Αξιοποίηση παραπροϊόντων και εναλλακτικών πρώτων υλών για την παραγωγή καινοτόμων προϊόντων ζύμης

(Utilization of by-products and alternative raw materials for production of innovative bakery products)

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Είμαι ο αποκλειστικός συγγραφέας της υποβληθείσας Διδακτορικής Διατριβής με τίτλο «Αξιοποίηση παραπροϊόντων και εναλλακτικών πρώτων υλών για την παραγωγή καινοτόμων προϊόντων ζύμης / Utilization of by-products and alternative raw materials for production of innovative bakery products». Η συγκεκριμένη Διδακτορική Διατριβή είναι πρωτότυπη και εκπονήθηκε αποκλειστικά για την απόκτηση του Διδακτορικού διπλώματος του Τμήματος Επιστήμης Τροφίμων και Διατροφής. Κάθε βοήθεια, την οποία είχα για την προετοιμασία της, αναγνωρίζεται πλήρως και αναφέρεται επακριβώς στην εργασία. Επίσης, επακριβώς αναφέρω στην εργασία τις πηγές, τις οποίες χρησιμοποίησα, και μνημονεύω επώνυμα τα δεδομένα ή τις ιδέες που αποτελούν προϊόν πνευματικής ιδιοκτησίας άλλων, ακόμη κι εάν η συμπερίληψή τους στην παρούσα εργασία υπήρξε έμμεση ή παραφρασμένη. Γενικότερα, βεβαιώνω ότι κατά την εκπόνηση της Διδακτορικής Διατριβής έχω τηρήσει απαρέγκλιτα όσα ο νόμος ορίζει περί διανοητικής ιδιοκτησίας και έχω συμμορφωθεί πλήρως με τα προβλεπόμενα στο νόμο περί προστασίας προσωπικών δεδομένων και τις αρχές Ακαδημαϊκής Δεοντολογίας.

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Abstract

Bakery products are popular snack foods, consumed worldwide, which contain refined wheat flour as their main ingredient. Due to their low nutritional value, they could be used for enrichment with health-promoting local raw materials and by-products (functional ingredients) in order to make them healthier and more sustainable. The main goal of the thesis was to investigate the effects of partial wheat substitution with alternative ingredients (barley, chickpea, lupin, cowpea, and yellow split pea flours) and agricultural by-products (grape seed and olive stone flours) on the properties and quality characteristics of bakery products. The thesis focused mainly on crackers as commonly consumed bakery goods. Specifically, the research evaluated the properties of the products throughout their production/processing stages (flours, dough, and final product). In addition, the use of sustainable packaging materials, in conjunction with enrichment with functional ingredients, was investigated for testing their impact on product shelf life. Sensory effects of sustainable packaging were also evaluated, as well as material strength, to assess the feasibility of packaging materials. Furthermore, eye-tracking technology was employed for assessing consumer acceptance and focus of functional crackers. The study findings show that understanding the functional properties of alternative to wheat flours and their effects on dough and final product can help in the design and optimization of food products, targeting desired processing and quality outcomes. Enrichment with barley and chickpea flour resulted in crackers with harder texture and equivalent lightness values to control, while crackers with grape seed and the higher level (30%) of olive stone flour had softer texture and high total color difference. In cases where the texture or color of the product was affected, this was dependent on the type of flour and the level of wheat substitution. Most functional flours resulted in darker and diverse color profiles. The use of sustainable materials (such as edible chitosan films) for the packaging of crackers preserved the characteristics of the products and their stability throughout their shelf life to almost the same extent as conventional packaging. The results showed that information about packaging sustainability results in positive consumer perception and emotions. Nutritional information was also found to be important based on eye-tracking findings. The nutritional label of crackers attracted most of the attention of consumers. Although the control sample (100% wheat cracker) was visually attended the longest, consumer acceptability was similar between all products. As the nutritional content in the GDA labels of all crackers was similar, consumers may have perceived all products as of equivalent nutritional value. Visual attention appeared to be driven by personal motives. The study provides evidence that the right combination of functional flour type and ratio can be used to improve product quality and specific cracker characteristics. Sustainable packaging materials as well as appropriate nutritional and sustainability labelling can be used to further enhance product sustainability and help attract attention of consumers.

Περίληψη

Τα προϊόντα ζύμης είναι δημοφιλή σνακ, που καταναλώνονται παγκοσμίως, και τα οποία περιέχουν ως κύριο συστατικό τους το επεξεργασμένο (λευκό) αλεύρι σίτου. Λόγω της χαμηλής διατροφικής τους αξίας, θα μπορούσαν να αποτελέσουν ιδανικούς υποψήφιους για εμπλουτισμό με τοπικές πρώτες ύλες και αγροτικά υποπροϊόντα που προάγουν την υγεία (λειτουργικά συστατικά) καθιστώντας τα πιο υγιεινά και βιώσιμα. Ο κύριος στόχος της διατριβής ήταν η διερεύνηση των επιπτώσεων της μερικής υποκατάστασης σιταριού με εναλλακτικά συστατικά (αλεύρι από κριθάρι, ρεβίθι, λούπινο, ασπρομύτικο φασόλι και άφκο/φάβα Λήμνου) και γεωργικά υποπροϊόντα (αλεύρι από κουκούτσι σταφυλιού και πυρήνα ελιάς) στις ιδιότητες και τα ποιοτικά χαρακτηριστικά των προϊόντων αρτοποιίας. Η διατριβή επικεντρώθηκε κυρίως στα κράκερ ως είδη αρτοποιίας ευρείας κατανάλωσης. Συγκεκριμένα, η έρευνα αξιολόγησε τις ιδιότητες των προϊόντων σε όλα τα στάδια παραγωγής/επεξεργασίας τους (άλευρα, ζύμη και τελικό προϊόν). Επιπλέον, η χρήση βιώσιμων υλικών συσκευασίας, σε συνδυασμό με τον εμπλουτισμό με λειτουργικά συστατικά, διερευνήθηκαν για τον έλεγχο της επίδρασής τους στη διάρκεια ζωής των προϊόντων. Αξιολογήθηκαν επίσης η αισθητηριακή αντίληψη των βιώσιμων συσκευασιών, καθώς και η αντοχή των υλικών, για την εκτίμηση της εφαρμοσιμότητας των υλικών συσκευασίας. Επιπλέον, χρησιμοποιήθηκε καινοτόμος τεχνολογία σιωπηρών μετρήσεων (eye-tracking) αντιδράσεων του καταναλωτή για την αξιολόγηση της αποδοχής και της εστίασης των ματιών. Τα ευρήματα της μελέτης δείχνουν ότι η κατανόηση των λειτουργικών ιδιοτήτων των εναλλακτικών αλεύρων και των επιπτώσεών τους στη ζύμη και το τελικό προϊόν μπορεί να βοηθήσει στο σχεδιασμό και τη βελτιστοποίηση των προϊόντων διατροφής, στοχεύοντας στην επιθυμητή επεξεργασία και ποιοτικά αποτελέσματα. Ο

εμπλουτισμός με αλεύρι κριθαριού και ρεβιθιού οδήγησε σε κράκερ με πιο σκληρή υφή και ισοδύναμες τιμές φωτεινότητας με το δείγμα ελέγχου (κράκερ από 100% σιτάρι), ενώ τα κράκερ με κουκούτσια σταφυλιού και με υψηλότερο επίπεδο (30%) αλεύρου από πυρήνα ελιάς είχαν πιο μαλακή υφή και μεγαλύτερη συνολική διαφορά χρώματος (ΔΕ). Στις περιπτώσεις όπου η υφή ή το χρώμα του προϊόντος επηρεάστηκε, αυτό εξαρτήθηκε από το είδος του αλεύρου καθώς και από το επίπεδο υποκατάστασης του αλεύρου σιταριού. Τα περισσότερα λειτουργικά άλευρα οδήγησαν σε σκουρότερο και διαφορετικά χρωματικά προφίλ προϊόντος. Η χρήση βιώσιμων υλικών (όπως εδώδιμα φιλμ χυτοζάνης) για τη συσκευασία των κράκερ διατήρησε τα χαρακτηριστικά των προϊόντων και τη σταθερότητά τους καθ' όλη τη διάρκεια ζωής τους, σχεδόν στον ίδιο βαθμό με τις συμβατικές συσκευασίες. Τα αποτελέσματα έδειξαν ότι η πληροφόρηση σχετικά με τη βιωσιμότητα συσκευασίας είχε ως αποτέλεσμα την θετική αντίληψη καταναλωτή και την δημιουργία θετικών συναισθημάτων. Οι διατροφικές πληροφορίες προϊόντος βρέθηκαν επίσης να αποτελούνε σημαντικό παράγοντα σύμφωνα με τα ευρήματα της εστίασης των ματιών. Η διατροφική ετικέτα των κράκερ τράβηξε το μεγαλύτερο μέρος της προσοχής των καταναλωτών. Αν και το δείγμα ελέγχου παρατηρήθηκε οπτικά περισσότερο, η αποδοχή από τον καταναλωτή ήταν παρόμοια μεταξύ όλων των κράκερ. Καθώς η διατροφική πληροφόρηση στις ετικέτες GDA όλων των κράκερ ήταν παρόμοια, είναι πιθανό οι καταναλωτές να αντιλήφθηκαν όλα τα προϊόντα ως ισοδύναμης θρεπτικής αξίας. Γενικά, η οπτική εστίαση φάνηκε να καθοδηγείται από τα προσωπικά κίνητρα των καταναλωτών. Η παρούσα έρευνα αποδεικνύει ότι ο σωστός συνδυασμός είδους και αναλογίας αλεύρων μπορεί να αξιοποιηθεί για τη βελτίωση της ποιότητας καθώς και συγκεκριμένων χαρακτηριστικών των κράκερ. Τα βιώσιμα υλικά συσκευασίας σε συνδυασμό με την κατάλληλη επισήμανση διατροφικής αξίας και βιωσιμότητας μπορούν να χρησιμοποιηθούν για την περαιτέρω ενίσχυση της βιωσιμότητας των προϊόντων και την προσέλκυση της προσοχής των καταναλωτών.

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Abbreviations

AOI: *Area of interest*

BA100: *Barley flour 100%*

BA10: *Barley flour 10%*

BA20: *Barley flour 20%*

BA30: *Barley flour 30%*

BD: *Bulk density*

CA: *Correspondence analysis*

CATA: *Check all that apply*

CH100: *Chickpea flour 100%*

CH10: *Chickpea flour 10%*

CH20: *Chickpea flour 20%*

CH30: *Chickpea flour 30%*

CW100: *Cowpea flour 100%*

CW10: *Cowpea flour 10%*

CW20: *Cowpea flour 20%*

CW30: *Cowpea flour 30%*

FCQ: *Food choice questionnaire*

GDA: *Guideline daily amounts*

GS100: *Grape seed flour 100%*

GS10: *Grape seed flour 10%*

GS20: *Grape seed flour 20%*

GS30: *Grape seed flour 30%*

LU100: *Lupin flour 100%*

LU10: *Lupin flour 10%*

LU20: *Lupin flour 20%*

LU30: *Lupin flour 30%*

OAC: *Oil absorption capacity*

OS100: *Olive stone flour 100%*

OS10: *Olive stone flour 10%*

OS20: *Olive stone flour 20%*

OS30: *Olive stone flour 30%*

PCA: *Principal component analysis*

PLA: *Polylactic acid*

PPL: *Polypropylene*

SC: *Swelling capacity*

TPA: *Texture profile analysis*

TVD: *Total visit duration*

WAC: *Water absorption capacity*

WF: *Wheat flour (control)*

YP100: *Yellow split pea flour 100%*

YP10: *Yellow split pea flour 10%*

YP20: *Yellow split pea flour 20%*

YP30: *Yellow split pea flour 30%*

ΔE: *Total color difference*

CHAPTER 1

Introduction

1.1 Bakery products: composition, nutritional and environmental aspects

1.1.1 History of bakery products

Bakery products vary in complexity and include a wide range of items, such as bread, cakes and biscuits (crackers and cookies). They all share wheat flour as their primary ingredient, providing bulk and structure, and are manufactured through the baking process. Given their significance in human nutrition, bakery products are consumed extensively on a daily basis (Martins et al., 2017; Giannou et al., 2014). Evidence suggests that the practice of baking may have originated as far back as 23,000 years ago (approximately 21,000 BC) during the Paleolithic Period. During the Paleolithic, people discovered wheat and learned to mix it with water and bake it on stones heated by fire. This gave rise to the first flatbread. Between 2600–3000 BC, ancient Egyptians began using yeast to produce fermented bread. More recently, in the 1900s, white bread became common for people from all walks of life. Presently, there is an abundance of bakery products available, ranging from simple ingredients used in plain pastries to the more complex components required to make a cake (Zhou et al., 2014).

1.1.2 Milling of wheat flour

Wheat holds a significant position as a major cereal crop in numerous regions around the globe. It belongs to the Triticum family, which comprises thousands of species, with the *T. aestivum* subspecies Vulgare and the hard wheat *T. durum* being of utmost commercial importance (McKevith, 2004). Wheat stands out as the most versatile cereal, primarily because its storage proteins have the ability to interact and create the gluten network, serving as the foundational structure in numerous bakery products. To extract flour from wheat kernels, a milling process consisting of a sequence of breaking, sieving, and size-reducing is employed (Zhou et al., 2014). The primary aim of milling is to isolate the bran and germ from the starchy endosperm, enabling the endosperm to be finely ground into flour (Dewettinck et al., 2008). In the early days of milling, flour was rich in bran parts and had low processing capacity. However, this changed in the 1800s with the introduction of new machinery. Some machines were designed for grinding grains (roller mill), while others focused on sifting the crushed material (sifter and purifier), enabling the production of refined flours (high milling processing) in significant quantities. These technological developments gave rise to white refined flours, altering the sensory properties of bread, particularly in terms of softness, color, and flavor of the crumb (Pagani et al., 2014).

1.1.3 Composition of bakery products

Bakery products rely on both the quantity and quality of gluten present. When wheat flour is mixed with water and kneaded, gluten proteins result in the creation of a cohesive and viscoelastic dough which is capable of retaining the gases produced during proofing. This process leads to well-formed and well-structured products, as is evident in bread and other leavened products, which exhibit desirable traits such as increased loaf volume. On the other hand, the absence of gluten in dough significantly affects its rheological properties. Gluten-free doughs, for example, are significantly less cohesive and elastic compared to wheat-based doughs (Cappelli et al., 2020).

Despite its abundant availability, the significance of water as a baking ingredient should not be underestimated. The quantity of water utilized in baking plays a critical role in determining various aspects of bakery products, including their quality, texture, taste, odor, volume, flavor, and mouthfeel. Hence, determining the correct amount of water is an essential aspect of baking (Zhou et al., 2014). The quantity of water used in dough significantly influences its consistency and the fermentation process. Within the dough, water is responsible for the most fundamental impact on its rheological properties, influencing the conformational state of biopolymers and interactions between various constituents, and ultimately contributing to the overall dough structure. In bread, the water content and its distribution play a crucial role in the product's textural properties, crumb softness, crispness, and shelf life. Moreover, the water's role in starch gelatinization directly affects the performance of the final product, making it a critical factor in achieving a desirable outcome (Cappelli et al., 2020).

The widespread proliferation of the yeast *Saccharomyces cerevisiae* has played an important role in the production of fermented bakery products since ancient times and continues to do so in the modern baking industry (Zhou et al., 2014). Leavening agents, which include yeast, other microorganisms, and chemical leaveners, are very important due to their ability to generate gas. This gas generation is responsible for the fermentation and expansion of dough, ultimately influencing the crumb structure of the baked product. Achieving the right crumb structure is vital for key product properties such as volume, shape, flavor, tenderness, and brittleness of the final products. Although the primary gas involved in leavening is carbon dioxide, other gases, such as ammonia gas, steam from water, and incorporated air (added during the mixing process), also play a role in the expansion of baked goods (Zhou et al., 2014). Each leavening agent (also known as "baking powders") has specific characteristics that makes it suitable for the application in certain products. However, each leavening agent also has limitations. Chemical leaveners constitute a category of leavening agents primarily composed of inorganic salts. They can be classified into three distinct groups, depending on the type of gas-producing component they contain: sodium bicarbonate, potassium bicarbonate, and ammonium bicarbonate (De Leyn, 2014).

Sweeteners represent another important ingredient in bakery products. In addition to the level of sweetness, sweeteners also influence the fermentability, appearance, flavor, color, structure, and texture of the final products. Among the various sweeteners, sucrose is of special importance and is widely used both domestically and commercially. However, due to the increasing public interest in lower calorie products, there has been a rising demand for sucrose substitutes in recent times (Zhou et al., 2014).

Fats represent another important element of bakery products with effects on sensory attributes such as creaminess, mouthfeel, spreadability, melting profile, cohesiveness, and structure. (Marangoni et al., 2014). Shortening in baking refers to the capacity of fats to impact the final product by lubricating, weakening, or shortening the food components' structure, resulting in desirable textural properties. The primary aims for shortening include providing tenderness, flavor, mouthfeel, and shortening to the gluten network. Additionally, using shortening in baking results in dough with improved handling qualities, a higher melting point, and an extended shelf life for the final product (Marangoni et al., 2014).

1.1.4 Mixing and creation of dough

The most crucial phase of the entire baking process is mixing. Failing to complete it correctly, makes correction impossible at a later stage (Zhou et al., 2014). The addition of water triggers a rapid and uniform hydration of the protein macromolecules due to the vast surface area of the flour particles. This initial and essential phenomenon facilitates the formation and development of the dough. What makes wheat important is its proteins' unique capability to create a viscoelastic dough. In practice, this means that the dough can be stretched and shaped without breaking, offering distinctive rheological properties (Pagani et al., 2014). The mixing stage plays a critical role in shaping most of the final product's characteristics, whether directly or indirectly. Appropriately mixing the dough is essential to ensure suitable handling properties. If the dough is undermixed or overmixed, it can have adverse effects on the final product, negatively affecting its texture and crumb grain. The objectives of dough mixing include uniform incorporation of all ingredients, efficient hydration of the flour and other dry raw materials, and the development of gluten (Haegens, 2014).

1.1.5 The process of baking

During baking, heat and mass transfer occur simultaneously and interactively within the dough, resulting in four significant changes: 1) As the temperature increases, gases within the dough are vaporized, causing the gas cells to expand; 2) With increasing temperature, starch undergoes gelatinization to a degree influenced by the local water availability, and proteins coagulate, limiting the dough's extensibility; 3) The initial structure characterized by closed gas cells separated by dough walls transforms into a porous structure with interconnected pores, known as the dough-crumb transition; 4) High temperatures in the oven cause water to vaporize at the boundaries. Depending on the product's thickness and baking conditions, this process supports the formation of a dry, firm crust in the case of dessert or bread dough and may also lead to complete drying in the case of biscuits and cookies (Zhou et al., 2014).

1.1.6 Categorization of bakery products

Bakery products comprise of a large range of goods, distinguished by their diverse characteristics such as size, shape, color, texture, taste, and flavor. These products can be categorized based on their formula, primarily focusing on sugar content or leavening type, as well as their pH level, moisture content, or water activity (a_W). They can be categorized into:

- unsweetened (bread, rolls, crackers, pizza base, crumpets, and bagels), sweet (cakes, biscuits, cookies, muffins, pancakes, dough nuts, and waffles), and filled goods (fruit and meat pies, pastries, sandwiches, cream cakes, pizza, and quiche);
- leavened with yeast (such as *S. cerevisiae*) or chemical agents (baking powders such as baking soda, acid salts, and inert fillers), or unleavened (without fermentation or gas producing agents) products;
- high acid ($pH < 4.6$), low acid ($pH > 4.6$ and $pH < 7$), and nonacid or alkaline bakery products ($pH > 7$) (Giannou et al., 2014).

Furthermore, based on their moisture content, bakery products are classified as:

- low moisture ($a_W < 0.6$) products (cookies and crackers);
- intermediate moisture ($a_W > 0.6$ and $a_W < 0.85$) products (soft cookies, doughnuts and Danish pastries); and
- high moisture ($a_W > 0.85$) bakery products (bread, cakes, and pies) (Giannou et al., 2014).

Low moisture bakery products are typically characterised by higher fragility and hygroscopicity, which contribute to their crisp or crunchy texture. If adequately packaged and stored, they have a longer shelf life. For low and intermediate moisture bakery products, their shelf life is often limited by physical factors such as moisture, staling, as well as chemical factors like rancidity. On the other hand, high moisture products are primarily at risk of microbiological spoilage, which is caused by bacteria, yeasts, and molds (Giannou et al., 2014).

1.1.7 Biscuits and crackers

The term "biscuit" originates from the Latin word "*panis biscotis*", which translates to "twice-cooked bread." This is because the traditional process involved baking the biscuits in a hot oven and then drying them in a cool oven. The word "biscuit" carries two meanings: (i) a small round bread leavened with baking powder or soda, and (ii) any of the various small flat sweet cakes. In its original British usage, "biscuits" include a wide range of small baked products, usually flat, made from wheat flour and containing various ingredients like fat, sugar, and others. These biscuits are characterized as cereal-based products with a moisture content of less than 5%. The cereal component is enriched with two key ingredients, fat, and sugar (Zhou et al., 2014). Biscuits play a significant role in the confectionery industry internationally and their popularity is continuously growing. These versatile products have wide appeal to all age groups, leading to extensive market penetration and ultimately representing a food of common dietary consumption. The biscuit sector is divided into five main categories: crackers, everyday varieties, chocolate-containing biscuits, healthier options, and other types (Konstantas et al., 2019). Crackers are thin and dry products with a moisture content typically ranging from 2% to 3%. They come in various shapes and can be either leavened or unleavened. When bitten or broken, crackers produce a distinct cracking or snapping sound. The main ingredients used in cracker production include wheat flour, fat (or shortening), salt, leavening agents (such as yeast, chemical leaveners, or a combination), and water (Giannou et al., 2014).

1.1.8 Shelf life of bakery products

From the moment bakery products are baked, their quality begins to deteriorate, resulting in staling, loss of moisture and flavor, and limited shelf life. Overall, the most important factors which affect the shelf life of food products are: raw material quality, product formulation, processing method, water activity, pH, oxygen, nutrient content, presence of microorganisms, packaging, preservatives, storage conditions and consumer handling (Giannou et al., 2014).

Bakery products with low moisture content, like crackers and cookies, are vulnerable to losing their crispness and are sensitive to interactions with water vapor and oxygen. Extended exposure to ambient storage conditions can result in the absorption of water from the environment, causing the product to become soft, soggy, and, overall, less appealing to consumers. The moisture threshold at which crispness is affected typically ranges between 4% and 6%. Furthermore, moisture absorption by dry bakery products can lead to changes in their dimensions. The most common type of change observed is an increase in size, often in terms of diameter or length, though an increase in thickness is also possible (Giannou et al., 2014).

Due to their normally higher fat content, bakery products are susceptible to experiencing rancidity when exposed to moisture and atmospheric conditions. Even at relatively low levels, the taste and odor of rancidity can be easily detected. Rancidity can manifest in two forms: oxidative and hydrolytic. Oxidative rancidity occurs when unsaturated fatty acids break down due to oxygen, via an autolytic free-radical mechanism. This subsequently leads to the formation of malodorous aldehydes, ketones, and short-chain fatty acids. With regards to hydrolytic rancidity, this occurs in the absence of oxygen and involves the hydrolysis of triglycerides, causing release of glycerol and malodorous fatty acids. This type of rancidity is worsened by the presence of moisture and certain endogenous enzymes, such as lipases and lipoxygenases (Giannou et al., 2014).

Exposure to light can also have adverse effects on the color of food products, leading to fat oxidation, rancidity, and undesirable off-flavors. During storage, it can lead to various negative reactions, including the degradation of carotenoids and other pigments, product discoloration, and non-enzymatic browning (Giannou et al., 2014).

Packaging on the other hand is a protective factor for the shelf life of food. In addition, it plays a crucial role in providing customers with safe, wholesome, and visually appealing products. Beyond its functional aspects, packaging can also serve other goals such as attracting customer attention, aiding in promotional efforts, facilitating traceability, identification (barcodes), conveying essential or additional information to the consumer, and assisting in product utilization. Overall, the most important functions of packaging are product protection, containment, storage, convenience, loading and transport (Giannou et al., 2014).

1.1.9 Nutritional properties of bakery products

Most bakery products on the market use white wheat flour as their main ingredient. White flour usually corresponds to 75% of the whole wheat grain, with most of the bran and wheat germ removed as part of milling. During the milling process, the separation of the germ and bran (regions containing various bioactive and functional compounds) unavoidably leads to a significant change in the nutritional value of the final product. In particular, white flour tends to have lower concentration of ashes, protein, vitamins, and soluble sugars compared to the original grain, but higher levels of starch. (Pagani et al., 2014). The concentration of fiber, vitamins, and minerals in grains is typically higher in the outer bran and aleurone layers. Therefore, the nutrient content of the final product depends on the extent to which these parts of the grain are removed during milling. Generally, higher processing of the grain results in lower quantities of vitamins in the flour. For this reason, white flour may contain less than one-third of the original mineral and vitamin content. The milling process may also lead to a reduction of bioactive substances, otherwise known as phytochemicals. For example, it was reported that certain phytochemicals like lignans and phenolic acids decrease in content after milling (McKevith, 2004).

White flour is produced when the extraction rate of milling is 75% or less. If the extraction rate exceeds 80%, the resulting flour will still contain some of the bran. When the extraction rate approaches 100%, a whole meal flour is produced, which retains most of the wheat kernel's components. Lower extraction rates, as in white flour, also lead to lower wheat flour protein content. Additionally, the protein composition is affected, as the proteins (albumins and globulins) in the outer layer are higher in certain amino acids, including lysine, arginine, aspartic acid, glycine, and alanine. On the other hand, the proteins in white flour generally contain higher levels of glutamine and proline (Dewettinck et al., 2008). Based on postprandial utilization in humans, wheat protein was reported to have a lower nutritional quality, with only 66% retention, compared to proteins found in milk (74%), soy (71%), pea (70%), and lupin (74%). Lysine is the most limited essential amino acid found in wheat protein. Although its concentration is still sufficient to meet the nutritional requirements of adults, it may not be adequate for the needs of children. In contrast, the lysine requirement of older children can be met by incorporating alternative cereals such as rye, barley, and oats in their diet (Dewettinck et al., 2008).

Another problem with bakery products based on white flour is the refined flour's higher glyceamic index (GI) (McKevith, 2004). Numerous studies have demonstrated the therapeutic potential of low-GI diets for individuals with diabetes and dyslipidemia. Also, research suggests that adopting a low-GI diet may play a preventive role in reducing the risk of developing type 2 diabetes and cardiovascular disease. White wheat flour's lack of dietary fiber may also add to the adverse health effects for consumers, as dietary fiber is linked with reductions in bowel transit time, incidences of cardiovascular disease, type 2 diabetes, colorectal cancer, hypertension, and risk of premature death (Dewettinck et al., 2008).

1.1.10 Environmental impact of bakery products

Food chains are significant contributors to waste generation and environmental impact throughout their various stages, including agriculture, animal farming, food processing, transport, retail, and consumption. These stages result in the disposal of organic residues, packaging, creation of greenhouse gases, wastewater, and other waste. As a result, they represent missed opportunities for utilizing valuable resources. In 2019, the carbon footprint of the global food chain was calculated to be 13.35% from farming-related greenhouse gas emissions, 6.49% from land use change, and 10.79% from pre- and post-production processes, making them the primary contributors to environmental emissions in the food chain. To address these issues, global initiatives aim to reduce environmental damage and promote more sustainable production systems. One such initiative is the Paris Agreement, established in 2015, where signatory countries committed to providing financial, technical, and infrastructural support to mitigate global climate change and work towards achieving a 2 °C reduction by 2030. This agreement aims to promote technological advancements and societal changes in order to achieve lower carbon emissions. To help tackle this problem, the concept of the circular economy (CE) has also gained popularity among researchers, governments, national and international organizations. Unlike the conventional economic and production model, where resources are utilized for product manufacturing and then discarded, leading to waste, pollution, and net carbon emissions, the circular economy model offers an alternative approach. With this approach, resources are reused, concentrating on minimal or zero use of non-renewable resources and minimizing waste generation. By adopting the CE model, producers can ensure sustainable production without harming the environment (Gonçalves and Maximo, 2022).

Recently, Konstantas et al. (2019) conducted a study to assess the environmental impact of biscuit production through a comprehensive evaluation of the environmental sustainability of six major biscuit types available in the market. Biscuit types considered included crackers, low fat/sugar, semi-sweet, chocolate-coated, and sandwich biscuits with chocolate or vanilla cream. The study took account of the entire life cycle of these biscuits. The study's findings revealed that the primary life cycle stage responsible for the most significant environmental impact in all types of biscuits was raw materials production, accounting for 41%-61% of the total impacts. Specifically, wheat flour, sugar, and palm oil were identified as the key raw materials in this stage. Wheat emerged as the most impactful raw material, significantly contributing to nine impact categories, including global warming potential. It also ranked as the second most relevant activity concerning fossil fuel depletion, terrestrial acidification, and natural land transformation. On average, wheat constituted 28% of the total impact across all categories and biscuit types, revealing it as the most environmentally impactful raw material in the study.

The environmental impact of wheat extents also and includes supply chain interruption issues. Recently, a combination of factors, including the pandemic, the war in Ukraine, inflation, and rising energy prices, has resulted in a significant surge in bread prices. This is evident in the average price of bread which saw a substantial increase of 18% in August 2022, compared to the same month in 2021 (Nicolosi et al., 2023). The increasing impact of climate change and energy crises has made NPK (nitrogen, phosphorus, and potassium) fertilizer supply chains more susceptible to volatility. A significant global event was the war in Ukraine, which led to a rise in gas prices and a decline in fertilizer supplies. As a result, this led to the higher likelihood of an energy and wheat resource crisis in global supply chains. As the conflict escalated, prices of natural gas, oil, and coal soared. Even before the conflict, the agricultural sector had been facing challenges due to unprecedented increases in the costs of natural gas and raw materials required for fertilizer production. Geopolitical tensions, including military aggression and economic embargoes, can trigger wheat supply crises. Ukraine, as one of the world's largest wheat exporters, experienced a notable increase in wheat prices during the war. To address these issues, it is crucial to reduce food production's over-reliance on imports. Reducing imports of wheat could, therefore, help reduce potential consequences of dependence such as famine and migration (Halecki and Bedla, 2022).

1.2 The rising popularity of functional foods and sustainability

1.2.1 Demand for healthier and sustainable foods

Chronic diseases, such as heart disease, cancer, and diabetes, present significant public health challenges. As the world's population continues to age, the risk of developing these chronic conditions increases significantly (Baker et al., 2022). Aging constitutes one of the most significant social and economic challenges of the 21st century across continents like Europe, North America, Japan, and Australia. It is estimated that in 2080, approximately 29.5% of Europe's population will be 65 years old or older. Aging is accompanied by reduced physical activity and notable alterations in body composition. Decreased bone mass, lean body mass, and water content, as well as an increase in fat mass, are common physiological changes caused by aging. These changes may contribute to frailty. Moreover, aging is often associated with a decline in nutritional status (Jędrusek-Golińska et al., 2020).

In light of modern developments, consumers increasingly express a strong preference for sustainably produced and processed foods that are considered safe, fresh, and natural, while also offering higher nutritional value (Granato et al., 2020). Consumers are increasingly showing strong interest in the nutritional profiles of food items, including bread and bakery products, as they focus more on their health. Modern consumers are in fact more conscientious in their purchasing decisions. They seek food that not only meets safety standards, but is also in accordance with principles of environmental, social, and economic sustainability. Ethical considerations, such as support of the local economy, biodiversity, working conditions, and resources for future generations, are also considered. An increasing number of consumers is prioritizing the sustainability of production processes in order to support local communities and businesses. Many of them now choose products not solely based on personal preferences but, more importantly, on what they perceive as beneficial for their health or society. This dietary shift is evident in the popularity of products made with unrefined flour, whole-meal bread and biscuits, and those with low gluten content. The food industry has also responded by becoming more attentive to nutritional aspects, even for traditional products like bread and bakery items. This trend is creating new models of consumption and leading to the development of novel food products (Nicolosi et al., 2023).

1.2.2 Functional foods

The development of functional foods is becoming popular as a possible solution for tackling chronic diseases, improving metabolic health and biochemical parameters in the human body, and helping in the prevention of noncommunicable diseases such as cardiovascular diseases (CVDs), cancer, diabetes type 2, and osteoporosis (Jędrusek-Golińska et al., 2020). Back in 1994, the Food and Nutrition Board of the U.S. National Academy of Sciences' Institute of Medicine provided a definition for functional foods, defining them as " *any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains*". Later, in 2005, the Institute of Food Technologists described functional foods as those that provide a health benefit beyond their basic nutrition. While definitions may vary, experts typically agree that functional foods comprise of ingredients that offer health benefits beyond the food's basic nutritional components (Baker et al., 2022).

The development of functional ingredients has been continuous over time. Initially, ingredients were mostly limited to vitamins (such as vitamin C, E, and folic acid) and minerals (like zinc, calcium, and iron). However, recently, more targeted micronutrients such as omega-3 fatty acids, phytosterols, and insoluble fibers are being used. Research is ongoing about the effects of various ingredients that are rich in fiber, including both soluble and insoluble types, as well as essential amino acids and phenolic compounds (Subiria-Cueto et al., 2022). Incorporating bioactive components (substances derived from plants, animals, or microorganisms, either naturally present in food or added in small amounts to the food matrix) has also become a common practice. Some examples of these bioactive components include phytoestrogens, polyphenols, lycopene, carotenoids such as alpha- and beta-carotene, lutein and zeaxanthin, pre-, pro-, and synbiotics, as well as plant sterols and stanols (Jędrusek-Golińska et al., 2020).

Functional foods have been introduced in many product categories, such as dairy products, confectionery, beverages, baked goods, and baby foods. Among these, juices and bakery products are highly consumed, known for their elevated carbohydrate content and, at times, a significant presence of fats in their composition. However, their flexible nature makes them excellent candidates for enrichment with functional ingredients (Subiria-Cueto et al., 2022). The functional food market, covering large areas globally such as the United States, Japan, Asia Pacific, and the European Union, represents a profitable segment within the food industry and is expected to experience a global growth rate of 8.5% (Granato et al., 2020). In Europe, the value of the functional food market reached an impressive €117 billion ($129 billion) in 2016. Among EU countries, dairy products account for 49% of the main categories of functional food, followed by cereals at 30% (Jędrusek-Golińska et al., 2020).

1.2.3 Ingredients for functional food enrichment

1.2.3.1 Agricultural by-products: a nutritious solution to food waste

Several studies have linked higher intake of fruits and vegetables to a reduced risk of all-cause mortality, particularly cardiovascular mortality (Granato et al., 2020). However, despite their health benefits, fruits and vegetables are significant contributors to food waste, accounting for approximately 44% of the total waste generated (Subiria-Cueto et al., 2022). Food waste is a substantial source of greenhouse gas emissions, contributing to over 20% of the total global production of greenhouse gases, including methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) (O'Connor et al., 2021). Food waste consists of organic matter which, if not handled properly, can present significant risks to both the environment and human health. Consequently, the proper management and disposal of food waste has become an urgent global challenge (Liu et al., 2023).

To achieve the goal of sustainable production chains, the most effective approach is waste prevention or minimization, with options like reuse, recycling, and energy recovery following suit. On the contrary, disposal, via the use of landfills or incineration, is regarded as the worst environmental option (Lavelli et al., 2016). Another solution for addressing the food-waste issue is valorisation. Valorisation represents a sustainable technique which helps transform food waste into value-added products, thus reducing the need for disposal in landfills and minimizing greenhouse gas emissions. Through the valorisation of food waste, economic and environmental opportunities arise, further mitigating the challenges associated with conventional waste disposal. This approach yields value-added products, contributes to reduction of environmental pollution, odor, and microbial overgrowth. Moreover, food waste valorisation creates opportunities for "green" jobs, addresses public concerns about environmental issues like climate change, facilitates the development of renewable resources, ensures product supply security, and generates valuable products and fertilizers for industrial and agricultural sectors. Additionally, it encourages research and development in technologies for the future (O’Connor et al., 2021).

Various types of food waste, including that from fruits, vegetables, and grains, consist of important nutrients and bioactive compounds such as polyphenols, dietary fiber, proteins, lipids, vitamins, organic acids, and minerals. Interestingly, some of these compounds are present in higher quantities in the discarded portions compared to the parts accepted by the market. These valuable compounds present an opportunity to transform food waste into value-added products suitable for human consumption. The primary goal of this approach is not only to reduce food waste but also to generate new sources of income and promote the development of a circular economy (Liu et al., 2023). Food waste by-products constitute approximately 38% of the total food production (Subiria-Cueto et al., 2022). These by-products retain high levels of bioactive compounds and other nutrients with health-enhancing properties, such as anti-inflammatory, antioxidant, and cardioprotective effects (Subiria-Cueto et al., 2022).

Olive and wine making by-products are generated abundantly in Mediterranean countries and represent good examples of unexploited health enhancing ingredients. Winemaking by-products serve as remarkable sources of phenolic compounds and dietary fiber, offering numerous health benefits. In addition, they exhibit antioxidant, coloring, antimicrobial, and texturizing properties (Lavelli et al., 2016). Grapes hold significant value as a highly cultivated and produced crop worldwide, making it the largest fruit crop globally. In Mediterranean countries, grape pomace production can reach substantial volumes of up to 1200 tonnes per year. However, the disposal of by-products in open areas poses both environmental and economic challenges. The primary winemaking waste consists of grape pomace, clarification sediment like lees, and yeast sediment (Kalli et al., 2018). Among these by-products, grape pomace, also known as grape marc, accounts for the majority of winemaking solid waste by weight (averaging around 60%), followed by lees (approximately 25%), and stalks (approximately 14%). Other minor solid by-products include wine filtration residues. On average, the processing of 100 kg of grapes results in 20-25 kg of pomace, which is a mixture of skins and seeds, 3-5 kg of stalks, and 8-10 kg of lees. The exact amounts may vary depending on the grape variety and the winemaking process used (Lavelli et al., 2016). These grape by-products offer an economical and cost-effective source for extraction. Furthermore, they are a rich source of health promoting ingredients, such as hydrocolloids, dietary fiber, lipids, proteins, and natural antioxidants, with flavonoids primarily present in the form of phenolic compounds (Kalli et al., 2018).

Olive oil by-products are another rich source of bioactive compounds, particularly polyphenols, which offer potential health benefits. Some of the phenolic compounds present in olive by-products are hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, syringic acid, gallic acid, luteolin, quercetin, cyanidin, verbascoside, and certain polymeric compounds. These by-products hold promise as low-cost raw materials for extracting antioxidant compounds, suitable for use in food, nutraceutical, or cosmetic applications. They represent a valuable resource for transforming agro-industrial waste into healthy ingredients that could be used for enrichment of commonly consumed products such as cereals (Cedola et al., 2020).

1.2.3.2 Dietary fiber

Dietary fiber, a diverse group of bioactive compounds, is abundantly found in various fruits and vegetables. As a vital component of plant cell walls, dietary fiber plays a crucial role in providing structural support to fruits and vegetables. From a chemical perspective, it consists of several carbohydrate polymers, including homopolysaccharides, heteropolysaccharides, lignins, oligosaccharides, resistant starches, gums, and mucilages (Subiria-Cueto et al., 2022). When included in the diet, there is evidence that it helps in the prevention of several chronic diseases. Dietary fiber plays a vital role in reducing blood glucose and cholesterol levels by effectively binding to bile acids and cholesterol. Consuming a diet rich in soluble fiber has been linked to a decreased risk of cardiovascular disease (CVD), type 2 diabetes, and obesity. Notably, incorporating barley products, or β-glucan from barley, into the diet has shown to lead to a significant reduction in total cholesterol and low-density lipoprotein (LDL) blood cholesterol levels. A comprehensive systematic review and meta-analysis examining the effects of different types of dietary fiber on blood pressure found that diets rich in β-glucan contributed to reducing systolic blood pressure. The high viscosity of β-glucan was also associated with a decline in postprandial blood glucose levels. Furthermore, whether naturally present in foods or taken as supplements, dietary fiber is effective in alleviating constipation (Jędrusek-Golińska et al., 2020). Due to these health benefits, dietary fiber has emerged as a promising option for the development of innovative foods, supplements, and functional ingredients (Subiria-Cueto et al., 2022).

1.2.3.3 Antioxidants and phenolic compounds

Oxidative stress represents a crucial factor in the development of various chronic diseases, such as cancer, cardiovascular disease (CVD), arthritis, autoimmune disorders, and neurodegenerative diseases. The oxidation of lipids and lipoproteins, particularly the LDL fraction, significantly contribute to the process of atherosclerosis in the human body. Incorporating antioxidant substances into the diet could help delay the onset of cardiovascular and neurological disorders, certain types of cancer, cataracts, and other noncommunicable diseases which are prevalent in older adults (Jędrusek-Golińska et al., 2020). For instance, resveratrol, a well-known phenolic compound found in red wine, possesses high antioxidant activity and offers many health benefits. It helps in reducing oxidative stress, inhibiting vascular inflammation, and providing protective effects for cardiovascular health. Some studies suggest that resveratrol may also contribute to reducing blood pressure (Jędrusek-Golińska et al., 2020).

Phenolic compounds, including flavonoids, phenolic acids, stilbenoids, coumarins, and lignoids, are considered antioxidant agents. Other antioxidant substances in food include carotenoids like carotenes and xanthophylls, terpenoids such as monoterpenes, triterpenes, and sesquiterpenes, and certain lipids like tocopherols and tocotrienols (Granato et al., 2020). Phenolic compounds are plant-derived secondary metabolites that have been the subject of extensive research due to their various physiological effects in the human body. These compounds play essential roles in pigmentation, UV protection, internal signaling, and protection against pathogens. Moreover, phenolic compounds contribute to the organoleptic characteristics of food, including color, flavor, and aroma, and significantly contribute to the functional properties of fruits and vegetables (Subiria-Cueto et al., 2022).

1.2.4 Functional bakery products

Bakery products hold an important position in the diets of various cultures worldwide, making them highly consumed goods. As a result, attempts have been made recently to use them for enrichment with health promoting by-products, such as potato peel powder, apple pomace, mango peel powder, plantain peel flour, jackfruit rind powder, and wine grape pomace, among others (Subiria-Cueto et al., 2022). Food by-products are abundant sources of functional ingredients, including fiber, minerals, phytochemicals, and so on. These by-products vary in origin and include a wide range of components, such as peel, stem, leaf, seed, shell, bran, kernel, pomace, and oil cake. Some examples of functional bakery products reported to have been enriched with by-products are: banana peel bread (5% and 10% level), pomegranate bagasse bread (5% and 15%), mango peel cakes (5% - 30%), orange peel cakes (12.5%), guava pomace cupcakes (5% - 20%), grape pomace muffins (5% - 10%), and cookies made with pomegranate peel (1.5% - 7.5%) and orange pulp (5% - 25%) (Martins et al., 2017).

1.3 Research objectives

The aim of the dissertation was the development and study of novel bakery products, namely crackers, through partial substitution of wheat flour with healthy local raw materials and agricultural by-products (functional ingredients).

More specifically, the objectives of the study aimed to:

- **(i)** Evaluate the properties of flours and blends of flours from alternative raw materials.
- **(ii)** Study the behaviour and qualities of dough consisting of functional flours.
- **(iii)** Investigate the effect of wheat flour substitution with functional flours on the final product (crackers).
- **(iv)** Study the shelf life of crackers made with functional flours and sustainable packaging.
- **(v)** Assess consumer acceptance and sensory aspects of sustainable packaging (biodegradable and edible); evaluate the robustness and strength of packaging materials.
- **(vi)** Evaluate sensory perception of functional crackers with eye-tracking technology.

CHAPTER 2

Properties of alternative flours and effects on cracker dough

2.1 Literature review

Crackers are popular bakery products widely consumed due to their convenience and long shelf life. Until recently their production relied mainly on refined wheat flour, which is characterised by its low nutritional value compared to whole grains (Agrahar-Murugkar et al. 2015). Wheat is an important dietary staple, averaging 65.6 kg annual per capita consumption globally, which amounts to 37% of the annual cereal consumption (Erenstein et al. 2022). However, relying on a single crop does not support food security and could potentially lead to problems in the supply chain of countries heavily dependent on imports. Furthermore, the development of a circular economy model in agriculture requires the promotion of natural biodiversity and the utilisation of local crops in products. Thus, the use of composite flours (blends of different flours with or without wheat) to partially or fully substitute wheat in bakery products became popular in recent years.

Consumers nowadays are health conscious and increasingly demand foods of higher nutritional value with established health benefits (Czeczotko et al. 2022). Thus, attempts have been made to substitute wheat flour in bakery products with flours from various sources such as spirulina (biscuits), barley and rye (biscuits), pomegranate peel (muffins), wine and olive by-products (crackers) and pulses (crackers) (Singh et al. 2015; Drakos et al. 2019; Topkaya and Isik 2019; Giannoutsos et al. 2023;

Koukoumaki et al. 2022), to assess their effect on the products and acceptability by the consumers.

Barley is another type of cereal, grown in several countries, mainly used as a substitute of wheat in bakery products due to the high fiber, b-glucan, and phenolic content (Drakos et al. 2019). Alongside cereal intake, pulses are also recognised for their health benefits, high protein and fiber levels (Millar et al. 2017). In Greece, health authorities recommend a minimum of 2 servings of pulses per week (CIHEAM 2010). Lemnos is a Greek island, located in the North Aegean Sea, with unique soil properties that allow the cultivation of different local varieties of pulses, namely afkos (yellow split pea; Lathyrus ochrus (L.) D.C.) and aspromytiko (cowpea; Vigna unguiculata (L.) Walp.). Consumption of these local pulses could help consumers meet the guidelines whilst supporting the local biodiversity. Additionally, agricultural by-products such as olive stone, a potential source of phenol rich antioxidants (Bolek et al. 2020), and grape seeds, a rich source of bioactive compounds such as resveratrol, phenolic acid, flavonoids, procyanidins as well as minerals and fiber (Antonic et al. 2021) have been successfully used to produce products such as functional biscuits (Bolek et al. 2020) and waffles (Antonic et al. 2021). These specific by-products are abundant in Lemnos due to the large production of olive oil and wine and can be easily obtained from olive mills and wineries.

As widely consumed snacks, crackers can be used for promoting the use of healthy ingredients. However, it is crucial to test the effects that each ingredient may have on product quality in response to the substitution of wheat. The research aimed to investigate the effects on the properties of flours and intermediate product (cracker dough) of wheat substitution by: 1) conventional flour derived from barley, 2) local pulse varieties of emerging use (chickpea, lupin, yellow split pea, and cowpea), and

3) less tested agricultural by-products, olive stone and grape seeds. Wheat substitution was selected at 10%, 20% and 30%, as this range was determined to be feasible in preliminary baking trials, and analyses across all stages of production: flour, dough and final product.

2.2 Materials and methods

2.2.1 Raw materials and samples

Wheat flour (Triticum durum, '*Limnos'*), barley flour (Hordeum vulgare, '*Panagia'*), yellow split pea flour (Lathyrus ochrus, '*Afkos'*), cowpea flour (Vigna unguiculata, '*Aspromytiko'*), grape seed flour (Vitis vinifera, '*Moschato Alexandrias'*) and olive stone flour (Olea europea, '*Koroneiki'*) were obtained locally from Lemnos, Greece (harvest of 2020 - 2021). Chickpea flour (Cicer arietinum) and Lupin flour (Lupinus albus) were purchased from local retailers. Alternative to wheat flours were tested at 100% and at 10%, 20% and 30% concentration in mixture with wheat flour.

2.2.2 Dough preparation

Product formulation was 100g of flour, 40g of water, 20g of canola oil, 1.85g of baking powder, 1g of salt and 2g of sugar. First, dry ingredients were mixed in a mixer (heavy duty stand mixer, KitchenAid) for 1 minute, whilst the liquid ingredients (along with sugar) were mixed separately to form an emulsion. Subsequently, all ingredients were mixed together (6 minutes) to make the dough. The dough was briefly kneaded and left to rest at 25 °C for 30 minutes. For dough analysis, samples were tested immediately (Table 2.1). The recipe resulted as part of optimization during preliminary trials.

Table 2.1. Dough samples and functional flour ratios.

Dough samples (flours)	Amount in dough (%)
Wheat (control)	100%
Lupin flour	10%
	30%
Chickpea flour	10%
	30%
Yellow split pea flour	10%
	30%
Cowpea flour	10%
	30%
Barley flour	10%
	30%
Olive stone flour	10%
	30%
Grape seed flour	10%
	30%

2.2.3 Analysis of functional properties of flours

Water absorption capacity (WAC), oil absorption capacity (OAC) and swelling capacity (SC) were determined as described by Chandra et al. (2015) with small modifications. For the WAC, one gram of sample was mixed with 10 mL of distilled water and allowed to stand at ambient temperature for 30 minutes, then centrifuged for 30 minutes at 3,000 rpm. Water absorption was examined as per cent water bound per gram of flour; for OAC, one gram of sample was mixed with 10 mL of sunflower oil and allowed to stand at ambient temperature for 30 minutes, then centrifuged for 30 minutes at 300 rpm. Oil absorption was examined as percent oil bound per gram of flour; and for SC, a 100 mL graduated cylinder was filled with the sample to 10 mL mark. Distilled water was added to give a total volume of 50 mL. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 minutes and left to stand for a further 8 minutes. The volume occupied by the sample was taken after the 8th minute. Bulk density (BD) was measured according to Yadav et al. (2012). Samples (50 g) were put into a 100 ml graduated cylinder and tapped 20–30 times. BD was calculated as weight per unit volume of sample. All measurements were conducted in triplicate.

2.2.4 Texture Profile Analysis (TPA) of cracker dough

Dough samples were molded to a cylindrical shape (30 mm diameter, 20 mm height) with a mold. In order to relieve residual stresses produced during sample preparation, the cylindrical dough was placed immediately in a plastic container to avoid dehydration and left to rest for one hour before testing. Samples were placed on a flat base of a texture analyser (TA. XT Plus, Stable Micro Systems) and were compressed by a probe up to the distance of 10 mm (50 % strain level) for two times which results in two curves (two bite test). The condition-set up for measuring textural properties was as follows: Pre-test speed: 1.00 mm/s; test speed: 2.00 mm/s; post-test speed: 2.00 mm/s; load cell: 50 kg; probe: 75 mm compression platen.

Four textural parameters were determined from each TPA curve: hardness (g), cohesiveness, adhesiveness, and springiness (%). Hardness represents the maximum compression force during the first cycle in the TPA curve. Cohesiveness is obtained from the ratio between the positive force area during the second compression cycle to that during the first compression cycle and shows how well the product withstands a second deformation relative to how it behaved under the first deformation. Adhesiveness is the negative force area of the first compression and springiness refers to how well a product physically springs back after it has been deformed during the first compression. Springiness is calculated as the distance ratio between the beginning and the maximum force of the second and first peaks. The data were collected for three replicates and the mean value was reported.

2.2.5 Β-glucan content analysis

Wheat (from Lemnos and commercial), barley (from Lemnos and commercial), whole wheat (from Lemnos and commercial), grape seed, olive stone, chickpea and lupin flours were analyzed for β-glucan content. The purpose of the analysis was to investigate possible benefits of substituting wheat flour in crackers with these flours, due to the potentially higher content of this specific nutrient. After the analysis, from the aforementioned flours, those with the highest level (local and commercial barley) were selected for further examination of the β-glucan level in the final product after 30% substitution of wheat flour. The control sample was a cracker made from 100% Lemnos wheat.

To conduct the β-glucan analysis, the methodology proposed by Megazyme (Mixed-linkage beta-glucan assay procedure – McCleary method) was followed. Samples were suspended and then rehydrated in a pH 6.5 buffer. This was followed by incubation with pure lichenase enzyme and filtration of the samples. An aliquot of the filtrate was hydrolyzed with β-glucosidase, which was determined using glucose oxidase/peroxidase reagent. Samples were analysed in duplicate.

2.2.6 Statistical Analysis

Each sample was analysed in triplicate (with the exception of data from β-glucan analysis) and differences among the values were analysed using a one-way analysis of variance (ANOVA). Post-hoc comparisons were performed using Tukey's HSD test adjustment. Pearson's correlation coefficient was used to measure the strength of a linear relationship between two variables. Presented values were based on flour type regardless of the level of substitution. Normality was assessed with graphical methods. All statistical analyses were two-sided, with the significance level set at 0.05, and carried out using XLSTAT software (Version, 2018.1, Addinsoft).

2.3 Results and discussion

2.3.1 Functional properties of composite flours

Functional properties such as swelling capacity, water absorption capacity, oil absorption capacity and bulk density are influenced by various components of food and help to evaluate and predict how flours may affect processing as well as final product characteristics (Awuchi et al. 2019). Composite flours presented with lower swelling capacity (SC) compared to WF, with the exception of olive stone flour (higher SC) (Figure 2.1), indicating that substitution of wheat can reduce swelling in crackers, likely due to the absence of gluten network formation. The use of olive stone flour resulted to increase in SC. SC describes the ability of flour to increase in volume when soaked in water. A lower SC could be helpful for crackers where, unlike bread, swelling of product is not desirable.

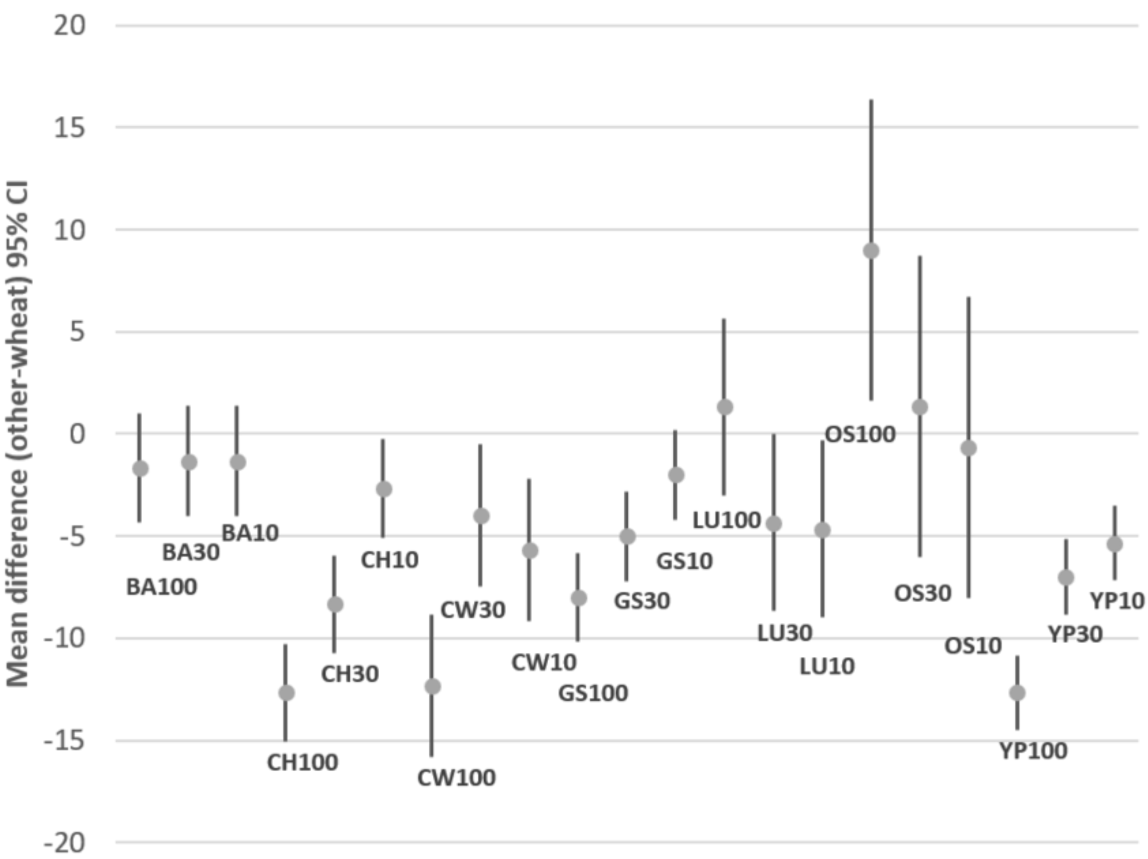

Figure 2.1. Mean differences in swelling capacity between composite flours and WF.

*Positive values represent higher SC while negative values represent lower SC than WF, with results expressed as means, n=3.

Samples LU100, CW100, OS100 and GS100 had significantly higher water absorption capacity (WAC) than WF (Figure 2.2). WAC refers to the ability of flour to hold water against gravity and is an important property for baking applications as it affects dough handling and consistency. Excessive or low absorption of water can cause negative effects, as the correct amount of hydration is necessary for achieving desirable dough and finished product quality (Awuchi et al. 2019). In composite flours blended with wheat, WAC was only higher for LU30 and OS30 suggesting that there was no impact at lower levels (<30%) of concentration. For olive stone flour, it was reported that its higher WAC is due to its high fiber content, which allows more water interactions through hydrogen bonding (Bolek et al. 2020). WAC has also been reported to be higher in other pulses such green-pea, yellow-pea (Millar et al. 2017) and pea flour (Kohajdová et al. 2013). It has been suggested that the WAC of pulse flours is due to their high fiber, protein and polysaccharide content (Du et al. 2014). However, this was not confirmed by the results for chickpea flour, in any concentration, which displayed significantly lower values than WF despite its reported higher fiber and protein content. Lower fat content also helps maintain a higher WAC (Yadav et al. 2012).

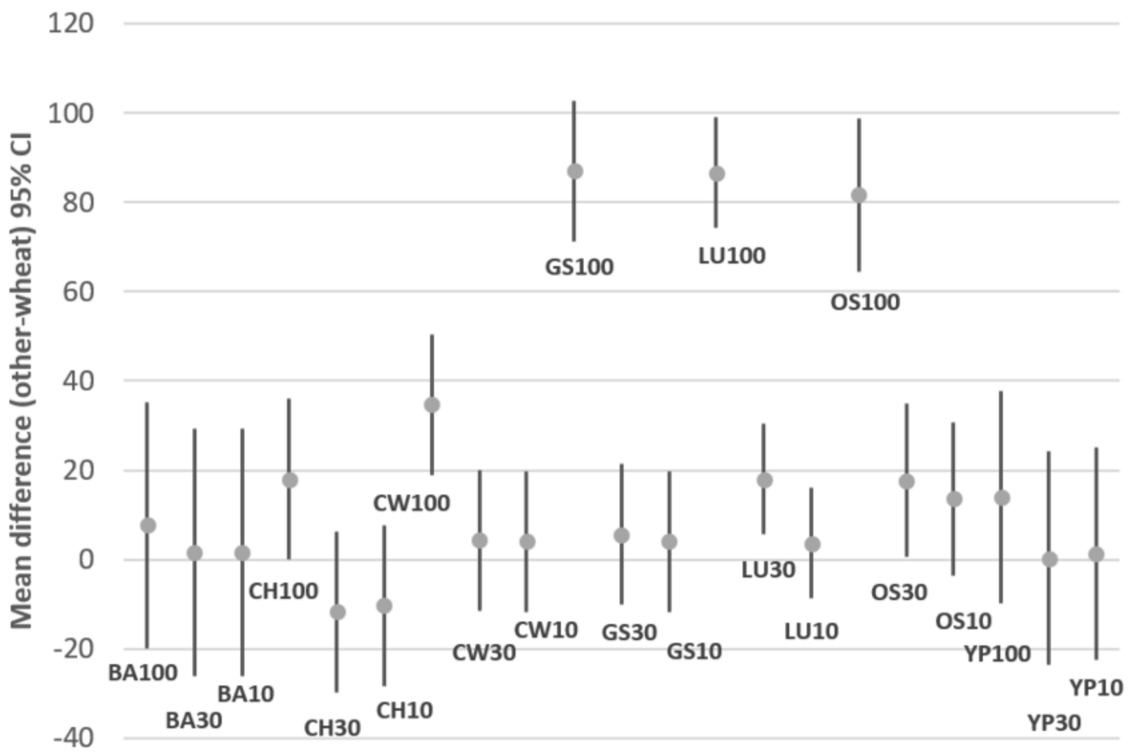

Figure 2.2. Mean differences in water absorption capacity between composite flours and WF.

*Positive values represent higher WAC while negative values represent lower WAC than WF, with results expressed as means, n=3.

Oil absorption capacity (OAC) capacity was highest for BA100 and lowest for GS100 and CW100 (Table 2.2). When yellow split pea flour was blended with wheat, OAC became significantly lower than WF. OAC refers to the rate at which protein binds to fat in food and is an important indicator for flavor retention, mouthfeel and product shelf life (Hasmadi et al. 2020). Results for barley flour suggest that its use could possibly enhance the sensory perception of wheat crackers. OAC is positively affected by hydrophobic proteins as well as other food characteristics, such as particle size and starch content (Du et al. 2014). The high fat content of specific flours, such as olive stone for instance, can have an adverse effect on OAC (Hasmadi et al. 2020). Our data showed a small, but nonetheless non-significant, decline in OAC level in olive stone flour. Flour OAC results offer a possible explanation for the lower reported flavor rankings received by wheat crackers enriched with olive stone flour and grape seed flour as opposed to those enriched with barley flour (Giannoutsos et al. 2023).

Table 2.2. Functional properties of flours.

Samples	OAC (%)	Bulk Density (g/ml)
WF	125.00	0.65
LU100	129.00	0.50*
CH100	110.33	0.66*
YP100	109.33	0.71*
CW100	97.33*	0.63
BA100	160.33*	0.55*
OS100	102.33	0.59*
GS100	94.66*	0.66*
LU10	108.66	0.64
LU20	109.33	0.63
LU30	106.33	0.61*
CH10	109.66	0.66*
CH20	116.00	0.65*
CH30	109.00	0.70*
YP10	97.66*	0.70*
YP20	96.66*	0.71*
YP30	93.66*	0.73*
CW10	102.00*	0.70*
CW20	100.00*	0.70*
CW30	100.00*	0.70*
BA10	126.33	0.64*
BA20	119.66	0.65*
BA30	127.33	0.62*
OS10	107.66	0.62*
OS20	110.66	0.60*
OS30	104.00	0.60*
GS10	116.33	0.65

GS20	106.00	0.66*
GS30	102.00*	0.65

Results are expressed as means, n=3.

Asterisk (*) indicates a statistically significant difference, at 5% level, compared to the control.

LU100, BA100 and OS100 had significantly higher bulk density (BD) than WF, whereas YP100 had the lowest BD. The BD of a sample is indicative of its density without the influence of any compression. It reflects the heaviness, as well as how much of a load a product can carry and can be used to determine a product's packaging requirements (Hasmadi et al. 2020). Drakos et al. (2019) reported higher BD for composite flours with barley. This is in line with this study's results for BA10 and BA30. Barley flour, as well as other flours with similar effect (lupin and olive stone) could, therefore, be used in future product formulations to help maximize packaging efficiency and reduce waste.

2.3.2 The effect of composite flours on dough texture

Compared to control, dough hardness was significantly higher in LU30, and GS30 (Figure 2.3). Bolek et al. (2020) reported that high fiber content can lead to harder dough. However, all barley and yellow split pea flour formulations presented significantly lower hardness, suggesting that fiber is not the only determining factor for dough hardness. Dough from chickpea flour was also softer than control, but only at higher substitution levels. Substituting wheat with cowpea flour also led to softer dough, but only the difference with at lower levels was of statistical significance. This may be due to higher water retention of the dough, resulting from the gluten network disruption (Agrahar-Murugkar et al. 2015). Interestingly, WAC of flours seems to be associated with higher dough hardness, specifically as observed with lupin and grape seed flours, while the inverse was observed with chickpea flour. This may denote that higher WAC leads to better water retention in the dough, creating thus a drier and more rigid dough profile. Overall, the results of our study indicate that dough hardness can be influenced by the type of flour and the level of wheat flour substitution and seems to be mediated by the flours' WAC (r=0.857 for grape seed flour; r=0.954 for lupin flour).

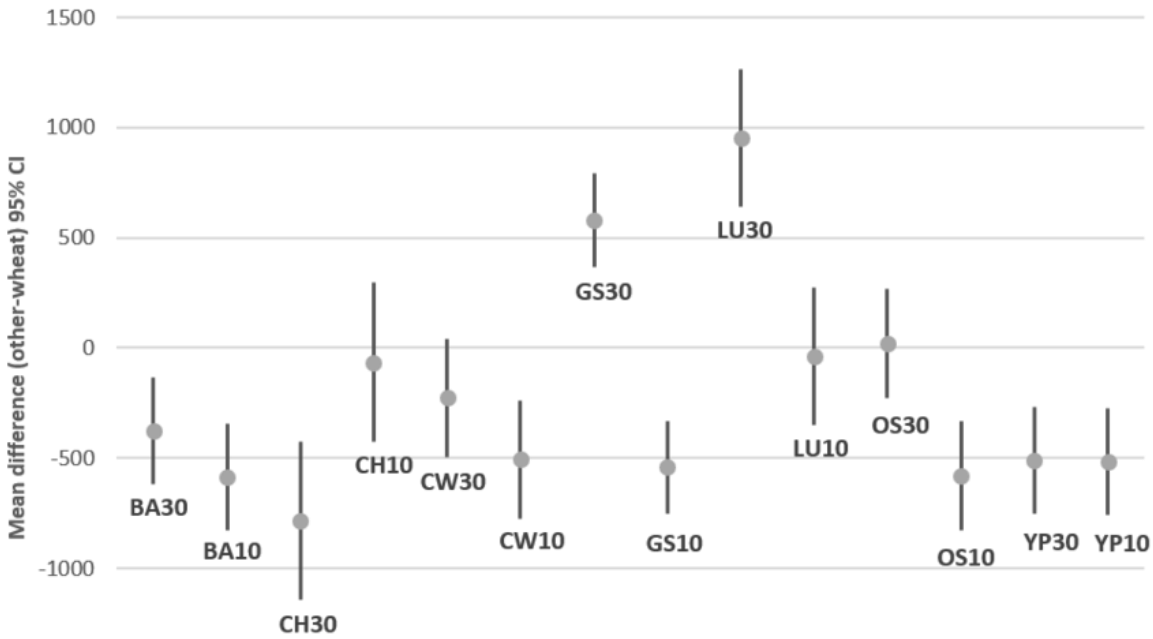

Figure 2.3. Mean differences in dough hardness between composite flours and WF.

*Positive values represent higher dough hardness while negative values represent lower dough hardness than WF, with results expressed as means, n=3.

No significant differences were seen for dough springiness between control and composite flour samples (Table 2.3). GS30 was the only sample with significantly lower dough adhesiveness and cohesiveness than the control, suggesting a possible link between the two parameters. However, no significant difference was seen at lower levels of wheat flour substitution (10% and 20%) indicating the effect to be amount dependent. High adhesiveness can have a negative impact during processing, due to dough stickiness, making handling difficult and potentially causing problems to machinery (Agrahar-Murugkar et al. 2015). Dough hardness, as observed from GS30, appears to have an inverse relationship with dough adhesiveness.

Table 2.3. Texture Profile Analysis of dough from composite flours.

Samples	Cohesiveness	Springiness (%)	Adhesiveness
WF	0.19	20.01	-252.52
LU10	0.18	20.39	-183.23
LU20	0.18	18.68	-184.05
LU30	0.18	18.57	-128.61
CH10	0.20	20.05	-368.57*
CH20	0.14	14.83	-153.17
CH30	0.14	14.99	-158.77
YP10	0.18	20.02	-209.35
YP20	0.17	19.21	-230.09
YP30	0.16	17.98	-163.85
CW10	0.19	19.94	-159.56
CW20	0.17	17.80	-205.92
CW30	0.16	17.60	-192.99
BA10	0.17	19.78	-211.39
BA20	0.18	20.49	-218.74
BA30	0.16	17.16	-144.26
OS10	0.16	18.02	-163.56
OS20	0.16	17.30	-155.80
OS30	0.17	17.39	-139.73
GS10	0.18	20.52	-196.67
GS20	0.16	17.93	-150.08
GS30	0.15*	16.31	-83.67*

Results are expressed as means, n=3.

Asterisk (*) indicates a statistically significant difference, at 5% level, compared to the control.

2.3.3 B-glucan content in alternative flours and raw materials

The results of the analysis (Table 2.4), initially on the raw materials (flour), showed that the alternative flours, with the sole exception of barley, had a very low β-glucan content. Barley flour had a significantly higher β-glucan level compared to wheat flour (local and commercial). It is however noteworthy that the Megazyme reference sample had a significantly higher level of β-glucan than the Lemnos and commercial barley flour samples. Interestingly, after substituting wheat flour with barley at a level of 30%, only the Lemnos barley flour cracker showed significantly higher β-glucan values compared to the control sample. This result is possible to indicate some advantage regarding the influence of baking (high oven temperatures) or β-glucan bioavailability in Lemnos barley compared to its commercial counterpart.

Table 2.4. B-glucan content of alternative flours.

Samples	**B-glucan content (g/100g)**
Barley control (flour)	1.527 a
Barley commercial (flour)	0.877 b
Barley Lemnos (flour)	0.867 b
Barley Lemnos (cracker)	0.417 c
Barley commercial (cracker)	0.264 cd
Wheat Lemnos (cracker)	0.158 de
Wheat Lemnos (flour)	0.131 de
Wheat commercial (flour)	0.113 de
Wholewheat Lemnos (flour)	0.080 de
Wholewheat commercial (flour)	0.061 de
Chickpea (flour)	0.030 e
Lupin (flour)	0.005 e

Grape seed (flour)	-0.009 e
Olive stone (flour)	-0.038 e
P value	< 0.0001

* Mean values followed by different letters are significantly different at a=0.05 significance level (P≤0.05).

2.4 Conclusion

The study findings show that understanding the functional properties of flours can help in the design and optimization of food products, targeting desired processing and quality outcomes. In cracker doughs, higher levels (30%) of lupin and grape seed flours resulted in significantly higher hardness. In contrast, barley flour dough had significantly lower hardness, indicating that factors other than fiber content contribute to hardness of the dough. Chickpea flour resulted in softer dough only at higher levels of substitution. The water absorption capacity of flours appears to be related to hardness, revealing that higher WAC levels in flour leads to drier and harder dough. Dough elasticity did not differ significantly between samples; however, dough with grape seed flour (30%) displayed significantly lower adhesiveness, suggesting a possible inverse relationship with dough hardness. The findings of the present research indicate that barley flour can be used to replace wheat in cracker-type products offering potential benefits to the consumer's health due to its higher β-glucan content. Lemnos barley presented with a higher level of β-glucan compared to its commercial counterpart, highlighting the benefits derived from the use of local raw materials.

CHAPTER 3

Substitution of wheat flour in crackers with alternative functional flours

3.1 Literature review

The world snack market grew 6.2% annually and was expected to reach 639 USD billion by 2023 (Batista et al., 2019). Sales of crackers, in particular, have been growing at faster rate within the snack food market (Millar et al., 2017). Crackers are defined as thin, dry and crisp baked foods, made predominantly with soft wheat flour, and contain low levels of sugar, fat and moisture. Nowadays, it has become popular to incorporate alternative and health ingredients as raw materials for the production of snacks, such as pulses and by-products of the agricultural production.

Functional bakery products could be defined as products with similar appearance to conventional but with demonstrated health benefits beyond those of traditional products (Kumar et al., 2017). Bakery products' main ingredient is refined wheat flour, which is considered to be a poor source of vitamins, minerals and fiber and has high glycemic index (GI) (Seal et al., 2021). Pulses, barley and polyphenol rich by-products of the food industry could represent good options for product enrichment. Pulses are a good source of complex carbohydrates and also are rich in dietary fiber, protein, vitamins, minerals and polyphenols (Mudryj et al., 2014) and could support plant-based diets which tend to lack in 'complete' proteins (Lynch et al., 2018). Barley is a rich source of fiber, such as β-glucan, and phenolic compounds (Drakos et al., 2019).

In recent years, interest has been growing in employing ingredients rich in phytochemicals, which would otherwise end up as waste, for fortifying food and enriching its health promoting properties. As a by-product of wine, grape seed flour is a rich source of various bioactive compounds such as resveratrol, phenolic acid, flavonoids, procyanidins, minerals and fiber (Antonic et al., 2021). Grape by-products have high content of phenolic compounds, such as flavanols (catechin, epicatechin, procyanidins B1, B2, B3 and B4), flavonols (quercetin, kaempferol) and phenolic acids (gallic, protocatechuic, syringic, vanillic and ellagic acids) (Mildner-Szkudlarz et al., 2013). Phenolic compounds have received a lot of attention with regards to their potential therapeutic role and health benefits, showing promise against several diseases such as, among others, cancer, diabetes, obesity, cardiovascular diseases, neurodegenerative diseases and osteoporosis (de Araújo et al., 2021).

Furthermore, the olive oil industry generates large amounts of olive cake (pulp, skin and stone). Olive by-products are very high in polyphenols, a group of phytochemicals widely studied for their antioxidant, antimicrobial and anti-inflammatory properties (Karnopp et al., 2015). Recently, olive stone has received attention due to being rich in phytochemicals, fiber ($\cong$ 47% seed dry weight), healthy fats, essential amino acids and minerals (Bolek, 2020; Maestri et al., 2019).

Over the last years, a number of studies have focused on the effect of substituting wheat with functional flours on crackers' nutritional, physical, chemical and sensory properties. Although many early and comprehensive studies have been performed with alternative flours to wheat in various snacks, much less attention has been given to crackers where publications are limited. Flours and quantities used included dietary fiber from defatted press meals of orange and grapefruit seeds (Yilmaz and Karaman, 2017) at 2.90%; flour from microalgae at 2% and 6% (Batista et al., 2019); multi-grain flour containing finger millet, brown rice and flour from pulses in different ratios at 30%, 40% and 50% (Herath et al., 2018); and flours originating from broad-bean, yellow-pea and green-pea at 40% (Millar et al., 2017). Results from these studies have revealed that functional flours have the potential to cause significant changes to the properties of wheat crackers, leading to several positive as well as negative effects. In some cases, the sensory profile of crackers was negatively affected by addition of functional flours (Yilmaz and Karaman, 2017; Batista et al., 2019).

There appears to be lack of research on crackers regarding the effects from the use of flours from barley and by-products of wine and olive processing such as grape seed and olive stone. It is important, however, to ensure that addition of functional flours does not result in compromised quality. Quality is affected by properties of food such as texture and color (Tunich, 2011; Luo et al., 2019). The objective of the research was to investigate the effect of replacing wheat flour with non-conventional and healthy flours in crackers on two crucial quality aspects: texture and color. Wheat replacement was attempted by the addition of varying quantities of barley, grape seed, olive stone, lupin flour (10 and 30%) and chickpea flour (30 and 50%) in order to evaluate the potential application of these flours in cracker formulations.

3.2 Materials and methods

3.2.1 Materials

Wheat (WF) from *Lemnos* variety and Barley flour (BF) from *Panagia* variety were obtained locally from Lemnos, Greece (harvest of 2020). Chickpea flour (CF) was obtained from *Bioagros* (Pella, Greece) and Lupin flour (LF) was purchased from *Lup'Ingredients* (Martigne-Ferchaud, France). Grape Seed Flour (GSF) was purchased from *PaleoCentrum* (Budapest, Hungary) and Olive Stone Flour (OSF) was purchased from *Nutexa* (Valencia, Spain).

3.2.2 Cracker Preparation

Cracker formulation was 100g of flour, 40g of water, 20g of canola oil, 1.85g baking powder, 1g of salt and 2g of sugar. The flour in the formulation of the control cracker (100% wheat flour; WF) was substituted with flour from pulses (chickpea, lupin) cereals (barley) and flours derived from polyphenol rich by-products (grape seed; olive stone). The substitution of wheat flour was 10% and 30% (W/W %), except in the case of chickpea flour which was 30 and 50%. Minimum and maximum substitutions were determined following preliminary trials to ensure appropriate baking times and dough consistency.

First, dry ingredients were mixed in a mixer, whilst the liquid ingredients (along with sugar) were mixed separately to form an emulsion. Subsequently, all ingredients were mixed together to form a dough (6 minutes). The dough was briefly kneaded and left to rest at room temperature for 30 minutes. Sheeting of the dough was done using a roller and pieces (10cm x 7.5cm) were cut with a mold. The crackers were baked in a professional baking oven at 170 °C for 15 minutes. They were allowed to cool at room temperature, packed in polyethylene wraps and stored before analysis.

3.2.3 Texture Analysis of Crackers

The texture of the crackers was analysed by using a TA. XT Plus Texture Analyzer (Stable Micro Systems) equipped with a 50kg load cell. Textural attributes measured were the following: peak force (N) which indicates hardness; first break distance (mm) indicating fracturability; and total area of work (J) which indicates the total energy used or toughness (Han et al., 2010). These were measured by carrying out the snap test with the use of the blade set (HDP/BS). For executing the test, the cracker is placed onto two support beams, while a third moves down in parallel, causing the sample to fracture in the middle (Batista et al., 2019). The parameters used for testing were: pre-test speed (1,00 mm/sec), test speed (1,00 mm/sec), post-test speed (10,00 mm/sec), descending distance (15mm) and trigger force (5g). The crackers were supported across two beams spaced at 6.5cm apart on the base plate. Each sample was analysed in 6 replicates.

3.2.4 Color Analysis of Dough and Crackers

A Lovibond LC 100 Spectrocolorimeter (The Tintometer Limited) was used for the measurement of color in the CIELAB system. The results were expressed in terms of L*, lightness (values increasing from 0% to 100%); a*, redness to greenness (positive to negative values, respectively); b*, yellowness to blueness (positive to negative values, respectively); Chroma, C* (saturation), hue angle and total color difference (ΔE). ΔE was calculated using the Equation 3.1 (letters *c* and *s* next to L*, a* and b* values indicate, respectively, control sample and sample under investigation).

$$\Delta E = \sqrt{(Lc^* - Ls^*)^2 + (ac^* - as^*)^2 + (bc^* - bs^*)^2} \qquad (3.1)$$

Measurements were taken from three different surface points, from the dough samples and the crackers, respectively. Values reported for dough and crackers are the mean of the aforementioned measurements.

3.2.5 Statistical Analysis

Data from lab analyses were processed in batches with 6 replicates for cracker texture analysis and in triplicate (3 different surface points) for color analysis of dough. Color analysis for the cracker samples was performed in 6 replicates per sample, on 3 different surface points. Differences among the values were analysed using a one-way analysis of variance (ANOVA). Mean comparisons were performed using Tukey's HSD test adjustment at significance level $\alpha=0.05$ ($P\leq0.05$). All the above-mentioned statistical analyses were carried out using XLSTAT v.2021.3.1 software (New York, Addinsoft).

3.3 Results and discussion

3.3.1 Effect of functional flours on the texture of crackers

Texture is a highly important property for crackers, as it is indicative of their freshness and quality. In particular, the hardness and fracturability of crackers offer a good idea about whether a product is perceived by consumers as having a crisp and crunchy texture, both highly desirable properties for this type of product (Batista et al., 2019; Millar et al., 2017; Yilmaz and Karaman, 2017).

OS30 was the only formulation which presented significantly lower hardness (43% lower) than the control sample (Table 3.1). Nevertheless, there was a noticeable, however, non-significant trend of decreasing hardness for OS10 and BA30. This result indicates that substitution of wheat does not affect product hardness. However, with particular flour types, hardness can be affected by the level of wheat substitution. Toughness presented a trend similar to hardness. Specifically, OS30 presented significantly lower toughness (52% less) than the control. Fracturability was not significantly affected.

Table 3.1. Textural properties of crackers as affected by different flour substitutions.

	Hardness (N)	Fracturability (mm)	Toughness (J)
WF (Control)	11.019 ± 2.96 ^{a}	2.151 ± 0.69^{a}	14.110 ± 3.20^{a}
BA10	11.144 ± 2.15^{a}	2.161 ± 0.78^{a}	14.871 ± 3.79^{a}
BA30	8.404 ± 1.37^{ab}	1.961 ± 0.45^{a}	11.251 ± 1.56^{ab}
LU10	10.549 ± 1.91^{a}	1.813 ± 0.38^{a}	12.601 ± 4.20^{ab}
LU30	9.973 ± 3.31^{ab}	1.743 ± 0.64^{a}	9.906 ± 2.22^{ab}
CH30	9.965 ± 2.62^{ab}	3.502 ± 4.91^{a}	10.303 ± 3.53^{ab}
CH50	9.392 ± 2.25^{ab}	2.010 ± 0.87^{a}	10.811 ± 5.46^{ab}
GS10	10.555 ± 1.60^{a}	1.724 ± 0.38^{a}	11.600 ± 2.16^{ab}
GS30	9.393 ± 1.22^{ab}	1.786 ± 0.74^{a}	10.718 ± 1.65^{ab}

OS10	8.559 ± 1.57[ab]	1.765 ± 0.51[a]	9.072 ± 1.32[ab]
OS30	6.238 ± 1.63[b]	1.450 ± 0.25[a]	6.813 ± 1.52[b]
P-value	0.012	0.735	0.003

Results are expressed as means ± standard deviation, n=6.
Mean values, within a column, followed by different letter(s) are significantly different at significance level a=0.05 (P≤0.05), according to Tukey's HSD test.

Our results are in agreement with a study of biscuits substituted with barley flour (10% - 40% substitution level), which showed that higher substitution of wheat led to decreased hardness (Drakos et al., 2019). Similarly, in a study of cookies, where wheat flour was substituted with grape skin and grape seeds flour (5% - 15% substitution level), hardness and fracturability were decreased when substitution levels increased (Kuchtová et al., 2018). These results were justified due to possibly increased water content which persisted baking and reduced gluten. Another possible explanation offered was that phenolic compounds from grapes may have contributed to the reduction in hardness due to inhibiting amylase activity. Mildner-Szkudlarz et al. (2013) showed that substitution of wheat with grape pomace at 30% in biscuits increased total phenolic content significantly from 0.85 mg GAE g^{-1}DM to 4.45 mg GAE g^{-1}DM. The gluten content of wheat directly contributes to product hardness and fracturability, leading to better gas entrapment and more cohesive and viscoelastic dough (Nammakuna et al., 2016). Furthermore, it has been argued that dietary fiber has the potential to reduce crispness, as it negatively affects the rheological behaviour and the uniformity of dough structure (Venkatachalam and Nagarajan, 2017). It is possible that the increased amount of the phenolic content and/or fiber in OS30 may have contributed to its softer texture, however, from the current data, it is not feasible to directly establish a causal relationship.

On the other hand, it has been suggested that added fibers actually lead to crackers with higher hardness and fracturability (Yilmaz and Karaman, 2017). For example, in a study of biscuits with added olive stone flour, increasing substitution of wheat led to increased hardness and fracturability (Bolek, 2020). According to the authors of this study, this result could be due to the increased fiber content, which in turn resulted in higher water absorption capacity. Specifically, fiber content increased significantly from 0.88% to 11.22% at 15% olive stone. In a case of wheat substitution in biscuits by up to 50% lupin flour, hardness and fracturability increased proportionally to substitution (Jayasena and Nasar-Abbas, 2011). When wheat was substituted with broad-bean flour and yellow pea flour in crackers and biscuits respectively, increasing replacement led to increased hardness, possibly due to the higher fiber and protein content of the product (Millar et al., 2017; Zhao et al., 2019). In contrast, grape seed flour used in waffles did not significantly affect hardness; however, this is a very different food matrix and substitution of wheat did not exceed 10% (Antonic, 2021). Interestingly, these observations are not in line with the findings from this study, where hardness was reduced (albeit not significantly) across all samples at higher levels of wheat substitution. In fact, results seem to indicate that hardness is proportionally linked to the level of wheat in crackers, possibly explained by its gluten content.

3.3.2 Effect of flours on dough color and baking

Color was reported to directly affect consumer perception and acceptability of crackers (Millar et al., 2017). Research shows that food color can exert such a powerful effect, to the point that by changing a food’s color, one can directly affect its taste, flavor intensity and even flavor identification (Spence, 2019). For this reason, the color of dough and final baked products was examined in this study. Based on L* values, the control sample (WF) was found to be the brightest (Table 3.2). Brightness decreased in all crackers with increased levels of wheat substitution.

Table 3.2. Differences in the color of crackers and dough as affected by wheat substitution with functional flours.

	Crackers						Dough					
	L*	a*	b*	C*	h°	ΔE	L*	a*	b*	C*	h°	ΔE
WF	74.12 ± 1.02^{a}	3.80 ± 0.48^{e}	29.28 ± 0.97^{d}	29.53 ± 1.01^{d}	82.61 ± 0.73^{a}	0.00^{i}	61.86 ± 1.13^{a}	1.20 ± 0.36^{e}	23.10 ± 0.85^{c}	23.16 ± 0.85^{de}	87.03 ± 0.83^{a}	0.00^{g}
BA10	73.03 ± 1.65^{ab}	4.22 ± 0.62^{e}	27.50 ± 1.15^{e}	27.82 ± 1.23^{e}	81.25 ± 0.88^{a}	2.75 ± 0.70^{h}	55.90 ± 0.91^{bc}	1.70 ± 0.60^{de}	21.40 ± 0.26^{c}	21.46 ± 0.32^{de}	85.50 ± 1.65^{a}	6.24 ± 0.83^{ef}
BA30	67.30 ± 1.52^{c}	5.34 ± 0.85^{d}	26.83 ± 1.56^{e}	27.38 ± 1.53^{e}	78.72 ± 1.74^{b}	7.50 ± 0.85^{g}	50.43 ± 0.80^{d}	3.60 ± 1.47^{cd}	18.96 ± 1.02^{cd}	19.36 ± 0.89^{ef}	79.30 ± 4.73^{a}	12.44 ± 1.29^{d}
LU10	72.21 ± 1.06^{b}	5.67 ± 0.73^{d}	36.18 ± 0.84^{b}	36.63 ± 0.89^{b}	81.10 ± 1.01^{a}	7.43 ± 0.82^{g}	54.26 ± 0.40^{c}	3.90 ± 0.52^{cd}	29.26 ± 2.71^{b}	29.53 ± 2.56^{bc}	82.30 ± 1.65^{a}	10.29 ± 1.65^{de}
LU30	66.11 ± 1.92^{c}	11.19 ± 1.39^{b}	41.39 ± 0.74^{a}	42.88 ± 0.66^{a}	74.87 ± 1.91^{c}	16.38 ± 1.32^{e}	50.86 ± 1.72^{d}	7.20 ± 0.40^{b}	36.56 ± 3.13^{a}	37.30 ± 3.10^{a}	78.80 ± 1.01^{ab}	18.42 ± 1.01^{c}
CH30	71.36 ± 2.49^{b}	8.16 ± 1.64^{c}	33.18 ± 0.92^{c}	34.21 ± 1.22^{c}	76.24 ± 2.35^{c}	6.52 ± 1.74^{g}	57.83 ± 0.51^{b}	1.60 ± 0.30^{de}	23.80 ± 1.47^{c}	23.90 ± 1.47^{de}	86.23 ± 0.47^{a}	4.29 ± 0.43^{fg}
CH50	64.18 ± 2.27^{d}	13.00 ± 1.67^{a}	32.68 ± 1.62^{c}	35.20 ± 1.68^{c}	68.30 ± 2.63^{d}	14.02 ± 1.57^{f}	57.60 ± 0.10^{b}	4.20 ± 0.43^{c}	31.46 ± 0.92^{ab}	31.73 ± 0.98^{b}	82.43 ± 0.58^{a}	9.85 ± 0.91^{de}
GS10	42.60 ± 1.57^{f}	9.00 ± 0.26^{c}	13.61 ± 0.48^{h}	16.31 ± 0.45^{h}	56.50 ± 1.00^{f}	35.58 ± 1.32^{c}	32.23 ± 1.49^{f}	8.43 ± 0.87^{b}	12.90 ± 1.27^{ef}	15.46 ± 1.51^{fg}	56.86 ± 1.75^{c}	31.84 ± 1.26^{b}
GS30	29.71 ± 1.15^{h}	8.84 ± 0.29^{c}	8.33 ± 0.58^{i}	12.15 ± 0.53^{i}	43.26 ± 1.73^{g}	39.79 ± 1.15^{b}	22.83 ± 1.72^{h}	9.10± 0.91^{ab}	9.70 ± 1.70^{f}	13.33 ± 1.61^{g}	46.63 ± 5.05^{d}	42.03 ± 2.02^{a}

OS10	49.63 ± 0.86^{e}	11.0 ± 0.32^{b}	23.48 ± 0.33^{f}	25.94 ± 0.38^{f}	64.90 ± 0.67^{e}	26.17 ± 0.57^{d}	41.90 ± 0.63^{e}	8.60 ± 0.78^{ab}	23.50 ± 1.34^{c}	25.06 ± 1.51^{cd}	69.96 ± 0.78^{b}	21.35 ± 0.43^{c}
OS30	37.05 ± 1.13^{g}	11.80 ± 0.39^{b}	17.22 ± 0.77^{g}	20.91 ± 0.74^{g}	55.55 ± 1.22^{f}	39.79 ± 1.13^{b}	28.76 ± 1.32^{g}	10.96 ± 1.15^{a}	15.43 ± 2.34^{de}	19.03 ± 1.15^{ef}	54.30± 7.06 ^{cd}	35.38 ± 1.88^{b}

*Results are expressed as means ± standard deviation, n=6 for crackers and n=3 for dough (3 different locations).

Mean values, within a column, followed by different letter(s) are significantly different at significance level a=0.05 (P≤0.05), according to Tukey's HSD test.

L*: Lightness; a*: Red/Green value; b*: Blue/Yellow value; C*: chroma; h°: hue; ΔE: Total color difference was measured in comparison to WF (those with a ΔE > 3 exhibit a color difference from WF which is visible to the eye).

These results are in line with a study of biscuits where L* decreased at ≥30% substitution of wheat with lupin flour (Jayasena and Nasar-Abbas, 2011). Decrease in brightness was also reported following wheat substitution with barley flour in biscuits (Drakos et al., 2019) and enrichment of whole wheat cookies with encapsulated grape skin extract (Dordoni et al., 2019). Similar results were generated when wheat in crackers was replaced by pulse flours (Millar et al., 2017), with the authors suggesting that color was affected by the flours' botanical source and natural pigmentation. In addition, baking leads to enzymatic browning (Maillard reaction and sugar caramelisation) as well as starch dextrinization (Drakos et al., 2019). Thus, the higher protein content of functional flours would result to higher availability of free-amino acids and hence Maillard-browning (Nammakuna et al., 2016).

Increased substitution of wheat flour led to higher values of a* (redness), except for GS30, which presented lower values than GS10. Pulse flours (LU and CH) resulted to significantly higher b* values (yellower) compared to control, which may be explained by their higher carotenoid content (Jayasena and Nasar-Abbas, 2011). A similar effect was seen in studies with yellow pea flour (biscuits), different blends of pulse flours (crackers) and lupin flour (biscuits) (Zhao et al., 2019; Millar et al., 2017; Jayasena and Nasar-Abbas, 2011). On the other hand, the OS and GS samples presented significantly lower b* values than the control. Decrease of b* values was also seen in biscuits with added olive stone powder (Bolek, 2020) and grape skin (Kuchtová et al., 2018). This is likely due to the natural pigmentation of olive stones and grape seeds, specifically the rich anthocyanin content with regards to the latter (Kuchtová et al., 2018). Chroma (c*) values were significantly different for all functional crackers and the control. Hue values for BA10 and LU10 were not significantly different from the control.

ΔE values showed an increase with the increase in the level of wheat flour substitution and based on flour type both in cracker and dough samples. For BA10 crackers, ΔE values indicated that color differences were not perceptible to the eye (ΔE of <3). These findings suggest that cracker color is significantly influenced by the type of flour and, especially, the level of flour used. Higher levels of substitution seem to have a stronger impact.

Baking led to significant differences in the color between the dough (unbaked) and the baked samples (cracker). All baked samples were brighter than dough. This result may be partly due to the natural pigment degradation as a result of high temperatures. In addition, a* values increased in all baked samples, besides GS30, possibly due to Maillard reaction. Overall, however, the effect of wheat substitution with functional flours resulted in a similar pattern between the unbaked and baked samples, indicating that color is determined by the type of flour used.

According to Spence (2019), the impact of food color on tasting depends both on what taste and flavor expectations a color signifies to the consumer, as well as on how close, or far, the actual tasting experience is from the visually induced expectation. For example, independence or even contrast may be observed when there is a significant difference between the tasting experience and the visually induced expectation. For functional crackers, larger differences in color, from what consumers would typically expect, could potentially lead to stronger contrast between visually induced expectations and actual tasting experiences.

3.4 Conclusion

Substitution of wheat with functional flours led to several changes in the properties of crackers. The study provides evidence that functional flours can be used as alternatives to wheat, however, the product's physicochemical properties (texture and color) can be affected depending on the type of flour and level of substitution. These results are important as only a limited number of studies so far have focused on production of wheat crackers with functional flours and in these studies the types of flours and levels used were different compared to our research. Our study aimed, therefore, to cover this gap in the literature by examining the substitution of wheat in crackers with various flours, separately (not blended together), and at low to high levels, ensuring that results are attributed to specific flour types and effects at different concentrations are identified. The proposed methodology of wheat flour substitution with functional flours could pave the way for the production of healthy and differentiated in taste snacks.

CHAPTER 4

Quality and shelf life of functional crackers packaged with sustainable materials

4.1 Literature review

Functional crackers offer promising health benefits; however, studies are lacking on their quality properties and effects on shelf life. Belonging in the dry biscuits category, crackers are traditionally considered to have a long shelf life, especially because of their low water activity (Romani et al., 2014). They are typically packed with plastic materials, due to their functionality, light weight, low cost, high performance and good processability (Sangroniz et al., 2019). However, packaging materials have different properties, and this can have an additional effect on their shelf life, independent from their formulation. Such an example are the packaging's barrier properties (Baele et al., 2020). Barrier properties, such as permeability of gases (e.g., O2), water, light and vapour, represent crucial factors for the quality of packaged goods. More specifically, although traditional methods of packaging such as metal cans and glass containers are considered impermeable, plastic packaging provides varying degrees of protection (Robertson, 2013).

Packaging, as a crucial factor for a product's shelf life, is important to provide good barrier properties, in order to minimize high oxygen permeability and moisture uptake, which can have detrimental effects on a product's quality during storage. Oxygen, light and moisture uptake are a cause of oxidation reactions in the product as well as loss of crispness. Lipid oxidation is responsible for the development of rancidity, resulting in off-odors and off-flavors. These can have a significantly negative effect on the product's shelf life and sensory acceptability (Zabihzadeh Khajavi et al., 2020; Romani et al., 2014).

Plastic, as a petroleum-based material, is increasingly recognised for its negative effect on the environment. For this reason, moving away from plastic packaging and focusing on more sustainable packaging materials is becoming a global trend (Romani et al., 2014). Such an example are biodegradable materials, like polylactic acid (PLA), which are considered better alternatives for the environment and are derived from bio-renewable sources can be enzymatically or hydrolytically degraded (Sangroniz et al., 2019). Edible options of packaging are also becoming increasingly researched as potential sustainable alternatives to conventional packaging. However, their commercial applicability is so far limited (Kowalska et al., 2020). Chitosan, a natural biopolymer, represents a promising edible packaging option, due to is non-toxicity, biocompatibility, high biodegradability, excellent film forming quality, and even antimicrobial activity (Tan et al., 2015).

Crackers produced with alternative flours, and in particular from legumes and local by-products of the food industry at 10% and 30% levels, were packed in three different types of packaging (plastic, biodegradable and edible) and were stored for 80 days in order to study the effect of functional ingredients and different packaging materials on the quality and shelf life of the new products. Additionally, crackers packaged in conventional and sustainable (biodegradable) packaging were shipped to two different locations, overseas (London, UK) and domestically (Samos). The purpose was to evaluate the protection offered by different packaging materials to the products during transport. Furthermore, the durability and strength of conventional and sustainable (edible) packaging was evaluated with a texture analyzer tensile strength test.

4.2 Materials and methods

4.2.1 Raw materials

Wheat flour (Triticum durum, ‘*Limnos’*), barley flour (Hordeum vulgare, ‘*Panagia’*), yellow split pea flour (Lathyrus ochrus, ‘*Afkos’*), cowpea flour (Vigna unguiculata, ‘*Aspromytiko’*), grape seed flour (Vitis vinifera, ‘*Moschato Alexandrias’*) and olive stone flour (Olea europea, ‘*Koroneiki’*) were obtained locally from Lemnos, Greece (harvest of 2020 - 2021). Chickpea flour (Cicer arietinum) and Lupin flour (Lupinus albus) were purchased from local retailers.

4.2.2 Cracker preparation

Product formulation was 100g of flour, 40g of water, 20g of canola oil, 1.85g of baking powder, 1g of salt and 2g of sugar. First, dry ingredients were mixed in a mixer (heavy duty stand mixer, KitchenAid) for 1 minute, whilst the liquid ingredients (along with sugar) were mixed separately to form an emulsion. Subsequently, all ingredients were mixed together (6 minutes) to make the dough. The dough was briefly kneaded and left to rest at 25 °C for 30 minutes. For the preparation of crackers, sheeting of the dough was done using a manual dough moulding machine and pieces (10cm x 7.5cm) were cut with a mold. The samples were baked in a professional baking oven (North, FK-60W) at 170 °C for 17minutes. They were allowed to cool at room temperature, packed in polyethylene wraps and stored for 24 hours before analysis. Crackers were tested at 10% and 30% concentration in mixture with wheat flour (Figure 4.1).

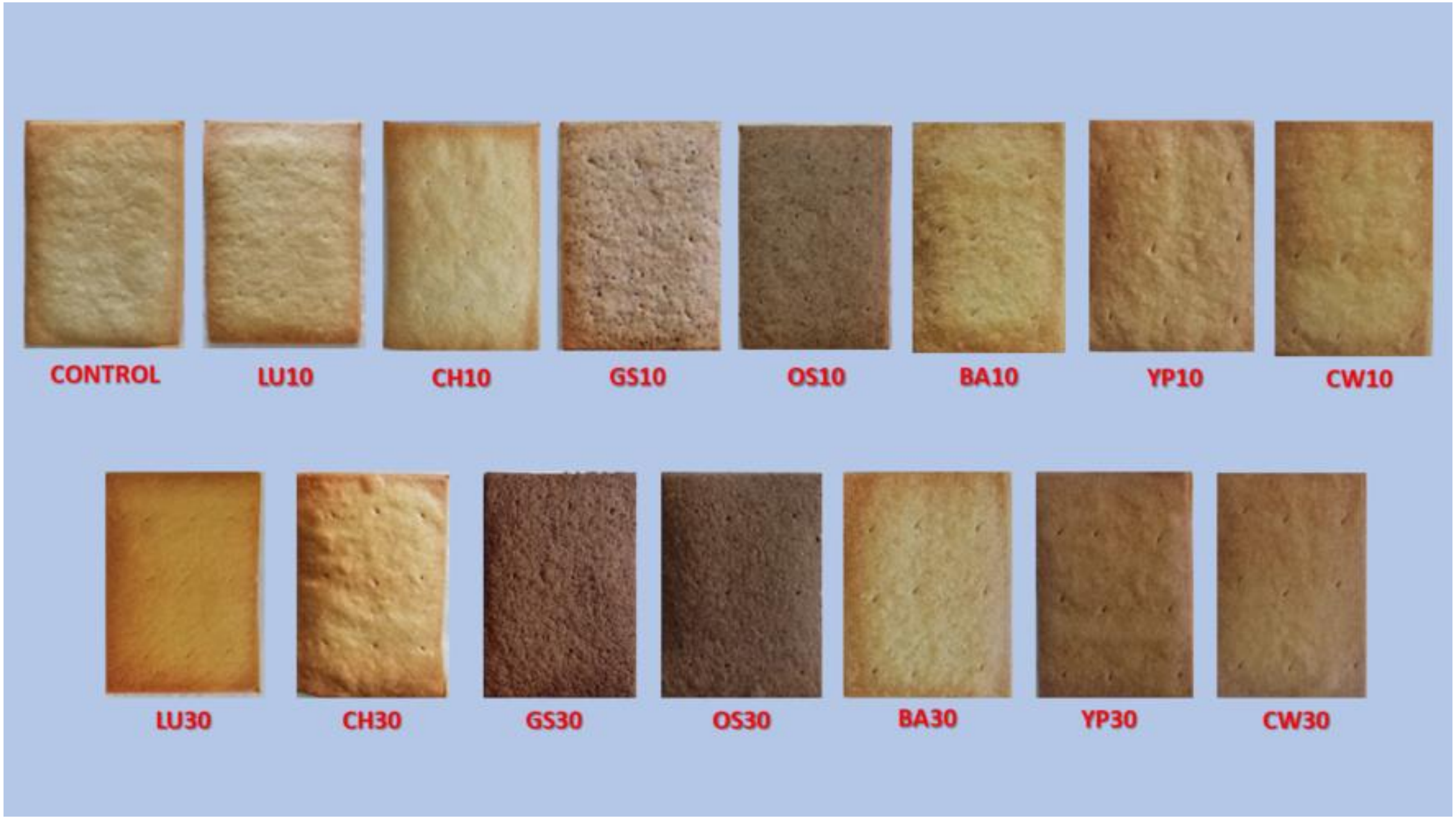

Figure 4.1. Crackers made with functional flours.

4.2.3 Packaging of crackers

Crackers made from 100% wheat flour, and 10% and 30% lupin, chickpea, grape seed and olive stone flours were packaged in three different types of packaging: conventional/plastic (material: polypropylene), biodegradable (material: polylactic acid) and edible (material: chitosan). The crackers were packed with a Tahipack THP-350 heat sealing machine. The conventional and biodegradable packaging were supplied by commercial companies, while the edible packaging was produced based on a protocol developed by the research group of Associate Professor Ioannis Mourtzinos (Aristotle University, Department of Agriculture).

4.2.4 Water activity and moisture content of crackers

Water activity (a_W) was measured using a water activity meter (Lab Touch-aw, Novasina). The moisture content of crackers was calculated based on the AOAC official method 930.15 (2005). Determinations were carried out in triplicate.

4.2.5 Physical parameters of crackers

Cracker dimensions, including length, width and thickness, were measured with a digital caliper and the spread ratio was calculated according to Equation 4.1.

$$Spread\ ratio = \frac{width}{thickness} \quad (4.1)$$

The weight before and after baking was measured and bake loss was determined based on Equation 4.2.

$$Bake\ loss = \frac{weight\ before\ baking - weight\ after\ baking\ and\ cooling}{weight\ before\ baking} \times 100 \quad (4.2)$$

Puffiness (%) of crackers was determined according to Equation 4.3.

$$Puffiness\ (\%) = \frac{thickness\ of\ baked\ cracker - thickness\ of\ cracker\ dough}{thickness\ of\ cracker\ dough} \times 100 \quad (4.3)$$

All measurements were done in triplicate and the mean value was used.

4.2.6 Texture analysis of crackers

The texture of the crackers was analysed using a TA. XT Plus Texture Analyzer (Stable Micro Systems) equipped with a 50kg load cell. Textural attributes measured were the following: peak force (N) which indicates hardness; first break distance (mm) indicating fracturability; and total area of work (J) which indicates the total energy used or toughness. These were measured by carrying out the snap test with the use of the blade set (HDP/BS). For executing the test, the cracker is placed onto two support beams, while a third moves down in parallel, causing the sample to fracture in the middle. The parameters used for testing were: pre-test speed (1,00 mm/sec), test speed (1,00 mm/sec), post-test speed (10,00 mm/sec), descending distance (15mm) and trigger force (5g). The crackers were supported across two beams spaced at 6.5 cm apart on the base plate.

4.2.7 Color analysis of crackers

A spectrocolorimeter (LC 100, Lovibond) was used for the measurement of color in the CIELAB system. The results were expressed in terms of L*, lightness (values increasing from 0% to 100%); a*, redness to greenness (positive to negative values, respectively); b*, yellowness to blueness (positive to negative values, respectively); Chroma, C* (saturation), hue angle, h° and total color difference (ΔE). ΔE was calculated using the Equation 4.4.

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \qquad (4.4)$$

Measurements were taken from three replicates. Data are reported as mean values.

4.2.8 Sample storage

To evaluate the shelf life and stability of the products, after production and packaging, crackers made from 100% wheat flour (control), and 10% and 30% lupin, chickpea, grape seed and olive stone flours were stored at ambient temperature, in the same room, under dry and dark conditions. The total duration of storage was 80 days; however, samples were also analysed on day 1 and day 40 of production. Samples analysed on day 1 were not packaged as this was not necessary.

4.2.9 Analysis of oxygen content O_2 (%)

Before opening, 3 packages for each type of cracker were analysed, at 40 and 80 days of storage, for the content (%) of O_2 in the headspace, using a gas analyzer device (Check mate II PBI-Dansensor A/S, Ringsted, Denmark). Since oxidation reactions are triggered by the presence of oxygen and light, it is important that the packaging provides a high barrier to water vapor, light, and oxygen to prevent or slow the rate of degradation of crackers during storage.

4.2.10 Packaging transportation stress test

100% wheat crackers were packaged in conventional polypropylene packages and in biodegradable PLA packages. For each type of packaging, 2 bags of (4) crackers were stored throughout the study in the same place, at ambient temperature, under dry and dark conditions (control plastic and control biodegradable). In addition, for each type of package, 2 bags of (4) crackers were sent from Myrina, Lemnos domestically (in Samos) and abroad (in London). For transport, the samples were placed in a box (small cardboard box). Upon return, the samples (along with control samples in storage) were analysed for package breakage percentage, cracker breakage percentage, package O_2 oxygen content, texture, color and water activity of the crackers. Calculations were made based on three replicates from each sample.

4.2.11 Edible packaging durability test

To evaluate the durability and strength of the packaging made of edible chitosan film, a tensile strength test was performed on the texture analyzer (TA. XT Plus Texture Analyzer, Stable Micro Systems). The parameters set for the test were as follows: pre-test speed (0.60 mm/sec), test speed (0.80 mm/sec), post-test speed (10.00 mm/sec), distance (30.00 mm/sec) and trigger force (0.049 N). Polypropylene plastic packaging was used as a control sample and each sample was analysed 3 times (3 replicates).

4.2.12 Statistical Analysis

Differences between values were analysed by one-way analysis of variance (ANOVA). Mean comparisons were performed using Tukey's HSD test adjustment at a significance level of $\alpha=0.05$ ($P\leq0.05$). Pearson’s correlation coefficient was used to measure the strength of a linear relationship between two variables. In some cases, statistical significance ($P < 0.05$) was calculated by the Kruskal-Wallis test, which is a nonparametric test for comparing samples from two or more groups of independent observations. This test was chosen because it does not require a normal distribution of groups and is more robust to extreme values. All aforementioned statistical analyses were performed using XLSTAT v.2021.3.1 software (New York, Addinsoft). Calculations were made based on three replicates from each sample.

4.3 Results and discussion

4.3.1 Effect of functional flours on physicochemical parameters of crackers

Crackers were tested for bake loss, dimensions, puffiness, spread ratio, water activity (a_W) and moisture (Table 4.1). No significant differences were observed for bake loss between composite flours and WF. Crackers with yellow split pea, lupin, and grape seed flours had higher spread ratio (Table 4.1) and lower puffiness at higher levels of wheat substitution (30% concentration), indicating a high linear association (r=0.984) between these two properties. Cracker puffiness may also be affected by flour swelling capacity, as observed by the flours' lower SC values. Bake loss and spread ratio are important quality indicators for bakery products as they are both linked to sensorial perception. Spread ratio is positively associated with texture, grain finesse, bite and mouthfeel and a lower bake loss is desirable, as it directly affects a product's shape (Agrahar-Murugkar et al. 2015).

Table 4.1. Physicochemical and mechanical properties of crackers made from composite flours.

Samples	aw	Moisture (%)	Puffiness (%)	Bake Loss (%)	Spread Ratio	Hardness (N)	Fracturability (mm)	Total Energy (J)
WF	0.245	4.27	50.00	28.37	23.83	11.65	3.02	20.80
LU10	0.267	4.45	45.00	28.23	24.81	12.31	1.44	14.40
LU30	0.191*	2.94*	28.16*	28.18	27.54*	13.26	1.52	17.24
CH10	0.224	3.25*	43.33	29.50	23.53	13.50	1.70	15.46
CH30	0.182*	2.79*	34.66	30.17	25.63	16.66	1.32*	15.59
GS10	0.136*	2.68*	46.16	29.80	23.75	12.34	2.31	14.88
GS30	0.136*	1.82*	24.33*	29.66	27.70*	6.51*	1.83	8.16*
OS10	0.162*	2.59*	53.83	29.37	22.65	13.03	1.82	14.22
OS30	0.131*	1.84*	22.00	29.83	29.35	9.87	2.70	14.03
CW10	0.143*	2.53*	44.00	27.13	24.49	13.90	2.08	15.60
CW30	0.135*	1.60*	23.00	28.53	28.52	12.63	1.96	15.91
YP10	0.151*	2.81*	41.33	22.13	24.78	14.28	1.88	22.92
YP30	0.137*	1.84*	14.00*	28.91	29.97*	8.09	2.15	11.61
BA10	0.143*	2.47*	48.83	25.21	23.22	18.65*	2.22	19.33
BA30	0.151*	2.69*	54.83	26.86	22.49	14.61	2.11	21.32

Results are expressed as means, n=3.

Asterisk (*) indicates a statistically significant difference, at the 5% level, compared to the control.

Composite flours led to lower a_W and moisture level in the final product. Moisture was found to be significantly reduced in biscuits enriched with composite flours from cereals, pulses, millets and wheat germ (Kumar et al. 2015). As the amount of water used in all formulations was the same, the reduced moisture and water activity of the crackers with composite flours indicates that water was less available in the dough. A a_W of <0.5 is required in crackers for preservation of microbiological safety and sensory acceptability (Millar et al. 2017). This result suggests that the use of composite flours could help increase the microbiological stability and shelf life of crackers, making them a viable alternative to wheat for commercial utilization.

4.3.2 Effect of wheat flour substitution on the texture and color of crackers

GS30 was presented with lower hardness and total energy values. This negative effect could partly be explained by the composite flour's observed dough hardness (see Chapter 2 / Figure 2.3). An inverse relation has been reported between hardness of dough and biscuit hardness (Agrahar-Murugkar et al. 2015), which is also supported by our cracker results (r=0.935 for grape seed flour). On the contrary, BA10 had significantly higher hardness values than WF. BA30 also had higher values, however, the result was not statistically significant. Results for barley flour also revealed an inverse correlation (r=0.836) between dough and final product hardness. A higher level of hardness and fracturability is desirable as they are attributes of a cracker's crunchy and crisp texture (Batista et al. 2019; Millar et al. 2017). It has been reported that hardness is increased by a flour's protein and fiber content (Agrahar-Murugkar et al. 2015; Bolek et al. 2020). This is confirmed by data for barley flour, a rich source of b-glucan fiber. Overall, texture was not significantly affected by enrichment with most types and levels of composite flours.

The lightness (L*) of crackers was significantly reduced in most samples besides lupin and barley flours (Table 4.2). Crackers with grape seed and olive stone flours were the darkest, especially at higher levels (30%). The same result was observed when wheat flour was substituted with olive stone flour in biscuits (Bolek et al. 2020) and with encapsulated grape skin extract in whole wheat cookies (Dordoni et al. 2019). The darkening effect on crackers seems to be proportional to the level of composite flour used. Redness (increased a* values) was significantly increased from addition of composite flours and could be due to increased protein content, which has been reported to increase a* values because of Maillard reaction (Mancebo et al. 2016). The Maillard reaction is likely to have also contributed to the observed decrease in L* values. B* values were affected in most samples, besides chickpea composite flours. The yellower color observed for lupin flour samples may be attributed to internal pigment components such as carotenoids and lutein (Zhao et al. 2019). Conversely, grape seed and olive stone flour formulations presented with significantly reduced b* values, indicating they were bluer. The same effect was observed by substitution of wheat with olive stone flour in biscuits (Bolek et al. 2020). C* values were also significantly influenced by wheat flour substitution with most samples presenting with higher values of C*, except from olive stone and grape seed flours. There seems to be a tendency for olive stone and grape seed flours to group together across different color parameters, highlighting their proximity, possibly explained by their polyphenol rich composition. C* values are indicative of colourfulness and intensity (Millar et al. 2017). H° was significantly reduced in all flour formulations. H° values were also reduced in biscuits when wheat flour was substituted by various cereals, pulses, millets and wheat germ (Kumar et al. 2015).

Table 4.2. Color analysis of crackers.

Samples	L*	a*	b*	C*	h°	ΔE
WF	70.60	2.46	28.26	28.43	85.00	0.00
LU10	69.66	5.63*	33.30*	33.76*	80.36	6.68*
LU30	64.76	10.73*	41.03*	42.40*	75.36*	16.50*
CH10	71.20	6.10	29.56	30.23	78.50	4.37*
CH30	64.46*	12.56*	31.86	34.26*	68.50*	12.54*
GS10	53.53*	7.23*	22.03*	23.26*	71.96*	18.93*
GS30	39.06*	11.00*	15.56*	19.10*	54.83*	35.03*
OS10	46.00*	5.63	17.50*	18.36*	72.16*	27.02*
OS30	30.83*	5.06	10.66*	11.83*	64.83*	43.56*
CW10	62.63*	12.23*	33.10*	35.30*	69.73*	13.66*
CW30	49.23*	17.20*	28.30	33.10*	58.66*	26.08*
YP10	61.20*	12.10*	32.63*	34.83*	69.63*	14.24*
YP30	46.66*	16.80*	25.03*	30.13*	56.20*	28.16*
BA10	66.80	8.73*	33.33*	34.53*	75.33*	9.15*
BA30	67.86	7.36*	31.30*	32.13*	76.76*	6.48*

Results are expressed as means, n=3.

Asterisk (*) indicates a statistically significant difference, at the 5%, compared to the control.

L*: Lightness; a*: Red/Green value; b*: Blue/Yellow value; C*: chroma; h°: hue; ΔE: Total color difference was measured in comparison to WF (those with a ΔE > 3 exhibit a color difference from WF which is visible to the eye).

Observed ΔE values demonstrate that color differences between all composite flour formulations and WF can be perceived by the eye. The highest color differences were observed by GS30 and OS30. Overall, color analysis results indicate that the color of crackers is affected by the type of flour used and the level of substitution. Changes to color as a result of wheat substitution could possibly lead to altered sensory perception. However, although it has been reported that color may influence perception of taste and flavor, it is not yet fully clear when or how this happens (Spence

2015). Shankar et al. (2010) suggested that the degree of discrepancy between an individual's actual and expected experience can significantly affect the extent to which color influences flavor. Intense color changes, therefore, as those indicated by ΔE values, could be used as a guide in evaluating and detecting thresholds for altered product perception.

4.3.3 Shelf life and stability of crackers

4.3.3.1 Package oxygen content O_2 (%)

As can be seen from the tables below (Table 4.3 and 4.4) the oxygen content (O_2) at 40 and 80 days of storage was slightly higher for the crackers packed in plastic bags and for the control samples of all packages. However, it is important that for all samples the O_2 content was very close to the O_2 level of atmospheric air, which is 20.95%, indicating the high gas barrier properties of all packaging materials, throughout the shelf life of the crackers and with all types and ratios of flours. This finding is in line with the results of a study which investigated the effects of polypropylene and polylactic acid packaging on the shelf life of biscuits for 105 days. The study revealed similar O_2 content during the storage period at 35 C for both packages (Balestra et al., 2019). However, there is some evidence that the O_2 content may be product dependent. For example, cooked potato slices packed in OPA/PP bags and OPA-EVOH/PP bags presented with a sharp decline of O_2 toward zero after the third day of storage. In this study also, packaging did not have an effect (Baele et al., 2020).

Table 4.3. Oxygen content O_2 (%) at 40 days of storage.

O_2 Content (40 days)	
Control plastic	20.800
OS10 plastic	20.800
OS30 plastic	20.800
Control biodegradable	20.767
CH10 plastic	20.700
CH30 plastic	20.700
GS10 plastic	20.700
GS30 plastic	20.700
LU10 plastic	20.700
LU30 plastic	20.700
Control edible	20.667
LU30 edible	20.667
CH10 biodegradable	20.500
CH30 biodegradable	20.500
GS10 biodegradable	20.500
GS30 biodegradable	20.500
LU10 biodegradable	20.500
OS30 edible	20.500
LU10 edible	20.467
LU30 biodegradable	20.467
GS10 edible	20.400
GS30 edible	20.400
OS10 biodegradable	20.400
OS10 edible	20.400
OS30 biodegradable	20.400
CH10 edible	20.267
CH30 edible	20.267

Table 4.4. Oxygen content O_2 (%) at 80 days of storage.

O_2 Content (80 days)	
Control edible	20.933
Control plastic	20.900
LU30 plastic	20.800
OS10 plastic	20.800
CH10 plastic	20.700

OS30 plastic	20.700
CH10 edible	20.600
CH30 plastic	20.600
GS10 plastic	20.600
GS30 plastic	20.600
LU10 plastic	20.600
LU10 edible	20.567
LU30 edible	20.567
OS10 biodegradable	20.567
OS10 edible	20.500
Control biodegradable	20.467
OS30 biodegradable	20.433
GS30 edible	20.400
OS10 edible	20.400
CH10 biodegradable	20.367
CH30 biodegradable	20.300
CH30 edible	20.300
OS30 edible	20.300
GS10 biodegradable	20.267
GS30 biodegradable	20.267
LU10 biodegradable	20.267
LU30 biodegradable	20.267

4.3.3.2 Textural properties of crackers in different packages

The texture of crackers packaged in plastic packaging was not affected throughout the shelf life of the products (Figure 4.2). However, for the crackers packaged in biodegradable packaging, it appears that in some cases, at 80 days of storage, there was some impact on the texture and specifically on the hardness of the products (Figure 4.3). It is important, nonetheless, to mention that this negative effect seems to be based on the type of alternative flour used. In particular, the affected products were crackers with lupin (10% and 30%), olive stone (30%) and grape seed (10%). In a study with biscuits packed in polypropylene and PLA based packaging, none of the packaging showed any effect on the product's textural properties (Balestra et al., 2019). For the crackers packaged in edible packages, hardness at 80 days appears to be negatively affected in lupin (30%) and olive stone (10%) crackers (Figure 4.4). It is noteworthy that the texture of the control sample was not affected in any of the packages.

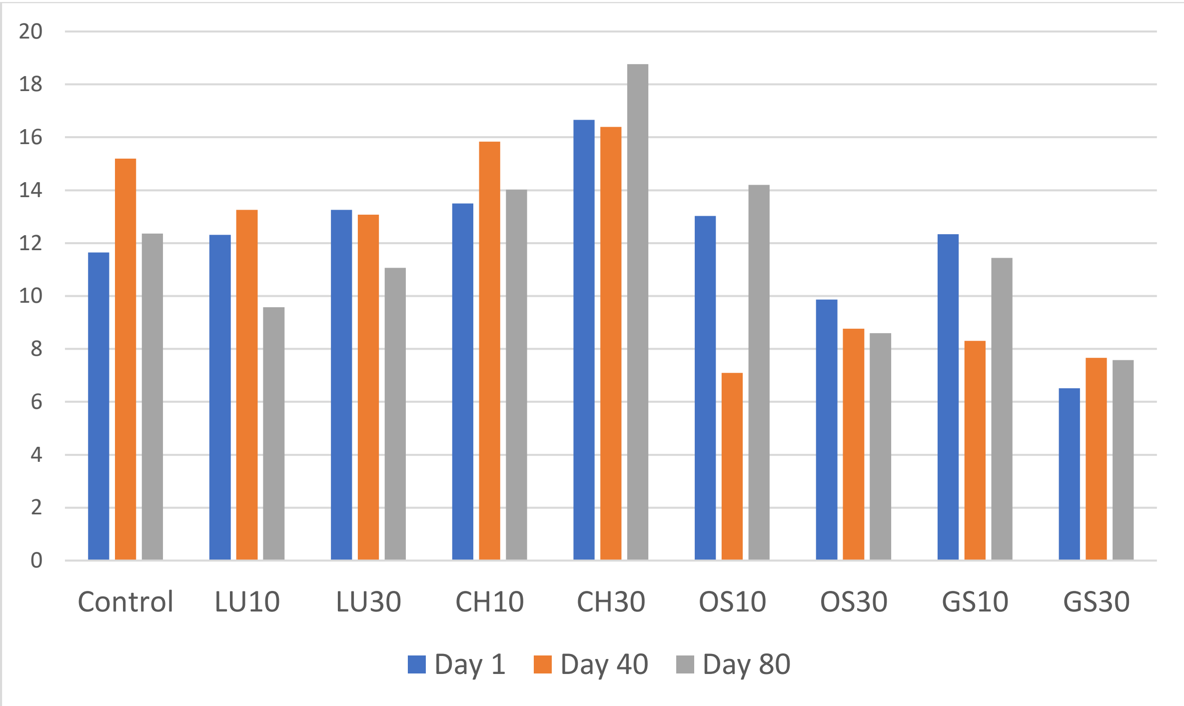

Figure 4.2. Hardness (N) of crackers in plastic packaging.

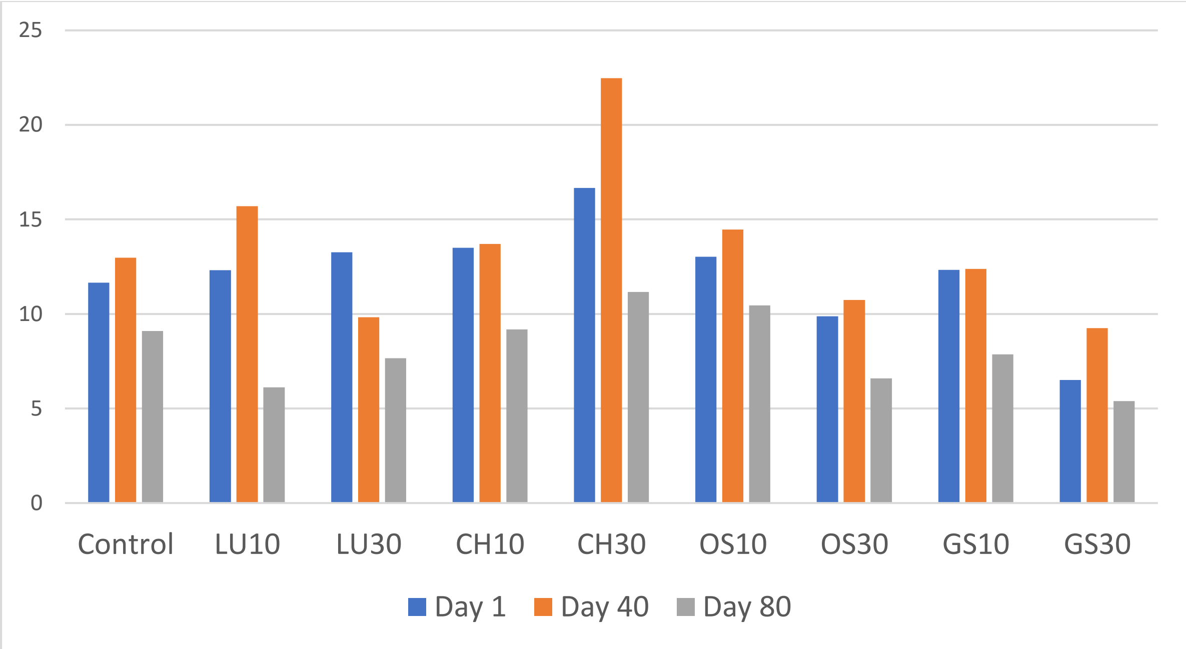

Figure 4.3. Hardness (N) of crackers in biodegradable packaging.

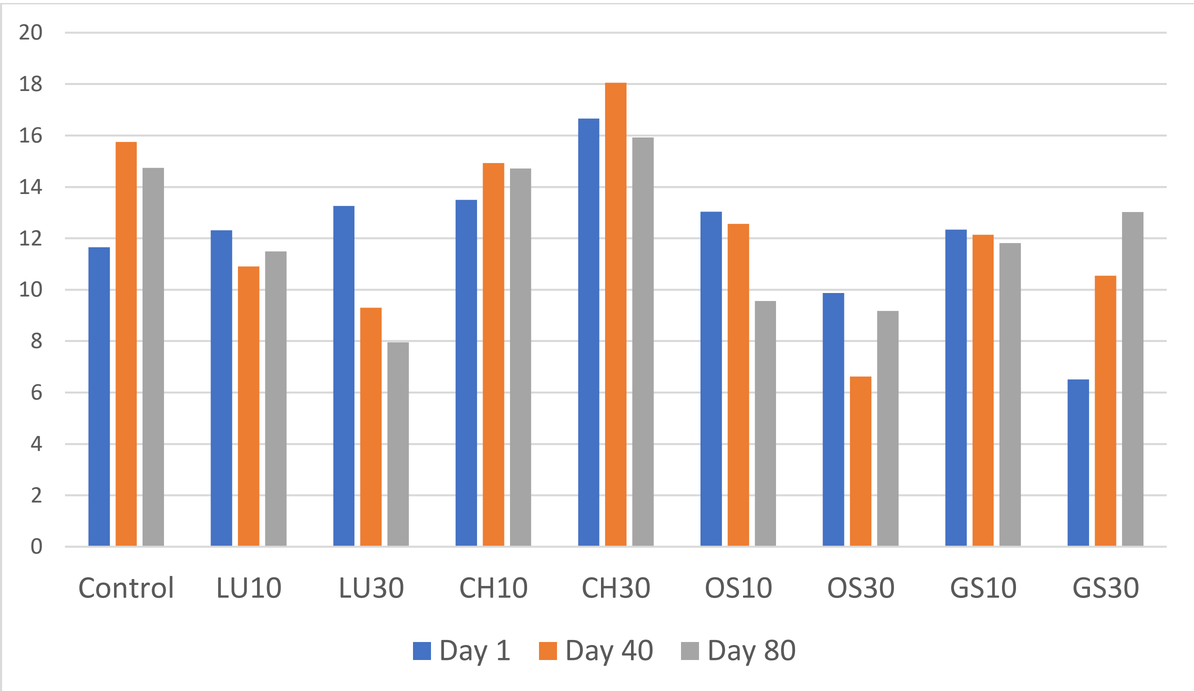

Figure 4.4. Hardness (N) of crackers in edible packaging.

4.3.3.3 Color of crackers in different packages

No significant changes during their shelf life were observed in the color of crackers of all types stored in plastic and biodegradable packaging. There were, however, some changes in color characteristics in some samples stored in edible packaging. In particular, lupin crackers (30%) showed higher a* values and lower b* values and chickpea crackers (10%) had higher a* and b* values at 80 days of storage. Crackers with olive stone (10% and 30%) and grape seed (10% and 30%) showed changes in all color parameters at 40 and 80 days of storage (Figures 4.5 – 4.9). Interestingly, while in the crackers with olive stone the color parameters a* and b* were increased compared to the samples at the beginning of their shelf life (day 1), in the sample with grape seed (30%), at 80 days, there was the opposite trend, i.e., the reduction of a*, b* and C values. According to Haile et al. (2013), a decrease in a*, b*, C values and/or an increase in lightness (L*) indicates a loss in color (discoloration) as a negative effect of storage time. Some color discoloration was also revealed in gluten free oat biscuits packed for 3 months in PET/CPP, BOPP, PVC, BOPP/CPP and PE/EVOH/PP (Duta et al., 2019).

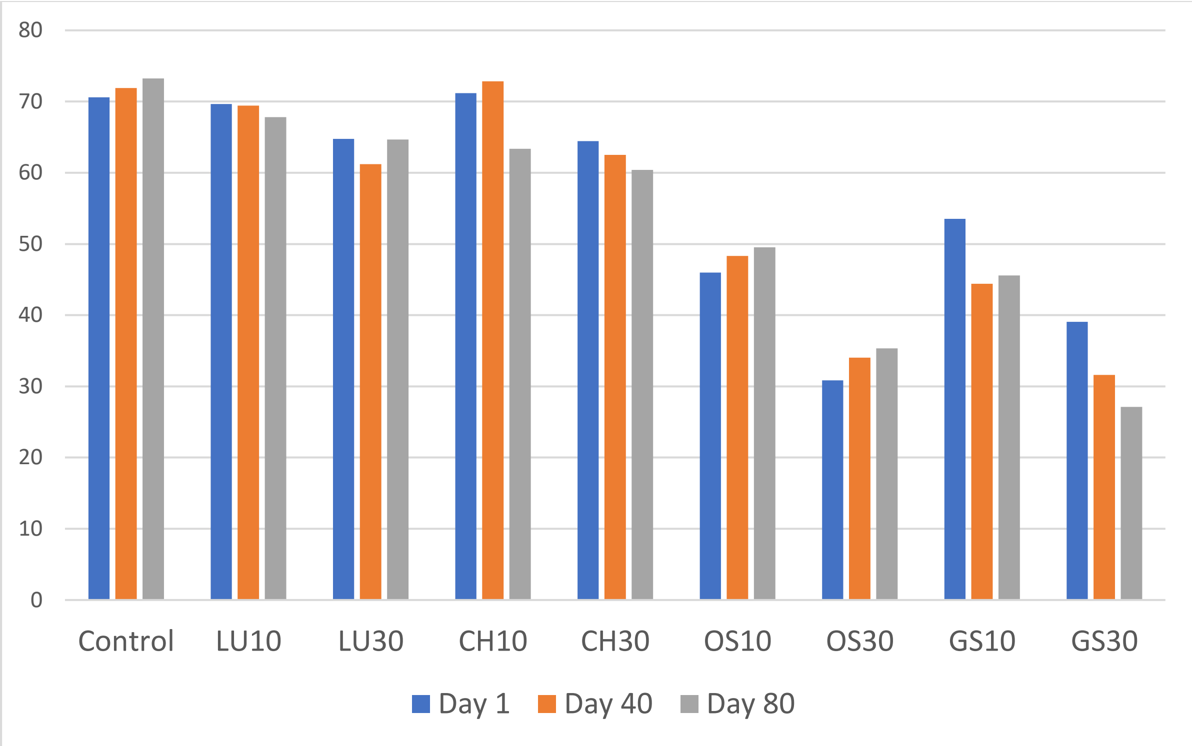

Figure 4.5. Cracker L* values (lightness) - edible packaging.

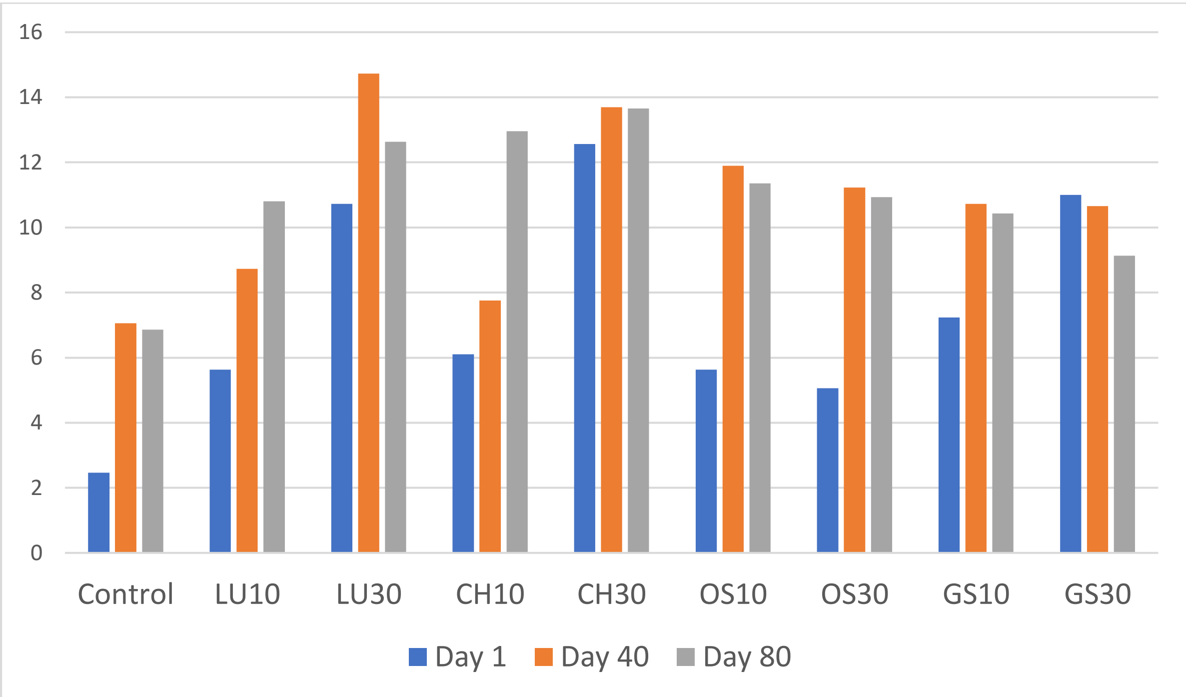

Figure 4.6. Cracker a* values - edible packaging.

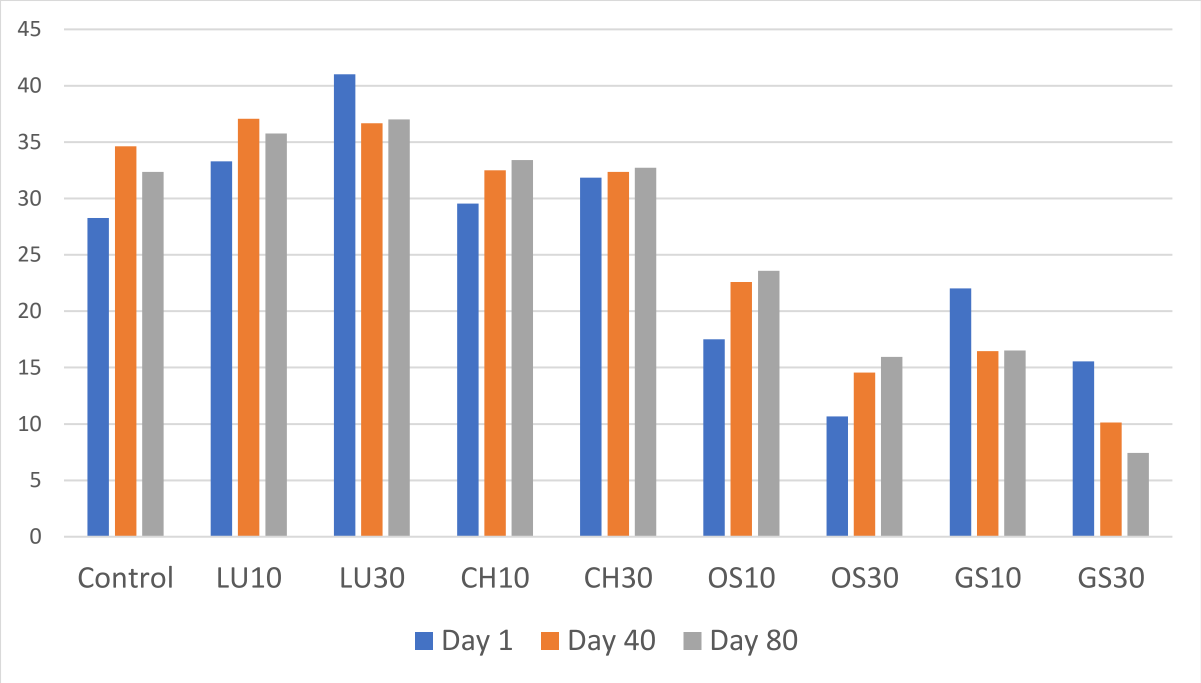

Figure 4.7. Cracker b* values - edible packaging.

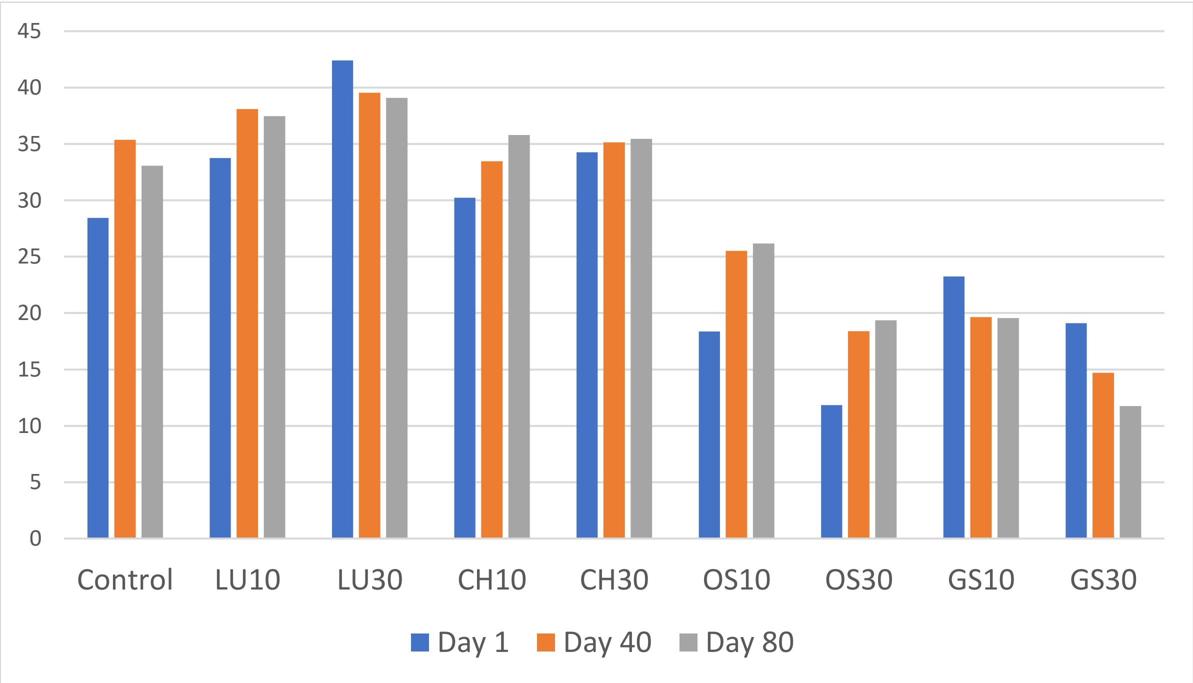

Figure 4.8. Cracker C (Chroma) values - edible packaging.

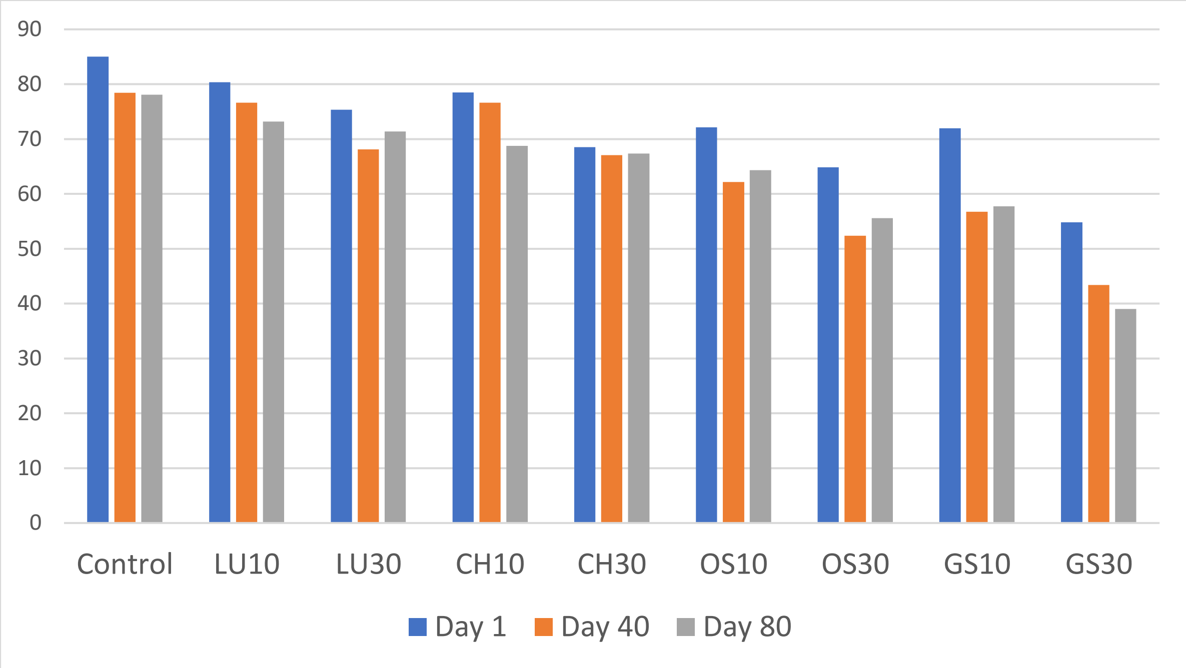

Figure 4.9. Cracker h (hue) values - edible packaging.

4.3.3.4 Water activity and moisture content of functional crackers during their shelf life

In most crackers, in all three package types, there were significant increases in water activity and moisture level during storage (40 and 80 days) (Figures 4.10 – 4.15). The highest increases, with a significant difference from the other two types of packaging, however, were presented in the crackers packaged in the biodegradable packaging. The same effect of PLA biodegradable packaging was reported on a 92-day shelf life study of biscuits, revealing highest water activity and moisture levels compared to other types of packaging (metalized orientated polypropylene (OPP)/paper (control); metalized poly-lactic acid (PLA)/paper; metalized OPPwith ethylene vinyl acetate pro-oxidant additive (EVA-POA)/paper). However, there was no effect on other product quality parameters (Romani et al., 2014). According to Sangroniz et al. (2019), the high permeability of PLA material is a limiting factor in its potential application. In contrast to biodegradable packaging, the crackers packed in edible packaging showed in some cases, at 40 and 80 days, lower water activity and/or moisture values compared to crackers in conventional packaging. However, in general, crackers packed in edible packaging had higher values than their counterparts in plastic packaging. Given the relationship of water activity and moisture content of a product to maintaining quality and shelf life stability, the sample values of biodegradable packaging may indicate some potential negative impact on the product, particularly near 80 days of storage. This, nonetheless, was not evident from other tests such as texture and color analysis.

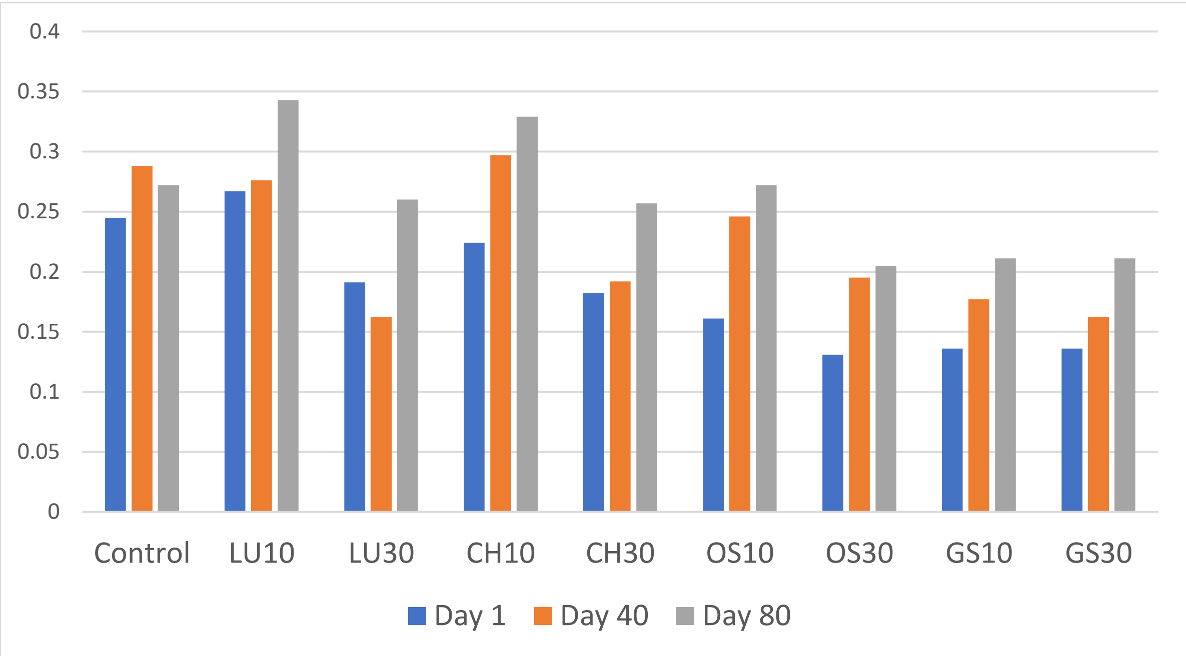

Figure 4.10. Water activity of crackers in plastic packaging.

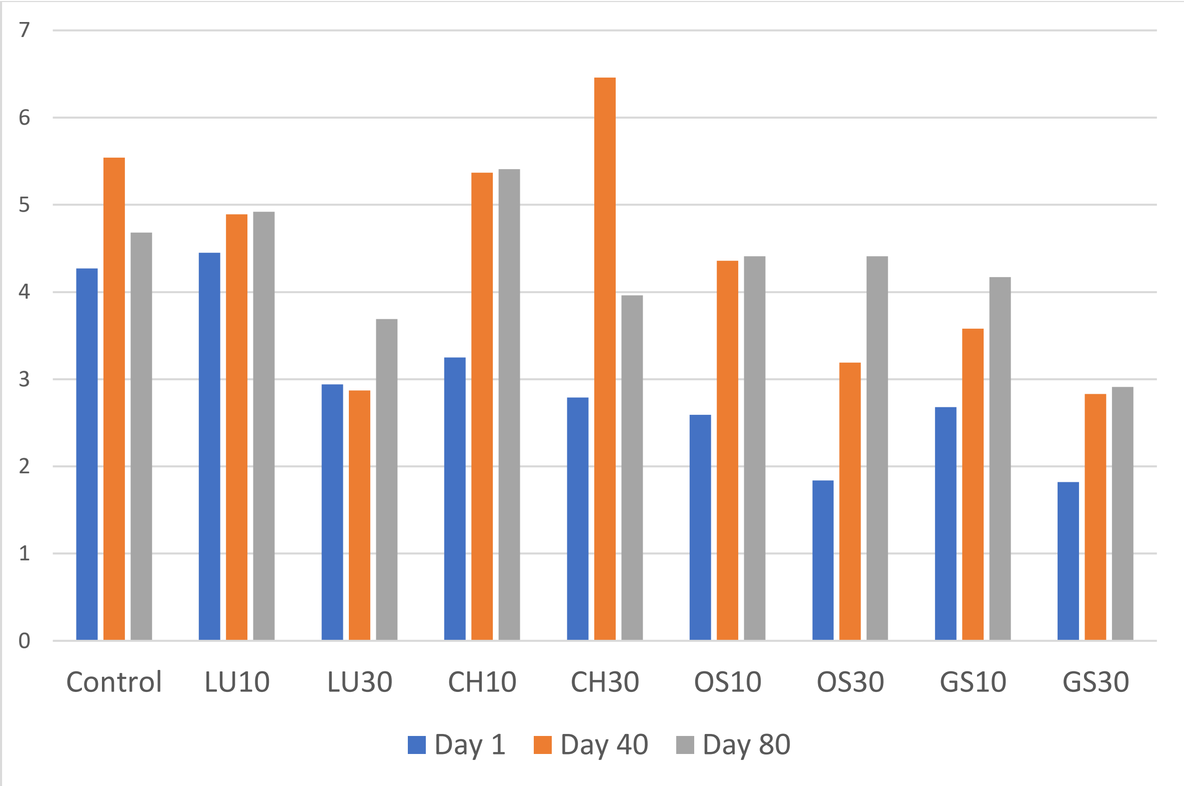

Figure 4.11. Moisture content (%) of crackers in plastic packaging.

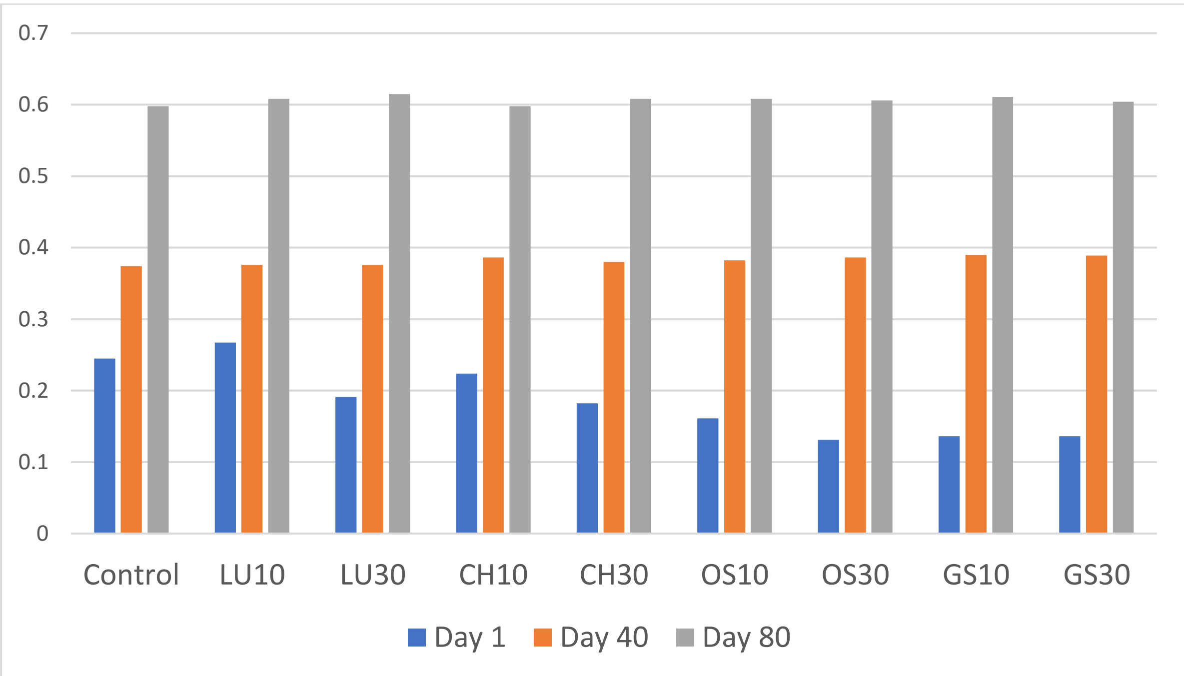

Figure 4.12. Water activity of crackers in biodegradable packaging.

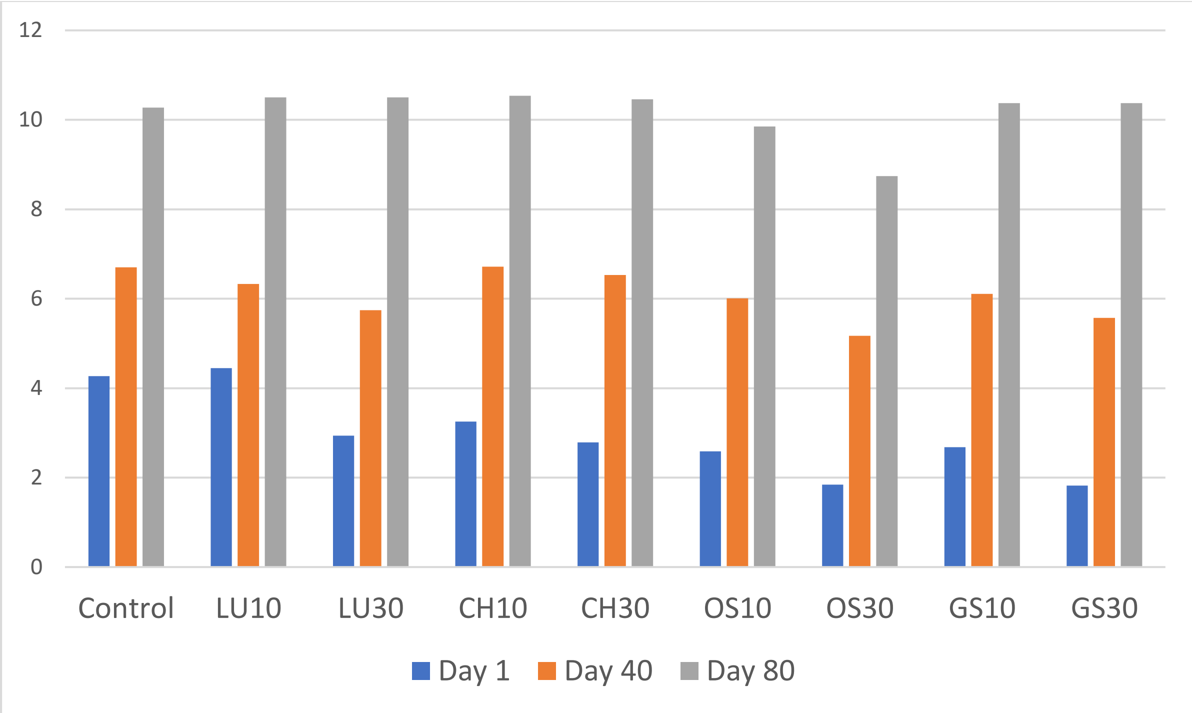

Figure 4.13. Moisture content (%) of crackers in biodegradable packaging.

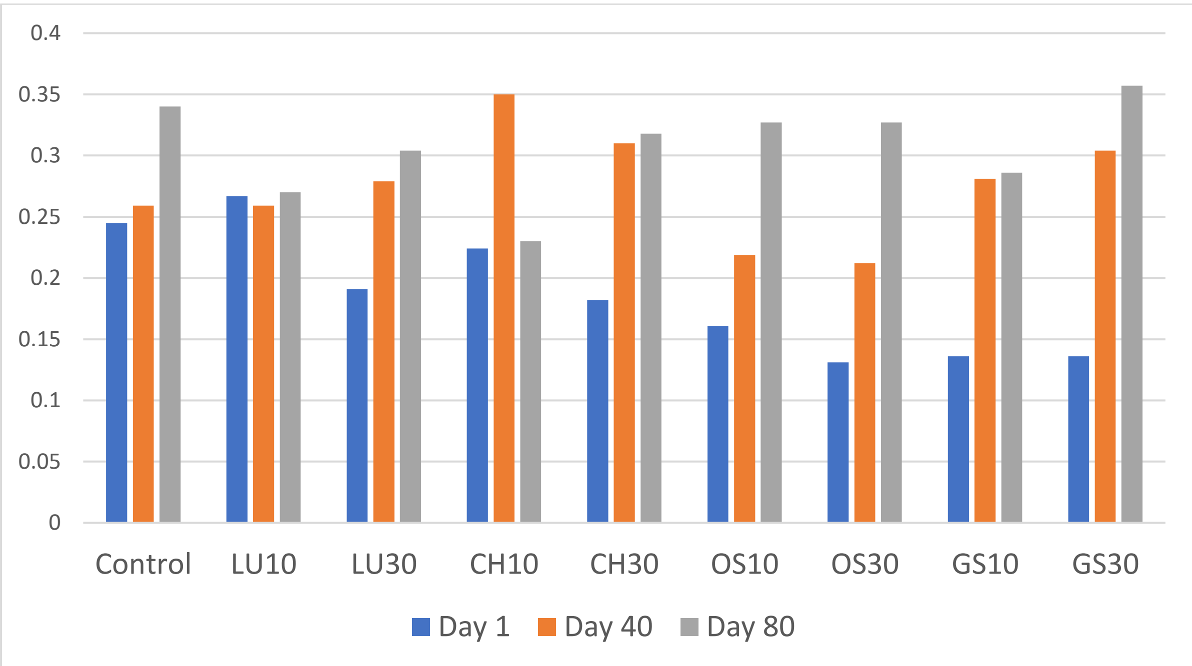

Figure 4.14. Water activity of crackers in edible packaging.

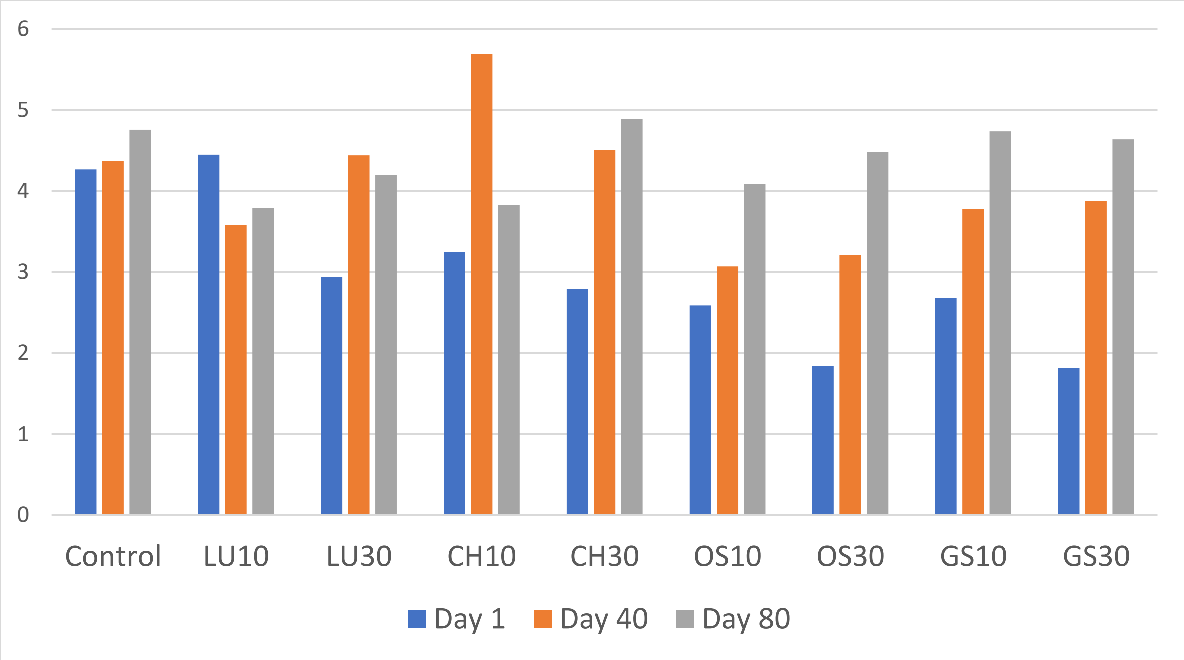

Figure 4.15. Moisture content (%) of crackers in edible packaging.

4.3.4 Durability assessment of conventional and sustainable packaging

4.3.4.1 The effect of transportation

As can be seen from the graph below (Figure 4.16), the samples that travelled outside Lemnos (Samos and London) in biodegradable packaging showed a particularly high breakage rate (100% level). In contrast, the plastic packaging appeared to be very durable, revealing a 0% breakage rate for all samples. This fact indicates that the type of packaging, as well as the factor of transportation, play an important role in the integrity of the package and/ or in its deterioration. Interestingly, in terms of degree of breakage, the crackers (50% breakage level) with the highest deterioration were the samples that travelled to Samos in plastic packaging. The lowest percentage of cracker breakage was shown by the samples kept in a storage condition (control) for both types of packaging (11.1% breakage percentage). However, the oxygen content was similar in all samples. Despite the high breakage rate in the biodegradable packages and crackers that travelled to Samos, the textural properties were not affected (Table 4.5). However, the water activity values in all biodegradable packages were higher than the plastic ones. This data supports the findings of the shelf life analysis. Regarding the color of the samples, there were no notable differences between them, with the only possible exception being the reduced b* and C values for the biodegradable packaging that travelled to Samos. This may indicate some discolouration tendency as a result of the aforementioned packaging damage.

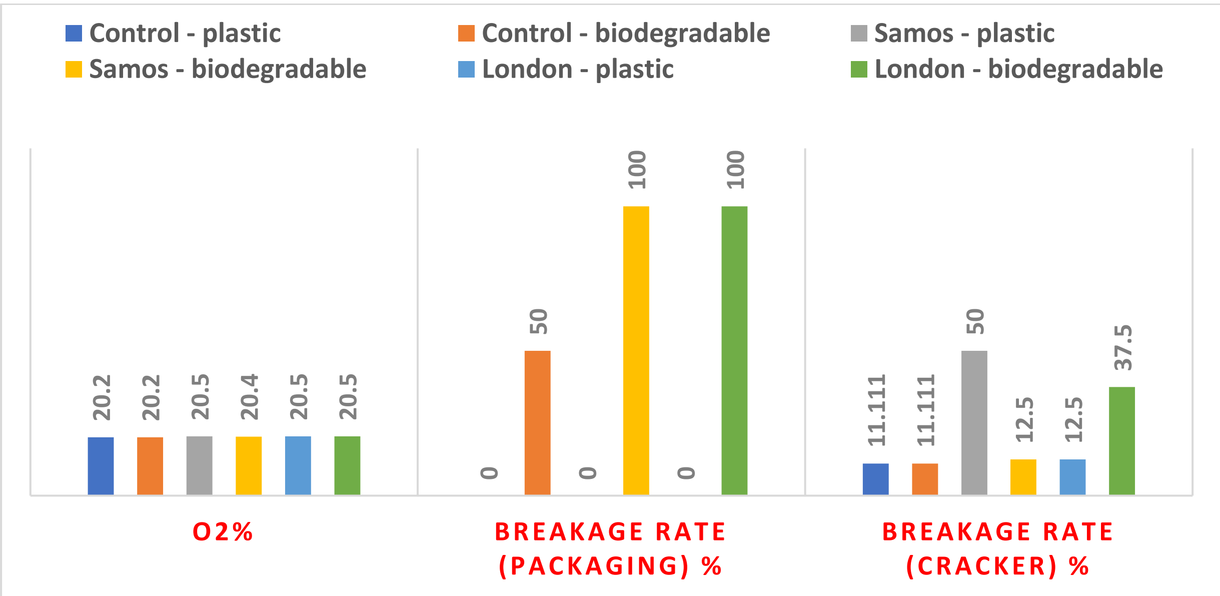

Figure 4.16. Oxygen content (%) and breakage rate (%) for packaging and crackers.

Table 4.5. Physicochemical properties and color of crackers in conventional and biodegradable packaging.

Samples	Water activity	Hardness	Fracturability	Toughness	Lightness (L*)	a*	b*	C	h
London - biodegradable	0.455 b	11.875 a	2.938 a	21.832 a	74.167 a	4.267 a	31.367 abc	31.700 abc	82.267 a
Samos - plastic	0.389 d	17.680 a	2.396 a	27.993 a	71.433 a	4.067 a	30.467 bc	30.800 abc	82.333 a
Control - plastic	0.442 b	13.940 a	1.753 a	21.696 a	71.267 a	6.533 a	31.867 ab	32.667 ab	78.567 a
London - plastic	0.410 c	14.695 a	1.932 a	19.240 a	71.233 a	6.000 a	32.967 a	33.533 a	79.700 a
Control - biodegradable	0.502 a	11.309 a	2.737 a	27.053 a	70.900 a	4.533 a	30.033 bc	30.367 bc	81.467 a
Samos - biodegradable	0.439 b	17.384 a	2.074 a	25.382 a	72.467 a	4.967 a	29.400 c	29.833 c	80.533 a
P value	< 0.0001	0.318	0.234	0.252	0.824	0.597	0.001	0.005	0.715

*Mean values, within a column, followed by different letters are significantly different at a=0.05 significance level (P≤0.05),

according to Tukey's HSD test.

4.3.4.2 Strength test of edible films

The durability results for packaging showed that plastic was significantly more durable than the edible chitosan packaging ($p = 0.001$). In particular, the mean average value (from three repetitions) of tensile strength for the edible packaging was 5.23 (N) while for the plastic packaging it was 11.25 (N), i.e., more than double (Figure 4.17). This effect may be limited to polypropylene, as a study which compared chitosan-based composite films with commonly used synthetic polyethylene film revealed comparable tensile strength between the two (Tan et al., 2015). It is important that the findings are interpreted in isolation, but in conjunction with the results from the packaging shelf life study.

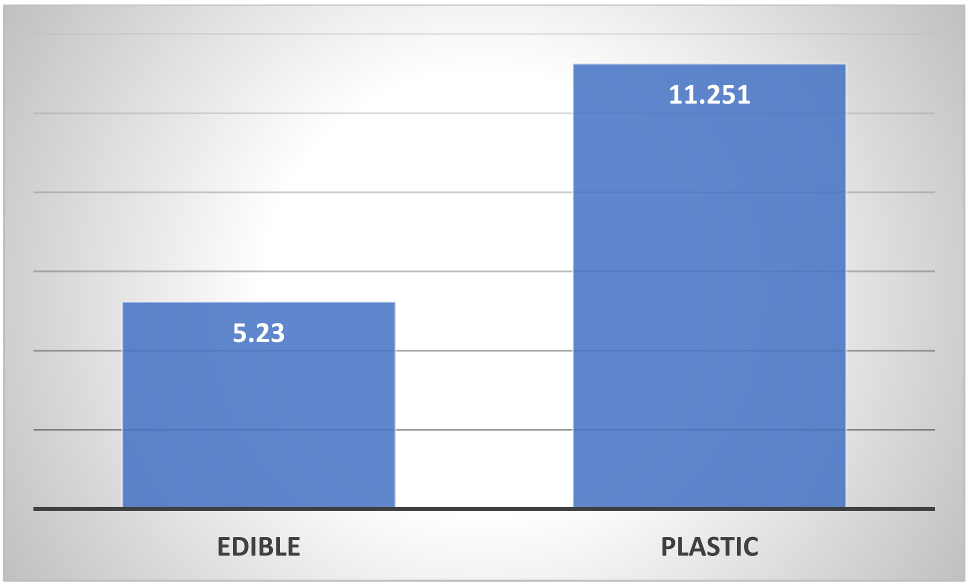

Figure 4.17. Tensile strength (N) for edible and plastic packaging.

4.4 Conclusion

The partial substitution (10% and 30%) in crackers of wheat with alternative flours and local raw materials from by-products does not negatively affect the shelf life nor the quality characteristics of the products. The use of sustainable materials (such as edible chitosan films) for the packaging of crackers preserves the characteristics of the products and their stability throughout their shelf life to almost the same extent as conventional packaging. However, some disadvantages were observed regarding the use of biodegradable polylactic acid packaging, mainly due to the tendency of the material to deteriorate during transport, the negative impact on water activity and moisture content, as well as, in specific cases, on the texture of the products. The edible packaging was less durable than the conventional one, without nonetheless significantly affecting the product negatively.

CHAPTER 5

Sensory and emotional reactions of sustainably packaged traditional bakery products

5.1 Introduction

Consumers' perception is a determining factor for a product's popularity and is influenced by intrinsic as well as extrinsic product cues (Krishna & Elder, 2021). Intrinsic cues refer to product characteristics such as texture, aroma, and flavor, whereas extrinsic cues are associated with characteristics outside of the product such as brand, price, packaging, and information (Gunaratne et al., 2019). The influence of extrinsic cues is particularly interesting in the case of traditional products, where there is little space for change in recipe, as well as in intrinsic cues during production and trading.

Although intrinsic product cues have been researched extensively as regards their effect on consumer acceptance, the study of extrinsic cues is a developing area and requires careful consideration. A number of studies have recently shown that external product cues can be equally important in influencing the sensory perception of food (Piqueras-Fiszman and Spence, 2015). Examples of product extrinsic factors that have proven to impact the sensory perception of food are brand name and sounds, nutrition and ingredient labels, price, package design, label size, lighting and even social context (Krishna & Elder, 2021; Wang et al., 2019; Symmank, 2019; and Malekpour et al., 2022).

Recent research shows that product information is another important external product cue with direct effect on consumers' acceptance. A study on crisps reported visual and verbal information on packaging to have an effect on sensory as well as non-sensory aspects and willingness to buy (Rebollar et al., 2017). In another study, informational attributes about freshness, shelf life, convenience and taste of fresh cod affected consumers' evaluations (Heide & Olsen, 2017). Product designation of origin (PDO) labelling (Savelli et al., 2021), organic food information (Asioli et al., 2018), brand information (Schouteten et al., 2017) and various packaging information (Włodarska et al., 2019) influenced the sensory perception of products.

Environmental awareness has grown, and people internationally have become more sensitive to issues affecting the environment. A recent global survey by the United Nations Development Programme (UNDP) (2021) revealed that there is strong support for action across different countries and research has shown that consumers are willing to pay more for sustainable packaging (Herrmann et al., 2022). Given the importance of packaging for food and its widespread use, it would be very useful to investigate how information on sustainable packaging affects consumers' perception of food. It has been suggested that, as consumers are likely to judge a food product and its packaging as a whole, any information linked to it prior to consumption may create expectations as regards quality and sensory perception (Pramudya & Seo, 2019). Previous research has shown that information on packaging can affect consumers' emotional responses and that emotional attachment to food influences repeated purchases (Gunaratne et al., 2019).

The increased interest in focusing on total consumer experience when examining consumer responses to food, has led to the development of various instruments to capture emotional responses elicited by food, beyond sensory liking (e.g., sensory evaluation, acceptance, liking, hedonic or preference measurements). Ways to assess food related emotions include numerous measurement methods and instruments. These are grouped into explicit and implicit methods depending on the way of assessment. The literature review confirms the dominance of explicit methods to investigate emotional responses in relation to food (Lagast et al., 2017). Implicit measurements such as facial expression, brain activity, eye movements etc, have been applied less; however, their use is increasing due to the wider accessibility of the technologies, and the evolution of interdisciplinary techniques that offer new and promising approaches to capture emotional responses.

Frequently, explicit methods of emotions measurement are based on a list of emotional terms that can be checked (i.e., selected) or rated in a questionnaire [check-all-that-apply (CATA) /rate-all-that-apply (RATA)]. The emotional lexicon can be predefined, consumer-defined, or a combination of both. Lists of emotions and emotion measurement tools have been developed for a wide variety of products, such as wine [e.g., (Danner et al., 2017; Ferrarini et al., 2010; Mora et al., 2018; Silva et al., 2014)], coffee [e.g., (Bhumiratana et al., 2014; Hu & Lee, 2019; Labbe et al., 2015)], beer [e.g., (Beyts et al., 2017; Chaya et al., 2015; Desira et al., 2020; Orth et al., 2004; van Zyl & Meiselman, 2015)], but also general non food-specific lists and tools, such as EsSense Profile (King & Meiselman, 2010; Nestrud et al., 2016), EmoSemio (Spinelli et al., 2015; Spinelli et al., 2014), Global Profile (Spinelli et al., 2019), EmoSensory Wheel (Schouteten et al., 2015, 2017), Empathic Food Test (Geier et al., 2016), Emotional Circumplex Model (Jaeger et al., 2018). The EsSense Profile is a commercially used emotion measurement tool applied internationally, translated in various languages, used as the basis for new measurement tools, and it is concise relatively to others (Panagiotou & Gkatzionis, 2022).

Traditional products could be more susceptible to the influence of sustainability claims as regards packaging due to limited changes in their production process. To our knowledge, the interaction between information, packaging, and food is an area that has received limited attention. Paximathia are rusks of Greek origin, common in Greece, a central component of the Mediterranean diet, and widely exported to numerous countries. In recent years, paximathia of several Greek regions were accredited PDO status and the profile of the product was standardised. Efforts have been made to modernise their packaging and add sustainability features. The aim of the study was to test how sustainable packaging information affects: (a) consumers' acceptability and (b) emotional responses to paximathia. To assess sensory perception of paximathia in relation to packaging, samples were assessed on hedonic scales and CATA questionnaires. The objective of the study was to identify how packaging affected liking of taste and emotional reactions. Responses under informed conditions on packaging were compared to uninformed tasting.

5.2 Materials and methods

5.2.1 Samples, stimuli, and experimental design

The study was conducted in three parts as previously described by Gunaratne et al. (2019) with slight modifications. First, under blind conditions to study the effect of sensory characteristics of paximathi on the sensory perception by consumers without packaging. Second, under packaging conditions to study the effect of packaging characteristics on consumers' visual perception of rusks. Third, under informed conditions to study the combined effect of sensory and packaging characteristics on consumers' sensory perception. The duration of all sessions in total was 30 minutes, with two 5-minute breaks in between.

Paximathia, Greek rusks (hereafter referred to as rusks) were supplied by a commercial producer in Lemnos, Greece. Their nutritional value was: (per 100g) 17g fat, 64g carbohydrates, 3.8g fiber, 11g protein and 1.1g salt. Each rusk was 4cm x 3cm and weighted ~ 30 g. Conventional packaging film was made from polypropylene (PPL) with 1.7 mm thickness and size of 30cm x 10cm and biodegradable was made from polylactic acid (PLA) and of the same size and thickness. Packaging films were obtained from commercial retailers: Plastimak (PPL) and Clear Bags (PLA). Foot sealer Tahipack THP-350 was used for the sealing of packages, and each package contained three rusks.

5.2.2 Participants

One hundred and four (N=104) participants, 75% women and 25% men, were recruited for the study, aged between 18 – 80 years old. They were recruited via e-mail, phone, or in person communication. All participants were consumers of bakery products and rusks; however, there were no prerequisites for participation such as consumption frequency or liking. Participants were not informed that the objective of the study was to assess the impact of packaging sustainability on the sensory perception of food. The experimental procedures were approved for ethics by the Department of Food Science and Nutrition at the University of Aegean. The sessions were carried out in individual sensory booths at the Laboratory of Consumer and Sensory Perception of Food & Drinks of the University of the Aegean. The booths were closed sensory analysis cabins ensuring soundproofing. Room temperature was controlled at 22 °C and testing in booths operated under white lighting conditions.

5.2.3 Blind Condition

Under blind conditions, the participants' sensory perception of each rusk sample was tested without packaging or information. Consumers received verbal and written instructions before they were led into the testing room. They were informed that they would receive rusk samples to taste. The samples (3 rusks) were provided on a white plate. After tasting the samples, participants assessed overall liking using a 7-point hedonic scale (1= dislike extremely to 7= like extremely). Subsequently, they reported their emotions by selecting the most suitable terms in a CATA questionnaire based on EsSense Profile (King and Meiselman, 2010).

In this study, a Greek lexicon-based emotion measurement tool was used to measure emotions elicited by rusks. The lexicon in Greek and the respective tool was developed based on the EsSense Profile methodology (King and Meiselman, 2010) as a guide (unpublished work). This choice was made based on the fact that EsSense Profile is a tool commercially used internationally, translated in various languages, used as the basis for new measurement tools (Panagiotou & Gkatzionis, 2022).

The Greek tool consisted of 33 emotions elicited by food consumption in general, namely: sensual, optimistic, relieved, unrestrained, energetic, grateful, happy, pleased, pleasant, calm, satisfied, cheerful, whole, privileged, healthy, relaxed, glad, good-looking, stressed, weak, disgusted, cheerless, unsatisfied, disappointed, dissatisfied, guilty, angry, tired, nervous, ashamed, resentful, sad, uninterested. To this list, 4 food-specific emotions were added, based on research, related to rusks and crackers consumption: self-restrained, prudent, condescending, lonely. Participants could also add emotions if they wished to the list of 37 terms.

5.2.4 Packaging Condition

It has been reported that the sense of touch is an important factor in the evaluation and decision-making regarding purchase and product usage (Pramudya & Seo, 2019). After a short break, participants received verbal and written instructions about the next session. This session aimed to investigate the direct effect of packaging on the sensory perception of the product. Consumers examined rusks in two different packaging materials without tasting the rusks. Participants were asked to assess the two different packages, without receiving any further information regarding their packaging materials or rusk. Packaging consisted of two materials: conventional plastic (polypropylene) and biodegradable (polylactic acid). Samples were coded with different random three-digit numbers and were filled with 3 rusks which were the same with the blind condition. Both packages were completely transparent and identical in shape and size. Consumers were instructed to evaluate each sample separately by handling it with their hands for approximately 30 seconds. Participants were instructed not to open or taste the product inside. After examining each sample, participants were asked to assess overall liking using a hedonic scale and emotions by using a CATA questionnaire, the same as used under blind conditions.

5.2.5 Informed Condition

Following a short break, participants received verbal and written instructions for the third session. Participants were instructed to evaluate rusks packed in three different packages. The first was conventional plastic (polypropylene), the second was biodegradable (polylactic acid), and the third was communicated to the consumers as 'edible' packaging; however, it was the same as the biodegradable package. The consumers were not informed that the rusks in the three packages were identical. Written and verbal information was provided to all participants about the differences between the three packaging options and about the potential environmental benefits of the biodegradable and edible packages. More specifically, participants were informed that the conventional packaging consisted of plastic materials, its usage is associated with negative environmental impact and that the sustainable and the edible packaging was more easily degradable. Samples were coded with different random three-digit numbers and included 3 rusks each. Consumers were instructed to evaluate each sample separately by opening the package and tasting the rusk in it. After examination of each sample, participants were asked to assess overall liking using a hedonic scale and emotions using a CATA questionnaire as described in the previous conditions. Water was provided to the participants to cleanse their palate between samples. Samples were presented in random order.

5.2.6 Statistical Analysis

Overall liking data were analysed with one-way analysis of variance (ANOVA). Mean comparisons were performed using Tukey's HSD test adjustment at significance level $\alpha=0.05$ ($P \leq 0.05$). CATA data were analysed with correspondence analysis (CA) and Principal Component Analysis (PCA) in order to explore relationships among the selected emotion-based terms and between the different conditions. Cochran's Q test was carried out for elucidating differences between the frequencies of the selected emotion-based CATA terms. Significance was tested at a significance level of 0.05.

All statistical analyses were performed using XLSTAT software (Version, 2018.1., Addinsoft).

5.3 Results

5.3.1 Consumer liking under different evaluation conditions

The results of liking evaluation were expressed on a 7-point hedonic scale (Table 5.1). Rusk samples evaluated under blind condition scored the highest level of liking, while the sample with claim of 'edible' package in the informed condition received a similar liking score. Samples in both conventional and biodegradable packaging received lower liking scores than rusks free of packaging; however, the liking of rusks in edible packaging was similar to the product without packaging.

Table 5.1. Mean consumer liking scores of samples evaluated in blind, packaging, and informed conditions.

Condition-Sample	Liking Score
First session	
Blind condition	5.5^{a}
Second session	
Packaging condition-Conventional	4.9^{b}
Packaging condition -Biodegradable	3.4^{c}
Third session	
Informed condition -Conventional	4.7^{b}
Informed condition-Biodegradable	5.0^{b}
Informed condition -'Edible'	5.1^{ab}

* Different superscript letters indicate statistically significant difference according to Tukey (HSD) test for a confidence level of 95%.

In the second session, the products were evaluated externally with no tasting of the rusks, and the conventional packaging was strongly preferred compared to biodegradable. However, during the informed condition, when consumers received information about the environmental impact of each packaging, the biodegradable sample scored higher than the conventional one, while the ‘edible’ scored the highest (albeit differences were not statistically significant).

5.3.2 Emotional profiles of samples under different evaluation conditions

Emotion-based terms in the form of a CATA questionnaire were presented to the participants in order to provide insight into consumer perception of the samples. Under the blind condition, the terms ‘pleased’ (51.9%), ‘calm’ (44.2%) and ‘healthy’ (30.7%) were chosen with the highest frequency (Table 5.2). Under the packaging condition, for the conventional package, the most frequently chosen term was ‘calm’ (43.2%), whereas for the biodegradable package, the most frequently selected terms were ‘nervous’ (38.4%), ‘unsatisfied’ (29.8%) and ‘worried’ (28.8%).

Table 5.2. Frequency (%) with which emotion-based terms were used or generated by consumers in order to describe their emotional responses to samples under different evaluation conditions and results from Cochran's Q test for comparison between samples.

CATA term	First session	Second session		Third session		
	Blind condition	**Packaging condition - Conventional**	**Packaging condition- Biodegradable**	**Informed condition - Conventional**	**Informed condition - Biodegradable**	**Informed condition - 'Edible'**
worried[1]	3.8^{a}	2.8^{abc}	28.8^{de}	4.8^{ab}	5.7^{abcd}	6.7^{abcd}
indifferent[1]	10.5^{abc}	26.9^{def}	26.9^{cde}	20.1^{bcd}	17.3^{bcdfeg}	11.5^{abcdef}
weak[1]	0.9^{a}	0.9^{ab}	1.9^{a}	4.8^{ab}	0^{a}	0.9^{ab}
disgusted[1]	0^{a}	0^{a}	3.8^{a}	0.9^{a}	0.9^{a}	0^{a}
sensual[1]	3.8^{a}	0.9^{ab}	0.9^{a}	0.9^{a}	2.8^{ab}	3.8^{abc}
optimistic[1]	15.3^{abcd}	10.5^{abc}	5.7^{a}	8.6^{abc}	24^{fgh}	25.9^{efgh}
moody[1]	0^{a}	3.8^{abc}	7.6^{ab}	3.8^{a}	0.9^{a}	2.8^{abc}
relieved[1]	3.8^{a}	6.7^{abc}	4.8^{a}	5.7^{ab}	6.7^{abcde}	18.2^{cdefgh}
unsatisfied[1]	4.8^{a}	12.5^{abcd}	29.8^{de}	15.3^{abc}	5.7^{abcd}	2.8^{abc}
disappointed[1]	1.9^{a}	4.8^{abc}	13.4^{abc}	10.5^{abc}	4.8^{abc}	5.7^{abcd}
uncontrollable[1]	2.8^{a}	2.8^{abc}	3.8^{a}	2.8^{a}	2.8^{ab}	1.9^{abc}
displeased[1]	0^{a}	5.7^{abc}	22.1^{bcd}	8.6^{abc}	3.8^{abc}	2.8^{abc}
restrained[1]	10.5^{abc}	8.6^{abc}	8.6^{ab}	15.3^{abc}	19.2^{cdefg}	15.3^{abcdefg}
energetic[1]	10.5^{abc}	5.7^{abc}	4.8^{a}	2.8^{a}	12.5^{abcdef}	9.6^{abcde}
guilty[1]	0.9^{a}	1.9^{ab}	3.8^{a}	6.7^{abc}	0^{a}	0^{a}
grateful[1]	9.6^{ab}	8.6^{abc}	2.8^{a}	8.6^{abc}	8.6^{abcdef}	14.4^{abcdefg}
happy[1]	9.6^{ab}	3.8^{abc}	1.9^{a}	7.6^{abc}	9.6^{abcdef}	14.4^{abcdefg}
pleased[1]	51.9^{f}	34.6^{fg}	12.5^{abc}	36.5^{e}	40.3^{i}	50^{j}
pleasant[1]	26.9^{cd}	18.2^{cde}	5.7^{a}	15.3^{abc}	21.1^{defg}	17.3^{bcdefg}
calm[1]	44.2^{ef}	43.2^{g}	11.5^{ab}	32.6^{de}	28.8^{ghi}	34.6^{hij}
angry[1]	0^{a}	0^{a}	13.4^{abc}	4.8^{ab}	3.8^{abc}	1.9^{abc}
satisfied[1]	58.6^{f}	32.6^{fg}	12.5^{abc}	33.6^{de}	39.4^{hi}	46.1^{ij}

affable[1]	6.7^{a}	16.3^{bcd}	5.7^{a}	14.4^{abc}	12.5^{abcdef}	10.5^{abcde}
merry[1]	11.5^{abc}	8.6^{abc}	6.7^{a}	7.6^{abc}	12.5^{abcdef}	15.3^{defgh}
tired[1]	0^{a}	0^{a}	3.8^{a}	0.9^{a}	0.9^{a}	0.9^{ab}
lonely[1]	1.9^{a}	2.8^{abc}	0.9^{a}	0^{a}	0^{a}	0^{a}
nervous[1]	1.9^{a}	4.8^{abc}	38.4^{e}	10.5^{abc}	12.5^{abcdef}	5.7^{abcd}
embarrassed[1]	0^{a}	0^{a}	1.9^{a}	3.8^{a}	0.9^{a}	0.9^{ab}
discontented[1]	0.9^{a}	4.8^{abc}	10.5^{ab}	6.7^{abc}	3.8^{abc}	2.8^{abc}
complete[1]	12.5^{abc}	8.6^{abc}	4.8^{a}	8.6^{abc}	$15.3^{abcdefg}$	18.2^{cdefgh}
privileged[1]	3.8^{a}	3.8^{abc}	0^{a}	0^{a}	2.8^{ab}	4.8^{abc}
sad[1]	0.9^{a}	0.9^{ab}	2.8^{a}	5.7^{ab}	0^{a}	2.8^{abc}
prudent[1]	7.6^{ab}	6.7^{abc}	2.8^{a}	7.6^{abc}	10.5^{abcdef}	12.5^{abcdef}
healthy[1]	30.7^{de}	9.6^{abc}	6.7^{a}	15.3^{abc}	22.1^{efg}	27.8^{fgh}
relaxed[1]	50^{f}	34.6^{fg}	9.6^{ab}	22.1^{cde}	24^{fgh}	29.8^{ghi}
joyful[1]	24^{bcd}	10.5^{abc}	6.7^{a}	10.5^{abc}	$15.3^{abcdefg}$	22.1^{defgh}
beautiful[1]	11.5^{abc}	9.6^{abc}	3.8^{a}	4.8^{ab}	6.7^{abcde}	12.5^{abcdef}
emotional arousal[2]	0.9^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
impressed[2]	0^{a}	2.8^{abc}	0^{a}	0^{a}	0^{a}	0^{a}
certain[2]	0^{a}	0.9^{ab}	0^{a}	0^{a}	0^{a}	0^{a}
confused[2]	1.9^{a}	3.8^{abc}	0.9^{a}	1.9^{a}	0.9^{a}	0^{a}
nostalgic[2]	0^{a}	0^{a}	0.9^{a}	0^{a}	0^{a}	0^{a}
pessimistic[2]	0^{a}	0^{a}	0.9^{a}	0.9^{a}	0^{a}	0^{a}

*Different superscript letters in columns indicate statistically significant difference ($P \leq 0.05$). Pairwise comparison was performed using the Critical difference (Sheskin) procedure. Additional emotion-based terms reported by participants are included in the table.

[1] Terms originally based on Essence profile

[2] Terms generated by consumers"

Under the informed condition, for the conventional package, the terms with the highest frequencies were ‘pleased’ (36.5%), ‘satisfied’ (33.6%) and ‘calm’ (32.6%). For the biodegradable and ‘edible’ packages, the terms which were preferred most frequently were ‘pleased’ (40.3% and 50%, respectively), ‘satisfied’ (39.4% and 46.1%) and ‘calm’ (28.8% and 34.6%). However, this is in stark contrast, with the emotional profile formed for the biodegradable package in the second part of the study which seemed to generate very negative emotional reactions and is in agreement with the comparison of liking scores between informed and uninformed conditions.

Figure 5.1 shows the correspondence analysis (CA) and the association of the samples under different evaluation conditions with emotion-based terms. The first (F1) and second dimension (F2) accounted for 89.80% of the total data variability (79.56% and 10.24% accordingly). Both the biodegradable and ‘edible’ packages, in the informed condition, were grouped together and were heavily associated with terms such as ‘optimistic’, ‘happy’, ‘joyful’, ‘healthy’ and ‘grateful’. The sample under blind conditions was also associated with positive emotions, such as ‘beautiful’, ‘pleasant’, ‘relaxed’ and ‘calm’; however, it was grouped separately. Under packaging conditions, the biodegradable package stood in the opposite direction and was strongly associated with negative emotions such as ‘angry’, ‘worried’ and ‘nervous’, unlike the conventional package, which was more associated with positive emotions.

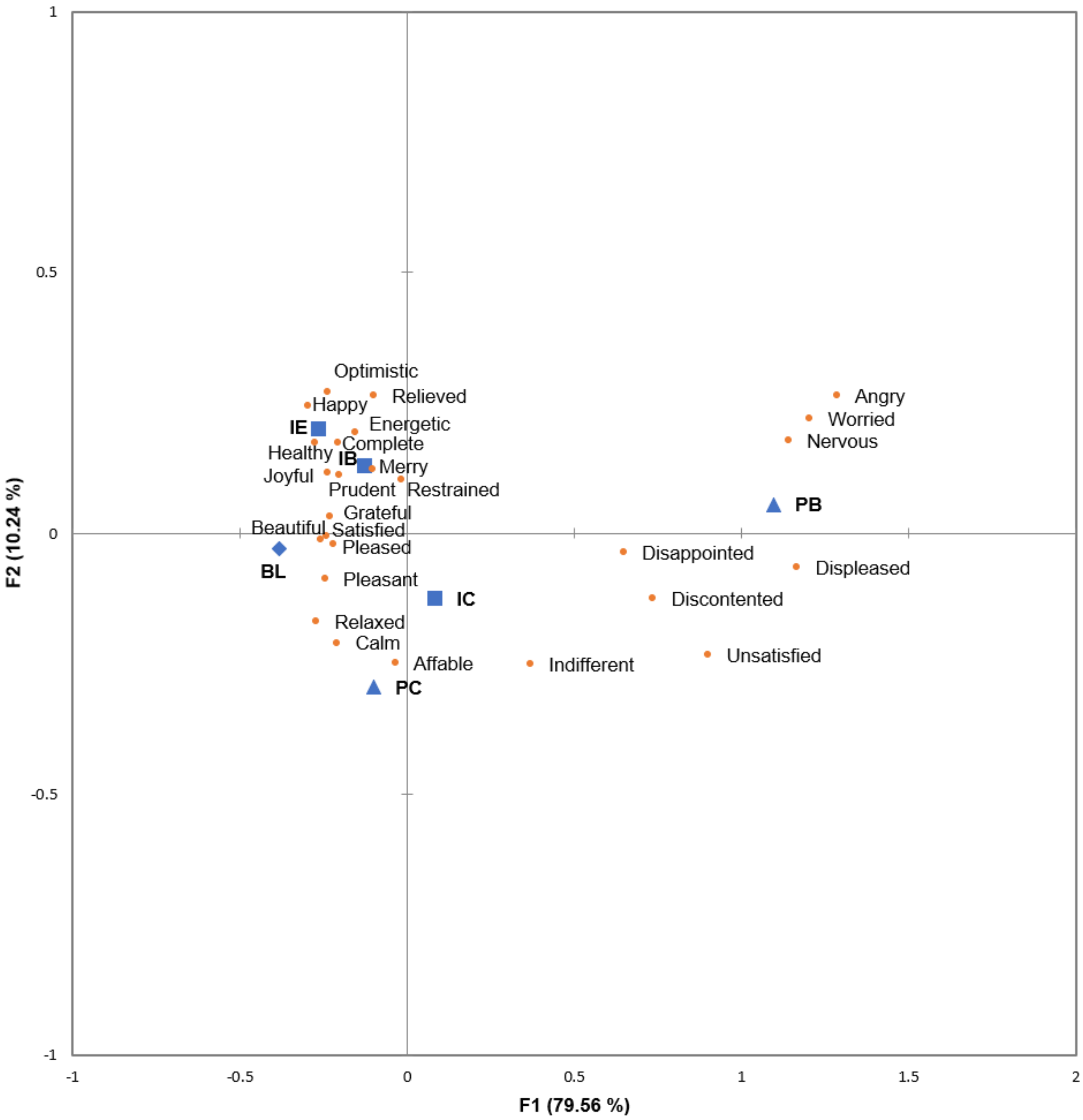

Figure 5.1. Correspondence analysis obtained from the blind, packaging, and informed conditions. Evaluation conditions appear in blue [symbol: diamond (◆) for session one; triangle (▲) for session two; and square (■) for session three] while emotion-based terms appear in red color [symbol: bullet (•)]. Only emotion-based terms with a selection frequency of ≥10% under any condition were selected for the analysis.

* Blind condition: BL; Packaging condition -Conventional: PC; Packaging condition-Biodegradable: PB; Informed condition -Conventional: IC; Informed condition -Biodegradable; IB; Informed condition -'Edible'; IE.

The results of the PCA (Figure 5.2) confirmed the positive relation between the biodegradable and 'edible' packages under informed conditions and their association with positive emotion-based terms, and the more negative emotional associations of the conventional packaging. Likewise, for the biodegradable package, under the packaging conditions, PCA results revealed that it stood in the opposite direction and was associated with negative emotions. The samples under blind conditions and the conventional packaging under packaging conditions were grouped separately.

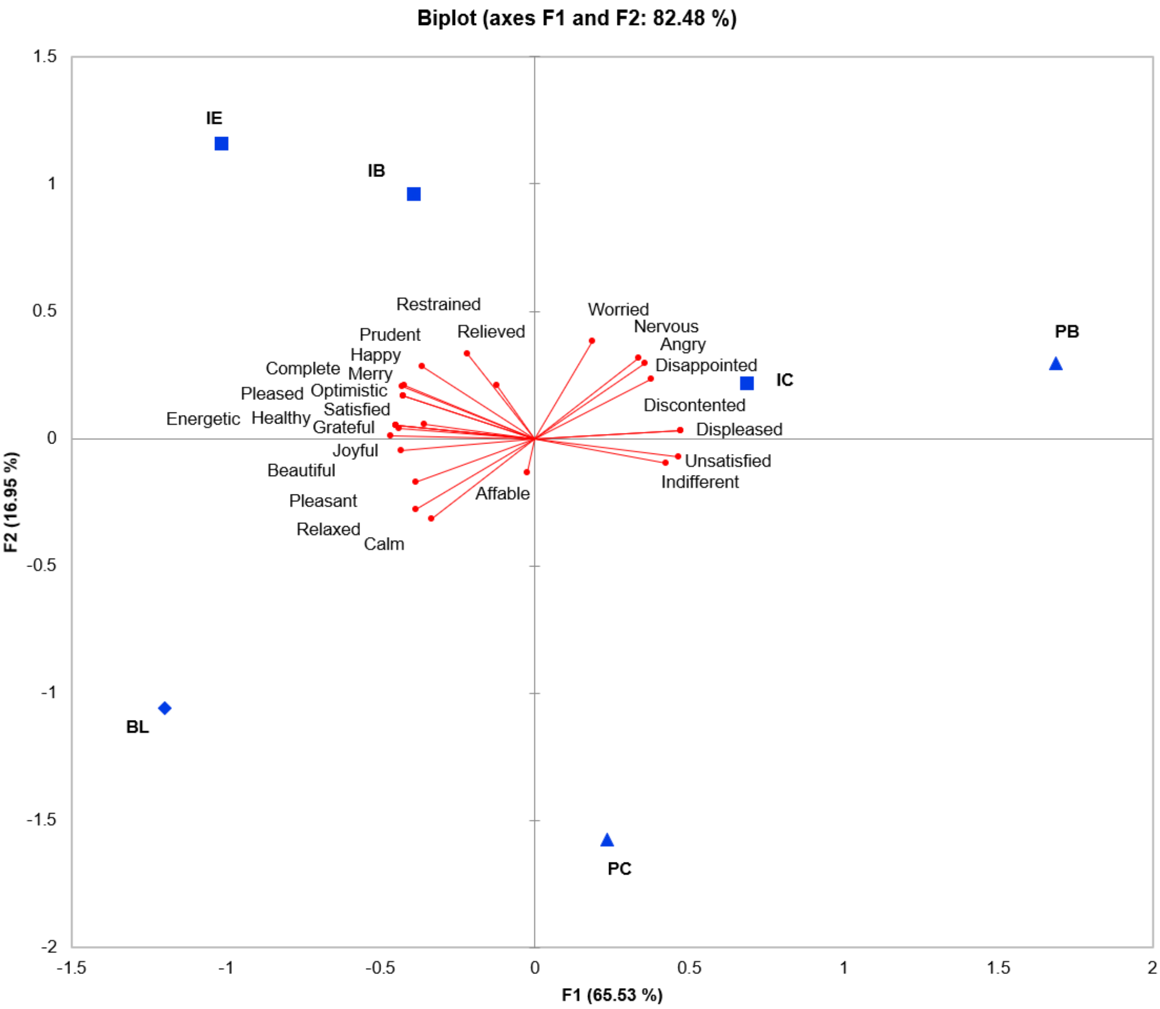

Figure 5.2. Biplot from Principal Component Analysis (PCA) obtained from the blind, packaging, and informed conditions. Evaluation conditions appear in blue [symbol: diamond (◆) for session one; triangle (▲) for session two; and square (■) for session three] while emotion-based terms appear in red color [symbol: bullet (•)]. Only emotion-based terms with a selection frequency of ≥10% under any condition were selected for the analysis.

* Blind condition: BL; Packaging condition -Conventional: PC; Packaging condition-Biodegradable: PB; Informed condition -Conventional: IC; Informed condition -Biodegradable; IB; Informed condition -‘Edible’; IE.

5.4. Discussion

5.4.1 Consumer liking under different evaluation conditions

The results of liking evaluation revealed that packaging had a negative effect on the sensory perception of the rusk. However, the lower liking of biodegradable packaging observed in the second session was masked by the effect of its environmental benefit in the third session. Participants’ comments collected during the session, made it apparent that the package labelled as ‘edible’ was perceived as the most environmentally friendly. Overall, expectations generated by communicating packaging sustainability were not sufficient to make sustainable packaging stand out, demonstrating that scoring was mainly sensory (taste) driven. This result is in contrast to Włodarska et al., (2019) who showed that liking scores of various apple juices were significantly affected by packaging information such as brand, type of production and nutritional value. However, the type of information under examination in that study was different. Research with yogurt revealed that brand information had an impact on the sensory profile of the product without nevertheless influencing liking (Schouteten et al., 2017), suggesting that liking scores alone may not be sufficient for the sensory assessment of foods. Interestingly, another study (Magnier et al., 2016) reported that packaging sustainability did not have an effect on the perception of product quality if the food was already perceived by consumers as intrinsically sustainable. Therefore, it is possible that paximathia, as they are considered a traditional product, may have been already perceived as inherently sustainable. Thus, it was concluded that emotional profiling may be a more suitable tool for predicting actual consumers' food choice. Research has shown that evoked emotions are considered to be better predictors of food choice than liking scores alone (Dalenberg et al., 2014).

5.4.2 Emotional profiles of samples under different evaluation conditions

The results for the conventional and biodegradable packages, under the packaging condition, indicate that the two packages caused diametrically opposing emotions to consumers. However, under the informed condition, both biodegradable and 'edible' packages shared a similar emotional profile. The stark contrast with the emotional profile formed for the biodegradable package in the second part of the study could possibly be explained by the notes collected from participants, where it became clear that this was due to the distinct sound of polylactic acid (PLA) packaging film, a feature of the material which has also been previously reported (Granato et al., 2022). Most importantly, this effect appears to be completely masked under informed conditions for both the biodegradable and 'edible' samples, indicating that claims of sustainability features result in higher satisfaction.

Our findings are in agreement with previous research on raisins and chocolate bars (Magnier et al., 2016), and frozen cod fillets (Donato and D'Aniello, 2022), which showed that food packed in sustainable packaging is perceived to be of higher quality. However, these studies did not test products under different evaluation conditions to combine sensory with emotional assessment. Magnier et al. (2016) compared packaging made of white plastic (conventional) with sustainable package which had a recycled cardboard look and used raisins and chocolate bars as food products. Other elements (i.e., brand, image, product description) were the same for both packages. In the second study (Donato and D'Aniello, 2022), participants were assigned at random to one of five experimental scenarios: a picture of a pack of frozen cod fillets of a fictitious brand with (vs without) an eco-label and presenting (vs not presenting) an ecological claim close to the eco-label. Results for the first study showed that packaging sustainability positively influences the perceived quality of a food product, however, this effect was moderated by the sustainability of the product. Specifically, when food is already seen as sustainable, the sustainability of the package does not have an added effect. In addition, perceived quality was mediated by the perceived naturalness of the product. The second study also showed that quality is positively affected by food packages presenting food-related eco-labels. According to the study's results, the presence of ecological claims makes both food- and packaging-related eco-labels equally effective in influencing consumers' food evaluations.

5.5 Conclusion

The results show that claims about package sustainability result in higher prevalence of positive emotions. The results of this study add to previous research and provide insights on the importance of sustainability as an external product cue. As we focused only on a particular product category, bakery products, due to their widespread popularity in Greece, future studies could confirm if the effect is reproducible in different food categories and cultural contexts. Different packages could lead to very divergent and strong emotional reactions and liking. Although biodegradable films are visually similar to PPL, other features, such as sound, could equally affect their perception by the consumer. However, negative perceptions could be completely reversed by information about their sustainability and their positive environmental impact. Communication of information about the environmental sustainability of packaging results in positive product-evoked emotions. Future studies could focus on the direct impact of food packaging materials, as well as of specific packaging properties such as sound and labelling, on the sensory evaluation of food. Although the objective of the present study did not include the investigation of the effect of packaging sound, it became apparent by the participants' comments that consumer perception was affected by it. The study provides further evidence of the strong impact of external product cues on consumer perception and shows that sustainability claims could be used as part of the strategy for developing a product; however, such developments should be utilised after consideration of the sensorial perception of the food in order to complement it.

CHAPTER 6

Evaluation of sensory effects of functional crackers with eye-tracking technology

6.1 Introduction

Functional crackers could offer a healthier alternative to widely consumed conventional ones. However, it is important to ensure that these products have sensorially acceptable properties and are recognised by consumers for their health offering features. Consumer choices are influenced by extrinsic as well as intrinsic product characteristics. Extrinsic characteristics include aspects of products such as packaging, labels, claims, brand and price, while intrinsic characteristics refer to properties of food such as taste and aroma. From those, it is in fact extrinsic characteristics that are primarily used by consumes to make a food choice, as intrinsic characteristics can only be evaluated after purchase and consumption (Ballco et al., 2020). Some of the extrinsic factors that have been found to play a role in food choice are desire for social approval, technological developments, income, environmental impact, nutritional status, health, convenience, mood, cultural factors, and self-image. Food choice, therefore, is a reflection of consumers' attitudes, ideals and philosophy of life (Cunha et al., 2018; Marsola et al., 2020).

One important question for policy makers and the food industry is how to better understand and predict consumer food choices in order to help promote healthier alternatives. It is known that food choices are influenced by a range of homeostatic and hedonic processes such as pleasure, metabolic hunger state, as well as knowledge about food. Most frequently, researchers focus on hedonic aspects of food which rely on self-reporting. However, there is a risk of missing out on less conscious aspects of food choice or factors of which consumers are unaware, unable or unwilling to articulate. These are known as implicit aspects of motivation. To better capture these factors, several biometric techniques have been developed in recent years which rely more on biological and physiological characteristics of consumers instead of self-reporting. Such an example is the eye tracking technique (Pedersen et al., 2021).

Eye tracking relies on visual attention as an important factor in the food decision making process. Specifically, the technique focuses on real-time measures of eye movements, pupil responses, gaze patterns and visual fixation of consumers' attention, providing calculations of the exact time, location, and duration of eye fixations. Eye tracking can provide valuable information to researchers as eye movements are believed to be very important in food choice. (Peng et al., 2021; Morquecho-Campos et al., 2022).

One way of driving consumers' attention to healthier food choices is the use of labelling. Food labels can be used to help attract attention to nutrition and health related information for example through the use of front-of-pack or back-of-pack information (Van Loo et al., 2021). In fact, recent research suggests that healthy food choices might be encouraged by optimizing nutrition labelling (Bialkova et al., 2014). For example, as back-of-pack nutrition information may be less easily visible by consumers, front-of-pack information summarizing nutrition information may be a better alternative in the identification of healthier food choices at the point of sale (Kelly et al., 2009). Such is the case of traffic light / Guideline Daily Amounts (GDA) labels. GDA labels provide information to consumers on calories, fat, saturated fat, sugar and salt (Bialkova et al., 2014).

The overall goal of this study is to examine consumer acceptance of functional crackers and how visual attention, product cues and personal consumer motives may relate to it. Knowing how much attention consumers pay to various product details and whether attention affects liking could assist government institutions and industry representatives in developing effective communication approaches in order to better promote the adoption of healthier dietary choices.

6.2 Methods

6.2.1 Participants

Fifty-four (N=54) persons (65% female and 35% male) of different ages (18 – 70 years old) and occupational backgrounds were recruited for the study. People were invited to attend the study via email, phone and / or in person. Participants received study invitations, which included information about the research, ethical considerations, and data protection. All participants signed consent forms and were informed that they were allowed to withdraw from the study at any point. There were no inclusion criteria other than participants had to be consumers of cracker products.

6.2.2 Stimuli

Six wheat crackers, made with different flour proportions, were used in the study – i.e., 100% wheat, 30% chickpea, 30% lupin, 30% grape seed, 30% olive stone, and 30% barley. For each product, the following five types of stimuli were included: product name, picture of raw material, picture of finished product (cracker), nutritional information (GDA / traffic light system), and price (Figure 6.1, 6.2 & 6.3). Nutritional information was calculated based on official data from the U.S. Department of Agriculture (FoodData Central, 2019). Price was estimated based on current market ingredient pricing. All images were processed via Microsoft Power Point (version 2306) to standardise object size and position across stimuli arrangements.

Figure 6.1. Products 100% wheat, 30% chickpea, and 30% lupin, displaying five types of stimuli: product name, picture of raw material, picture of finished product (cracker), nutritional information (GDA / traffic light system), and price.

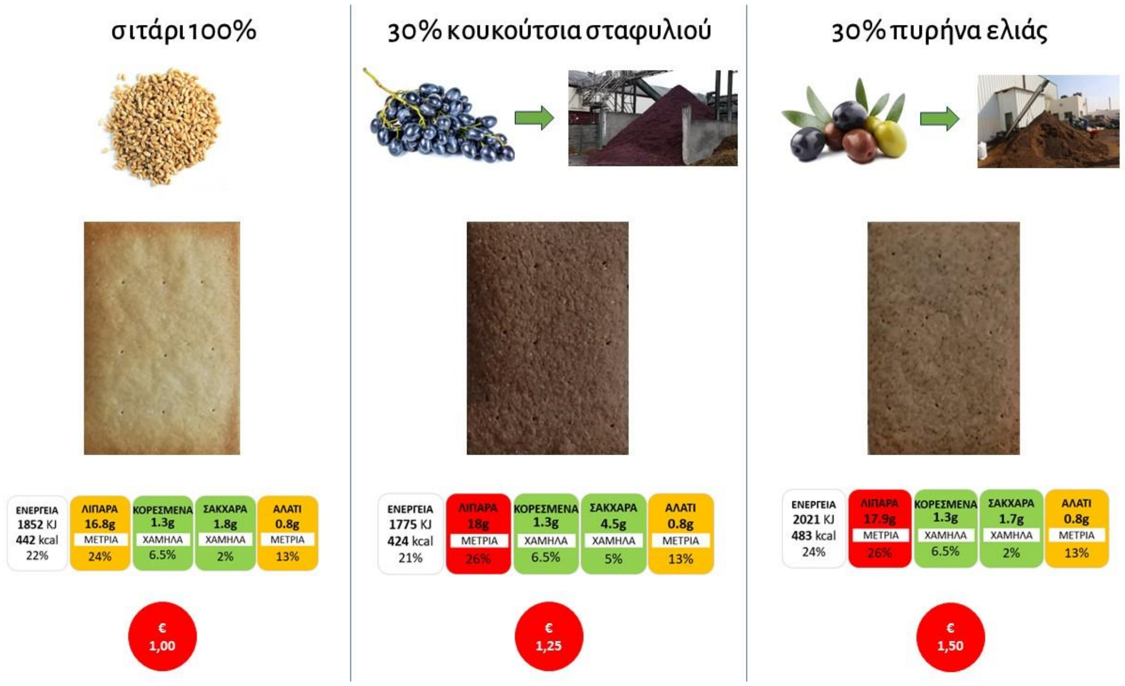

Figure 6.2. Products 100% wheat, 30% grape seed, and 30% olive stone, displaying five types of stimuli: product name, picture of raw material/ by-products, picture of finished product (cracker), nutritional information (GDA / traffic light system), and price.

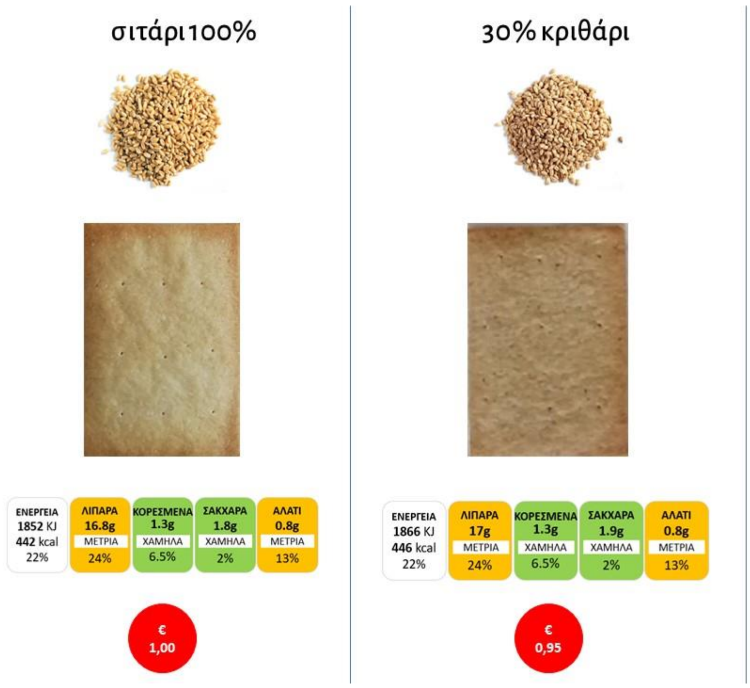

Figure 6.3. Products 100% wheat and 30% barley, displaying five types of stimuli: product name, picture of raw material, picture of finished product (cracker), nutritional information (GDA / traffic light system), and price.

6.2.3 Procedure

The study comprised of one session lasting approximately 10 minutes. After consenting to participate in the study, participants were seated 40 to 60 cm in front of a computer monitor. The monitor was equipped with a Tobii Pro Spark eye-tracking camera attached to the base. Eye-tracking cameras measured participant visual attention to stimuli by collecting data for fixation and total visit duration (TVD). TVD is the total amount of time the eye fixates on an area of interest (AOI) and is a relevant measure for analyzing attention. According to research, TVD was found to be correlated with cognitive processing, decision making, and subjective attribute importance. The areas of interest (AOIs) included in the study were: product name, depiction of raw material, picture of finished product (cracker), nutritional information, and price. AOIs are specific regions of the visual stimuli defined by a researcher and allow for the collection of gaze metrics within the specified area. Before every session, the eye tracker was calibrated using a 5-point system for each participant. Calibration is necessary because of slight variances in the geometry of the visual axis across participants. The calibration points direct eyes to specific locations and then triangulates eye movement and fixation to record visual attention accurately. After calibration, participants completed the study independently to minimize distractions that could impact visual attention. Participants were asked to carefully observe all product details appearing on the screen and then to record “liking”, assessing each product separately. Finally, participants filled out a Food Choice Questionnaire (FCQ), which is designed to evaluate food choice based on personal preferences and incentives. The session was carried out in individual sensory booths at the Laboratory of Consumer and Sensory Perception of Food & Drinks of the University of the Aegean. The booths were closed, sensory analysis cabins ensuring soundproofing. Room temperature was controlled at 25 °C and testing in booths operated under white lighting conditions.

6.2.4 Data collection

Three methods were used for collecting data from participants: (a) Tobii Pro Lab (version 1.217.49450) software was used for processing gaze and pupil data from the eye-tracking device and calculating TVD; (b) a liking questionnaire based on a 7-point hedonic scale (1= dislike extremely to 7= like extremely) to assess consumer acceptance; and (c) the Food Choice Questionnaire (FCQ) for evaluating food choice based on personal motives and preferences. The FCQ is a 36-item instrument assessing the importance of several factors that influence food choice, namely “health”, “sensory appeal”, “price”, “convenience”, “mood”, “natural content”, “weight control”, “familiarity” and “ethical concern” (Figure 6.4). It has been applied in numerous studies internationally and is translated in over 20 languages (Cunha et al., 2018). Participants were asked to assess the importance of each of these 36 FCQ items for “the food I eat on a typical day”, on a 4-point scale (1 = “not at all important” to 4= “very important”).

Items	Factor/dimension
Contains a lot of vitamins and minerals Keeps me healthy Is nutritious Is high in protein Is good for my skin/teeth/hair/nails etc Is high in fibre and roughage	Health
Helps me cope with stress Helps me to cope with life Helps me relax Keeps me awake/alert Cheers me up Makes me feel good	Mood
Is easy to prepare Can be cooked very simply Takes no time to prepare Can be bought in shops close to where I live or work Is easily available in shops and supermarkets	Convenience
Smells nice Looks nice Has a pleasant texture Tastes good	Sensory appeal
Contains no additives Contains natural ingredients Contains no artificial ingredients	Natural content
Is not expensive Is cheap Is good value for money	Price
Is low in calories Helps me control my weight Is low in fat	Weight control
Is what I usually eat Is familiar Is like the food I ate when I was a child	Familiarity
Comes from countries I approve of politically Has the country of origin clearly marked Is packaged in an environmentally friendly way	Ethical concern

Figure 6.4. Structure of the Food Choice Questionnaire as developed by Steptoe et al., (1995), with its 36 items associated with the sentence: “It is important to me that the food I eat on a typical day” (reproduced by Cunha et al., 2018).

6.2.5 Data analysis

Statistical analysis of data was carried out with one-way analysis of variance (ANOVA). Post-hoc comparisons were performed using Tukey’s HSD test adjustment. For FQC data, the Bonferroni correction was used to adjust for different comparisons.

All statistical analyses were two-sided, with the significance level set at 0.05, and carried out using XLSTAT software (Version, 2018.1, Addinsoft).

6.3 Results and discussion

6.3.1 Total visit duration (TVD) for cracker products

Across all products, the nutritional information stimulus attracted the most attention (Figures 6.5 – 6.7). As shown by Figures 6.8 - 6.10, the nutritional stimulus in all products received the highest number and duration of fixations. Total visit duration (mean) was the longest for nutritional information (4823.15 ms), followed by picture of cracker (1411.53 ms), picture of the raw material (843.03 ms), picture of by-products for crackers with grape seed and olive stone (719.89 ms), price (440.06 ms), and product name (421.33 ms) (Figures 6.11 – 6.16). This information shows that from all AOIs, participants focused the most on the products' nutritional aspect, followed by product appearance (picture of cracker), indicating that nutritional and sensory (visual) evaluation was most important. Interestingly, price was attended less, possibly due the artificial laboratory setting not reflecting realistic conditions (e.g., not conducted in a retail environment). An eye-tracking study conducted using granola bar concepts showed that participants focused the longest on health claims, followed by sustainability claims; whereas price was attended the shortest (Van Loo et al., 2021). According to Van Loo et al (2021), spending longer time at a certain attribute (AOI) is associated with higher valuation of that particular attribute. Consequently, higher valuation leads to higher likelihood of choice.

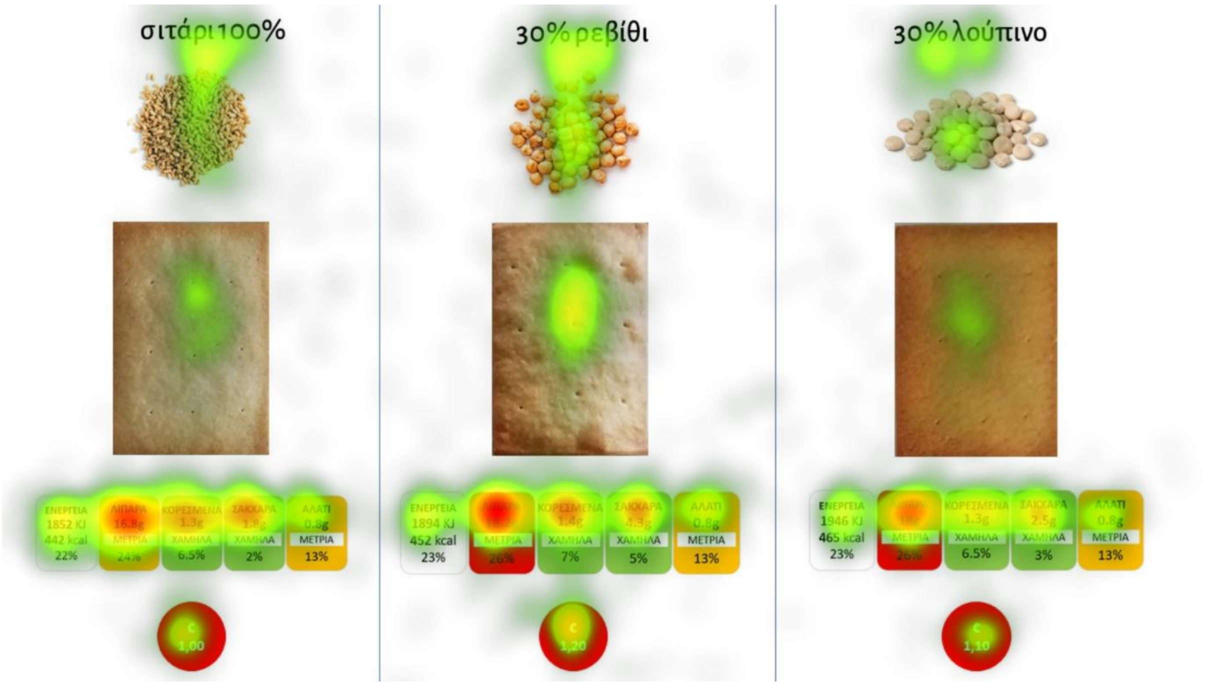

Figure 6.5. Heat map of crackers 100% wheat, 30% chickpea, and 30% lupin. Heat maps show how looking is distributed over the stimuli. Highlighted areas indicate the focus of visual attention for all participants, accumulated over maximum time.

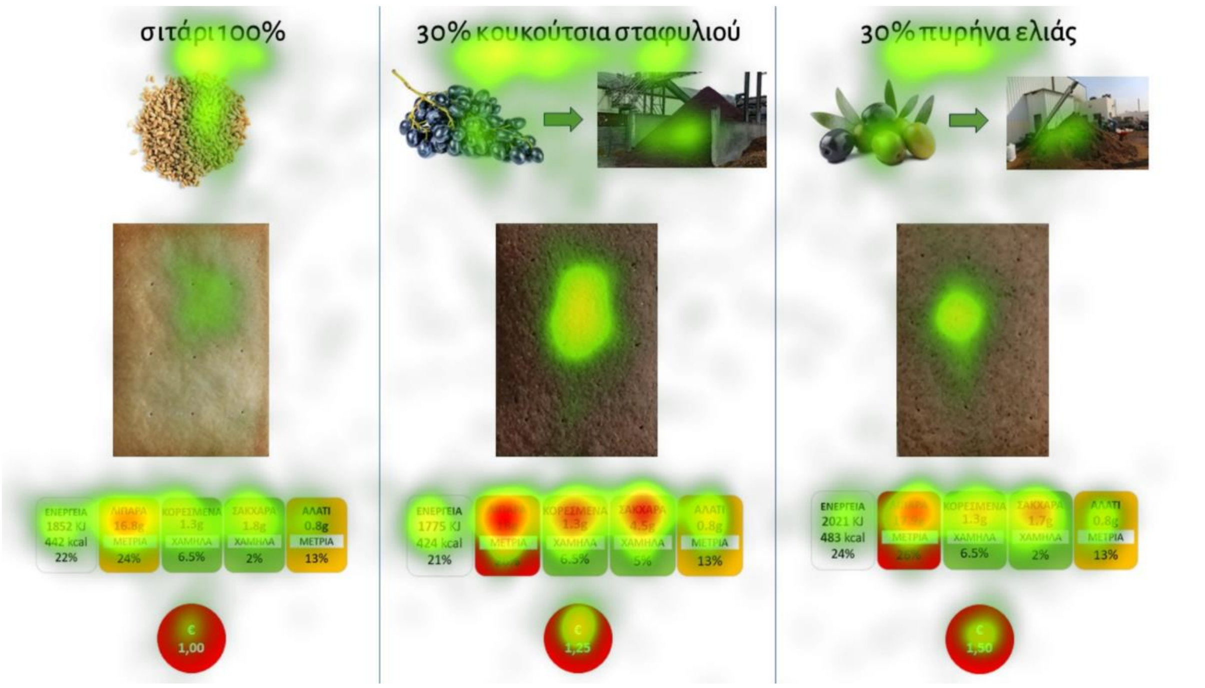

Figure 6.6. Heat map of crackers 100% wheat, 30% grape seed, and 30% olive stone. Heat maps show how looking is distributed over the stimuli. Highlighted areas indicate the focus of visual attention for all participants, accumulated over maximum time.

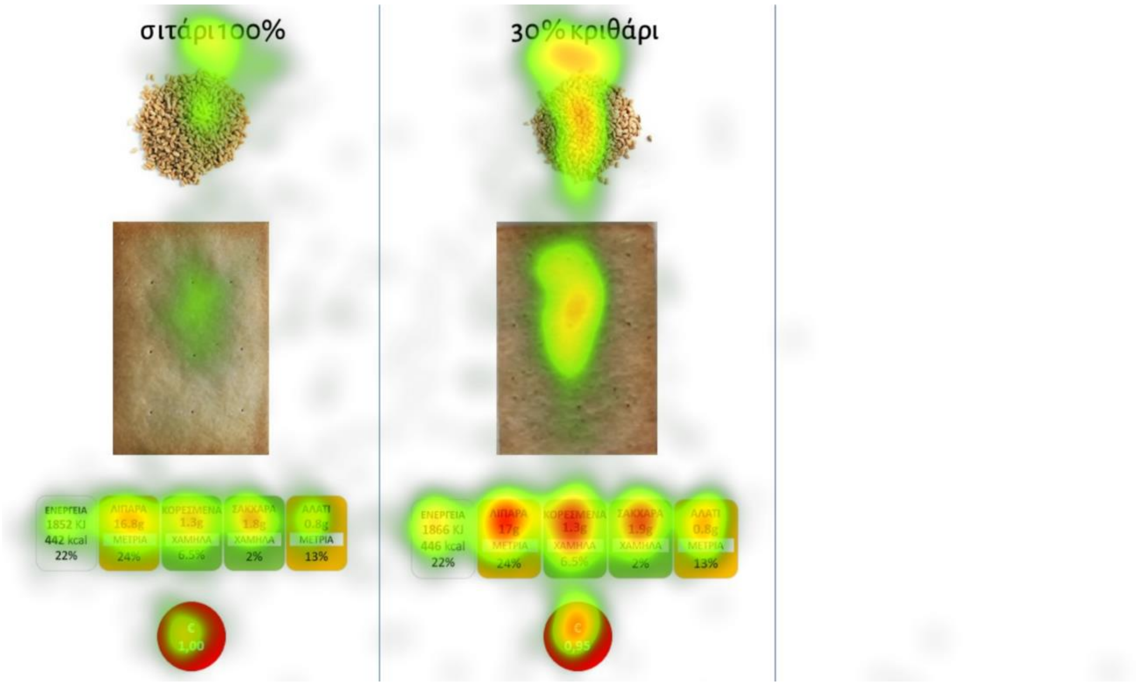

Figure 6.7. Heat map of crackers 100% wheat and 30% barley. Heat maps show how looking is distributed over the stimuli. Highlighted areas indicate the focus of visual attention for all participants, accumulated over maximum time.

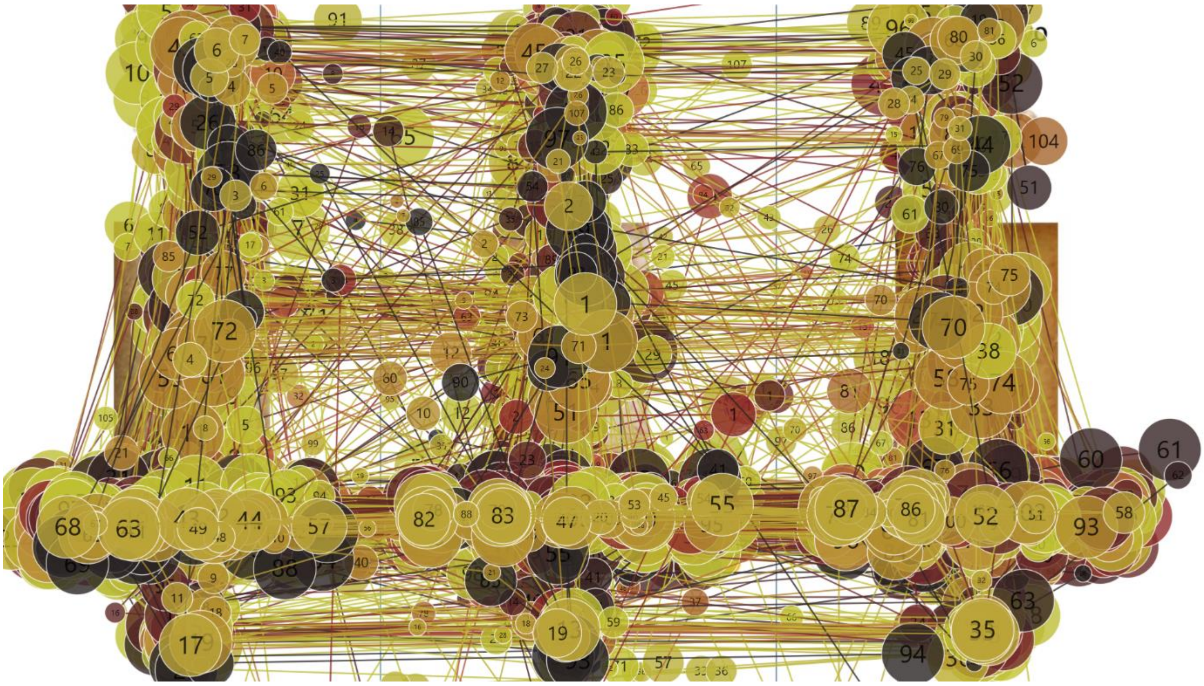

Figure 6.8. Scan path of crackers (from left to right): 100% wheat, 30% chickpea, and 30% lupin. Scan paths show the sequence and position of fixations (dots) on a stimulus. The size of the dots indicates the fixation duration and the numbers inside the dots represent the order of the fixations.

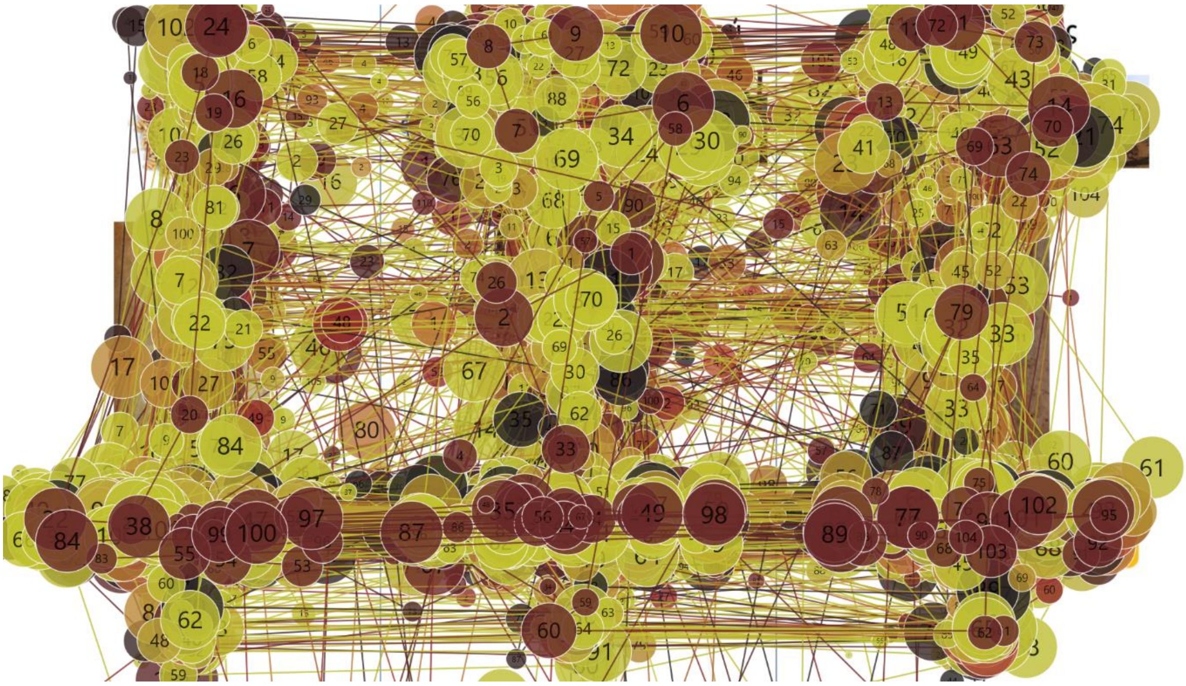

Figure 6.9. Scan path of crackers (from left to right): 100% wheat, 30% grape seed, and 30% olive stone. Scan paths show the sequence and position of fixations (dots) on a stimulus. The size of the dots indicates the fixation duration and the numbers inside the dots represent the order of the fixations.

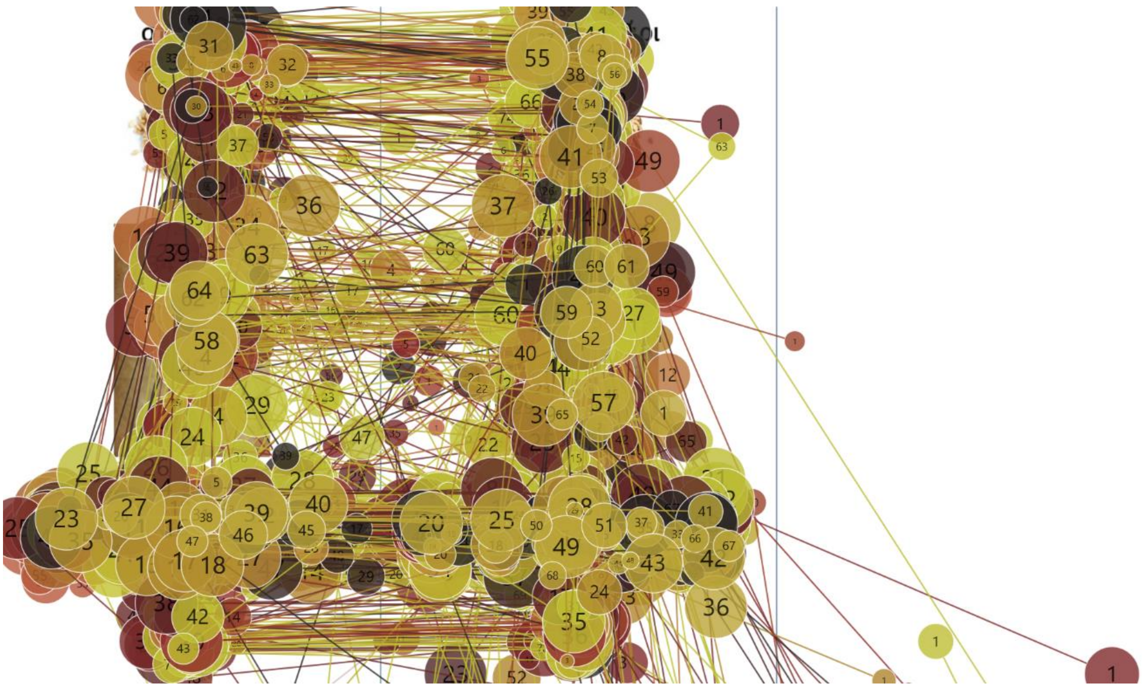

Figure 6.10. Scan path of crackers (from left to right): 100% wheat and 30% barley. Scan paths show the sequence and position of fixations (dots) on a stimulus. The size of the dots indicates the fixation duration and the numbers inside the dots represent the order of the fixations.

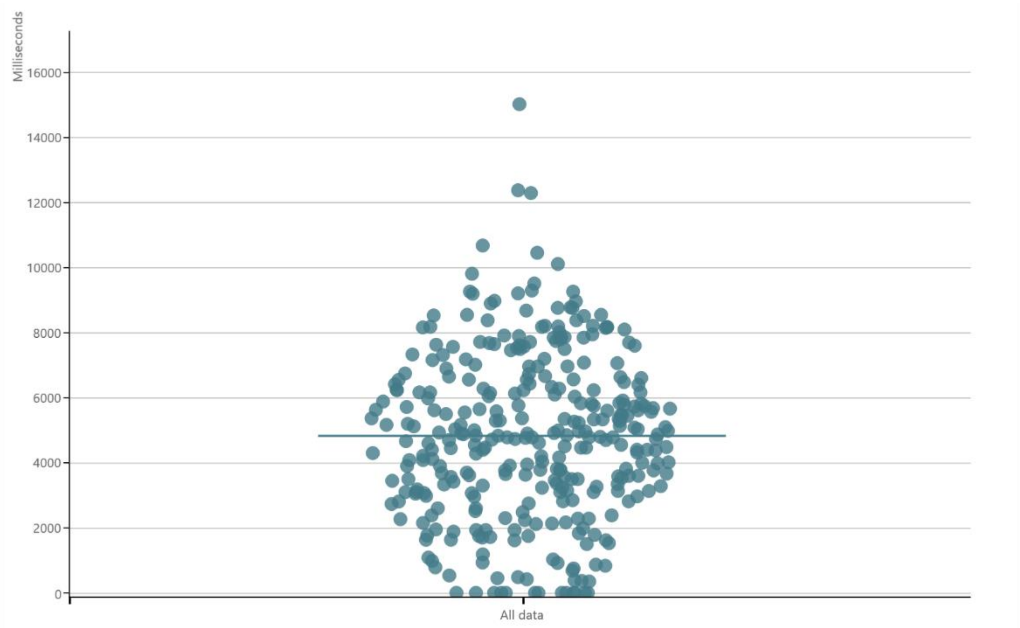

Figure 6.11. Nutritional information's (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

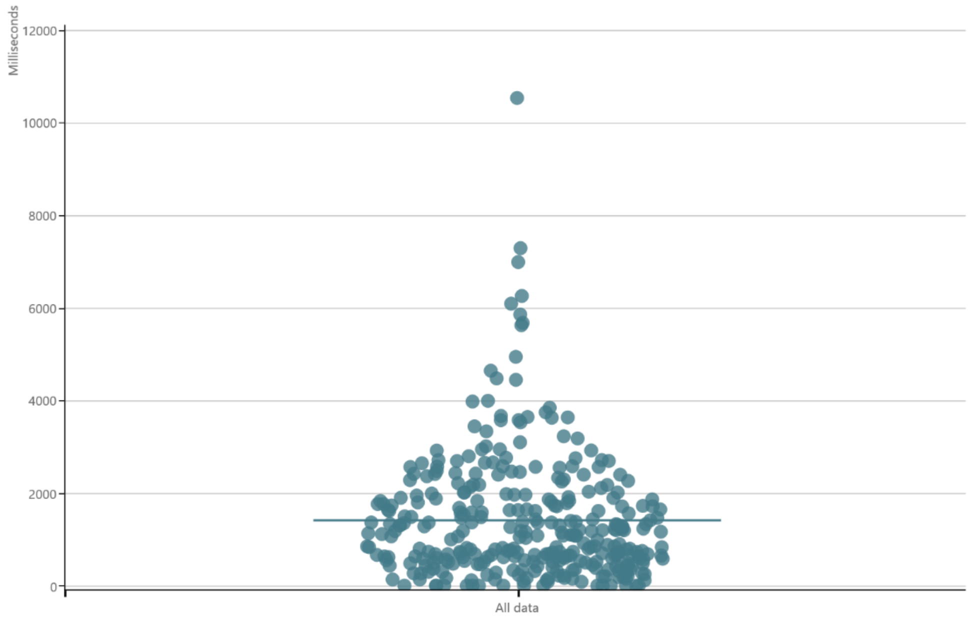

Figure 6.12. Picture of cracker's (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

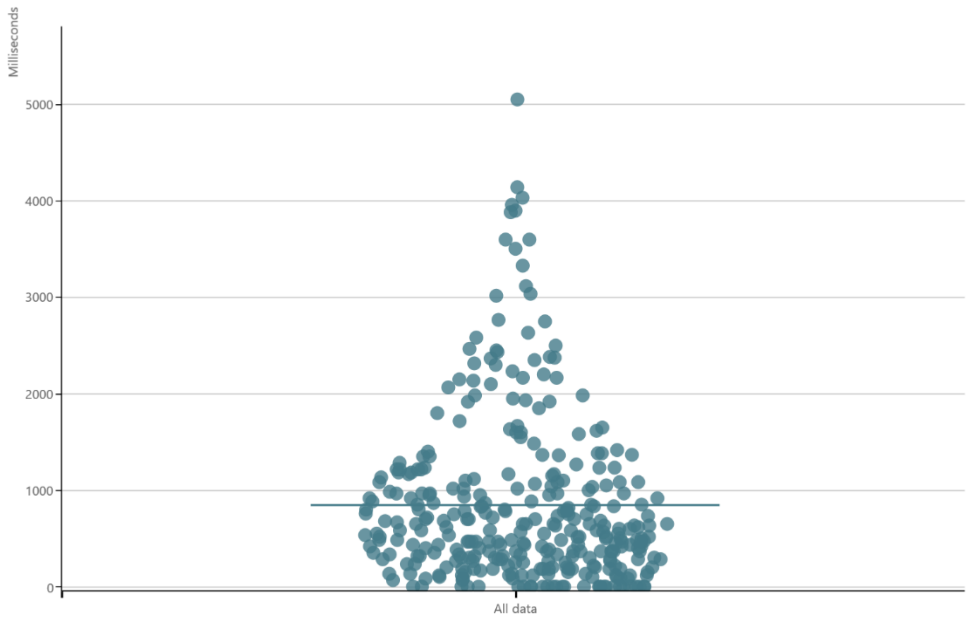

Figure 6.13. Picture of raw material's (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

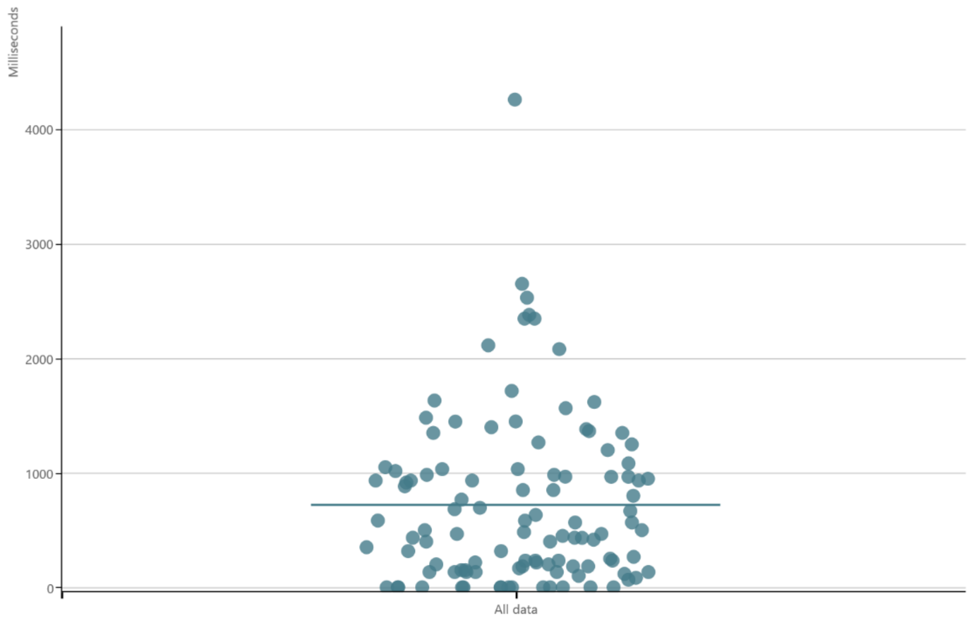

Figure 6.14. Picture of by-products' (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

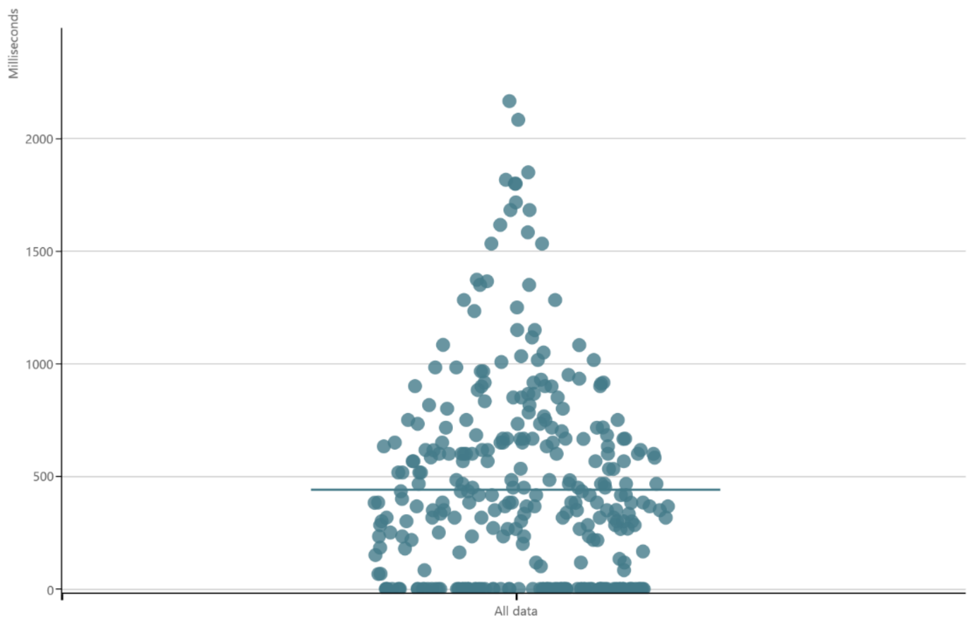

Figure 6.15. Picture of price's (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

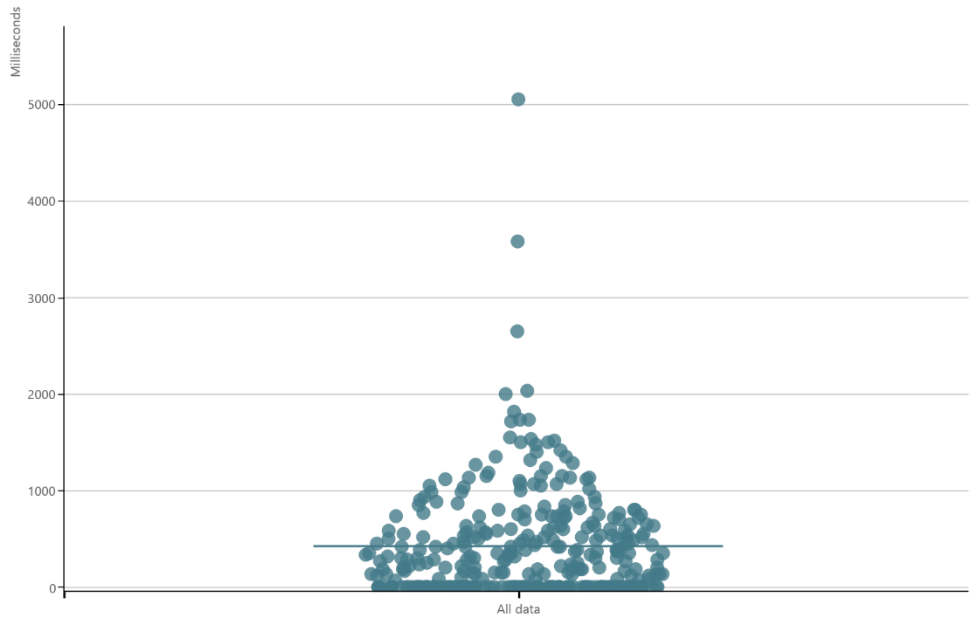

Figure 6.16. Picture of product name's (AOI) total visit duration for all crackers. The line in the graph represents the products' mean value.

Research shows that TVD is associated with food choice. For example, when participants were asked to evaluate a range of sweet and savoury snack food options, they chose the ones they looked at the longest, indicating longer examination and verification of the ultimate choice (Morquecho-Campos et al., 2022). As highlighted by the red circle of Figure 6.17, the highest TVD of any stimuli was encountered for the nutritional information stimulus of 100% wheat cracker. Mean TVD for 100% wheat cracker's nutritional information was the longest (6052.19 ms) compared with the other products. For the rest of the crackers, mean TVD was 4909.43 ms for 30% chickpea, 4208.19 ms for 30% lupin, 5099.41 ms for 30% grape seed, 4030.96 ms for 30% olive stone, and 4638.74 ms for 30% barley. These results reveal that the cracker made with 100% wheat was attended the longest while 30% olive stone was attended the least.

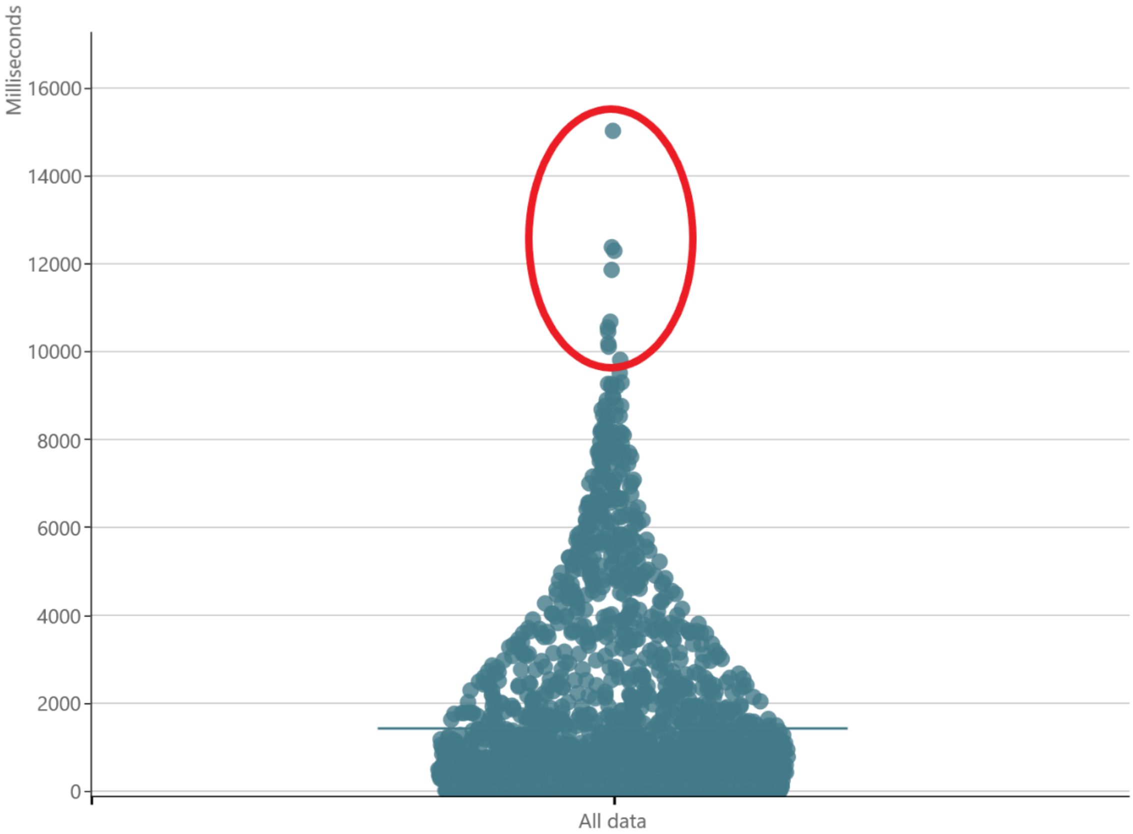

Figure 6.17. Total visit duration for all AOIs and crackers combined. The red circle at the top highlights the highest values received for 100% wheat cracker's nutritional information. The line in the graph represents the products' mean value.

It is worthwhile mentioning that a comment made by several participants was that the red color in the GDA label strongly attracted their attention and even drove their decisions in terms of product liking. This observation seems to be reflected in the literature, with consumers reporting preference for color-coded traffic light labels (Kelly et al., 2009). The reason for this is that a color-coded GDA label may attract attention because of its color. When Bialkova et al. (2014) compared different front-of-pack nutritional label systems it was the color-coded GDA label that was fixated the longest, in comparison to other labels such as monochrome GDAs and the Choices logo. The study also found that the product fixated the longest had the highest likelihood of being chosen, revealing that attention mediates the effect of nutrition labels on choice. This is very important as most consumers do not even attend to nutrition labels when grocery shopping (Van Loo et al., 2021).

6.3.2 Consumer acceptance of functional crackers

Product liking was the highest for 100% wheat (5.2) and lowest for 30% grape seed and olive stone (4.5) (Figure 6.18). It has been reported in recent research that liking is predictive of food choice and food intake (Recio-Román et al., 2020). This finding shows that the focus of attention (which was the longest for 100% wheat) could be predictive of product liking and, therefore, food choice. However, the result was not of statistical significance, indicating that, overall, consumer acceptance in terms of visual perception was not very different for the conventional and functional crackers. One possible explanation for this could be that because the nutritional content of all crackers was similar, consumers may have perceived all products as of equivalent nutritional value.

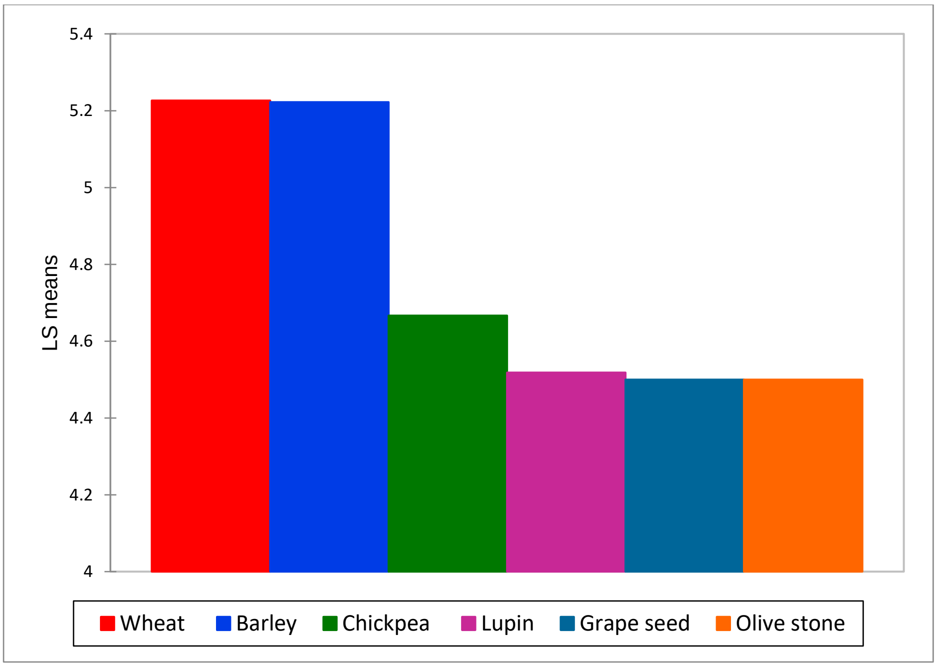

Figure 6.18. Liking results for all crackers.

*In red: 100% wheat; in blue: 30% barley; in green: 30% chickpea; in purple: 30% lupin; in blue: 30% grape seed; and in orange: 30% olive stone.

6.3.3 Participant food choices

Based on the participants stated food choices, the factors that appear to motivate them the most in choosing was health and natural content. On the other hand, familiarity with food and ethical concern was of least importance to them (Table 6.1). This result offers a possible explanation regarding the strong focus of the participants' visual attention on the nutritional information of products. It has been previously reported that attention to nutritional information and related choice is stronger for participants with stronger health goals (Van Loo et al., 2021). As expected, sensory appeal was also a very important factor for food choice, nonetheless not as important as health and natural content. In general, food choice seems to be strongly affected by culture. For example, consumers in Belgium, Hungary, Romania, and the

Philippines, rated the sensory appeal as the most important factor for food choice. On the other hand, for people in the Philippines the health factor was the most important. In addition, although in some populations, convenience is an important factor, for consumers in countries with high consumption of home-made foods, convenience does not seem to be important (Marsola et al., 2020).

Table 6.1. Participants' stated food choice factors (in order of importance) based on the FCQ.

Health	3.207 ^{a}
Natural content	3.121 ^{a}
Sensory appeal	3.060 ^{a}
Convenience	3.007 ^{ab}
Mood	2.866 ^{abc}
Price	2.818 ^{abc}
Weight control	2.788 ^{abc}
Familiarity	2.639 ^{bc}
Ethical concern	2.454 ^{c}
P value	< 0.0001

*Different superscript letters indicate statistically significant difference ($P \leq 0.05$).

6.4 Conclusion

The GDA nutritional information label attracted the most the attention of consumers. Although the conventional, 100% wheat, cracker was visually attended the longest, consumer acceptability was similar between all products. As the nutritional content in the GDA labels of all crackers was similar, consumers may have perceived all products as of equivalent nutritional value. Visual attention appears to be driven by personal motives, as indicated by participants' answers on the Food Choice Questionnaire rating health and natural content as most important food choice factors.

CHAPTER 7

Conclusion

The findings of the present research showed that local raw materials and by-products can be used successfully for the production of functional crackers. Although partial substitution of wheat led to some changes in the properties of crackers, in most cases the quality of the products remained unaffected. Barley flour and chickpea flour resulted in crackers with harder texture and equivalent lightness values to control, while crackers with grape seed and with higher level (30%) of olive stone flour had softer texture and high total color difference. In cases where the texture or color of the product was affected, this was dependent on the type of flour and the level of wheat substitution. Most functional flours resulted in darker and diverse color profiles. Barley flour from Lemnos presented with a higher level of β-glucan compared to its commercial counterpart, highlighting the benefits derived from the use of local raw materials in bakery products. Substitution of wheat with local raw materials and by-products did not negatively affect the physicochemical characteristics of the product throughout its shelf life and was even associated with positive changes such as lower water activity and moisture content. In addition, the use of sustainable materials, such as edible chitosan film, for the packaging of crackers preserved the stability of the products to almost the same extent as conventional packaging. The results of the research showed that claims about package sustainability result in higher prevalence of positive consumer emotions. Furthermore, even negative features of packaging materials (such as sound in certain cases) can be completely reversed by information about their sustainability and their positive environmental impact. The results of this study add to previous research and provide insights on the importance of sustainability as an external product cue. The use of eye-tracking technology revealed that consumers pay particular attention to a product's nutritional value. Among all products, the nutritional information GDA label attracted most of consumers' attention. Both nutritional information and sustainability labelling can be used in order to attract consumers' attention to these features of functional crackers.

PUBLICATIONS

- Giannoutsos, K., Zalidis, A.P., Koukoumaki, D.I., Menexes, G., Mourtzinos, I., Sarris, D. and Gkatzionis, K. (2023) Production of functional crackers based on non-conventional flours. Study of the physicochemical and sensory properties. *Food Chemistry Advances, 2*, https://doi.org/10.1016/j.focha.2023.100194.
- Giannoutsos, K., Koukoumaki, D.I., Panagiotou, M. and Gkatzionis, K. (2023) The effect of modern claim related to packaging sustainability on the sensory perception of traditional Greek rusks (paximathi). *Food Quality and Preference*, *106,* 104817, https://doi.org/10.1016/j.foodqual.2023.104817.
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- Chatziharalambous, D., Kaloteraki, C., Papagianni, O., Giannoutsos, K., Koukoumaki, D., Sarris D., Gkatzionis, K., Koutelidakis, A. (2023) Study of the total phenolic content, total antioxidant activity and in vitro digestibility of novel wheat crackers enriched with cereal, legume and agricultural by-products flours. Oxygen, 3(2), 256-273. https://doi.org/10.3390/oxygen3020017.

APPENDIX

A1. Liking questionnaire for session 1 (as issued, in Greek) for the sustainable packaging sensory trial.

Ονοματεπώνυμο:

Δοκιμάστε το δείγμα αρτοποιίας που παρέχεται. Μετά την δοκιμή υποδείξτε **πόσο σας άρεσε** το δείγμα **639** επιλέγοντας την πιο κατάλληλη φράση από τις παρακάτω:

639

__ Μου αρέσει πάρα πολύ

__ Μου αρέσει πολύ

__ Μου αρέσει

__ Μέτρια αρέσκεια

__ Δεν μου αρέσει

__ Δεν μου αρέσει πολύ

__ Δεν μου αρέσει καθόλου

Σχόλια:……

A2. CATA questionnaire for session 1 & 3 (as issued, in Greek) for the sustainable packaging sensory trial.

Παρακάτω δίνεται μια λίστα λέξεων που περιγράφουν διαθέσεις και συναισθήματα. Παρακαλούμε επιλέξτε όσες λέξεις θεωρείτε πως σας αντιπροσωπεύουν σχετικά με το δείγμα αρτοποιίας που δοκιμάσατε.

αγχωμένος	
αδιάφορος	
αδύναμος	
αηδιασμένος	
αισθησιακός	
αισιόδοξος	
άκεφος	
ανακουφισμένος	
ανικανοποίητος	
απογοητευμένος	
ασυγκράτητος	
δυσαρεστημένος	
εγκρατής	
ενεργητικός	
ένοχος	
ευγνώμων	
ευτυχισμένος	
ευχαριστημένος	
ευχάριστος	
ήρεμος	
θυμωμένος	
ικανοποιημένος	
καταδεκτικός	
κεφάτος	
κουρασμένος	
μοναχικός	
νευρικός	
ντροπιασμένος	
παραπονεμένος	
πλήρης	
προνομιούχος	
στεναχωρημένος	

συνετός	
υγιής	
χαλαρός	
χαρούμενος	
ωραίος	

Σχόλια:……

A3. Liking questionnaire for session 2 (as issued, in Greek) for the sustainable packaging sensory trial.

Ονοματεπώνυμο:

Σας παρέχονται δύο (2) συσκευασίες με τριψήφιο κωδικό:

348 821

Παρακαλώ επεξεργαστείτε για τουλάχιστον 30 δευτερόλεπτα την κάθε συσκευασία, **χωρίς να την ανοίξετε. Μην δοκιμάσετε το δείγμα αρτοποιίας που περιέχεται** στην κάθε συσκευασία.

Υποδείξτε πόσο σας άρεσε το δείγμα **348** επιλέγοντας την κατάλληλη φράση από τις παρακάτω:

348

__ Μου αρέσει πάρα πολύ

__ Μου αρέσει πολύ

__ Μου αρέσει

__ Μέτρια αρέσκεια

__ Δεν μου αρέσει

__ Δεν μου αρέσει πολύ

__ Δεν μου αρέσει καθόλου

Σχόλια:……

A4. CATA questionnaire for session 2 (as issued, in Greek) for the sustainable packaging sensory trial.

Παρακάτω δίνεται μια λίστα λέξεων που περιγράφουν διαθέσεις και συναισθήματα. Παρακαλούμε επιλέξτε όσες λέξεις θεωρείτε πως σας αντιπροσωπεύουν σχετικά με το δείγμα **348** που επεξεργαστήκατε.

αγχωμένος	
αδιάφορος	
αδύναμος	
αηδιασμένος	
αισθησιακός	
αισιόδοξος	
άκεφος	
ανακουφισμένος	
ανικανοποίητος	
απογοητευμένος	
ασυγκράτητος	
δυσαρεστημένος	
εγκρατής	
ενεργητικός	
ένοχος	
ευγνώμων	
ευτυχισμένος	
ευχαριστημένος	
ευχάριστος	
ήρεμος	
θυμωμένος	
ικανοποιημένος	
καταδεκτικός	
κεφάτος	
κουρασμένος	
μοναχικός	
νευρικός	
ντροπιασμένος	
παραπονεμένος	
πλήρης	
προνομιούχος	
στεναχωρημένος	
συνετός	
υγιής	

χαλαρός	
χαρούμενος	
ωραίος	

Σχόλια:…….

A5. Liking questionnaire for session 3 (as issued, in Greek) for the sustainable packaging sensory trial.

Ονοματεπώνυμο:

Σας παρέχονται τρεις (3) συσκευασίες με δείγματα αρτοποιίας με τριψήφιο κωδικό.

Παρακαλώ ανοίξτε τις συσκευασίες και δοκιμάστε τα δείγματα που περιέχονται στην κάθε συσκευασία με την σειρά που αναφέρεται παρακάτω, από τα αριστερά προς τα δεξιά. Χρησιμοποιείτε νερό, ανάμεσα από την δοκιμή των δειγμάτων

590 274 951

Υποδείξτε πόσο σας άρεσε το δείγμα **590** επιλέγοντας την κατάλληλη φράση από τις παρακάτω:

590

__ Μου αρέσει πάρα πολύ

__ Μου αρέσει πολύ

__ Μου αρέσει

__ Μέτρια αρέσκεια

__ Δεν μου αρέσει

__ Δεν μου αρέσει πολύ

__ Δεν μου αρέσει καθόλου

Σχόλια:…….

A6. Liking questionnaire (as issued, in Greek) for the eye-tracker functional crackers trial.

Ονοματεπώνυμο:

Παρατηρήστε τις πληροφορίες για το κάθε κράκερ και τις εικόνες που εμφανίζονται στην οθόνη. Μετά το τέλος της προβολής, σημειώστε με Χ για το **πόσο σας άρεσε** το κάθε εμφανιζόμενο προϊόν επιλέγοντας την πιο κατάλληλη φράση από τις παρακάτω:

Κράκερ από:	Δεν μου αρέσει καθόλου	Δεν μου αρέσει πολύ	Δεν μου αρέσει	Μέτρια αρέσκεια	Μου αρέσει	Μου αρέσει πολύ	Μου αρέσει πάρα πολύ
100% Σιτάρι							
30% Ρεβίθι							
30% Λούπινο							
30% Κουκούτσια Σταφυλιού							
30% Πυρήνα Ελιάς							
30% Κριθάρι							

Σχόλια: ……

A7. Food choice questionnaire (as issued, in Greek) for the eye-tracker functional crackers trial.

Ονοματεπώνυμο:

Επιλέξτε στον ακόλουθο πίνακα (σημειώνοντας με Χ) για τον κάθε παράγοντα πόσο σημαντικός είναι για εσάς.

«Είναι σημαντικό για εμένα το φαγητό που τρώω σε μια τυπική ημέρα να»:

	«καθόλου σημαντικό»	«λίγο σημαντικό»	«σημαντικό»	«πολύ σημαντικό»
Περιέχει πολλές βιταμίνες και μέταλλα				
Με κρατάει υγιή				
Είναι θρεπτικό				
Είναι υψηλό σε πρωτεΐνη				
Είναι καλό για το δέρμα/δόντια/μαλλιά/νύχια μου κλπ				
Είναι υψηλό σε φυτικές ίνες				
Με βοηθάει στην διαχείριση του στρες				
Με βοηθάει να ανταπεξέλθω στην ζωή				
Με βοηθάει να χαλαρώσω				
Με κρατάει σε εγρήγορση				
Με κάνει χαρούμενο/η				
Με κάνει να νιώθω καλά				
Είναι εύκολο στην προετοιμασία				
Μπορεί να μαγειρευτεί πολύ απλά				
Μην χρειάζεται χρόνος για προετοιμασία				

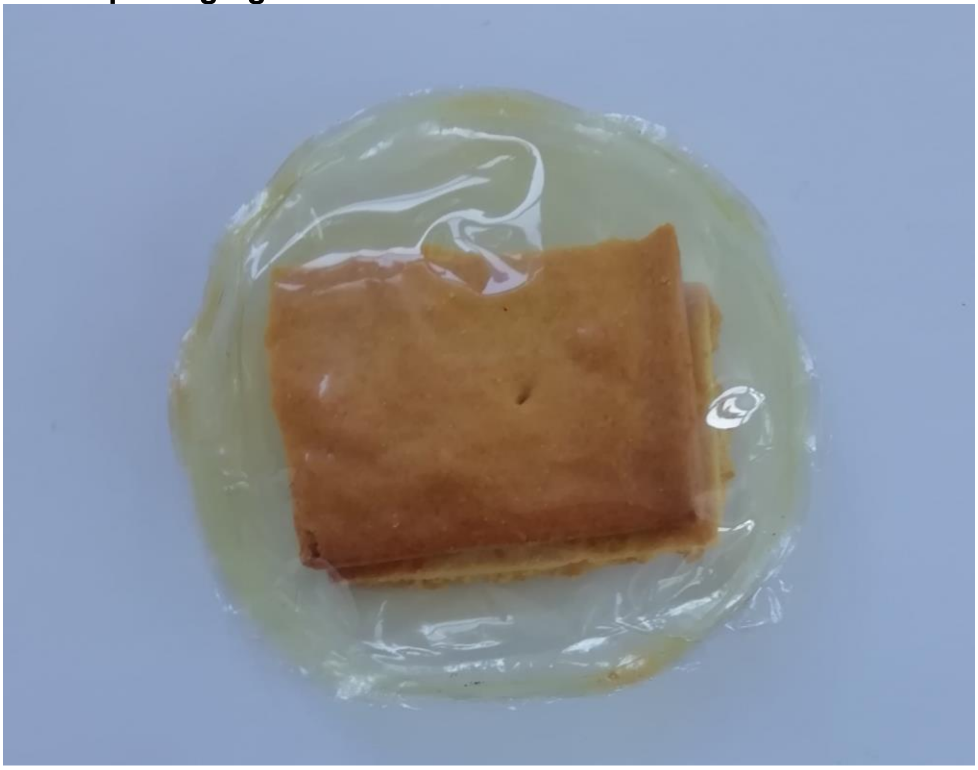
Μπορεί να αγοραστεί σε καταστήματα κοντά στο μέρος που μένω ή εργάζομαι				
Διατίθεται εύκολα σε καταστήματα και σούπερ μάρκετ				
Μυρίζει ωραία				
Φαίνεται ωραίο				
Έχει ευχάριστη υφή				
Έχει ωραία γεύση				
Μην περιέχει πρόσθετα				
Περιέχει φυσικά συστατικά				
Μην περιέχει τεχνητά συστατικά				
Μην είναι ακριβό				
Είναι φθηνό				
Έχει καλή σχέση ποιότητας τιμής				
Είναι χαμηλό σε θερμίδες				
Με βοηθά να ελέγξω το βάρος μου				
Είναι χαμηλό σε λιπαρά				
Είναι αυτό που τρώω συνήθως				
Είναι οικείο				
Είναι σαν το φαγητό που έτρωγα όταν ήμουν παιδί				
Προέρχεται από χώρες που εγκρίνω πολιτικά				
Γνωρίζω την χώρα προέλευσης				
Συσκευάζεται με τρόπο φιλικό προς το περιβάλλον				

A8. Pictures of plastic, biodegradable and edible packaging.

Plastic packaging:

Biodegradable packaging:

Edible packaging:



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