/1541-4337.12771

McClements, D. J., & Grossmann, L. (2021b). A brief review of the science behind the design of healthy and sustainable plant-based foods. *Npj Science of Food*, *5*, 17. https://doi.org/10.1038/s41538-021-00099-y

McClements, D. J., & Grossmann, L. (2022). *Next-Generation Plant-based Foods*. Springer International Publishing. https://doi.org/10.1007/978-3-030-96764-2

Mettler, A. E. (1986). The role of the food industry. *Acta Paediatrica Scandinavica Supplement*, *323*(SUPPL. 323), 84–95. https://doi.org/10.1111/J.1651-2227.1986.TB10354.X

Milani, M. A., Mizani, M., Ghavami, M., & Eshratabadi, P. (2013). The Physico-Chemical Influences of Yellow Mustard Paste - Comparison with the Powder in Mayonnaise. *Journal of Food Processing and Technology*, *4*(3). https://doi.org/10.4172/2157-7110.1000210

Ministry of Foreign Affairs. (n.d.). *History - About Portugal*. Permanent Mission of Portugal to the United Nations. Retrieved 16 May 2022, from https://onu.missaoportugal.mne.gov.pt/en/about-portugal/history

Morley, W. G. (2016). Mayonnaise. *Encyclopedia of Food and Health*, 669–676. https://doi.org/10.1016/B978-0-12-384947-2.00449-9

Moskowitz, H. R., & Arabie, P. (1970). Taste intensity as a function of stimulus concentration and solvent viscosity. *Journal of Texture Studies*, *1*(4), 502–510. https://doi.org/https://doi.org/10.1111/j.1745-4603.1970.tb00748.x

Nelson, D. L. (David L., Nelson, D. L. (David L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry*. W.H. Freeman.

Nicolle, D. (2012). *The Portuguese in the Age of Discovery c.1340–1665* (1st ed.). Osprey Publishing.

Nielsen, N. S., Petersen, A., Meyer, A. S., Timm-Heinrich, M., & Jacobsen, C. (2004). Effects of Lactoferrin, Phytic Acid, and EDTA on Oxidation in Two Food Emulsions Enriched with Long-Chain Polyunsaturated Fatty Acids. *Journal of Agricultural and Food Chemistry*, *52*(25), 7690–7699. https://doi.org/10.1021/JF0492316

Öhlund, K., Olsson, C., Hernell, O., & Öhlund, I. (2010). Dietary shortcomings in children on a gluten-free diet. *Journal of Human Nutrition and Dietetics*, *23*(3), 294–300. https://doi.org/10.1111/J.1365-277X.2010.01060.X

Orlich, M. J., & Fraser, G. E. (2014). Vegetarian diets in the Adventist Health Study 2: a review of initial published findings. *The American Journal of Clinical Nutrition*, *100*(suppl_1), 353S-358S. https://doi.org/10.3945/ajcn.113.071233

Orlich, M. J., Singh, P. N., Sabaté, J., Fan, J., Sveen, L., Bennett, H., Knutsen, S. F., Beeson, W. L., Jaceldo-Siegl, K., Butler, T. L., Herring, R. P., & Fraser, G. E. (2015). Vegetarian Dietary Patterns and the Risk of Colorectal Cancers. *JAMA Internal Medicine*, *175*(5), 767. https://doi.org/10.1001/jamainternmed.2015.59

Orlich, M. J., Singh, P. N., Sabaté, J., Jaceldo-Siegl, K., Fan, J., Knutsen, S., Beeson, W. L., & Fraser, G. E. (2013). Vegetarian Dietary Patterns and Mortality in Adventist Health Study 2. *JAMA Internal Medicine*, *173*(13), 1230. https://doi.org/10.1001/jamainternmed.2013.6473

Osborn, H. T., & Akoh, C. C. (2004). Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chemistry*, *84*(3), 451–456. https://doi.org/https://doi.org/10.1016/S0308-8146(03)00270-X

Pangborn, R. M., Trabue, I. D. A. M., & Szczesniak, A. S. (1973). Effect of hydrocolloids on oral viscosity and basic taste intensities. *Journal of Texture Studies*, *4*(2), 224–241. https://doi.org/https://doi.org/10.1111/j.1745-4603.1973.tb00666.x

Patwardhan, B., Mutalik, G., & Tillu, G. (2015). Concepts of Health and Disease. In B. Patwardhan, G. Mutalik, & G. Tillu (Eds.), *Integrative Approaches for Health* (pp. 53–78). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-801282-6.00003-6

Pieniak, Z., Verbeke, W., Vanhonacker, F., Guerrero, L., & Hersleth, M. (2009). Association between traditional food consumption and motives for food choice in six European countries. *Appetite*, *53*(1), 101–108. https://doi.org/10.1016/J.APPET.2009.05.019

Polo, A., Arora, K., Ameur, H., di Cagno, R., de Angelis, M., & Gobbetti, M. (2020). Gluten-free diet and gut microbiome. *Journal of Cereal Science*, *95*, 103058. https://doi.org/10.1016/J.JCS.2020.103058

Portugal Foods. (2021). *Trends 2021 - Setor Agroalimentar*.

Raikos, V., McDonagh, A., Ranawana, V., & Duthie, G. (2016). Processed beetroot (Beta vulgaris L.) as a natural antioxidant in mayonnaise: Effects on physical stability, texture and sensory attributes. *Food Science and Human Wellness*, *5*(4), 191–198. https://doi.org/10.1016/J.FSHW.2016.10.002

Richardson, A., Chu, S., Agapides, M., Adelstein, S., Wallman, L., & Wienholt, L. (2022). Gluten-free diet adherence and implications for the diagnosis of coeliac disease. *Pathology*, *54*(5), 606–610. https://doi.org/10.1016/j.pathol.2021.12.297

Rimac-Brnčić, S., Lelas, V., Rade, D., & Šimundić, B. (2004). Decreasing of oil absorption in potato strips during deep fat frying. *Journal of Food Engineering*, *64*(2), 237–241. https://doi.org/10.1016/J.JFOODENG.2003.10.006

Romanić, R. (2020). Cold pressed sunflower (Helianthus annuus L.) oil. In M. F. Ramadan (Ed.), *Cold Pressed Oils* (pp. 197–218). Academic Press. https://doi.org/10.1016/B978-0-12-818188-1.00017-7

Schneiderman, N., Ironson, G., & Siegel, S. D. (2005). Stress and Health: Psychological, Behavioral, and Biological Determinants. *Annual Review of Clinical Psychology*, *1*(1), 607–628. https://doi.org/10.1146/annurev.clinpsy.1.102803.144141

Schuppan, D., Mäki, M., Lundin, K. E. A., Isola, J., Friesing-Sosnik, T., Taavela, J., Popp, A., Koskenpato, J., Langhorst, J., Hovde, Ø., Lähdeaho, M.-L., Fusco, S., Schumann, M., Török, H. P., Kupcinskas, J., Zopf, Y., Lohse, A. W., Scheinin, M., Kull, K., … Greinwald, R. (2021). A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease. *New England Journal of Medicine*, *385*(1), 35–45. https://doi.org/10.1056/NEJMoa2032441

Shahidi, F. (2015). Handbook of Antioxidants for Food Preservation. In F. Shahidi (Ed.), *Handbook of Antioxidants for Food Preservation* (1st ed.). Woodhead Publishing.

Sharafi, Y., Majidi, M. M., Goli, S. A. H., & Rashidi, F. (2015). Oil Content and Fatty Acids Composition in Brassica Species. *International Journal of Food Properties*, *18*(10), 2145–2154. https://doi.org/10.1080/10942912.2014.968284

Sharma, M. K., & Shad, D. O. (1985). Introduction to Macro- and Microemulsions. In *Macro- and Microemulsions* (Vol. 272, pp. 1–18). American Chemical Society. https://doi.org/doi:10.1021/bk-1985-0272.ch001

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (2002). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*(6), 945–948. https://doi.org/10.1021/JF00018A005

Silva, S., Pinho, J. P., Borges, C., Santos, C., Santos, A., & Graça, P. (2015). *Linhas de Orientação para uma Alimentação Vegetariana Saudável* (Programa Nacional para a Promoção da Alimentação Saudável, Ed.). Direção-Geral da Saúde.

Singh, P. N., & Lindsted, K. D. (1998). Body Mass and 26-Year Risk of Mortality from Specific Diseases among Women Who Never Smoked. *Epidemiology*, *9*(3). https://journals.lww.com/epidem/Fulltext/1998/05000/Body_Mass_and_26_Year_Risk_of_Mortality_from.7.aspx

Skerrett, P. J., & Willett, W. C. (2010). Essentials of Healthy Eating: A Guide. *Journal of Midwifery & Women's Health*, *55*(6), 492–501. https://doi.org/10.1016/J.JMWH.2010.06.019

Snowdon, D. A., & Phillips, R. L. (1985). Does a vegetarian diet reduce the occurrence of diabetes? *American Journal of Public Health*, *75*(5), 507–512. https://doi.org/10.2105/AJPH.75.5.507

Sørensen, A. D. M., Nielsen, N. S., Hyldig, G., & Jacobsen, C. (2010). Influence of emulsifier type on lipid oxidation in fish oil-enriched light mayonnaise. *European Journal of Lipid Science and Technology*, *112*(9), 1012–1023. https://doi.org/10.1002/EJLT.200900288

Sørensen, A. D. M., Nielsen, N. S., & Jacobsen, C. (2010). Oxidative stability of fish oil-enriched mayonnaise-based salads. *European Journal of Lipid Science and Technology*, *112*(4), 476–487. https://doi.org/10.1002/EJLT.200900180

Štern, P., Valentová, H., & Pokorný, J. (2001). Rheological properties and sensory texture of mayonnaise. *European Journal of Lipid Science and Technology*, *103*(1), 23–28. https://doi.org/https://doi.org/10.1002/1438-9312(200101)103:1<23::AID-EJLT23>3.0.CO;2-P

Sung, W. C., & Chai, P.-S. (2017). Effect of Flaxseed Flour and Xanthan Gum on Gluten-Free Cake Properties. *Journal of Food and Nutrition Research, Vol. 5, 2017, Pages 717-728*, *5*(10), 717–728. https://doi.org/10.12691/JFNR-5-10-1

Świąder, K., & Marczewska, M. (2021). Trends of Using Sensory Evaluation in New Product Development in the Food Industry in Countries That Belong to the EIT Regional Innovation Scheme. *Foods*, *10*(446). https://doi.org/10.3390/foods10020446

Szalaj, D., Torres, M. A., Veiga-Malta, T., Angélico, M. M., Sobrinho-Gonçalves, L., Chaves, C., Alcoforado, B., Garrido, S., Ré, P., Cabral, H., & Silva, A. (2021). Food-web dynamics in the Portuguese continental shelf ecosystem between 1986 and 2017: Unravelling drivers of sardine decline. *Estuarine, Coastal and Shelf Science*, *251*, 107259. https://doi.org/10.1016/j.ecss.2021.107259

Takai, J. I., Endo, Y., Okuzaki, M., & Fujimoto, K. (2003). Autoxidation of Fish Oil in Mayonnaise-Like O/W Type Emulsion. *Food Science and Technology Research*, *9*(4), 383–386. https://doi.org/10.3136/FSTR.9.383

The Kraft Heinz Company. (2022). *Unpublished work*.

Thompson, T., Dennis, M., Higgins, L. A., Lee, A. R., & Sharrett, M. K. (2005). Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *Journal of Human Nutrition and Dietetics*, *18*(3), 163–169. https://doi.org/10.1111/J.1365-277X.2005.00607.X

Thomson, M. K., Jacobsen, C., & Skibsted, L. H. (2000). Mechanism of initiation of oxidation in mayonnaise enriched with fish oil as studied by electron spin resonance spectroscopy. *European Food Research and Technology 2000 211:6*, *211*(6), 381–386. https://doi.org/10.1007/S002170000199

Thorogood, M., Mann, J., Appleby, P., & McPherson, K. (1994). Risk of death from cancer and ischaemic heart disease in meat and non-meat eaters. *BMJ*, *308*(6945), 1667–1670. https://doi.org/10.1136/bmj.308.6945.1667

Toribio-Mateas, M. A., Bester, A., & Klimenko, N. (2021). Impact of Plant-Based Meat Alternatives on the Gut Microbiota of Consumers: A Real-World Study. *Foods*, *10*(9), 2040. https://doi.org/10.3390/foods10092040

Turner-McGrievy, G. M., Wirth, M. D., Shivappa, N., Wingard, E. E., Fayad, R., Wilcox, S., Frongillo, E. A., & Hébert, J. R. (2015). Randomization to plant-based dietary approaches leads to larger short-term improvements in Dietary Inflammatory Index scores and macronutrient intake compared with diets that contain meat. *Nutrition Research*, *35*(2), 97–106. https://doi.org/10.1016/j.nutres.2014.11.007

Uluata, S., McClements, D. J., & Decker, E. A. (2016). Riboflavin-induced oxidation in fish oil-in-water emulsions: Impact of particle size and optical transparency. *Food Chemistry*, *213*, 457–461. https://doi.org/10.1016/J.FOODCHEM.2016.06.103

Vaisey, M., Brunon, R., & Cooper, J. (1969). Some Sensory Effects of Hydrocolloid Sols on Sweetness. *Journal of Food Science*, *34*(5), 397–400. https://doi.org/https://doi.org/10.1111/j.1365-2621.1969.tb12788.x

van Hees, N. J. M., Giltay, E. J., Tielemans, S. M. A. J., Geleijnse, J. M., Puvill, T., Janssen, N., & van der Does, W. (2015). Essential Amino Acids in the Gluten-Free Diet and Serum in Relation to Depression in Patients with Celiac Disease. *PLOS ONE*, *10*(4), e0122619. https://doi.org/10.1371/JOURNAL.PONE.0122619

Venkateshwarlu, G., Let, M. B., Meyer, A. S., & Jacobsen, C. (2004a). *Chemical and Olfactometric Characterization of Volatile Flavor Compounds in a Fish Oil Enriched Milk Emulsion*. https://doi.org/10.1021/jf034833v

Venkateshwarlu, G., Let, M. B., Meyer, A. S., & Jacobsen, C. (2004b). *Modeling the Sensory Impact of Defined Combinations of Volatile Lipid Oxidation Products on Fishy and Metallic Off-Flavors*. https://doi.org/10.1021/jf0351321

Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological Implications of Lipid Oxidation Products. *Journal of the American Oil Chemists' Society*, *94*(3), 339–351. https://doi.org/https://doi.org/10.1007/s11746-017-2958-2

Vlontzos, G., Kyrgiakos, L., & Duquenne, M. (2018). What Are the Main Drivers of Young Consumers Purchasing Traditional Food Products? European Field Research. *Foods*, *7*(2), 22. https://doi.org/10.3390/foods7020022

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

Warner, K., Frankel, E. N., & Mounts, T. L. (1989). Flavor and Oxidative Stability of Soybean, Sunflower and Low Erucic Acid Rapeseed Oils 1. In *JAOCS* (Vol. 66, Issue 4, pp. 558–564). https://doi.org/10.1007/BF02885448

Weel, K. G. C., Boelrijk, A. E. M., Alting, A. C., van Mil, P. J. J. M., Burger, J. J., Gruppen, H., Voragen, A. G. J., & Smit, G. (2002). Flavor Release and Perception of Flavored Whey Protein Gels: Perception Is Determined by Texture Rather than by Release. *Journal of Agricultural and Food Chemistry*, *50*(18), 5149–5155. https://doi.org/10.1021/jf0202786

Woodfield, H. K., & Harwood, J. L. (2017). Oilseed Crops: Linseed, Rapeseed, Soybean, and Sunflower. In B. Thomas, B. G. Murray, & D. J. Murphy (Eds.), *Encyclopedia of Applied Plant Sciences* (2nd ed., Vol. 3, pp. 34–38). Academic Press. https://doi.org/10.1016/B978-0-12-394807-6.00212-4

World Health Organization. (2017, February 3). *Determinants of health*. Questions and Answers. https://www.who.int/news-room/questions-and-answers/item/determinants-of-health

World Health Organization. (2020, April 29). *Healthy diet*. https://www.who.int/en/news-room/fact-sheets/detail/healthy-diet

*World Population Prospects 2022: Summary of Results*. (2022).

Xu, L., Yu, X., Li, M., Chen, J., & Wang, X. (2017). Monitoring oxidative stability and changes in key volatile compounds in edible oils during ambient storage through HS-SPME/GC–MS. *International Journal of Food Properties*, *20*(sup3), S2926–S2938. https://doi.org/10.1080/10942912.2017.1382510

Yamauchi, R., Aoki, Y., Sugiura, T., Kato, K., & Ueno, Y. (1982). Effect of Sugars and Sugar Analogs on Autoxidation of Methyl Linoleate and Safflower Oil. *Agricultural and Biological Chemistry*, *46*(12), 2997–3002. https://doi.org/10.1080/00021369.1982.10865548

Zuccotti, G., Fabiano, V., Dilillo, D., Picca, M., Cravidi, C., & Brambilla, P. (2013). Intakes of nutrients in Italian children with celiac disease and the role of commercially available gluten-free products. *Journal of Human Nutrition and Dietetics*, *26*(5), 436–444. https://doi.org/10.1111/JHN.12026

## Appendix A. Mayonnaise formulations

### I. Natural antioxidants testing

Regarding extract L study, following the natural EDTA replacer, rapeseed oil used in the production of HSG and Vegan mayonnaises (Table A.1) had an average peroxide value of 2.02 and 1.92 meq $O_2$/kg, respectively.

*Table A.1. HSG and Vegan mayonnaise recipes for extract L study*

| Ingredients | HSG EDTA | HSG no EDTA | HSG L | Vegan EDTA | Vegan no EDTA | Vegan L |
|---|---|---|---|---|---|---|
| **Water phase** | | | | | | |
| Water | x | x | x | x | x | x |
| EDTA | x | | | x | | |
| Extract L | | | x | | | x |
| Sugar | x | x | x | x | x | x |
| Salt | x | x | x | x | x | x |
| Mustard | x | x | x | | | |
| Natural Flavour | | | | x | x | x |
| **Emulsifier** | | | | | | |
| Liquid Egg Yolk | x | x | x | | | |
| Modified starch | | | | x | x | x |
| **Oil phase** | | | | | | |
| Rapeseed Oil | x | x | x | x | x | x |
| Cold swelling starch 1 | x | x | x | | | |
| Cold swelling starch 2 | x | x | x | | | |
| **Vinegar** | | | | | | |
| Spirit vinegar 19.5 % | x | x | x | x | x | x |

For the extract X study, that used a rosemary and spinach extract in mayonnaise, the rapeseed oil included in the production of HSG (Table A.2) and Vegan mayonnaises (Table A.3) had an average peroxide value of 1.09 meq $O_2$/kg.

*Table A.2. HSG mayonnaise recipes for extract X study*

| Ingredients | HSG EDTA | HSG no EDTA | HSG X1 pH 3.5 | HSG X2 pH 3.5 | HSG X2 pH 4.0 |
|---|---|---|---|---|---|
| **Water phase** | | | | | |
| Water | x | x | x | x | x |
| EDTA | x | | | | |
| Extract X1 | | | x | | |
| Extract X2 | | | | x | x |
| Sugar | x | x | x | x | x |
| Salt | x | x | x | x | x |
| Mustard | x | x | x | x | x |
| Trisodium citrate | | | | | x |
| **Emulsifier** | | | | | |
| Liquid Egg Yolk | x | x | x | x | x |
| **Oil phase** | | | | | |
| Rapeseed Oil | x | x | x | x | x |
| Cold swelling starch 1 | x | x | x | x | x |
| Cold swelling starch 1 | x | x | x | x | x |
| **Vinegar** | | | | | |
| Spirit Vinegar 19.5 % | x | x | x | x | x |

*Table A.3. Vegan mayonnaise recipes for extract X study*

| Ingredients | Vegan EDTA | Vegan no EDTA | Vegan X2 pH 3.0 |
|---|---|---|---|
| **Water phase** | | | |
| Water | x | x | x |
| EDTA | x | | |
| Extract X2 | | | x |
| Sugar | x | x | x |
| Salt | x | x | x |
| Natural Flavour | x | x | x |
| **Emulsifier** | | | |
| Modified starch | x | x | x |
| **Oil phase** | | | |
| Rapeseed Oil | x | x | x |
| **Vinegar** | | | |
| Spirit Vinegar 19.5 % | x | x | x |

## I. Mayonnaise formulations – Ideal Recipe Design

Concerning the Ideal Recipe Design workstream, following two factors (i.e., egg type, oil type), oils with an average peroxide value as shown in Table A.4 were used in the production of the different HSG mayonnaises (Table A.5 and Table A.6).

*Table A.4. Peroxide value of different types of oils used in the production of mayonnaises for Ideal Recipe Design*

| Type of oil | PV (meq $O_2$/kg) |
|---|---|
| Sunflower | 2.55 |
| Rapeseed | 1.60 |
| High Oleic Rapeseed | 3.29 |
| Soybean | 2.00 |

*Table A.5. Design of experiments' mayonnaise formulations*

| Ingredients | DOE | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **Water phase** | | | | | | | | | | | | | | | | |
| Water | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Sugar | x | x | x | x | x | x | x | x | a | a | a | a | a | a | a | a |
| Salt | x | x | x | x | x | x | x | x | a | a | a | a | a | a | a | a |
| Mustard | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Emulsifier** | | | | | | | | | | | | | | | | |
| Liquid Egg Yolk | x | x | x | x | | | | | | | | | | | | |
| Heat Stable Egg Yolk | | | | | x | x | x | x | | | | | | | | |
| Powdered Egg Yolk | | | | | | | | | a | a | a | a | | | | |
| Whole Egg/Egg Yolk | | | | | | | | | | | | | a | a | a | a |
| **Oil phase** | | | | | | | | | | | | | | | | |
| Sunflower Oil | x | | | | x | | | | x | | | | x | | | |
| Rapeseed Oil | | x | | | | x | | | | x | | | | x | | |
| High oleic Rapeseed Oil | | | x | | | | x | | | | x | | | | x | |
| Soybean Oil | | | | x | | | | x | | | | x | | | | x |
| Cold swelling starch 1 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Cold swelling starch 1 | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Vinegar** | | | | | | | | | | | | | | | | |
| Spirit vinegar 20 % | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |

Legend: a – ingredient quantity in formulation adjusted so that final quantity of sugar and salt is common to every sample.

*Table A.6. Positive references mayonnaise formulations*

| Ingredients | Sunflower EDTA | Rapeseed EDTA | Soybean EDTA |
|---|---|---|---|
| **Water phase** | | | |
| Water | x | x | x |
| EDTA | x | x | x |
| Sugar | x | x | x |
| Salt | x | x | x |
| Mustard | x | x | x |
| **Emulsifier** | | | |
| Liquid Egg yolk | x | x | x |
| **Oil phase** | | | |
| Sunflower Oil | x | | |
| Rapeseed Oil | | x | |
| Soybean Oil | | | x |
| Cold swelling starch 1 | x | x | x |
| Cold swelling starch 1 | x | x | x |
| **Vinegar** | | | |
| Spirit Vinegar 20 % | x | x | x |

## Appendix B. Mayonnaise sensory analysis

### I. Natural Antioxidants Testing

**Instructions**
Compare the sample with **reference sample 000** for the indicated taste attributes and score the difference you perceived compared to the **reference 000.**

**Degree of difference to reference 000:**
**0:** the same as reference 000;
**2:** slight differences;
**4:** slight to moderate differences;
**6:** moderate differences;
**8:** large differences.
**Negative score means lower in intensity and positive score means higher in intensity compared to the reference 000.**

| Sample Code | Appearance Thickness | Taste | | | | | | | | | | | Off taste | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Painty | Sour | Egg | Sulphuric egg | Mustard | Fatty/oily | Buttery | Rancid oil | Green/grass | Aromatic herbs | Metal | | | |
| | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Score* | *Describe the off taste* | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |

### II. Ideal Recipe Design

**Instructions**
Compare the sample with **reference sample 000** for the indicated taste attributes and score the difference you perceived compared to the **reference 000.**

**Degree of difference to reference 000:**
**0:** the same as reference 000
**2:** slight differences
**4:** slight to moderate differences
**6:** moderate differences
**8:** large differences
**Negative score means lower in intensity and positive score means higher in intensity compared to the reference 000.**

| Sample Code | Taste Rancid Oil | Taste Metal | Taste Egg | Overall flavour | Comments |
|---|---|---|---|---|---|
| | *Score* | *Score* | *Score* | *Score* | |
| | | | | | |
| | | | | | |
| | | | | | |

## Appendix C. Results and Discussion

I. Natural Antioxidants Testing

### Extract L study – Sensory profile

*Table C.1. Extract L study : sensory scores of HSG mayonnaise samples until 3- and 4-months storage at 20°C and 30°C, respectively*

| Storage | | Sample | Appearance | Taste | | | | | | | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T (°C)** | **t (months)** | | **Thickness** | **Painty** | **Sour** | **Egg** | **Sulphuric egg** | **Mustard** | **Fatty/ oily** | **Buttery** | **Rancid oil** | **Green/ grass** | **Aromatic herbs** | **Metal** | |
| - | 0 | HSG no EDTA | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | | HSG L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 3 | HSG no EDTA | -2 | 1 | **3** | 1 | 1 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 2 |
| | | HSG L | -1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | **2** | 3 |
| 30 | 1 | HSG no EDTA | **-3** | **2** | 1 | 2 | 2 | -1 | 2 | 0 | 3 | 0 | 0 | 1 | 1 |
| | | HSG L | -1 | 0 | 1 | 1 | 3 | -1 | 1 | 0 | 3 | 1 | 1 | **4** | **3** |
| | 2 | HSG no EDTA | -1 | 1 | -1 | 2 | 2 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 |
| | | HSG L | -1 | 2 | -1 | 0 | 3 | -1 | 2 | 0 | **5** | 0 | 0 | 4 | **4** |
| | 3 | HSG no EDTA | -1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 3 | 0 | 0 | 0 | 2 |
| | | HSG L | **-4** | 2 | 1 | -1 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 1 | 3 |
| | 4 | HSG no EDTA | -2 | 1 | 2 | 1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 2 |
| | | HSG L | -1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 3 | 0 | 0 | **2** | 1 |

*Table C.2. Extract L study: sensory scores of Vegan mayonnaise samples until 3- and 4-months storage at 20°C and 30°C, respectively*

| **Storage** | | **Sample** | **Appearance** | **Taste** | | | | | | | | | | | **Off taste** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T (°C)** | **t (months)** | | **Thickness** | **Painty** | **Sour** | **Egg** | **Sulphuric egg** | **Mustard** | **Fatty/ oily** | **Buttery** | **Rancid oil** | **Green/ grass** | **Aromatic herbs** | **Metal** | |
| - | 0 | Vegan no EDTA | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 1 |
| | | Vegan L | **-2** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 20 | 3 | Vegan no EDTA | 0 | **3** | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 1 |
| | | Vegan L | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 |
| 30 | 1 | Vegan no EDTA | -2 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 2 |
| | | Vegan L | 1 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| | 2 | Vegan no EDTA | 1 | 4 | 2 | 0 | 0 | 0 | 2 | 0 | 2 | 4 | 1 | 2 | 3 |
| | | Vegan L | 0 | 4 | 2 | 0 | 0 | 0 | 2 | 0 | 2 | 4 | 2 | 3 | 3 |
| | 3 | Vegan no EDTA | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | **3** | 0 | 0 | 0 | 2 |
| | | Vegan L | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| | 4 | Vegan no EDTA | -1 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | 0 | 1 |
| | | Vegan L | 0 | 3 | 1 | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 0 | 1 | 2 |

**Extract X study – Physicochemical profile**

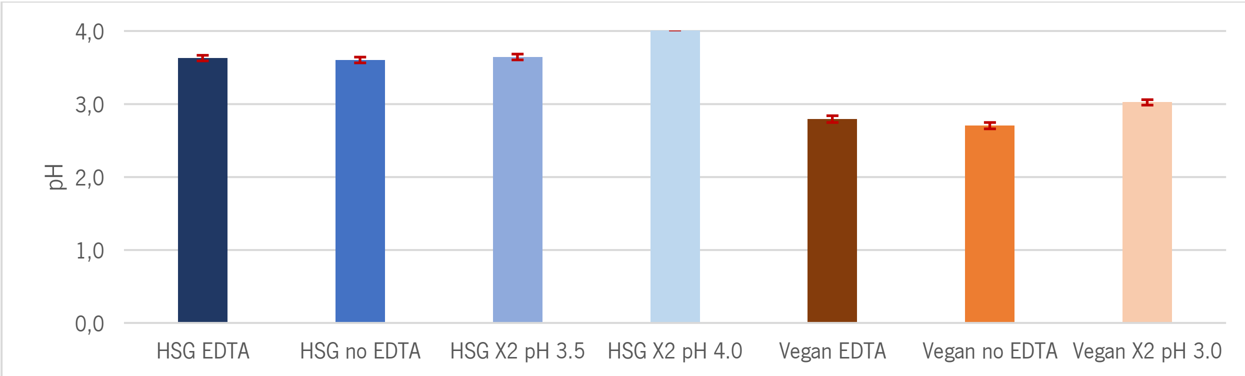



*Figure C.1. Average pH of HSG and Vegan mayonnaises after 4 months of storage at 30°C, with error bars showing the standard deviations.*

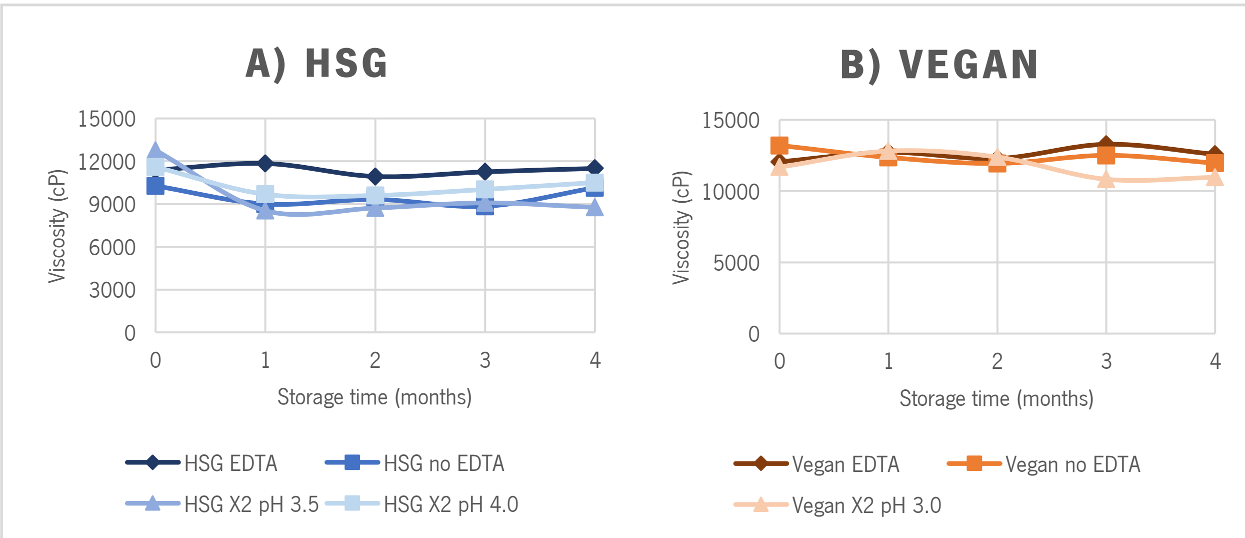



*Figure C.2. Viscosity of a) HSG and b) Vegan mayonnaises until 4 months of storage at 30°C.*

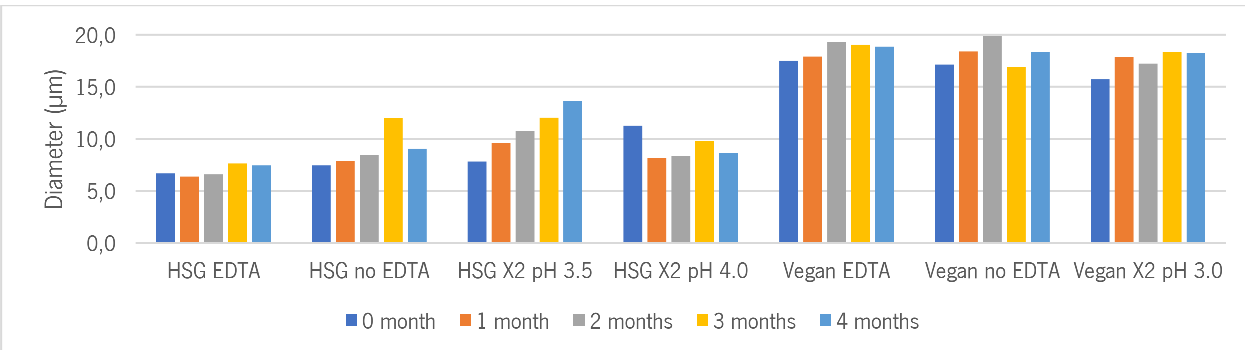



*Figure C.3. Particle size (in µm) of HSG and Vegan mayonnaise for 4 months of storage at 30°C.*

*Table C.3. Globule size images of HSG mayonnaise when fresh and after 6 and 9 months stored at 20 °C*

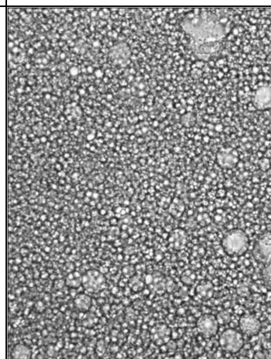
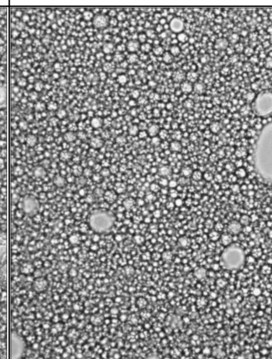
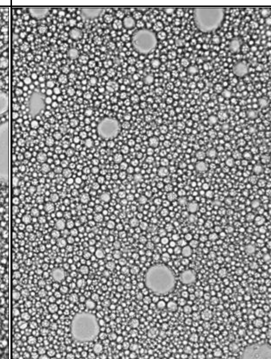
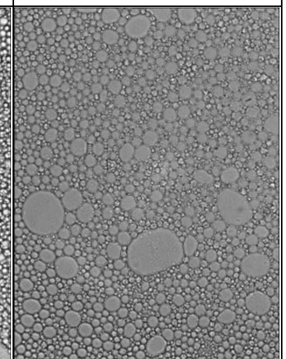
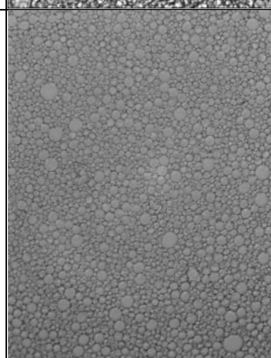
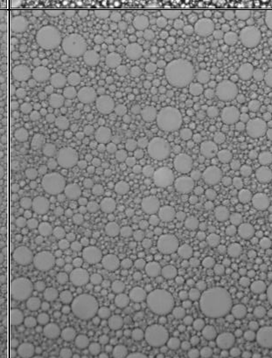
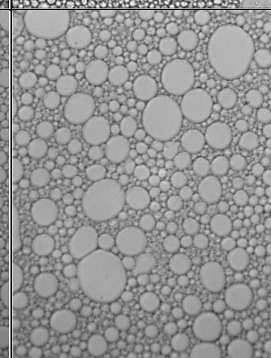
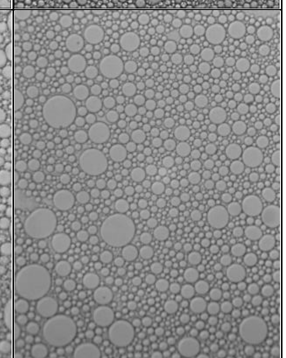
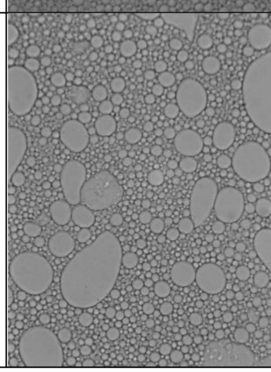
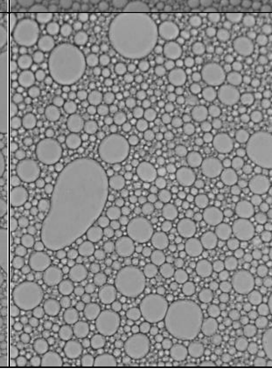
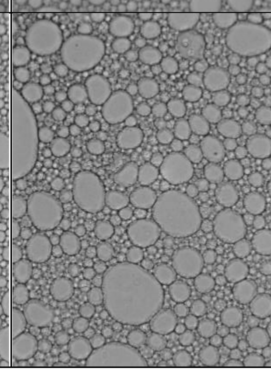
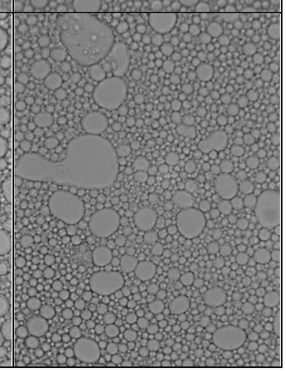
| Storage | | HSG EDTA | HSG no EDTA | HSG X2 pH 3.5 | HSG X2 pH 4.0 |
|---|---|---|---|---|---|
| T (°C) | t (months) | | | | |
| - | 0 |  |  |  |  |
| 20 | 6 |  |  |  |  |
| | 9 |  |  |  |  |

*Table C.4. Globule size images of HSG mayonnaise when fresh and after 1, 2 and 4 months stored at 30°C*

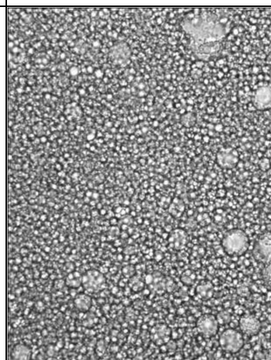
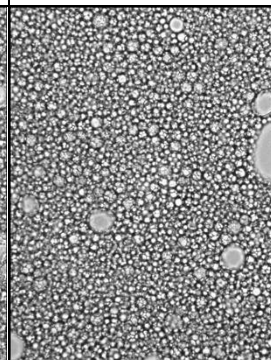
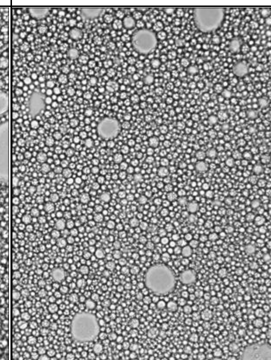
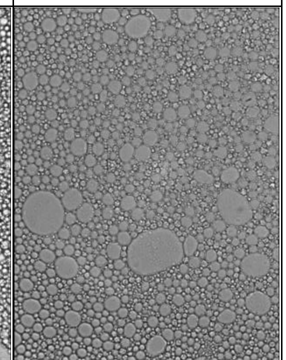
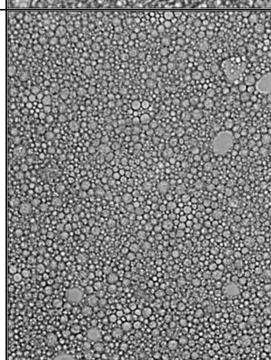
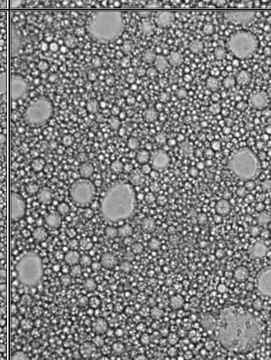
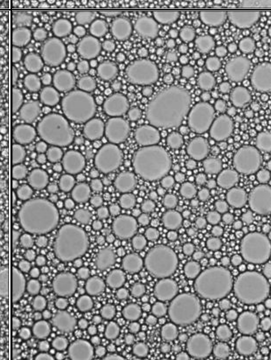
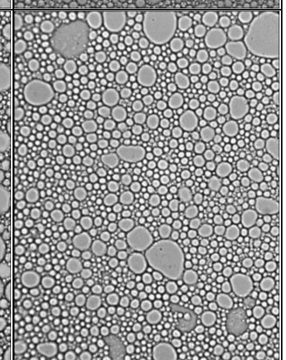
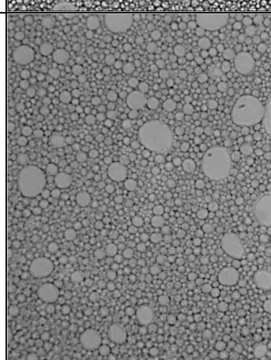
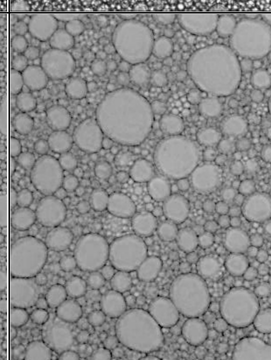
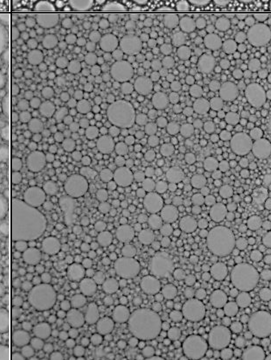
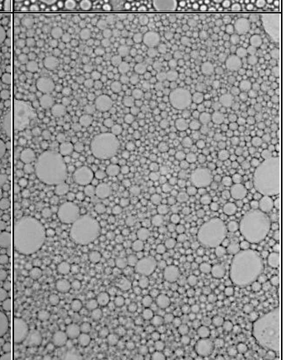
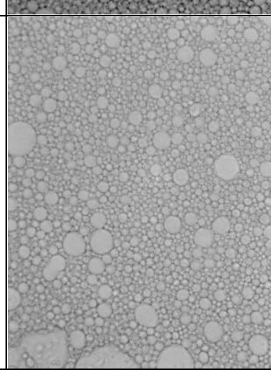
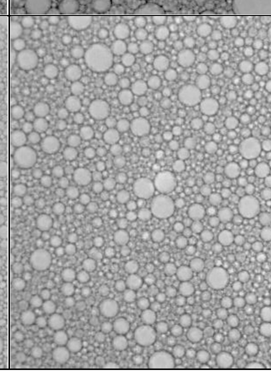
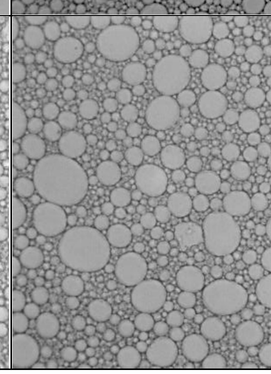
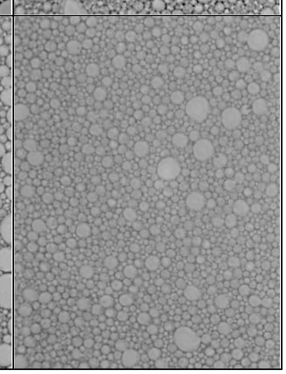
| Storage | | HSG EDTA | HSG no EDTA | HSG X2 pH 3.5 | HSG X2 pH 4.0 |
|---|---|---|---|---|---|
| T (°C) | t (months) | | | | |
| - | 0 |  |  |  |  |
| | 1 |  |  |  |  |
| 30 | 2 |  |  |  |  |
| | 4 |  |  |  |  |

*Table C.5. Globule size images of Vegan mayonnaise when fresh and after 6 and 9 months stored at 20°C*

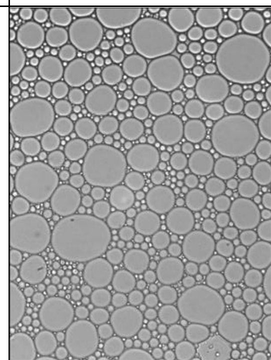
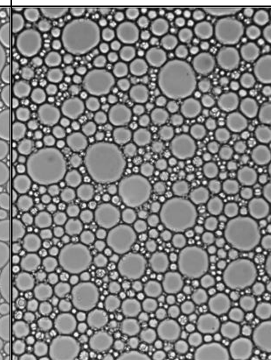
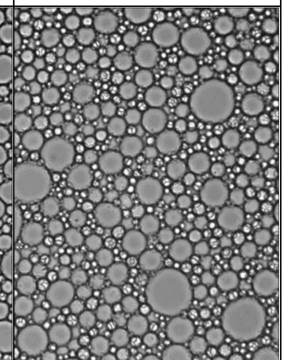
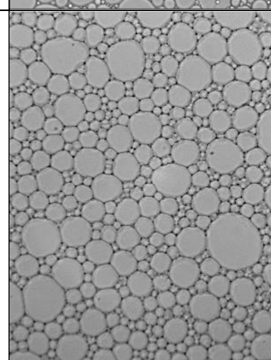
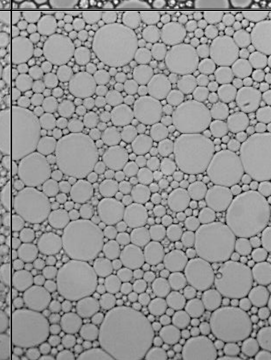
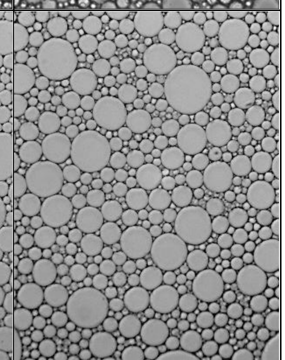
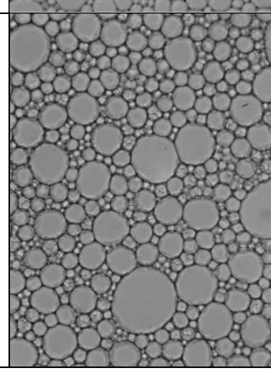
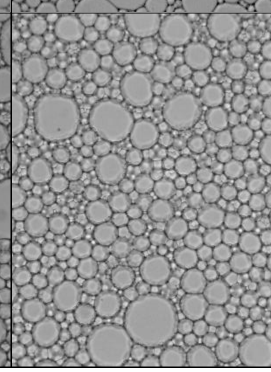
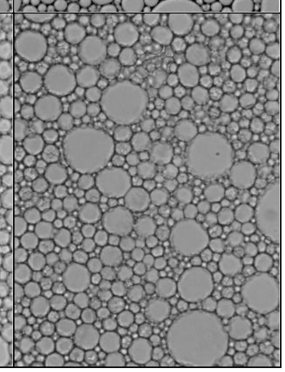
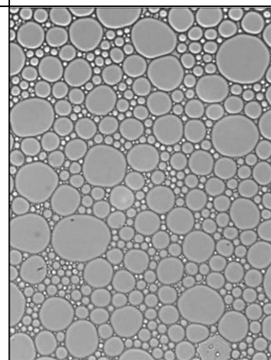
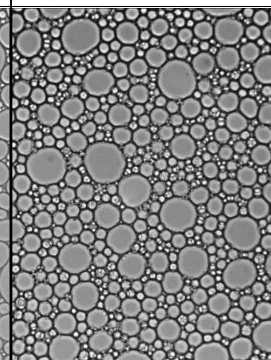
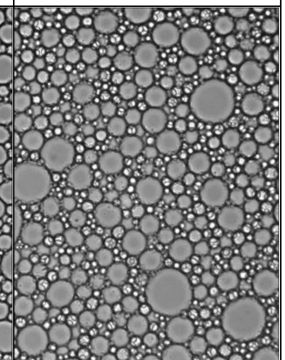
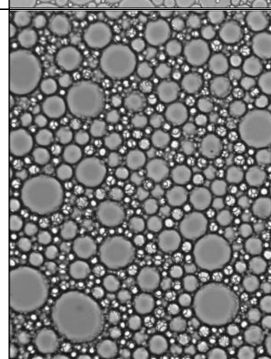
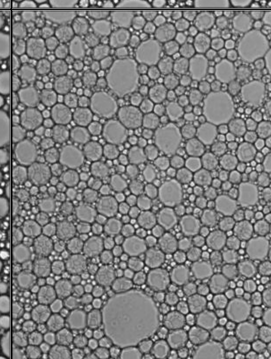
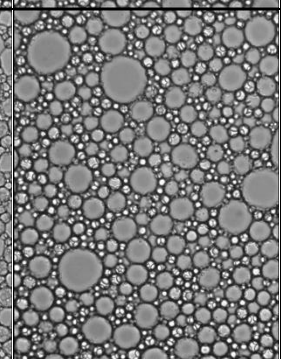
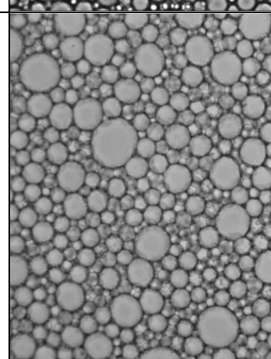
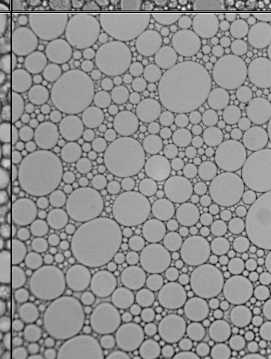
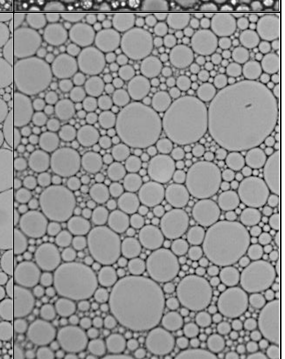
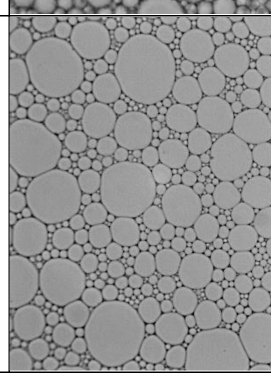
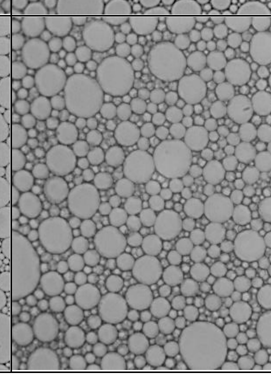
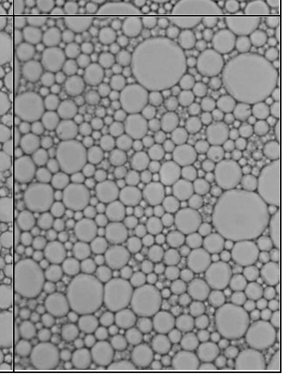
| **Storage** | | **Vegan EDTA** | **Vegan no EDTA** | **Vegan X2 pH 3.0** |
|---|---|---|---|---|
| **T (°C)** | **t (months)** | | | |
| - | 0 |  |  |  |
| 20 | 6 |  |  |  |
| | 9 |  |  |  |

*Table C.6. Globule size images of Vegan mayonnaise when fresh and after 1, 2 and 4 months stored at 30°C*

| **Storage** | | **Vegan EDTA** | **Vegan no EDTA** | **Vegan X2 pH 3.0** |
|---|---|---|---|---|
| **T (°C)** | **t (months)** | | | |
| - | 0 |  |  |  |
| 30 | 1 |  |  |  |
| | 2 |  |  |  |
| | 4 |  |  |  |

## Extract X study – Volatile profile

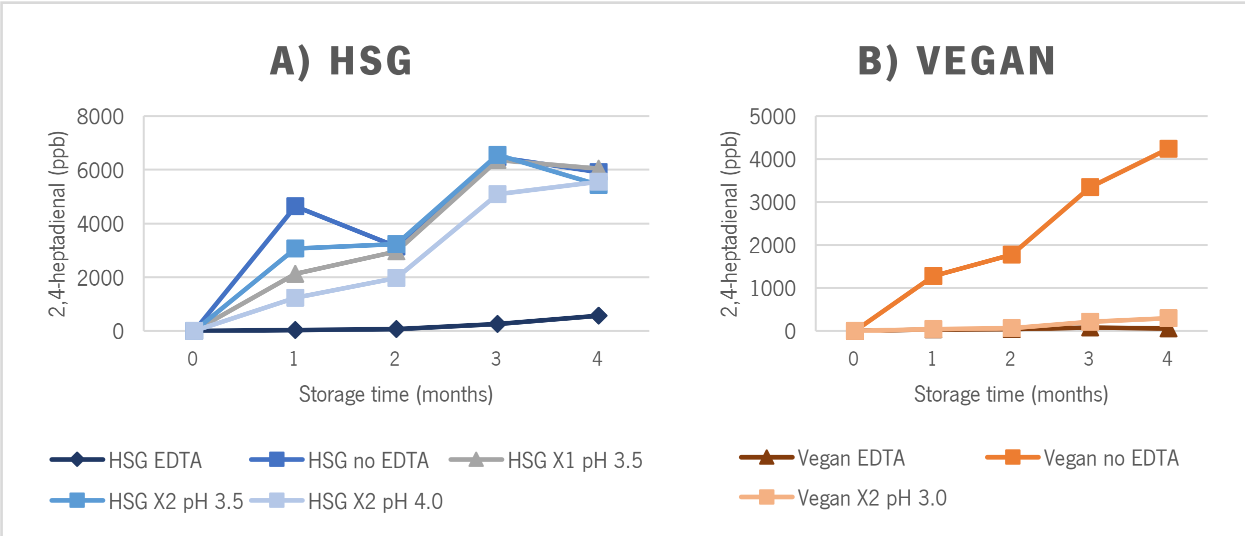



*Figure C.4. Quantification of (E,E)-2,4-heptadienal (in ppb) concerning a) HSG and b) Vegan mayonnaises stored at 30°C for 4 months.*

## Extract X study – Sensory profile

*Table C.7. Extract X study: Sensory scores of HSG mayonnaise samples until 9- and 4-months storage at 20°C and 30°C, respectively*

| Storage | | Sample | Appearance | Taste | | | | | | | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T (°C) | t (months) | | Thickness | Painty | Sour | Egg | Sulphuric egg | Mustard | Fatty/ oily | Buttery | Rancid oil | Green/ grass | Aromatic herbs | Metal | |
| - | 0 | HSG no EDTA | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 1 |
| | | HSG X1 pH 3.5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | **1** | 1 |
| | | HSG X2 pH 3.5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | **1** | 1 |
| 20 | 3 | HSG no EDTA | -2 | 2 | 1 | 2 | 2 | -1 | 2 | 0 | 2 | 0 | 0 | 1 | 2 |
| | | HSG X1 pH 3.5 | -1 | **0** | 2 | **0** | **0** | 0 | **0** | 0 | 1 | 0 | 0 | 1 | 2 |
| | | HSG X2 pH 3.5 | -1 | 2 | 1 | **0** | 2 | -1 | 2 | 0 | 2 | 0 | 0 | 1 | 1 |
| | 6 | HSG no EDTA | -1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 |
| | | HSG X1 pH 3.5 | -1 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 1 |
| | | HSG X2 pH 3.5 | -1 | 1 | **0** | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 0 | 1 |
| | 9 | HSG no EDTA | -3 | 2 | 1 | 1 | 2 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 2 |
| | | HSG X1 pH 3.5 | -2 | 2 | 1 | 2 | 1 | 0 | **1** | 1 | 3 | 0 | 0 | 0 | **4** |
| | | HSG X2 pH 3.5 | **0** | 1 | 1 | 1 | **0** | 0 | **1** | 0 | 2 | 0 | 0 | 0 | **0** |
| 30 | 1 | HSG no EDTA | -1 | 2 | 1 | 1 | 1 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 3 |
| | | HSG X1 pH 3.5 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | **1** |
| | | HSG X2 pH 3.5 | 0 | 1 | 0 | **3** | 1 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | **1** |
| | 2 | HSG no EDTA | -2 | 2 | 2 | 1 | 0 | 0 | 2 | 1 | 3 | 0 | 0 | 0 | 2 |
| | | HSG X1 pH 3.5 | -3 | 2 | 1 | 2 | **2** | 1 | 1 | 0 | **1** | 0 | 0 | 0 | 1 |
| | | HSG X2 pH 3.5 | -2 | **0** | 1 | 2 | 1 | **2** | 1 | 0 | **1** | 0 | 0 | 0 | 1 |
| | 3 | HSG no EDTA | -1 | 2 | 0 | 0 | 1 | -1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| | | HSG X1 pH 3.5 | -2 | **0** | 1 | 0 | 1 | 0 | 2 | 0 | 2 | 1 | 1 | 1 | 2 |
| | | HSG X2 pH 3.5 | -1 | 1 | **2** | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| | 4 | HSG no EDTA | -2 | 1 | 2 | 0 | 3 | -1 | 2 | 0 | 4 | 0 | 0 | 0 | 2 |
| | | HSG X1 pH 3.5 | -3 | 2 | **0** | 0 | **1** | -1 | 1 | 0 | **1** | 0 | 1 | 1 | **0** |
| | | HSG X2 pH 3.5 | -2 | 1 | 1 | 0 | **1** | -1 | 2 | 0 | **1** | 0 | 1 | 1 | 1 |

*Table C.8. Extract X study: Sensory scores of Vegan mayonnaise samples until 9- and 4-months storage at 20°C and 30°C, respectively*

| Storage | | Sample | Appearance | Taste | | | | | | | | | | | Off taste |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T (°C)** | **t (months)** | | **Thickness** | **Painty** | **Sour** | **Egg** | **Sulphuric egg** | **Mustard** | **Fatty/ oily** | **Buttery** | **Rancid oil** | **Green/ grass** | **Aromatic herbs** | **Metal** | |
| - | 0 | Vegan no EDTA | 0 | 0 | 2 | 0 | 0 | -1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 |
| | | Vegan X2 pH 3.0 | 0 | 0 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 20 | 3 | Vegan no EDTA | 0 | 4 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 4 | 0 | 0 | 2 |
| | | Vegan X2 pH 3.0 | 0 | **2** | 0 | 0 | 0 | 0 | **0** | 0 | **0** | **0** | 0 | 0 | **0** |
| | 6 | Vegan no EDTA | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 1 | 3 | 3 | 0 | 0 | 2 |
| | | Vegan X2 pH 3.0 | 0 | **1** | 0 | 0 | 0 | 0 | 1 | 0 | **0** | **1** | 0 | 0 | 1 |
| | 9 | Vegan no EDTA | -1 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 4 |
| | | Vegan X2 pH 3.0 | 0 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | **0** | 1 | 0 | 0 | **0** |
| 30 | 1 | Vegan no EDTA | 0 | 5 | -2 | 0 | 0 | 0 | 3 | 0 | 2 | 2 | 0 | 0 | 4 |
| | | Vegan X2 pH 3.0 | -1 | **1** | **0** | 0 | 0 | 0 | **0** | 0 | 1 | **0** | 0 | 0 | **0** |
| | 2 | Vegan no EDTA | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 1 | 3 |
| | | Vegan X2 pH 3.0 | 0 | **2** | 0 | 0 | 0 | 0 | 1 | 0 | **1** | **1** | 0 | 1 | 2 |
| | 3 | Vegan no EDTA | 0 | 4 | -1 | 0 | 0 | 0 | 1 | 0 | 2 | 4 | 0 | 0 | 2 |
| | | Vegan X2 pH 3.0 | 0 | **2** | -1 | 0 | 0 | 0 | 0 | 0 | **0** | **1** | 0 | 0 | 1 |
| | 4 | Vegan no EDTA | -3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 0 | 0 | 2 |
| | | Vegan X2 pH 3.0 | -4 | **0** | 0 | 0 | 0 | 0 | -1 | 0 | **0** | **1** | 0 | 0 | **0** |

## II. Ideal Recipe Design

*Table C.9. Sensory scores of mayonnaises from the design of experiment until 1 month at 30°C*

| Sample name | Rancid oil taste | | | Metal taste | | | Egg taste | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0 m** | **0.5 m** | **1 m** | **0 m** | **0.5 m** | **1 m** | **0 m** | **0.5 m** | **1 m** |
| DOE 1 | 0.0 | 2.4 | 3.1 | 0.9 | 0.5 | 0.5 | -0.2 | -1.4 | 0.3 |
| DOE 2 | 1.8 | 3.6 | 4.8 | 1.4 | 2.0 | 1.6 | 0.5 | 1.2 | 1.3 |
| DOE 3 | 1.9 | 3.3 | 1.9 | 1.3 | 3.4 | 2.1 | 0.3 | -0.9 | 1.3 |
| DOE 4 | 0.4 | 2.6 | 2.8 | 0.0 | 1.1 | 1.2 | 0.7 | 0.4 | 0.4 |
| DOE 5 | 0.2 | 0.9 | -0.5 | 0.7 | 1.5 | 0.0 | 0.1 | -0.4 | 0.0 |
| DOE 6 | 0.7 | 2.4 | 2.0 | 0.2 | 1.3 | 1.0 | 1.7 | -0.3 | 0.4 |
| DOE 7 | 1.9 | 2.7 | 1.4 | 3.0 | 4.2 | 2.8 | 0.3 | -0.1 | 0.0 |
| DOE 8 | 0.7 | 1.1 | 1.0 | 0.4 | 0.8 | 0.0 | 0.4 | -0.3 | 0.3 |
| DOE 9 | 1.8 | 1.7 | 1.8 | 0.4 | 1.7 | 0.9 | 1.2 | 0.8 | 1.6 |
| DOE 10 | 1.8 | 2.7 | 1.8 | 0.7 | 1.7 | 1.2 | 1.6 | 0.9 | 1.2 |
| DOE 11 | 2.0 | 2.7 | 2.1 | 2.6 | 3.0 | 1.2 | 0.2 | 0.4 | 0.2 |
| DOE 12 | 2.8 | 1.6 | 1.4 | 1.8 | 1.5 | 0.9 | 1.1 | 0.7 | 2.0 |
| DOE 13 | 0.7 | 0.7 | 1.4 | 0.7 | 1.5 | 0.0 | -0.3 | 0.2 | 1.5 |
| DOE 14 | 0.2 | 1.4 | 1.7 | 0.0 | 2.3 | 0.6 | 1.0 | 0.5 | -0.4 |
| DOE 15 | 2.0 | 1.5 | 1.4 | 1.9 | 3.7 | 1.5 | 1.1 | -0.6 | 0.6 |
| DOE 16 | 0.4 | 0.7 | 0.4 | 0.2 | 0.5 | 0.0 | 0.4 | 0.5 | 1.6 |

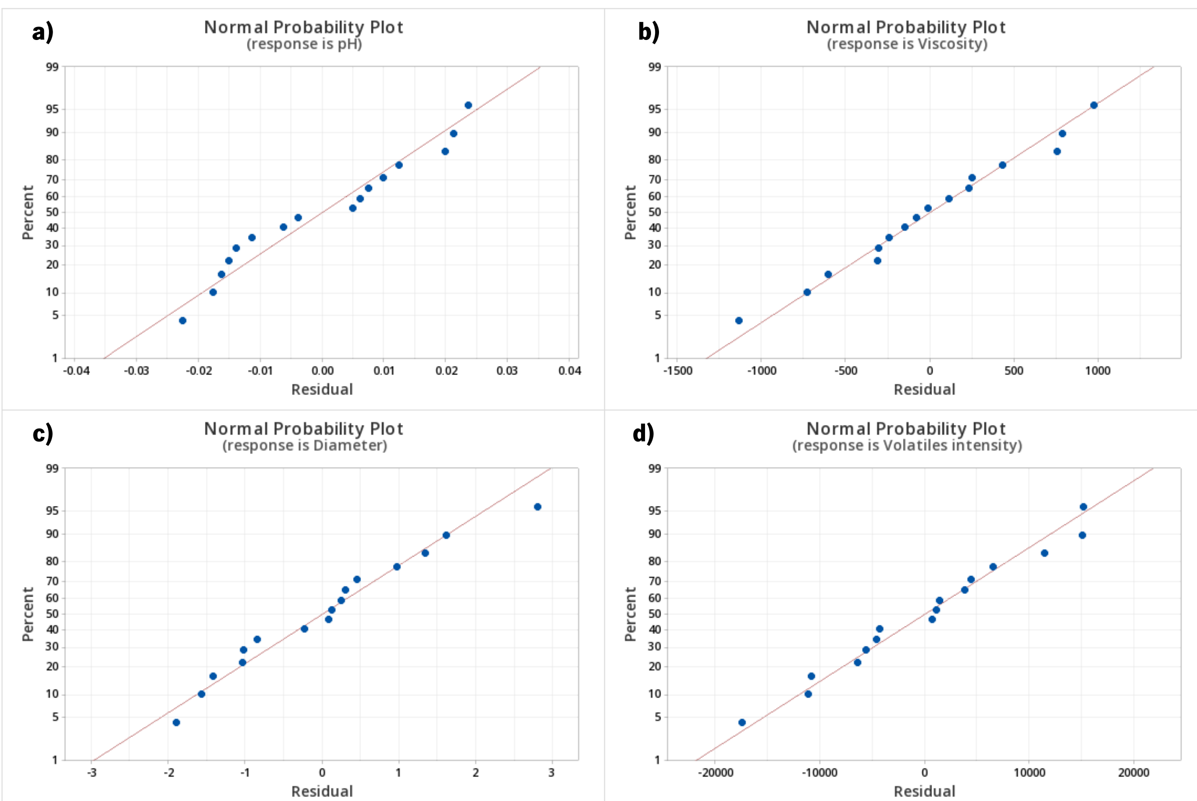



*Figure C.5. Normality probability plots for physicochemical properties and volatiles intensities of mayonnaise stored 1 month at 30°C: a) pH, b) viscosity and c) diameter and d) volatiles intensity.*

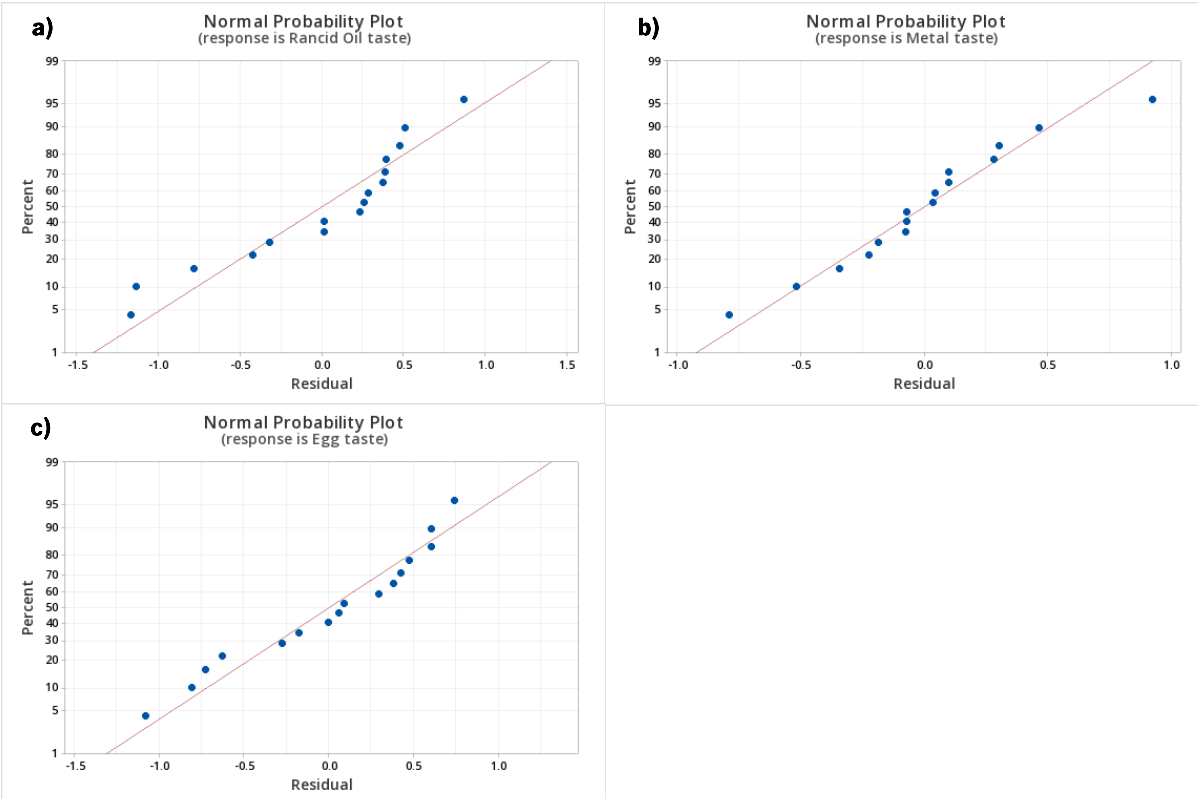



*Figure C.6. Normality probability plots for sensory properties: a) rancid oil taste, b) metal taste and c) egg taste of mayonnaise stored 1 month at 30°C.*

## Appendix D. *Pataniscas* sensory analysis

**Acceptance test**

**Name:________________________________________ Age:______**

**Instructions:**
Start by tasting the *Patanisca* that has been presented on your left, and evaluate each of the following parameters (A to F) according to the scale that has been provided. There is water and crackers available to cleanse the palate in samples' tasting.

**Sample's code:______**

A. Global acceptance

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

B. Global appearance

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

C. Odour

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

D. Texture

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

E. Taste

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

F. Intention of purchase

- ☐ 5 Definitely would buy
- ☐ 4 Probably would buy
- ☐ 3 Maybe/Maybe not
- ☐ 2 Probably would not buy
- ☐ 1 Definitely would not buy

Comments: ________________________________________________________

________________________________________________________________

**Instructions:**
After tasting the previous sample, now start by tasting the sample that has been presented on your right, and evaluate each of the following parameters (A to F) according to the scale that has been provided. There is water and crackers available to cleanse the palate in samples' tasting.

**Sample's code:______**

A. Global acceptance

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

B. Global appearance

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

C. Odour

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

D. Texture

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

E. Taste

- ☐ 9 Extremely pleasant
- ☐ 8 Moderately pleasant
- ☐ 7 Pleasant
- ☐ 6 Slightly pleasant
- ☐ 5 Neutral
- ☐ 4 Slightly unpleasant
- ☐ 3 Unpleasant
- ☐ 2 Moderately unpleasant
- ☐ 1 Extremely unpleasant

F. Intention of purchase

- ☐ 5 Definitely would buy
- ☐ 4 Probably would buy
- ☐ 3 Maybe/Maybe not
- ☐ 2 Probably would not buy
- ☐ 1 Definitely would not buy

Comments: ______________________________________________________________________

______________________________________________________________________________