UNIVERSITY OF BARI ALDO MORO

Department of Soil, Plant and Food Sciences (DiSSPA)

PhD PROGRAMME IN SOIL AND FOOD SCIENCES

XXXVII CYCLE

PhD Thesis in Food Science and Technology

AGRI-07/A

THE FLAT BREADS OF THE MEDITERRANEAN AREA: VALORIZATION AND INNOVATION

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FINAL EXAM 2025

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Summary (English)

This doctoral thesis is related to the PRIMA project "Flat bread of Mediterranean area; innovation and emerging process and technology" - Flat Bread Mine, aimed at the enhancement and innovation of flat breads funded within the PRIMA Call 2020 Section 1 Agrofood IA.

Flat breads are the oldest type of bread crafted globally. These breads are popular, widely consumed and appreciated for several reasons: i) they can be made from flours other than wheat, such as pseudocereals or legumes, promoting the use of sustainable local crops from marginal lands; ii) they do not necessarily require an oven for baking; iii) they can serve both as a plate and as cutlery; iv) they can be dehydrated through a second baking process, which prevents mould growth and extends their shelf life; and v) their compact shape makes them easy to transport. These attributes contribute to the enduring popularity of flat breads.

Flat breads are characterized by a significant historical background and have survived over the centuries due to their versatility. Indeed, they can be produced using the same methods as thousands of years ago or with modern, fully automated industrial equipment. Flat breads are also adaptable to different types of fortification, allowing tradition to merge with innovation.

This PhD project explores therefore the possibility to valorize the existing flat breads and then "reinterpreting" them in a new way, so the thesis is composed of two parts: Part I – Valorization, and Part II – Innovation.

The cornerstone of Part I was an overview of the different recipes, processes and product quality specifications of flat breads produced in the Mediterranean countries involved in the Flat Bread Mine project. The information collected on the 143 flat breads have been recorded in a public online database in the website of the Flat Bread Mine project and discussed in a scientific article. It turned out that the formulation is generally simple and includes flour, water, possibly yeast, and salt. Additional ingredients, characteristic of the so-called "garnished flat bread", are region-dependent, reflecting local availability. Moreover, many flat breads have achieved national or international quality recognition, while many others require actions to promote their dissemination, being extremely local and little known.

A specific focus on the Italian typologies has led to a second article to spread awareness of these products, information which was previously fragmentary and little known by the international scientific community. Flat breads, indeed, have a fundamental importance in the cultural heritage of Italian gastronomy, and their identity must be recorded and preserved. It appeared that each regional community has its unique traditional recipes, preparation methods, and baking systems. Besides soft wheat flour, durum wheat semolina is used in southern Italy and Sardinia, chestnut flour in Tuscany, rye flour in Alto Adige, chickpea flour in Liguria, and corn flour in central Italy. The consistency of dough varies from a pourable batter for unleavened flat breads, to a firm dough for the leavened ones, where the use of sourdough, predominant in the past, has been replaced by commercial baker's yeast. Flash baking (short time, high temperature) is typical of all flat breads. Ancient baking systems consisted of terracotta or metal plates to be placed in the fireplace, today replaced by metal plates used on the gas stove. Domed wood-fired ovens are also popular among Italian consumers, followed by modern

electric ovens. Italian flat breads are thus deeply rooted in their origin and linked to traditional practices. Nonetheless, there is room for technical improvement, which is not in contrast with their genuineness.

The Part II of this doctoral thesis, instead, is focused on the innovation of flat breads, with special reference to *focaccia*. In fact, based on the survey carried out on the Italian products, a reference flat bread has been selected that is sufficiently representative of national production and widely appreciated, namely *focaccia*. The *focaccia* is a typical Italian garnished flat bread, similar to pizza, consumed as street food, rich in oil and diversified for the different toppings used. This specific flat bread has been the subject of several studies aimed at innovating it, proposing the use of protein-rich legume flours or alternative oils, rich in antioxidants.

Flour, usually wheat flour, is the main ingredient in the formulation of *focaccia*. Therefore, the potential for innovation of this flat bread lies first of all in changing the type of flour used. By experimenting with different flours, including gluten-free options, new versions of *focaccia* can be developed to meet different food preferences and dietary needs.

In order to achieve nutritional improvement, especially in terms of increased protein and fiber, legume flours were considered the most suitable.

The use of pea-based type I sourdough was proposed. While exploiting the nutritional benefits of this reformulation, the effects on digestibility, volatile profile and antioxidant activity of *focaccia* were investigated, observing that the addition of pea sourdough resulted in an increase in the content of phenolic compounds and a concomitant enhancement of antioxidant activity. This was accompanied by a reduction in *in vitro* starch digestibility and an improvement in *in vitro* protein digestibility. Furthermore, it resulted in a more complex volatile profile, characterized by a higher presence of aldehydes, alcohols, and Maillard reaction products.

Dry-fractionated pea protein concentrate, instead, was tested as an innovative ingredient in gluten-free *focaccia*. It was included as a factor in experimental mixture designs with rice and corn flour to evaluate how different ratios affect the texture, color and sensory features of the final product. The midpoint of the experimental domain, represented by *focaccia* containing 5 g/100 g of pea protein concentrate and 20 g/100 g each of rice and corn flour, was identified as the optimal formulation. The sample demonstrated an optimal texture, minimal impact on color, and a moderate legume odor and flavor. Moreover, the sample would benefit from the addition of labelled nutritional claims, including "source of protein" (energy value provided by protein >12%), "source of fiber" (fiber >3 g/100 g) and "low-fat" (fat<3 g/100 g).

This section of the thesis highlights how legume flours and protein concentrates can enhance the protein and fiber content of end products. However, they may affect the odor and taste, reducing the pleasantness of the final product and thus its sensory acceptability.

In this context, a comprehensive evaluation of the de-flavouring strategies in the production of legume-based foods, with a particular focus on lentils, has been carried out. This analysis aims to identify key challenges and explore potential ways to improve the sensory qualities of these products, promoting sensory acceptance and wider consumption. The analysis demonstrated that heat treatments, alcohol washing, and high-pressure processing (HPP) are particularly fruitful in limiting off-flavors, which are olfactorily described as “beany”, “boiled legume” and “green” odor notes and have the potential to reduce the acceptability of legume-based products.

Although oil is considered a secondary ingredient in most flat breads, its abundance in *focaccia* formulation makes it significantly impacts texture, flavor and taste of the final product. Therefore, optimizing the type and quality of oil used is crucial in *focaccia* innovation and improvement. The principles of circular economy and strategies for by-product and waste up-cycling are becoming increasingly important, even in the milling industry. In this context, the oil recovered from durum wheat milling by-products, namely “durum wheat oil”, was proposed for *focaccia* preparation and its performance was compared with olive oil and sunflower oil. Overall, it was found that the *focaccia* with olive oil was the most stable at oxidation events due to the acidic composition, in which monounsaturated fatty acids prevailed, and the presence of phenolic compounds. The *focaccia* with durum wheat oil showed instead a prevalence of polyunsaturated fatty acids, but also a significant presence of tocotrienols, with antioxidant activity. Both the *focaccia* with olive oil and the one with durum wheat oil were more resistant to oxidation than *focaccia* with sunflower oil, as shown by the volatile profile, considering the volatile oxidation markers such as hexanal and nonanal.

In conclusion, the results of all the studies carried out during this PhD project demonstrate significant opportunities for improvement in the quality of *focaccia*, chosen as a paradigm of Italian flat breads, combining tradition and innovation. This strategy permits the development of products that not only meet the sensory expectations of consumers but also respond to current nutritional requirements, such as increased vegetable protein, fiber and healthy fats. Enhanced formulations have the potential to serve as valid dietary options, addressing the increasing demand for healthier and more sustainable products while respecting the essential attributes of palatability and quality.

Summary (Italian)

Tesi di dottorato in Scienze del suolo e degli alimenti

I pani piatti dell'area Mediterranea: valorizzazione e innovazione

Questa tesi di dottorato è legata al progetto PRIMA "Flat bread of Mediterranean area; innovation and emerging process and technology" - Flat Bread Mine, progetto Europeo finanziato nell'ambito del bando PRIMA 2020 Sezione 1 Agrofood IA, che mira alla valorizzazione e all'innovazione dei pani piatti.

I pani piatti sono i più antichi tipi di pane prodotti a livello globale. Questi pani sono largamente consumati e apprezzati per diversi motivi: i) possono essere prodotti con farine diverse dal grano, come pseudocereali o legumi, promuovendo l'uso di colture locali sostenibili provenienti da aree agricole marginali; ii) non richiedono necessariamente il forno per la cottura; iii) possono servire sia come piatto che come posate; iv) possono essere disidratati attraverso un secondo processo di cottura, impedendo la crescita di muffe e prolungandone la conservabilità; e v) la loro forma compatta li rende facili da impilare riducendo i costi di trasporto. Questi attributi contribuiscono alla loro persistente popolarità, tanto che ancora oggi sono molto diffusi.

I pani piatti sono caratterizzati da un importante background storico e sono sopravvissuti nei secoli grazie alla loro versatilità. Infatti, possono essere prodotti con gli stessi metodi di migliaia di anni fa o con moderne attrezzature industriali completamente automatizzate. I pani piatti sono anche adattabili a diverse variazioni innovative negli ingredienti utilizzati nella loro preparazione, permettendo alla tradizione di fondersi con l'innovazione.

Questo progetto di dottorato esplora quindi la possibilità di valorizzare i pani piatti esistenti per poi "reinterpretarli" in modo nuovo, per cui la tesi è composta da due parti: Parte I - Valorizzazione, e Parte II - Innovazione.

Il cardine della Parte I è stata una panoramica delle diverse ricette, dei processi e delle specifiche di qualità dei pani piatti prodotti nei Paesi del Mediterraneo coinvolti nel progetto Flat Bread Mine. Le informazioni raccolte su 143 pani piatti sono state riportate in un database disponibile online sul sito web del progetto Flat Bread Mine e sono state discusse in un articolo scientifico. È emerso che la formulazione di questi prodotti è generalmente semplice e comprende farina, acqua, eventualmente lievito e sale. Gli ingredienti aggiuntivi, caratteristici dei cosiddetti "pani piatti farciti", dipendono dalla regione e riflettono la disponibilità locale. Inoltre, molti pani piatti hanno ottenuto un riconoscimento di qualità a livello nazionale o internazionale, mentre molti altri richiedono azioni per promuoverne la diffusione, essendo estremamente locali e poco conosciuti.

Un focus specifico sulle tipologie italiane ha portato a un secondo articolo per diffondere la conoscenza di questi prodotti, le cui informazioni erano in precedenza frammentarie e poco conosciute dalla comunità scientifica internazionale. I pani piatti, infatti, hanno un'importanza fondamentale nel patrimonio culturale della gastronomia italiana e la loro identità deve essere registrata e preservata. È emerso che ogni comunità regionale ha ricette tradizionali, metodi di preparazione e sistemi di cottura unici. Oltre alla farina di grano tenero, si utilizzano la semola di grano duro nell'Italia meridionale e

in Sardegna, la farina di castagne in Toscana, quella di segale in Alto Adige, quella di ceci in Liguria e quella di mais nell'Italia centrale. La consistenza dell'impasto varia da una pastella semiliquida per i pani piatti non lievitati, a un impasto compatto per quelli lievitati, dove l'uso del lievito madre, predominante in passato, è stato sostituito dal lievito commerciale. La cottura per tempi brevi ad alta temperatura è tipica di tutti i pani piatti. Gli antichi sistemi di cottura consistevano in piastre di terracotta o di metallo da collocare nel camino, oggi sostituite da piastre metalliche utilizzate sui fornelli a gas. Anche i forni a legna a cupola sono molto diffusi in Italia, seguiti dai moderni forni elettrici. I pani piatti italiani sono quindi profondamente radicati alle proprie origini e sono legati a pratiche tradizionali. Tuttavia, c'è spazio per un miglioramento tecnico, che non è in contrasto con la loro genuinità.

La Parte II di questa tesi di dottorato, invece, si focalizza sull'innovazione dei pani piatti. In particolare, sulla base dell'indagine condotta sui prodotti italiani, è stato selezionato un pane piatto di riferimento, sufficientemente rappresentativo della produzione nazionale e ampiamente apprezzato, ovvero la focaccia. La focaccia è un pane piatto farcito tipico italiano, simile alla pizza. Questa è consumata come cibo di strada, è ricca di olio e diversificata per i diversi condimenti utilizzati. Questo pane piatto è stato oggetto di diversi studi volti alla sua innovazione, proponendo l'aggiunta di farine di legumi ricche in proteine e oli alternativi, ricchi di antiossidanti.

La farina, solitamente di grano, è l'ingrediente principale nella formulazione della focaccia. Pertanto, il potenziale di innovazione della focaccia risiede innanzitutto nella possibilità di cambiare il tipo di farina utilizzata. Sperimentando diverse farine, comprese quelle senza glutine, è possibile sviluppare nuove versioni della focaccia in grado di soddisfare le diverse preferenze alimentari e le esigenze dietetiche.

Per ottenere un miglioramento nutrizionale, soprattutto in termini di aumento del contenuto di proteine e di fibre, le farine di legumi sono state considerate le più adatte e sono state sperimentate sia nella focaccia convenzionale sia in quella gluten-free.

È stato proposto l'uso di un lievito madre di tipo I a base di piselli. Sfruttando i benefici nutrizionali di questa riformulazione, sono stati studiati gli effetti sulla digeribilità, sul profilo volatile e sull'attività antiossidante della focaccia, osservando che l'aggiunta di pasta madre di piselli ha determinato un aumento del contenuto di composti fenolici e un concomitante potenziamento dell'attività antiossidante. Ciò è stato accompagnato da una riduzione della digeribilità *in vitro* dell'amido e da un miglioramento della digeribilità *in vitro* delle proteine. Inoltre, si è osservato un profilo volatile più complesso, caratterizzato da una maggiore presenza di aldeidi, alcoli e prodotti della reazione di Maillard.

Il concentrato proteico di pisello frazionato a secco, invece, è stato testato come ingrediente innovativo per la focaccia senza glutine. Questo ingrediente è stato incluso come fattore nei disegni sperimentali di miscela con farina di riso e di mais per valutare come i diversi rapporti tra ingredienti influenzano la consistenza, il colore e le caratteristiche sensoriali del prodotto finale. Il punto intermedio del dominio sperimentale, rappresentato da una formulazione contenente 5 g/100 g di concentrato proteico di piselli e 20 g/100 g di farina di riso e di mais, è stato identificato come quello ottimale. La focaccia realizzata con questa formulazione mostrava una consistenza ottimale, con un impatto minimo sul colore e un moderato odore e sapore di legumi.

Inoltre, questa focaccia arricchita di concentrato proteico di pisello può beneficiare di vari claims nutrizionali in etichetta, quali "fonte di proteine" (valore energetico fornito dalle proteine >12%), "fonte di fibre" (fibre >3 g/100 g) e "a basso contenuto di grassi" (grassi<3 g/100 g).

Questa sezione della tesi evidenzia quindi come le farine di legumi e i concentrati proteici possano migliorare il contenuto di proteine e fibre dei prodotti finali. Tuttavia, tali farine possono influire sull'odore e sul gusto, riducendo la gradevolezza del prodotto finale e quindi la sua accettabilità sensoriale.

Partendo da queste considerazioni, è stata condotta una revisione della letteratura scientifica redigendo un articolo che analizzi tutte le possibili strategie di deodorazione nella produzione di alimenti a base di legumi, con particolare attenzione alle lenticchie. L'analisi mira a identificare gli approcci più idonei per migliorare le qualità sensoriali di questi prodotti, migliorandone l'accettabilità sensoriale e promuovendone un consumo più ampio. L'analisi ha dimostrato che i trattamenti termici, il lavaggio con alcool e i trattamenti ad alte pressioni (HPP) sono particolarmente utili per limitare gli off-flavor che tipicamente derivano dai legumi, che sono complessivamente descritti con le note di "beany", "legume bollito" e "vegetale".

Sebbene l'olio sia considerato un ingrediente secondario nella maggior parte dei pani piatti, la sua abbondanza nella formulazione della focaccia influenza significativamente la consistenza, il sapore e il gusto del prodotto finale. Pertanto, l'ottimizzazione del tipo e della qualità dell'olio utilizzato è fondamentale per il miglioramento della focaccia. Inoltre, i principi dell'economia circolare e le strategie di valorizzazione dei sottoprodotti e degli scarti stanno diventando sempre più importanti, anche nell'industria molitoria. In questo contesto, l'olio ottenuto dai sottoprodotti della macinazione del grano duro, denominato "olio di grano duro", è stato proposto per la preparazione della focaccia e le sue prestazioni sono state confrontate con l'olio di oliva e l'olio di girasole. Complessivamente, è emerso che la focaccia con olio di oliva era più stabile agli eventi ossidativi grazie alla composizione acidica, in cui prevalevano gli acidi grassi monoinsaturi, e alla presenza di composti fenolici. Le focacce con olio di grano duro, in cui si osservava una prevalenza di acidi grassi polinsaturi, erano anche caratterizzate da una significativa presenza di tocotrienoli, anch'essi aventi azione antiossidante. Entrambe le focacce, all'olio di oliva e all'olio di grano duro, erano migliori rispetto al campione con olio di girasole, come osservabile dal profilo dei composti volatili considerando i composti marker dell'ossidazione, quali esanale e nonanale.

In conclusione, i risultati di tutti gli studi condotti durante questo progetto di dottorato dimostrano significative opportunità di miglioramento della qualità della focaccia, scelta come paradigma dei pani piatti italiani, combinando la tradizione con approcci innovativi. Questa strategia permette di sviluppare prodotti che non solo soddisfano le aspettative sensoriali dei consumatori, ma rispondono anche alle attuali esigenze nutrizionali, come il maggiore contenuto di proteine vegetali, fibre e grassi "sani". Le formulazioni migliorate hanno il potenziale per fungere da valide opzioni dietetiche, rispondendo alla crescente domanda di prodotti più sani e sostenibili, rispettando al contempo gli attributi essenziali di palatabilità e qualità.

General introduction and thesis scope and aims

With deep social, symbolic and religious significance across civilisations, bread is one of the most traditional and culturally significant foods. Among the hundreds of different types of bread produced worldwide, the flat breads are the oldest, characterized by a thickness ranging from a few millimetres to a few centimetres. Flat breads offer numerous advantages related to the formulation and process, such as the use of cereal different from wheat, also gluten-free and cultivated in marginal lands. They are also compact, easy to transport, and offer substantial room for innovation.

This context forms the basis of the PRIMA project "Flat bread of Mediterranean area; innovation and emerging process and technology" (Flat Bread Mine) and of the related PhD project.

Both projects have two main aims: 1) valorize flat breads, and 2) innovate them, adapting their formulations to meet different market needs, nutritional aims and health requirements.

Accordingly, the thesis is divided into two parts. In the first, work was carried out to enhance the value of flat breads through their cataloguing and in-depth study of their characteristics, processes and occasions of consumption. In the second part, possible areas for improvement were highlighted, to diversify the offer and adapt to new nutritional needs, also taking into consideration environmental aspects.

To achieve these aims, information about the cultural and technical features of the flat breads produced and consumed in the Mediterranean basin was collected and recorded in an online available database. The latter is organized as an Excel spreadsheet and collects the information of 143 flat breads, providing a comparative chart based on their production processes, ingredients, baking systems, and key attributes (**Article no. 1**).

In addition, an in-depth study of Italian flat breads has been conducted to address the current lack of information in the international scientific literature (**Article no. 2**). The primary aim of this work was to make a catalogue of these flat breads in order to promote their recognition and explore the possible areas for improvement and innovation.

Linked to this last concept is the second objective of this thesis: the innovation of flat breads.

Firstly, *focaccia* was chosen as the flat bread to be improved due to its versatility and popularity, especially in Italy. *Focaccia* is a close relative of pizza, and like the latter is characterized by the possibility of using different ingredients in the topping, diversified according to consumer preferences.

In terms of amount, flour is the main ingredient in all flat breads. Therefore, the potential for innovation of this flat bread resides in the possibility of modifying this ingredient. While cereals are commonly used, pseudo-cereals and legumes can also be incorporated into flat breads, including gluten-free versions. Legumes, in particular, are of considerable interest due to their higher protein content compared to cereals and their ability to complement the amino acid profile by providing lysine, an essential amino acid in which they are particularly rich.

The first study focused on the use of pea flour in the formulation of a semolina-based *focaccia*. The pea flour was used to obtain a Type I sourdough to evaluate the ability of fermentation to reduce anti-nutrients and to assess the impact on the nutritional, digestibility and volatile profiles of the flat breads (**Article no. 3**).

For the gluten-free version of *focaccia*, the use of protein concentrates was experimented, in combination with rice and corn flour, using the experimental design for mixtures. A clean label *focaccia* was optimized using this approach to enrich the protein content while keeping optimal sensory characteristics (texture, color and odor) of the flat bread. The dry fractionated pea proteins were chosen for their higher environmental sustainability compared to wet concentrates, as they are obtained by physical separation without the use of water and solvents (**Article no. 4**).

Once the optimal ingredients had been identified, the focus shifted to addressing potential sensory challenges.

Both studies observed repercussions on the product's odor characteristics and sensory acceptability, respectively. Therefore, the issue of off-odors of legumes, characterized by the descriptors as “beany”, “rancid” and “green”, was evaluated. In this context, considering the scientific literature that is particularly extensive for soya and peas, but lacking for lentils, an overview was carried out of this issue. Strategies to mitigate the effects were examined (**Article no. 5**). This could also be of interest for the use of this legume in the formulation of future flat breads.

Another nutritionally and sensory significant ingredient in *focaccia* is oil. Therefore, in the last study, the lipid fraction of this flat bread was considered, investigating the effect of changing oil on product characteristics. In particular, three different vegetable oils were compared: olive oil, sunflower oil and durum wheat oil (**Article no. 6**).

Overall, this thesis offers an overview of some selected strategies to enhance the nutritional, cultural and economic value of flat breads, allowing these traditional staples to be transformed into innovative products that meet modern dietary needs while preserving their rich heritage.

List of publications

1. Pasqualone, A.; **Vurro, F.;** Summo, C.; Abd-El-Khalek, M.H.; Al-Dmoor, H.H.; Grgic, T.; Ruiz, M.; Magro, C.; Deligeorgakis, C.; Helou, C.; Le-Bail, P. The large and diverse family of Mediterranean flat breads: A database. *Foods* **2022**, *11*(15), 2326. **https://doi.org/10.3390/foods11152326**
2. **Vurro, F.**; Summo, C.; Squeo, G.; Caponio, F.; Pasqualone, A. The use of durum wheat oil in the preparation of *Focaccia*: effects on the oxidative stability and physical and sensorial properties. *Foods* **2022**, *11*(17), 2679. **https://doi.org/10.3390/foods11172679**
3. De Angelis, D.; **Vurro, F.**; Santamaria, M.; Garzon, R.; Rosell, C. M.; Summo, C.; Pasqualone, A. Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free *focaccia* flat bread. *LWT* **2023**, *182*, 114873. **https://doi.org/10.1016/j.lwt.2023.114873**
4. Mefleh, M.; **Vurro, F**.; Summo, C.; Pasqualone, A. Traditional Italian flatbreads: cultural diversity, processing technology and future perspectives. *J. Ethn. Foods* **2024**,*11,* 24. **https://doi: 10.1186/s42779-024-00238-2**
5. **Vurro, F**.; De Angelis, D.; Squeo, G.; Caponio, F.; Summo, C.; Pasqualone, A. Exploring volatile profiles and de-flavoring strategies for enhanced acceptance of lentil-based foods: A Review. *Foods* **2024**, *13*(16), 2608. **https://doi.org/10.3390/foods13162608**

In addition to the publications listed above, the following study has been submitted and is currently under review.

1. **Vurro, F**.; Santamaria, M.; Summo, C.; Rosell, C.M.; Pasqualone, A. Pea-based sourdough to improve nutritional features, digestibility and volatile profile of durum wheat *focaccia*, *J. Funct. Food,* **under review**.

Part I

Chapter 1. Bread and flat bread: Knowing to value

1. Bread and flat bread: Knowing to value

Bread is a staple food throughout the world, accompanying main meals and becoming an important source of essential macro- and micronutrients. The global average bread consumption per person is expected to be 24.8 kg in 2024, while in Italy it is estimated to be 46.7 kg. The global bread market is expected to grow by 6.34%, considering the period 2024-2029 [1].

The diversity of bread types is vast: the cereals used, the degree of refinement of the flour, the type of fermentation, the process, the other ingredients and the shape provide a basis for classification. Each type of bread has a history and the potential to be known and appreciated [2]. Probably the oldest processed food, bread has social, symbolic and religious significance. It has been associated with different historical events and has many references in art, music and literature.

In ancient Rome, bread was central to the daily diet and its free distribution to citizens, known as "*panem et circenses*," served as a means of social control to appease the masses and maintain public order [3]. During the French Revolution, bread became a symbol of popular discontent: its scarcity and high cost were key factors in the revolts that led to the fall of the monarchy. Restrictions in the availability of bread, such as during wars and economic crises, have often marked moments of significant social tension and political change [4]

In art and literature, bread has also acquired a strong symbolic and metaphorical value. In painting, works such as Caravaggio's The Supper at Emmaus and Leonardo da Vinci's The Last Supper use bread as a central element to represent sharing, sacrifice and the divine. In Italian literature, bread appears as a symbol of social justice and the fight against injustice as seen in The Betrothed (*I Promessi Sposi*)[5] of Alessandro Manzoni, where hunger and the lack of bread represent the suffering of the people under maladministration, or in "*Cristo si è fermato ad Eboli*"[6] of Carlo Levi, where the flat breads were often baked in ovens and eaten in simple forms, sometimes hard and dry, due to the lack of resources in the homes of Lucanian farmers.

Bread holds significant importance in the Bible, occurring in both the Old and New Testaments [7]. In Exodus, the Lord told the Israelites to keep the Passover by eating unleavened bread for seven days because the Jewish people did not have time to leaven the dough during the exodus from Egypt (Exodus 12:15). In the New Testament, bread takes on a metaphysical connotation as the Holy Eucharist instituted by Jesus and is also at the centre of the Lord's Prayer [8]. For Muslims, bread is considered a gift from Allah and there is a rituality which characterize the preparation. Bread making is a typically female activity, handed down through the generations [9].

The findings suggest that already 4000 years before the advent of Neolithic farmers, humans used cereals and wild tubers to produce the first forms of bread [10]. In this context, the oldest types of bread were characterized by a thickness of between a few millimetres and a few centimetres. For this reason, they are named "flat bread", in contrast to "voluminous" bread [11].

The spread of flat breads closely followed that of cereals, starting in the Fertile Crescent and gradually reaching the Mediterranean basin, the Arabian Peninsula, India, the Anatolian Peninsula, the Balkans and China [11]. Examples of them are archaeological finds and discoveries dating back to the Neolithic, Egyptian and Assyrian periods [10,12]. The ancient Egyptians cultivated wheat, barley, and sorghum and used them in bread formulations, while in Palestine, these same cereals were combined with other flours, such as rye, millet, and legumes [12].

Recently, during archaeological excavations in the Archaeological Park of Pompeii, an *affresco* was discovered: it is the reproduction of a precursor of the modern pizza [13] (Figure 1).

Figure 1. Reproduction of pizza, Archaeological Park of Pompeii.

Despite their very ancient origins, flat breads are among the best-known and most widely consumed foods. The global market size for flat breads reached 45.8 billion dollars in 2023 and is projected to reach 72.8 billion dollars with a compound annual growth rate (CAGR) of 5.14% from 2024 to 2032 [14,15].

The term that best describes flat breads is undoubtedly "versatility", in terms of recipes, preparation, baking methods and consumption habits. Another defining characteristic is "sustainability", due to the fewer ingredients required and the shorter baking times, resulting in a lower consumption of energy and resources compared to voluminous breads. For example, some flat breads are baked using unusual baking systems, such as under hot ashes and embers, or *pain de sable* or "ash-baked bread", typical of nomadic communities in Sinai, southern Tunisia and Algeria [11].

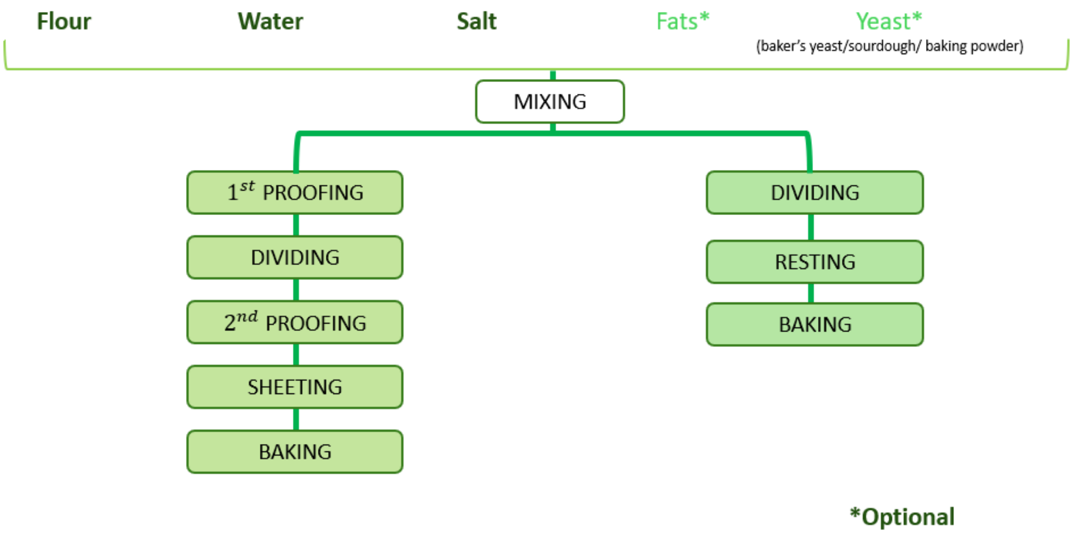

Figure 2. Production process of flat breads.

The main steps in the production process of flat breads are summarized in Figure 2. In particular, the first step involves manually or mechanically mixing of the ingredients to obtain a dough of the desired consistency. For the leavened flat breads, the volume of the dough increases, due to the microbial activity and CO_2 production. For the unleavened flat breads, the dough can be baked directly, making the production and baking process much quicker. Shaping is typically done manually using a rolling pin or finger pressure, although, on an industrial scale, laminating machines are often used to achieve a uniform and thin thickness. The final step is baking, which can be done in different methods, and is generally very rapid. For the flat breads characterized by a semi-liquid dough, the batter is poured directly onto the hot plate and evenly distributed to obtain a thin thickness. The bread with a consistent dough can be baked using fire plates and ovens. Traditional baking systems sometimes depend on the speed and manual skills of women, who are traditionally involved in their home production [11].

For flat breads, several approaches can be identified for classification, in relation to cultural and historical aspects, ingredient formulation and the production process. In first place, flat breads are divided into garnished, plain and fried (Figure 3). Garnished flat breads are topped before or after baking. Fried flat breads are prepared by dipping the dough into hot oil until the product takes on an even golden color. The plain flat breads are "simple" bread, and they can be classified according to the consistency of the dough [11].

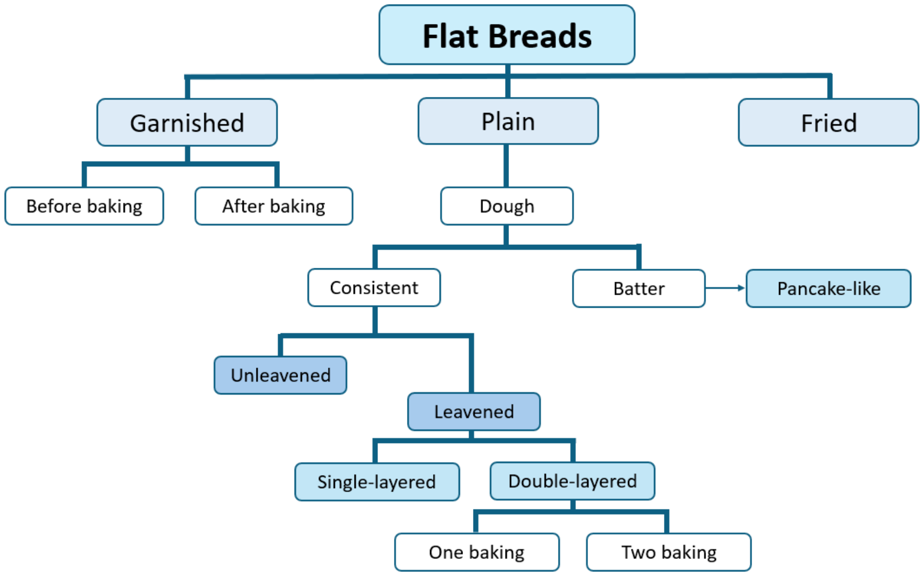

Figure 3. Classification of flat breads.

A distinction can be made between pancake-like breads, made from a semi-liquid batter, and those made with a more consistent dough. The batter of pancake-like flat breads can be obtained from gluten-free flours, such as chestnut, acorn, and legume flours, as they do not require a strong gluten matrix [11, 16]. This offers the possibility of overcoming food scarcity and using crops from rural and marginal areas [16]. The dough can generally be cooked on metal or terracotta hot plates. Examples of these breads are the Somali *Laxoox*, the Yemeni *Lahoh* formulated with sorghum [17], the Italian *Neccio*, made with chestnut flour. Historically, the chestnut flour was combined with acorn flour, due to the need to use wild plants in times of famine, poverty, and limited availability of alternatives [16]. Examples of different types of flat breads made from acorn flour are found in Syria, Afghanistan, Iran and Iraq [16].

For the flat breads with a consistent dough, the presence of a fermentation step allows a further classification. Flat breads can be unleavened or leavened. The latter are divided into single or double-layered (Figure 4). Single-layer flat breads consist of a single sheet and are easy to prepare. To prevent the dough from expanding, it can be pierced, with a fork's prongs [18]. Examples of this type of bread are *Tortillas*, *Paratha* and Scandinavian flat bread [18]. In contrast, the double-layered feature a characteristic "pocket" formed during baking. During baking, water partially evaporates, and fermentation gases expand due to high temperatures, creating a balloon-like separation between the top and bottom layers [11]. Examples of this type of bread include Greek *Pita* and Jordanian *Kmaj* [19].

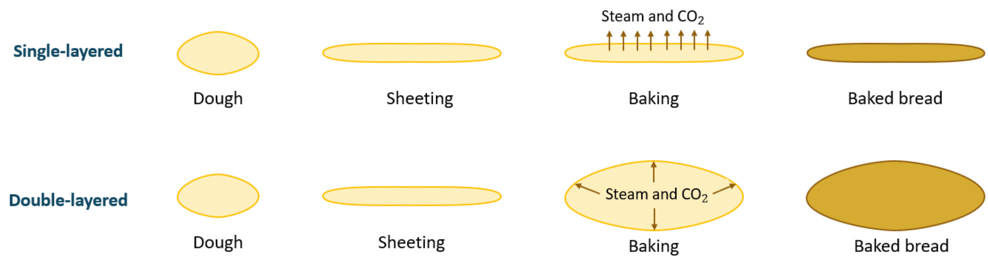

Figure 4. Differentiation between single and double-layered flat breads.

As previously mentioned, flat breads can be obtained from wheat flour, as well as other cereals, pseudo-cereals or legumes, allowing the inclusion of local or marginal crops, thereby reducing environmental impact [11].

Some Southern Italian and Greek flat breads use re-milled durum wheat semolina, reflecting the historical use of this ingredient in the semiarid regions of the Mediterranean basin [19]. Examples of this are the Apulian *Puccia*, Sardinian *Carasau* bread and the Greek *Koulouri* [19]. Other flours are minimally used supporting local and dietary diversity. In colder regions, rye flour was identified as a common staple. It is the principal ingredient of *Schüttelbrot* and *Puccia Iadina,* originating in the Dolomites. Similarly, corn, sorghum and chestnut and chickpea flours are key ingredients in other products, either alone or blended with other cereals [18]. For example, in Somali *Laxoox* flat bread different cereals such as red and white sorghum, millet (pearl and finger millet), barley, corn, wheat and legume flours are mixed [17].

In general, the leavening process in bread-making is mainly carried out with commercial yeast, which has largely replaced traditional sourdough. However, the latter is valued for its distinct nutritional benefits and unique organoleptic properties, which contribute to a richer flavor and texture profile. In addition to yeast, baking soda is sometimes used in bread recipes to enhance the bread's structure (e.g. in Italian *Piadina Romagnola*, Croatian *Zlevanka* and Greek *Plakopita*). Unlike yeast, baking soda does not initiate fermentation: it acts as a leavening agent by triggering a chemical reaction that helps the bread rise. This can be particularly useful in bread types that require a specific texture without the extended fermentation period needed for yeast [11].

Finally, the type of baking systems and times is at the heart of flat bread diversification. The baking system can be divided into plates, obtained with terracotta, metal or stone; vertical ovens, such as *tannur* and *tabun*; domed ovens, called in Italian "*forno a legna*" [11].

Each method contributes to the bread's characteristics in terms of crispiness, softness, and roasted aroma. This variety of baking techniques has given rise to a wide range of flat breads, each with its own cultural and culinary significance. Generally, the baking process is completed in a few minutes, though in some cases, it involves a two-stage process. *Carasatura* refers to the process of obtaining a thin, crisp bread by baking it at a high temperature. The technique of this second baking step is historically linked to pastoralism and transhumance, producing a dry and long-lasting bread, ideal for shepherds and herdsmen during their seasonal migrations [11]. Transhumance is a form of nomadism recognized by UNESCO World Heritage, based on the movement of pastures, due to the climatic conditions and cattle's nutritional needs [20].

Flat breads can act as containers for holding food, similar to wraps or sandwiches. Some flat breads are "edible plates" or can also be used as eating utensils, such as a spoon or fork, allowing diners to scoop portions of food from a container. This dual purpose not only enhances the dining experience but also reduces the need for additional utensils, making the meal quicker and more sustainable [21,22].

1.1 Mediterranean gastronomic culture: A *focus* on flat breads

The Mediterranean basin is the cradle of important gastronomic traditions, characterized by a mixture of peoples, tastes and smells. Common to the diets of Mediterranean populations is the high consumption of vegetables, cereals, legumes and olives, moderate consumption of fish, meat and dairy products, and low consumption of sugar and wine [23,24]. The Mediterranean diet has been influenced by the trade and cultural exchanges that have taken place over time. For example, the Arabs influenced the combination of sweet and sour, using honey or sugar and vinegar. Cinnamon, pepper, cloves and nutmeg became an integral part of recipes, thanks to trade routes through the Indian Ocean, the Persian Gulf or the Red Sea [25]. Ingredients such as potatoes, corn, beans, and tomatoes, that are consumed daily today, were discovered in America by Christopher Columbus [26].

The Mediterranean diet is therefore considered to be the result of the combination of many cultures, many peoples and many traditions that have shaped the eating habits of the Old Continent over the centuries. In this context, the Intergovernmental Committee of UNESCO recognized this dietary model as an Intangible Cultural Heritage of Humanity in 2020 [20].

One of the staples of the Mediterranean diet is bread, which has been diversified thanks to economic and cultural exchanges. Traditionally it was made from whole wheat flour and therefore it was rich in fiber but with a brown color. In the past, brown bread was far more common among the poor and rural populations [27]. White bread, on the other hand, was generally associated with the upper and economically affluent classes [28]. Today, however, whole grain consumption is being promoted to reap the benefits for the gut microbiota and to prevent several diseases [29].

In the Mediterranean basin, flat breads are locally ubiquitous and represent a staple food that is consumed daily. They are eaten dipped in *humus*, legume soups, and *baba ghanoush* (an aubergine-based dip) [30], to soak in a meat broth [31], and contain other foods such as roasted meat, falafel and vegetables [19].

Among the Mediterranean countries, there is an important tradition and linking food and religion. This is particularly true in Greece, especially for flat breads. For example, there are traditions linked to Christmas or Easter. For example, there are traditions connected to Christmas or Easter. The *Lambropsomo* (Easter bread) and the *Lagana* are the typical Easter breads decorated with red painted eggs, the symbol of life [32], while the *Christopsomo* is the typical Christmas bread decorated with walnuts, symbolyze rebirth. *Koulouri* is popular in Thessaloniki and characterized by sesame seeds, a symbol of abundance and fertility [33].

1.2 Italian flat breads

Italy is world-famous for its rich artistic and cultural heritage, which is the result of many peoples and contaminations that have significantly enriched the territory over time. Gastronomy is an expression of culture [34]. In Italy, the relationship between “poor” and “rich” cuisine, between “city” and “country”, and between “past” and “future” has created a unique gastronomic heritage. Even in times of famine, rationing, and difficulties, Italy has never lacked a food culture, with well-defined rules and ingenuity to make the most of the few resources available.

Diversification has given rise to what could be called a "bread geography", with over 200 regional types of bread, each distinguished by its artisanal qualities [35]. These include older breads with ingredients that are no longer common, such as acorn or flaxseed bread, and more common breads that are widespread and appreciated, even recognized with quality marks, such as Altamura DOP bread and Dittaino DOP bread [34,36]. Among the multitude of Italian breads, a good part are flat breads, diversified among regions and with specific peculiarities. Italian flat breads can be divided in plain and garnished.

Plain flat breads are "simple" products, generally made with flour, water, salt and sometimes yeast and fats. In contrast, garnished or stuffed flat breads represent a more complex formulation in terms of the variety of ingredients that can be added, making these breads for occasional consumption, often associated with special occasions and ceremonies [19].

Italian flat breads are widespread from the north to the south of the country. In the Emilia Romagna the most famous flat bread is the *Piadina Romagnola*, recognized as Protected Geographical Indication. *Piadina* is cooked on the traditional “*testo*”, stone or on clay pans, and traditionally garnished with curated meat, cheese and vegetables. The same area also produces other famous flat breads, such as the *Crescentina, Gnocco Fritto,* and *Crescentina Fritta* [33].

The *Schüttelbrot* is a flat bread produced in Trentino-Alto Adige and recognized with Protected Geographical Indication. It is obtained with rye and sourdough and is very dry, similar to the Scandinavian crisp flat namely *Hapankorppuja* and *Tosirukinen.*

The Sardinian flat breads are made with semolina and generally present a low moisture content. Examples include *Carasau* bread*, Guttiau* bread*, Pistoccu* and *Zichi*. Thanks to this, these breads can be stored for a long period and can be easily transported during transhumance or in the fields, during working days [33].

Likewise, in Apulia, a double-layered flat bread called *Puccia* is made from re-milled durum wheat semolina. It is similar to the Greek *Pita*, circular in shape and characterized by a pocket for filling with many ingredients [33].

In the collective imagination, the iconic foods of Italian cuisine are pizza, *focaccia* and pasta. These are among the most appreciated, consumed and reproduced, given the myriad combinations of fillings and toppings. There are many versions, varying according to the region, province, town, and family in which they are prepared. This is true not only of these products but of Italian gastronomy as a whole.

1.2.1 Pizza and focaccia: A comparative analysis of Italy's iconic flat bread

Pizza is one of the most popular foods in the world, with positive trend and perspectives [37]. The word “pizza” comes from "pizzicare", referring to the way the dough was pressed to form the flat bread. The history of pizza goes back thousands of years: Etruscans, ancient Greeks and ancient Romans all ate some form of ancient pizza. The practice of stuffing dough before baking is attributed to the Greeks.

Pizza is a simple food with humble origins that has grown in popularity and variety over time. Originating among the poor and working class, pizza became popular with the upper and the middle classes for its combination of taste, versatility and convenience.

Nutritionally, the pizza is an “edible plate”, a complete and nutritionally balanced meal [38]. The wheat-based dough provides carbohydrates, while the cheese provides protein and fats, together with the drizzle of oil that is generally added before and after cooking. The addition of ingredients such as tomato sauce or vegetables serves to enhance the overall palatability and sensory experience [38].

Basically, the dough is flattened with fingers, topped with various ingredients, and baked. However, there are different nuances of pizza, related to the thickness, topping and final characteristics of this bread [38]. Even when talking about a single flat bread, such as pizza, it is possible to make a further classification according to the region of origin. The *'pizza verace napoletana'* has a thickness in the centre of no more than 0.25 cm, while the edge of about 1-2 cm, called “*cornicione*”, is regular and puffy. The pizza in Bari, on the other hand, is thin, crispy and easy to fold [39], while the Roman pizza is drier and crispier and is therefore called “*scrocchiarella*”.

In Rome, pizza is also made *“in teglia”* (in a pan), with a rectangular shape; it is thicker and moister. The *Pinsa* is oval or rectangular, with a crispy outside and a soft inside, with a dough made with wheat, soya, rice flours and sourdough [40].

The most traditional pizza is the “Margherita”, dedicated to Queen Margherita of Savoy and topped with tomato, mozzarella, and basil, symbolizing the colors of the Italian flag. According to popular belief, it was invented by Raffaele Esposito during the Queen's visit to Naples in 1889. However, this story is likely a false history, which does not diminish the enduring popularity of this dish. The earliest versions of toppings, in contrast, were much simpler, consisting of lard, garlic, salt, basil, and occasionally, *caciocavallo* cheese when available [38].

Nowadays, pizzerias offer many combinations of fillings on the menu, in order to satisfy the tastes of all consumers and to meet new nutritional and ethical requirements. For example, the “pineapple pizza” is very popular outside Italy, but it is viewed with great scepticism by Italian pizza purists. Recently, however, a version has also been proposed by some Neapolitan pizza makers.

The art of Neapolitan pizza-making has been recognized by UNESCO as an Intangible Cultural Heritage [20]. It represents a culinary tradition passed down through generations and highlights the cultural and social significance of pizza in Naples and beyond.

A close relative of the pizza is the *focaccia*. The name comes from the Latin *focus*, meaning fireplace. *De Agricoltura* by Marco Porcio Catone (234-149 BC) is the first written Latin source to mention the *libum farrem*, the ancient Roman *focaccia* that was offered at sacrifices and weddings [42]. In the Middle Ages, *focaccia* was considered a poor food, made with leftover dough from bread and baked to test the temperature of wood-fired ovens before introducing bread.

The basic recipe is simple, consisting of flour, yeast, salt, oil and water [43]. In the south of Italy, such as Sicily and Apulia, re-milled durum wheat semolina is commonly used in the recipe, giving the crumb its typical golden color [43].

Historically, *focaccia* was made exclusively using a sourdough-based process that required a long fermentation period. Today, however, most modern bakeries now use fresh compressed baker's yeast: this change allows for a more efficient and faster production process that meets the demands of modern baking practices while preserving the traditional qualities of the bread.

The preparation of *focaccia* is similar to that of pizza in the initial step of mixing, but *focaccia* is made by incorporating an abundant amount of oil into the dough and poured onto its surface to confer the typical greasiness [44]. Olive oil, especially extra-virgin olive oil, is a central ingredient in the preparation of many types of *focaccia*. It enhances the bread's flavor and palatability, imparting a distinctive aroma and taste. The quantity and quality of the oil used in *focaccia* play a crucial role in its nutritional properties and oxidative stability. Studies have shown that the choice of oil, differentiated by its level of unsaturation and antioxidant content, can significantly influence these aspects. However, another factor that should not be overlooked is cost. Although extra virgin olive oil is the preferred oil, bakeries tend to opt for refined oils because they are cheaper [44]. Another difference between pizza and *focaccia* is the thickness: the former is thin and has no crumb, while the latter is 1-5 cm thick and has a crumb [43].

Focaccia is often topped before baking with a variety of ingredients such as fresh tomatoes, onions, potatoes, olives and cheese. It can also be flavored with aromatic herbs such as rosemary, sage and oregano [43].

Studies have shown that the degree of lipid degradation is influenced by the moisture content of the toppings used in the preparation of *focaccia* [44]. When the entire surface of the *focaccia* is covered with moist toppings, the temperature rise during baking is moderated by evaporation, reducing the heat stress of the oil. For example, a topping of sliced potatoes can help to protect the lipid fraction. In contrast, the highest level of oxidative degradation is observed when rosemary alone is used, probably because it is dry and does not limit oxidation and heat damage during baking [44].

1.3 How to valorize?

In the food sector and in flat bread panorama, the cultures and traditions play an important role in the gastronomic heritage. Traditional flat breads represent a high economic and social role, contributing to the development, sustainability, and attractiveness of the tourist experience [45]. To appreciate and preserve these traditions, it is essential to increase awareness and understanding through thorough documentation, educational programmes, and public relations. In addition, encouraging local communities to become actively involved in promoting and celebrating their unique flat bread varieties can foster a sense of pride and continuity [45].

For these reasons over the years, the national and international authorities and private organizations have established specific quality marks, that certify the specific origin and allow these products to be promoted and protected. In particular, the European Union has instituted the Protected Designation of Origin (PDO), the Protected Geographical Indication (PGI), and the Traditional Speciality Guaranteed (TSG) [45]. For the PDO, production, processing, and preparation take place in a specific geographical area, while for the second, at least one of the stages of takes place in a specific geographical area. Finally, for the TSG, the qualities are not linked specifically to the geographical area, but the traditional production method is detailed and must be followed to have a specific product.

In Italy, there are Traditional Agri-Food Products, which are linked to a territory and its history, with a requirement that they have existed for no less than 25 years [46]. The official list is published periodically by Mipaaft, the Ministry of Agricultural, Food, Forestry, and Tourism Policies. Instead, the acronym DECO stands for Municipal Designation of Origin. It is a brand to enhance and promote the products of a municipal area.

At the international level, the Slow Food Foundation, together with the Presidia, supports small-scale traditional production that is in danger of dying out, the upgrading of territories and the recovery of handicrafts and processing techniques. It has also created the "Ark of Taste", a catalogue of quality food products in danger of disappearing.

Finally, the United Nations Educational, Scientific and Cultural Organization (UNESCO) has included some food preparation as Intangible Cultural Heritage, to protect these heritages of humanity [20].

The existence of all these brands, which are now increasingly known and sought by consumers, and all the activities aimed at learning about and deepening the cultural, traditional and culinary aspects of these products, are fundamental to preserving their survival, protecting them from extinction, passing them on to future generations and promoting their consumption.

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Chapter 2. The Large and diverse family of Mediterranean flat breads: A database

This chapter has been previously published as Pasqualone, A.; **Vurro, F.**; Summo, C.; Abd-El-Khalek, M. H.; Al-Dmoor, H. H.; Grgic, T.; Ruiz, M.; Magro, C.; Deligeorgakis, C.; Helou, C.; Le-Bail, P. (2022). The large and diverse family of Mediterranean flat breads: A database. *Foods*, *11*(15), 2326. **https://doi.org/10.3390/foods11152326**

The Large and diverse family of Mediterranean flat breads: A database

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Abstract

An in-depth survey was conducted by collecting information from web sources, supplemented by interviews with experts and/or bakers, to identify all the flat breads (FBs) produced in the nine Mediterranean countries involved in the FlatBreadMine Project (Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta and Spain), and to have an insight into their technical and cultural features. A database with information on 143 FB types (51 single-layered, 15 double-layered, 66 garnished, 11 fried) was established. Flours were from soft wheat (67.4%), durum wheat (13.7%), corn (8.6%), rye, sorghum, chickpea, and chestnut (together 5.2%). The raising agents were compressed yeast (55.8%), sourdough (16.7%), baking powder (9.0%), but 18.6% of FBs were unleavened.

Sixteen old-style baking systems were recorded, classified into baking plates and vertical ovens (tannur and tabun). Artisanal FBs accounted for 82%, while the industrial ones for 7%. Quality schemes (national, European or global) applied to 91 FBs. Fifteen FBs were rare, prepared only for family consumption: changes in lifestyle and increasing urbanization may cause their disappearance. Actions are needed to prevent the reduction of biodiversity related to FBs. Information in the database will be useful for the selection of FBs suitable to promotional activities and technical or nutritional improvement.

Keywords: flat bread; flour quality; traditional bread; ethnic food; baking system; vertical oven; bread culture; food heritage; food diversity; quality schemes

2.1 Introduction

Bread is one of the basic components of the daily diet, and its history is linked to human history. The “flat” breads include a multitude of bread types different from each other but always relatively thin, ranging from a few millimeters to a few centimeters in thickness. These breads, whose origin is very ancient [1], are produced all over the world. Those spread from the Fertile Crescent reached the Mediterranean area (North Africa, Southern Europe, Middle East and Anatolian peninsula), the Indian subcontinent and the Caucasian region, up to Xinjiang [2], as well as the Arabian peninsula, with interlinks with the Horn of Africa [2,3] Flat breads are also produced in the American Continent, mostly in Central and South America [4], but also in the North, owed to Native Americans [5].

Flat breads meet the need of increasing the sustainability of food system for several reasons: i) can be obtained from cereals other than wheat, as well as pseudocereals or pulses, allowing the use of local productions from marginal lands; ii) require short baking times, eventually even without using an oven (under hot ashes); iii) can wrap around food or serve as a spoon, reducing tableware use and water consumption; iv) are transported with little encumbrance and reduced energy impact; v) if baked to dryness, have a quite long shelf life, reducing bread waste [2].

These strong points made flat breads very popular so that, though having an ancient origin, they have survived until today. Nowadays, these highly versatile breads can be produced either in the same way as they were made thousands of years ago or in modern fully automatic industrial lines. In addition, they can be seasoned or stuffed with a variety of ingredients becoming cheap, convenient and palatable street foods. Renowned examples of these are the *döner kebab* and the related *shawarma* and *gyro*, i.e. finely sliced roasted meat rolled, or stuffed, in a pocket-type flatbread (typically named “*pita*”) with salad and various sauces [6,7]. Other examples are the Italian *pizza* and *focaccia*, or the French *fougasse*, prepared by seasoning the surface of the flattened dough disc with several ingredients, before baking [8]. The fast pace of the modern lifestyle has led to a growing demand for ready-to-eat foods and a concomitant increase in the consumption of flat breads. The global market for these products accounted for $ 81,796.6 million in 2018 and is expected to grow to $ 145,180.9 million by 2027 [9].

Although flat breads are an ancient and consolidated product, there is still much room for technical and nutritional improvement. The baking step is typically very fast, being carried out at high temperature with direct heating. This process may cause quality and safety issues such as burned edges, due to non-uniform heat distribution, and the formation of combustion contaminants such as benzopyrenes and polycyclic aromatic hydrocarbons (PAHs) [7,10]. New baking systems have been recently proposed, such as an indirect heating plant with a rotary baking tray [11]. Also the Bake Off Technology (BOT), consisting of producing bread from industrial refrigerated, frozen or non-frozen bakery goods (partially-baked bread or “part-baked” bread) to be sold for domestic baking, has increased its market share indicating a growing interest by consumers [12], and could be applied to flat breads. Flat breads, which are a staple food of high nutritional importance in many countries, are also suitable for reformulation with a variety of fortifying ingredients of animal or plant origin, able to raise the content of proteins and micronutrients [13,14].

In this context, an international research project, namely “Flat Bread of Mediterranean area: Innovation & Emerging process & technology” (FlatBreadMine) has been recently financed by the European Union H2020-PRIMA, with the main aim of valorizing and innovating flat breads. However, to propose technical innovations (such as low-pressure baking and part-baking) and nutritional improvement (by incorporating flour of legumes, acorns, or carobs), a precise picture of the existing flat breads is needed, in order to select the most suitable ones.

The aim of this work was, therefore, giving an insight into the technical and cultural features of the flat bread types produced in each one of the Mediterranean countries involved in the FlatBreadMine project (namely Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta and Spain). The steps to achieve this goal have been: 1) to identify all the flat bread types produced in the selected countries; 2) to collect information on their main technical characteristics, from the starting ingredients to the end-product, including the cultural features; 3) to set up a database containing all the information; 4) to examine and interpret the information collected [15] in order to highlight the diversity of flat breads across the considered countries and to define the selection criteria.

2.2 Materials and methods

2.2.1. Surveyed area

Nine countries of the Mediterranean area, involved in the FlatBreadMine project, were objects of study: Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta, and Spain.

2.2.2 Subject of the survey

A survey was carried out to collect information on flat breads, including traditional and artisanal ones. The surveyed flat breads had to be original and native of each surveyed country. Therefore pizza, for example, which has an Italian origin, was listed as an Italian flat bread and surveyed only in Italy, although prepared also in the other countries object of the study.

For each flat bread, the following data were collected: (i) The regional area or town of origin, and the area of marketing and diffusion; (ii) the ingredients used in bread preparation (flour, yeast, additional ingredients, and their ratio); (iii) the raw material characteristics (type of flour and its quality features; type of yeast; information on any additional ingredient); (iv) the production process, step by step (kneading time and temperature; conditions of the first leavening step; shaping specifications in terms of average diameter and thickness; conditions of the second leavening step; time and temperature of baking; oven type); (v) the characteristics of bread (type and size; optimal quality features; artisanal or 35-industrial); (vi) the main references and sources of information.

2.2.3 Data collection

Data were collected between October 2021 and May 2022. The first step was the identification of all flat breads produced in each country, which was done by accessing the websites of bakers' associations, food blogs, and scientific literature; browsing the official lists of traditional food products uploaded onto the websites of the EU, the Italian Ministry of Agriculture, and Slow Food; consulting local experts. The latter were scholars involved in the protection and rediscovery of traditional food products including bread, who helped to uncover rare breads not regularly available in the market. They were selected via convenience sampling, based on direct knowledge with the researchers involved in the study, and were contacted by phone for advice.

The second step consisted in collecting the information referred to in Section 2.2. for each flat bread. Information was primarily retrieved from web sources: official technical datasheets of breads awarded of quality marks, scientific literature, websites of bakers' associations, and food blogs. Missing information in web sources was sought from the experts and/or from bakery managers/owners through structured face-to-face/phone interviews.

The recruitment of respondents (experts and/or bakers), according to convenience sampling, was based on direct knowledge with the researchers involved in the study, or supported by the bakers' associations, who introduced the researchers. Interviews were based on a questionnaire (Supplementary Table S1) composed by qualitative and quantitative open-ended questions, which was pre-tested with the president of the consortium of bakers specialized in the production of *Focaccia* barese flat bread (Bari, Italy), who was asked to answer the questions and comment on their feasibility, to avoid excessively generic, or too specific and technical questions, difficult to understand.

After pre-testing, technical questions regarding bread packaging, modified atmosphere and storage conditions were deleted. To reduce work, considering the great number of surveyed breads (n = 143), only the questions necessary to fill the information gaps with respect to web sources were asked. In addition, experts who knew more than one type of flat bread, as well as bakers who produced more than one type of flat bread, were asked to provide information on all of them. The first contact was by telephone, to present the study and to make an appointment for the face-to-face or phone interview, if the participant agreed. The choice between face-to-face and phone interview was made according to the interviewee's preference. The interviewers were the researchers involved in the study. They facilitated the comprehension, also linguistic, of the questionnaire, which was written in English.

The interviewers let the conversation flow naturally and took notes of the answers to each question. Data were anonymized and treated in an aggregated way.

2.2.4 Database structure

A database was set up by the Excel software (Microsoft Office, Version 2018 for Windows, Microsoft Corporation, Redmond, Washington, DC, USA) to gather information on the flat breads of each surveyed country. The database structure included one row per each bread type and 27 columns for the above reported information. In addition, a representative picture was included for each type of bread. The database was uploaded onto the FlatBreadMine project website and is publicly accessible at the link: https://flatbreadmine.eu/resources/ (accessed on 7 July 2022).

2.2.5 Data analysis

The distribution of data was analyzed as percent frequency by Excel software (Microsoft Office, Version 2018 for Windows, Microsoft Corporation, Redmond, Washington, DC, USA).

2.3. Results and discussion

2.3.1 Flat bread diversity in the surveyed area

A total of 143 different flat bread types were found to be produced in the surveyed countries, distributed as follows: 14 from Croatia, 8 from Egypt, 3 from France, 23 from Greece, 75 from Italy, 6 from Jordan, 7 from Lebanon, 2 from Malta and 5 from Spain (Figure 1) [16].

Figure 1. Geographical distribution of flat breads in the surveyed area (Croatia, Egypt, France, Greece, Italy, Jordan, Lebanon, Malta and Spain).

The high number of flat breads recorded in Italy was probably due to the existence of strong regional gastronomic differences within the Italian territory. Furthermore, the consolidated tendency to rediscover and keep alive the memory of small-scale, local and traditional food products, included flat breads, finds its maximum expression in Italy, home of the Slow Food Foundation for Biodiversity [17]. This trend, appreciated by modern consumers [18, 19], aligns with the European policies for promoting traditional foods and protecting their origin [20], and will be discussed more in depth in paragraph 3.9.

Another important factor is the different meaning that flat breads assume in different areas. In Italy these products are considered as a delicacy, admitting many variations on a regional basis, while in the areas where flat breads originated in the antiquity, i.e.

Middle East [1,2] (Jordan and Lebanon, for this survey), or in closer countries, such as Egypt, they represent staple foods that are consumed daily, therefore are less affected by variations.

Flat breads can be classified into plain (further categorized into single- or double-layered), garnished (seasoned or stuffed), and fried. Table 1 shows the occurrence of flat breads in the different categories through the surveyed countries. Garnished flat breads accounted for 46.2%, with a 27.3% contribution by Italy. These flat breads, prepared as specialties to be consumed occasionally, were seasoned or stuffed with several ingredients before baking. In Jordan and Egypt, instead, flat breads were only plain.

Table 1. Occurrence of flat breads in the different categories.

	Flat bread category							
	Plain				**Garnished (seasoned, stuffed)**		**Fried**	
Country	**Single-layered**		**Double-layered**					
	Total number	**%**	**Total number**	**%**	**Total number**	**%**	**Total number**	**%**
Croatia	5	3.5	2	1.4	7	4.9	-	-
Egypt	6	4.2	2	1.4	-	-	-	-
France	-	-	-	-	3	2.1	-	-
Greece	6	4.2	1	0.7	12	8.4	4	2.8
Italy	23	16.1	7	4.9	39	27.3	6	4.2
Jordan	5	3.5	1	0.7	-	-	-	-
Lebanon	3	2.1	1	0.7	3	2.1	-	-
Malta	-	-	1	0.7	1	0.7	-	-
Spain	3	2.1	-	-	1	0.7	1	0.7
Total	51	35.7	15	10.5	66	46.2	11	7.7

Among the plain types, the single-layered category, easier to be prepared, accounted for 35.7%. The double-layered (10.5%) are, instead, those characterized by the typical "pocket", such as the Jordan *Kmaj* [21] (Figure 2A), the Egyptian *Shamy* and *Baladi* [22], and the common Arabic bread or *Khobz* (*Khobz* means "bread" in Arabic) (Figure 2B), which are all known in western countries as "Pita" bread.

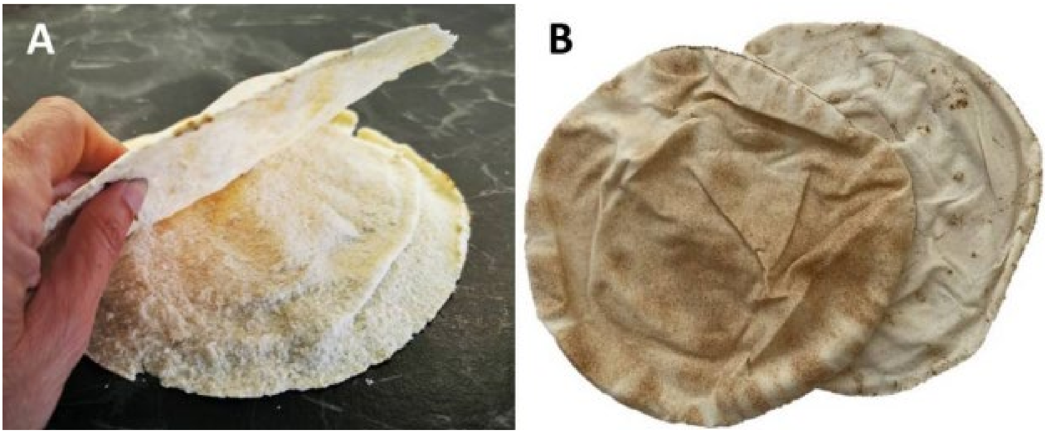

Figure 2. (A) Jordan *Kmaj* opened to show the internal "pocket". **(B)** Lebanese *Khobz.*

The pocket is the visible result of the thermal expansion of the fermentation gas into a thin dough layer, which takes place during baking (Figure 3).

Figure 3. Jordan *Kmaj* in an automatic baking line. The inflation of bread due to the thermal expansion of the gases is clearly visible.

Fried flat breads accounted for 7.7%. They were recorded in Italy (6 breads, namely *Gnocco fritto, Crescenta fritta* – also named *Crescentina fritta – Pinzini ferraresi, Cresciolina, Pizza fritta, Pitt'ajima*), Greece (4 breads: *Pisia, Tiganopsomo, Spargana tou Christou and Fyllota), and Spain (one bread: Arepas Canarias*).

Figure 4 shows, per each country, the number of flat bread types marketed outside the area of origin (town, subregion), compared to the total number flat breads produced in the country. In Italy, Greece and Croatia, only a minority of flat breads were found to be marketed through the entire country, outside the area where they are originally produced and consumed, which was generally a very limited geographic area.

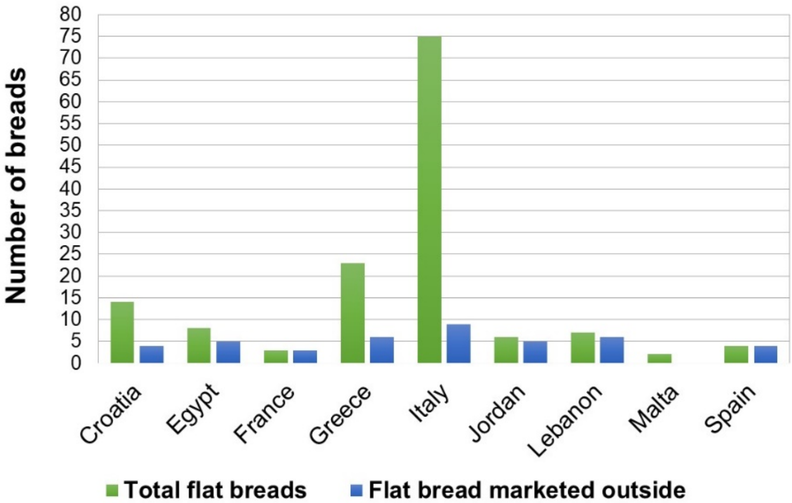

Figure 4. Number of flat bread types marketed outside the area of origin, compared to total flat breads per country (n = 143).

On the contrary, in countries such as Jordan, Lebanon and Egypt, the distribution and consumption of flat breads was homogenous throughout the entire country and three flat breads from these countries were also exported abroad (namely the Egyptian *Baladi* and Shamy, and the Lebanese *Khobz*). These findings agreed with the different character, previously discussed, that flat breads assume in different areas: local specialties vs. national staple foods.

2.3.2 Flour type and quality

Refined soft wheat flour was widely used (67.4%) in the preparation of the surveyed flat breads (Table 2).

Table 2. Type of flour used in the preparation of flat breads. Multiple answers were admitted, because breads could be prepared with different flours or with flour blends.

Country	Type of flour											
	Soft wheat				Durum wheat [a]		Corn [a]		Rye [a]		Other species [a,b]	
	Refined		Whole meal									
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	14	8.0	2	1.1	-	-	3	1.7	2	1.1	-	-
Egypt	5	2.9	1	0.6	-	-	2	0.6	-	-	2	1.1
France	3	1.7	-	-	-	-	-	-	-	-	-	-
Greece	20	11.4	2	1.1	4[c]	2.2	3	1.7	-	-	-	-
Italy	59	33.7	2	1.1	14	8.0	5	2.9	2	1.1	3	1.7
Jordan	6	3.4	-	-	5	2.9	-	-	-	-	-	-
Lebanon	7	4.0	1	0.6	1	0.6	1	0.6	-	-	-	-
Malta	2	1.1	1	0.6	-	-	-	-	-	-	-	-
Spain	3	1.7	-	-	-	-	2	1.1	-	-	-	-
Total	118	67.4	9	5.1	24	13.7	16	8.6	4	2.3	5	2.9

[a] Refined flour, unless otherwise specified. [b] Sorghum, chickpea, chestnut. [c] Durum wheat whole meal is used in the preparation of *Koulouri* (Greece).

Refined soft wheat flour was widely used (67.4%) in the preparation of the surveyed flat breads (Table 2).

Whole meal flour of soft wheat was used only in 5.1% of cases. Earlier studies, dating the late Nineties, reported that flat breads were commonly made of wheat flour at high extraction levels [4], so this flour has been progressively substituted by the refined one.

The use of durum wheat flour (more precisely, re-milled semolina) was reported in 13.7% of cases. Durum wheat cultivation is common in semiarid zones of the Mediterranean basin, and its use in bread making has been already reported [23]. In fact, durum wheat breads (not flat), such as Altamura bread [24] and Dittaino bread [25], have been awarded by the Protected Designation of Origin (PDO) European Union (EU) mark for their peculiar characteristics, such as a compact and yellowish crumb (due to carotenoid pigments). Durum wheat whole meal represented an exception and was found only in the preparation of the Greek *Koulouri*.

The use of corn refined flour accounted for 8.6%. In three cases it was subjected to thermal treatments: pre-cooked corn flour was recorded in the production of the Spanish *Arepa Canarias*, and up to 30% extruded-cooked corn flour could be optionally added to soft wheat refined flour to prepare the Croatian flat breads *Pogača* and *Kukuruzna miješana ciabatta* ("corn composite *ciabatta*"). The thermal treatment causes starch gelatinization, increasing dough viscosity [26]. In the presence of wheat flour, this effect is not strictly needed because good viscoelasticity is ensured by gluten; however, the thermal treatment could be made also because it is able to slow down bread aging [27,28]. Corn has long been cultivated in the Canary Islands and Eastern Europe, including Croatia [29], so in the past the exclusive use of corn in the preparation of these breads could be hypothesized, explaining the need of pre-gelatinizing flour. The use of pre-cooked corn flour in the preparation of *Arepa Canarias*, indeed, resembles the procedure adopted for its Venezuelan counterpart, Arepa, which is made from corn only [30]. Probably a return cultural contamination took place in the Canary Islands following migrations to America, including Venezuela. Without a thermal pretreatment, instead, corn flour is used in the preparation of the Egyptian Bataw and Meraharah, as well as in the Talo, a traditional bread from the Mungia subregion of Basque Countries. Interestingly, the latter has been associated by archeobotanists to an ancient flat bread of the same area, made of acorn [31], which is being rediscovered recently for its high nutritional value [32]. Corn flour can be used as an alternative to wheat flour in the Greek *Souvlakopita* and *Plakopita*, while *Bobota*, which was the most consumed bread in Greece during the German-Italian occupation during World War II, was and is still made exclusively from corn flour [16]. In Italy, corn flour is used to prepare the *Carchiola, Torta al Testo, Pizza di granone, Pizza con farina di mais,* and *Pizza di farinella bacolese*. Only a very small amount of corn flour, about 5%, is mixed with wheat flour for preparing the Lebanese *Markouk.* Rye flour was found to be used in colder areas - to which is more adapted [33] - such as the alpine Italian regions and part of Croatia. A mixture of rye and soft wheat flour, indeed, is used to prepare the Italian *Puccia ladina* (typically consumed in the mountain huts of the Dolomites) and *Schüttelbrot*. Rye flour is optionally added to soft wheat flour to prepare the Croatian *Pogaˇca* and *Kruh ispod peke* ("bread under the lid"). Like other commodities, cereals and particularly wheat, have long since become fully globalized. Egypt, for example, has become the world's largest importer of wheat, exposing the country to significant vulnerabilities, not to mention that more than half of the consumption of wheat in the Mediterranean countries comes from Russia and Ukraine [34]. The use of alternative flours should therefore be strengthened. Among them, sorghum flour was traditionally used to prepare the Egyptian *Khobz min el dorra al rafi'ah* and *Zallut;* however, these breads are now only prepared at home for family consumption and not for commercial purposes, with a serious risk of losing their knowledge. Pulses, though nutritionally valuable, with an amino acid profile complementary to that of cereals, are underrepresented. Chickpea flour is used only in the Italian *Farinata*, a typical street food from Liguria region with variants in Tuscany and Piedmont [35]. However, the addition of pulse flours to bakery products, including flat bread, has been the object of a rising attention in the recent years [36–39]. Chestnut flour is used for preparing the Italian *Neccio*. Besides their high antioxidant activity, chestnuts are rich in minerals, polyunsaturated fatty acids, fiber, and vitamins [40]. In Italy, indeed, this crop has an important economic value [41] and specific chestnut cultivars (Carpinese, Pontecosi, Capannaccia, and Morona) grown in the Garfagnana subregion of Tuscany are used to prepare the flour named "Farina di Neccio della Garfagnana" which has been recognized as a PDO food product. According to tradition, before milling, the chestnuts are dried in small stone buildings named "metati", where a hearth on the ground floor heats and dries the chestnuts placed on the upper floor [42].

Regarding the quality of flours used for flat breads, a limited technical knowledge was observed in all the surveyed countries, especially (but not exclusively) for the most artisanal productions. Consequently, this information often remained undefined in the database (where "not specified" is indicated), reaching 71.6% of missing data, which was the highest percentage among all collected data. Where information on the quality features was available, protein and gluten content and gluten quality were the most frequently reported. For refined wheat flour the quality parameters generally were: protein content ≥ 9%, wet gluten content ≥ 25%, alveograph W ≥ 180 × 10−4 J, and P/L ≤ 1. For Pizza Napoletana Traditional Specialty Guaranteed (STG) more detailed quality information was available: dry gluten 9.5–11%, alveograph W 220–380 × 10−4 J, P/L 0.50–070, water absorption 55–62%, farinograph stability 4–12 min, farinograph drop off of consistency ≤60 Brabender Units [43]. For corn flour only protein content ≥7% was reported. It should be mentioned that data collection at bakers faced obstacles related to the COVID-19 pandemic restrictions and to the successive flour shortage following the Ukraine crisis. The economic value of bread and its scarcity have always important social repercussions, as evidenced by past and recent history. Bread availability provides a sense of security, while the lack of bread can be the cause of violent social movements. For example, in Lebanon, where the COVID-19 pandemic economic loss overlapped with a pre-existing crisis [44], further wheat shortage exacerbated the situation, making wheat and bread availability an extremely hot topic. Especially small producers suffered from the financial crisis on full blow, which took its toll on their sales to the point that they cannot afford even proper maintenance for their equipment. A similar situation was observed in Egypt and Jordan [45,46]. At various levels, lockdown-related economic losses and flour shortage were common to all countries, making bakers not really inclined to cooperate with the interviews. These issues were reduced, but not totally solved, with the help of local associations of bakers which introduced the researchers, or relying on direct knowledge with them.

2.3.3 Additional ingredients

Though many plain flat breads (54.3%) did not contain any lipid, olive oil was used in 19 cases, 10 of which were characterized by the use of the extra virgin category (Table 3).

Table 3. Type of fat eventually used in plain flat breads. Multiple answers were admitted, because breads could be prepared with different oils and fats or with blends.

Country	Olive oil		Sunflower oil		Vegetable oil (not specified)		Lard		None	
	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	1	1.4	2	2.9	1	1.4	-	-	3	4.3
Egypt	-	-	-	-	1	1.4	-	-	7	10.0
France	-	-	-	-	-	-	-	-	-	-
Greece	2	2.9	-	-	-	-	-	-	5	7.1
Italy	13[a]	18.6	-	-	-	-	8	11.4	12	17.1
Jordan	-	-	-	-	-	-	-	-	6	8.6
Lebanon	1	1.4	-	-	1	1.4	-	-	3	4.3
Malta	1[a]	1.4	-	-	-	-	-	-	-	-
Spain	1	1.4	-	-	-	-	-	-	2	2.9
Total	19	27.1	2	2.9	3	4.3	8	11.4	38	54.3

[a] Extra virgin olive oil is used in 9 Italian flat breads and in the Maltese one.

Two flat breads included sunflower oil in their formulation, and three were prepared with lard, namely the Italian *Piadina Romagnola, Crescentina* di Modena, and *Torta al testo*, whose official technical sheets [8,47–49], however, report also the possibility to use olive oil. The use of lard is traditional in the area of origin of these three flat breads, which is approximately the same area of Prosciutto di Parma PDO ham and is characterized by the presence of numerous pig farms. However, besides the obvious nutritional and health implications related to the reduction of saturated fatty acids, the substitution of lard with olive oil (possibly extra virgin olive oil), could eventually overcome religious restrictions for pork-derived ingredients. All the garnished flat breads contained vegetable oils or lard. Vegetable oils, when specified, were represented by olive, sunflower, or rapeseed oil, and their blends (Table 4). Greek, Italian, and French garnished flat breads were prepared with olive oil, and in particular extra virgin olive oil was used in the Italian *Focaccia di Recco* and *Pizza Napoletana* STG, agreeing with their official technical sheet [43,50]. Sunflower oil was used in the Croatian *Rudarska greblica* and *Zlevanka*, as well as in the *Polji ˇcki soparnik*, where, however, it was used in 50:50 mixture with olive oil. The French *Flammekueche*, of Alsatian origin (with German influence), was prepared with rapeseed oil. Lard, instead, was used in two Croatian (*Rudarska greblica* and *Zlevanka*) and ten Italian garnished flat breads (*Gnocco ingrassato, Focaccia Novese, Focaccia di Voltri, Crescia d'la stacciola, Crescia brusca, Pizza a scannatur di Carbone, Scarcedda, Pizza con i cingoli di maiale, Pizza con le sfrigole, Crescenta bolognese*). During kneading and baking several reactions take place, including lipid oxidation. Studies carried out in several types of Italian *focaccia* have shown that the level of oxidation may change by varying the type of toppings, with moist ingredients able to mitigate the rise of temperature during baking, thus exposing the lipid fraction to a less severe heat stress [8].

Excluding oils and fats, the garnished flat breads contain also other ingredients which impart them well-defined and recognizable sensory characteristics and, by varying in nature and quantity, differentiate them into a myriad of nuanced variations. These ingredients are used to stuff or season the dough before baking, and their combination generates a pleasant palatability which makes the final product become much more than a staple food. The additional ingredients can be of plant and/or animal origin. The plant-based ones, which include spices, various vegetables, cereals, or seeds, accounted for 40.5%, while the sum of the ingredients having animal origin (dairy products, eggs, cured meat, canned fish, and meat) totalized 59.5%. Cured meat includes several charcuterie products such as salami and ham, while fresh meat is typically pork meat, added for example to Italian *Pizza a scannatur* di Carbone prepared on the day of the pig slaughter.

Table 4. Characteristic ingredients of garnished flat breads. Multiple answers were admitted, because breads could be prepared with different fats and ingredients.

Country	Fats				Additional ingredients											
	Vegetable oil[a]		Lard		Plant-based ingredients[b]		Dairy products		Eggs		Cured meat		Canned fish		Meat	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	5	8.3	2	3.3	7	6.3	3	2.7	2	1.8	-	-	3	2.7	-	-
Egypt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
France	3	5.0	-	-	2	1.8	1	0.9	-	-	1	0.9	1	0.9	-	-
Greece	10	16.7	-	-	9	8.1	5	4.5	6	5.4	-	-	-	-	-	-
Italy	28	46.7	10	16.7	23	20.7	12	10.8	5	4.5	15	13.5	6	5.4	1	0.9
Jordan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebanon	1	1.7	-	-	3	2.7	2	1.8	-	-	-	-	-	-	1	0.9
Malta	-	-	-	-	1	0.9	1	0.9	1	0.9	-	-	-	-	-	-
Spain	1	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	48	80.0	12	20.0	45	40.5	24	21.6	14	12.6	16	14.4	10	9.0	2	1.8

[a] When specified, olive oil, sunflower oil, rapeseed oil or blends of these were used. [b] Spices, vegetables, cereals, seeds.

2.3.4 Leavening

Leavening agents were: compressed bakers' yeast (*Saccharomyces cerevisiae*) (55.8%), sourdough (16.7%), and baking powder (9.0%), while 18.6% flat breads were unleavened (Table 5).

Table 5. Type of yeast, if any, used in the preparation of flat breads. Multiple answers were admitted, because breads could be prepared with different types of yeast or yeast mixtures.

Country	Type of yeast							
	Baking powder		**Compressed yeast**		**Sourdough**		**None**	
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Croatia	2	1.3	9	5.8	2	1.3	3	1.9
Egypt	1	0.6	5	3.2	1	0.6	-	-
France	-	-	3	1.9	-	-	-	-
Greece	1	0.6	17	10.9	1	0.6	5	3.2
Italy	7	4.5	40	25.6	19	12.2	16	10.3
Jordan	3	1.9	4	2.6	-	-	1	0.6
Lebanon	-	-	6	3.8	-	-	1	0.6
Malta	-	-	1	0.6	2	1.3	-	-
Spain	-	-	2	1.3	1	0.6	3	1.9
Total	14	9.0	87	55.8	26	16.7	29	18.6

Sourdough, indeed, despite being the most traditional leavening method in the past, has overtime been partly replaced by compressed yeast, which is easier to handle and reduces leavening times. However, there is a renewed interest by consumers toward sourdough-leavened flat breads, also based on research results that prove nutritional and qualitative improvements, such as a reduction of phytic acid in whole meal flat breads [51], an increase of selenium bioavailability [52], and improved shelf life [53], texture and sensory properties [54,55]. More "modern" leavening aids, such as baking soda, are used in the Italian flat breads *Piadina Romagnola, Crostolo, Torta al Testo, Pizza scima, Gnocco fritto, Crescenta fritta*, in the Croatian *Pogaca z oreji* and *Zlevanka*, and in the Greek *Plakopita* (*Pita* bread baked on a stone). In the preparation of *Crescentina* di Modena baking soda can be used as an alternative to the most used compressed yeast.

Similarly, the Egyptian *Shamy* bread is leavened with baking powder as an alternative to compressed yeast. In three Jordan breads *(Mashrouh, Tannur,* and *Taboun*), instead, baking soda is used together with compressed yeast. Among the unleavened flat breads there is an Italian one whose multiple names all remind the absence of fermentation: "Pizza azzima" (which is the original name, literally meaning "unleavened pizza" in Italian), and its naming variations "*Pizza ascima*", "*Pizza scive*" and "*Pizza scime*". Another unleavened Italian flat bread, similar to the former, is the "Pitt'ajima", whose name is also clearly related to the original "*Pizza azzima*". The Egyptian *Shamsi* bread (whose name derives from the Arabic word "shams", meaning "sun"), instead, is a leavened one. Its leavening phase is interesting from the ethnographic point of view, because traditionally it takes place with the help of sun heat, i.e., open air, under the direct sunlight [16] (Figure 5).

Figure 5. Preparation of Egyptian *Shamsi* bread: dough exposed to the sun for fermentation.

This bread is decorated by making three crescent-shaped cuts, that form three angles as the dough rises. Coptic Christians, instead, make four cuts to obtain a roughly cross-shaped loaf [56] (Figure 6).

Figure 6. Egyptian *Shamsi* bread.

2.3.5 Baking

When specified, the declared baking temperatures were 300 °C (28.7%) (Table 6).

Table 6. Baking temperature adopted in the baking process of flat breads.

Country	Temperature (°C)							
	< 250		250 – 300		> 300		Not specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	7	4.9	1	0.7	2	1.4	4	2.8
Egypt	-	-	1	0.7	7	4.9	-	-
France	3	2.1	-	-	-	-	-	-
Greece	13	9.1	-	-	2	1.4	8	5.6
Italy	48	33.6	4	2.8	22	15.4	1	0.7
Jordan	-	-	1	0.7	4	2.8	1	0.7
Lebanon	1	0.7	3	2.1	3	2.1	-	-
Malta	-	-	-	-	1	0.7	1	0.7
Spain	-	-	-	-	-	-	5	3.5
Total	72	50.3	10	7.0	41	28.7	20	14.0

In the past, flat breads were baked only in wood-burning ovens, at very high temperatures, above 300 °C. Nowadays, the adopted baking temperature tends to be lower, below 250 °C, due to greater awareness on the risks related to the formation of thermal contaminants, such as polycyclic aromatic hydrocarbons (PAHs) [10,57] and acrylamide [58]. Besides the modern electric or gas ovens (belt conveyor tunnel ovens or batch ovens), several traditional baking systems are still used in the preparation of flat breads. This survey recorded 16 different traditional ways to bake flat bread (Table 7).

The method that most resembles the way flat breads were probably baked in antiquity was recorded for the Jordanian *Arbood*, the traditional bread of the Bedouins. This bread is prepared in the easiest possible way, i.e., unleavened and baked under hot ashes (a very rational way to cook, when an oven is not available). A fire is lit in a sandy area and, after the wood has burned, the dough disc is placed over hot ashes and covered with other ashes. During baking, bread is turned with the help of a stick, to cook evenly on both sides. Another simple baking tool is a metal grill placed on the embers. The one used for baking the Italian *Carchiola*, named r'ticula, has a pivot in the center, so that it can be turned without removing it from the embers of the fireplace. The convex circular metal griddle (named Saj in Middle East and Egypt and Satsi in Greece) (Figure 7) is used in a similar way to the metal grills, being placed on the embers. It has a large diameter, approximately 50 cm, and it is suitable to bake very large flat breads such as the Jordan *Shrak* (also named *Saaj* bread from its baking system) and *Mashrouh* and the similar Lebanese *Markouk* (named also *Saj* bread).

Table 7. Traditional baking systems used in the preparation of flat breads.

Traditional baking system	Country and breads
On a hot sandy ground, under hot ashes and embers	Jordan: *Arbood*
Metal grill (*R'ticula*) on embers	Italy: *Carchiola, Crostolo*
In the fireplace (named *Komin* in Croatia, *Camino* in Italy), under hot ashes and embers	Croatia: *Poljički soparnik*; Italy: *Crescia sotto la cenere*
On the hearth, under a bell-shaped iron lid (*Peka*) covered with embers	Croatia: *Kruh ispod peke* ("Bread under the lid" or "The *Peka*")
In the fireplace, under a terracotta lid (*Coppo*) covered with embers	Italy: *Pizza scime (or Pizza scive, Pizza ascima, Pizza azzima); Pizza somma*
Baking stone	Greece: *Plakopita*, *Spargana tou Christou*
Terracotta plate (*Tégia*)[a]	Italy, *Piadina Romagnola*
Terracotta plate, smaller than *Tégia* (*Tigella*)[b]	Italy, *Crescentina di Modena*
Terracotta plate with a terracotta lid (*Testo*)[a]	Italy: *Testarolo Pontremolese*, *Panigaccio*[c], *Torta al Testo*[d], *Neccio*[d], *Crescia sfogliata*[d]
Iron griddle	Spain: *Talo*
Circular convex metal griddle (*Saj* or, only in Greece, *Satsi*)	Jordan: *Mashrouh, Saaj*; Lebanon: *Saj;* Egypt: *Farasheeh*; Greece: *Fylla Perek (Perek sheets)*
Metal pan	Greece: *Pisia, Tiganopsomo*, *Sfakianopita, Fyllota*
	Italy: *Farinata*
Tinned copper pan (*Sole*)	Italy: *Borlengo di Guiglia*
Igloo-shaped clay oven (*Tabun*)	Jordan: *Tabun*
Vertical, tubular-shaped clay oven (*Tannur*)	Jordan: *Tannur*; Lebanon: *Tannur*
Refractory stone oven, dome-shaped	All surveyed countries, with many breads each

[a] Modern versions made of metal are currently used; [b] Modern versions of this cooking system consist of two flat cast iron discs, between which the dough is cooked; [c] *Panigaccio* is cooked without the lid, between two *testo* plates. Multiple *testo* plates can be stacked; [d] *Torta al Testo*, *Neccio* and *Crescia sfogliata* are cooked without the lid, but have to be flipped during baking to cook homogeneously on both sides.

Figure 7. A domestic *saj.*

The most traditional types of oven are those which retained the greatest differences among countries, being tightly related to local history and habits. Basically, two main baking systems were observed: baking plates (originally made of raw clay or terracotta, but nowadays generally substituted by iron cast), either coupled with lids or not, and vertical ovens: *tannūr* (transcribed also as *tannur* or *tannour*; pl. *tananir*) and *tabūn* (or *tabun*, *taboun*; pl. *tawabeen)*.

Baking plates, i.e. large, open and shallow vessels, have been documented for cooking food in various contexts—temporal, spatial, and cultural, in parts of Europe and the eastern Mediterranean [59]. Late Neolithic baking pans of the Balkans (early 5th millennium B.C.) and the subsequent baking plates of central Europe (late 5th and early 4th millennia B.C), are small- and medium-sized flat trays interpreted as being used for baking bread [60]. Furthermore, baking trays (25–40 cm diameter) with elaborate molded rims, appear in Syria during the Early Bronze Age (3200-2000 B.C.) and are found throughout the Levant during the Middle (2000-1600 B.C.) and Late Bronze Ages (1600-1100 B.C.). They are interpreted as vessels used on special occasions for baking bread or flat pies [61].

Baking lids have a documented ancient root in the Roman *testum.* Indeed, Cato reports in the *Liber de agri cultura* (chapters 74 and 75) that bread and other foods were baked "*sub testum*", i.e. on the hearth and under a terracotta lid named *testum* [62]. Instead, terracotta baking plates to be placed on the embers, named *testelli* (sing. *testello*), were reported in the Middle Age in central-northern Italy [62]. The influence of the original Latin word on the name "*Testo*" given to the terracotta plate with a terracotta lid traditionally used to bake the Italian *Testarolo Pontremolese* and *Torta al Testo* is evident, as is the influence on the name of the corresponding flat breads.

Another naming similarity is between *tigella* (pl. *tigelle*), the terracotta plate used to bake the *Crescentina di Modena* (which is very often named also *tigella*, after its baking system), and *taguella*, the typical flat bread prepared by the Touareg of Central Sahara [63]. The *tigella* terracotta plate derives its name from the Latin verb *tegere*, meaning "to cover", because the traditional way of cooking the *Crescentina di Modena* involved to place the dough on the red-hot *tigella*, to cover it with another *tigella* and to stack several of them in the fireplace. Chestnut or walnut leaves were used to avoid the direct contact of the dough with terracotta, as well as to flavor it and keep it clean from the ash.

Tannur and *tabun*, instead, are vertical ovens [2]. The *tannur* consists of a truncated conical structure (Figure 8).

Figure 8. Dough discs pressed onto the inner walls of *tannur* for baking.

Archeological remains of these ovens are widespread in the Middle East, Central Asia, northern India, North Africa and along the Mediterranean coasts [2]. The *tannur* is still used in the rural areas of the Middle East, particularly in Syria and Iraq, whereas it disappeared in Egypt, where it was used until the 19th century [64]. Embedded in the masonry, which acts as a workbench to prepare the dough and lay the bread loaves for cooling, the *tannur* is placed in a slightly inclined position to facilitate the introduction of food to be baked, including bread, through the circular opening at the top, or "mouth" [2]. The dough discs are rapidly pressed onto the inner walls of *tannur* with the aid of a "bread cushion" or directly by hand. The adhesion to the vertical walls can be helped by wetting the surface of the dough discs just before slapping them into the oven [2]. After about 1–2 minutes of cooking, the bread is taken out by metal tongs. The vertical ovens, like all wood-burning ovens, reach very high temperatures, exceeding 300 °C.

The *tabun*, instead, has an upper opening as the *tannur*, but has an "igloo" shape, wider than tall [2] (Figure 9). This kind of oven, of Palestinian origin, is present in Jordan also due to large presence in this country of Palestinian refugees [46,65-67]. It is used in a different way than *tannur* because, instead of slapping onto the inner walls, the loaves are placed on the oven floor, next to the embers, usually on a layer of hot pebbles [2]. During baking, the top opening of the oven is closed with a metal lid.

Figure 9. *Tabun* oven.

The wood-fired refractory stone oven, dome-shaped, was present in all the surveyed countries, but with a different "perception". In countries such as Italy, for instance, the presence of a wood-fired refractory stone oven ("*forno a legna*") in a *pizzeria* is perceived very positively and attracts customers. On the contrary, the traditional "*furn fallahi*" (farmer oven) used to bake the *Bataw* bread in the rural areas of Egypt is perceived as obsolete and has been almost abandoned (although it will likely make a comeback due to the sharp rise in the price of gas following the current Ukraine crisis).

2.3.6 Bread characteristics

Regarding size and shape (Table 8), 50 breads had a diameter between 10 and 40 cm, while seven breads were larger than 40 cm: the Italian *Borlengo* and *Testarolo*, the Croatian *Poljički soparnik*, the Jordan *Saaj* (or *Shrak*) and *Mashrouh*, the Lebanese *Saj* (or *Markouk*) and *Khobz*. Only one bread was smaller than 10 cm, namely the Italian *Crescentina di Modena*. Not all flat breads are circular: 34.2% of them were oval or rectangular.

Table 8. Shape characteristics of flat breads.

Country	Circular (diameter, cm)								Other shapes [a]	
	< 10		10-40		> 40		Not specified			
	Number	%	Number	%	Number	%	Number	%	Number	%
Croatia	-	-	4	2.8	1	0.7	-	-	9	6.3
Egypt	-	-	5	3.5	-	-	3	2.1	-	-
France	-	-	-	-	-	-	-	-	3	2.1
Greece	-	-	18	12.6	-	-	-	-	5	3.5
Italy	1	0.7	17	11.9	2	1.4	28	19.6	27	18.9
Jordan	-	-	3	2.1	2	1.4	1	0.7	-	-
Lebanon	-	-	5	3.5	2	1.4	-	-	-	-
Malta	-	-	2	1.4	-	-	-	-	-	-
Spain	-	-	3	2.1	-	-	-	-	2	1.4
Total	1	0.7	57	39.9	7	4.9	32	22.4	46	32.2

[a] Rectangular or oval.

An important quality characteristic of flat breads was golden color (45.4%). Also texture was relevant, which should be crunchy (12.2%) for the hard flat bread types and soft (14.1%) for those pliable and rollable (Table 9).

Table 9. Principal quality characteristics of flat breads. Multiple answers were admitted, because breads could show more quality characteristics at the same time.

Country	Quality characteristics							
	Golden color		Crunchy texture		Soft texture and/or pliability		Not specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	13	8.6	2	1.2	1	0.6	-	-
Egypt	5	2.9	-	-	-	-	3	1.8
France	2	1.3	-	-	-	-	1	0.6
Greece	13	8.6	7	4.1	11	6.5	-	-
Italy	21	15.4	8	5.2	8	4.7	41	24.1
Jordan	5	2.9	-	-	-	-	1	0.6
Lebanon	6	3.6	1	0.6	3	1.8	-	-
Malta	1	0.7	2	1.2	1	0.6	-	-
Spain	2	1.3	-	-	-	-	2	1.2
Total	68	45.4	30	12.2	24	14.1	48	28.24

In the marketing classification, bread is included in the group of products of frequently purchased products, characterized by short shelf life and subject to high risk of waste [15,68,69]. The shelf life of flat breads ranged from shorter than 3 days (58%), between 3 and 7 days (27.3%) and up to one year (9.1%) in case of hard, dry flat breads (Table 10). Hard breads were obtained by means of a two-step baking: the first to cook and the second to dry them. Traditionally, this procedure was typical of breads to be carried during the transhumance of sheep [2]. These flat breads were the Croatian *Mlinci* (meaning “Mills”) and *Zagorski mlinci*, the Egyptian *Bataw* and *Merahrah*, the Greek *Fylla Perek*, the Italian *Pane Carasau*, *Schuttelbrot*, *Guttiau*, *Pistoccu*, *Zichi* and *Puccia ladina*, the Lebanese *Mullat al smeed* and the Spanish *Torta Cenceña* and *Torta de Gazpacho Manchego*. The latter only shares its name with the cold vegetable soup named *gazpacho* consumed during the summer mainly in the Andalusia region. *Gazpacho Manchego*, indeed, is a game meat stew from the Castilla La Mancha region, traditionally eaten with unleavened bread cakes. Its most peculiar ingredient is the unleavened bread cake (*Torta*) which, originally, was the plate for *Gazpacho Manchego*.

Table 10. Shelf life of flat breads.

Country	Shelf life							
	< 3 Days		3-7 Days		Up to 1 year[a]		Not specified	
	Number	%	Number	%	Number	%	Number	%
Croatia	3	2.1	1	0.7	2	1.4	8	5.6
Egypt	1	0.7	5	3.5	2	1.4	-	-
France	2	1.4	1	0.7	-	-	-	-
Greece	19	13.3	3	2.1	1	0.7	-	-
Italy	48	34.6	21	14.0	6	3.7	-	-
Jordan	5	3.5	1	-	-	-	-	-
Lebanon	3	2.1	3	2.1	1	0.7	-	-
Malta	2	1.4	-	-	-	-	-	-
Spain	-	-	4	2.8	1	0.7	-	-
Total	83	58.0	39	27.3	13	9.1	8	5.6

[a]Dry breads.

In 5.6% of cases the shelf life was not specified because the product was prepared at a very small scale level and marketed unpackaged.

Studies showed that modified atmosphere packaging (40% carbon dioxide and 60% nitrogen), coupled with an oxygen absorbent sachet, prolonged the shelf life of *pita* bread up to 28 days [70]. Alternatively, sodium propionate (0.3%) can be added [71]. Innovative solutions for extending the shelf life of bread are under study, such as ethanol and/or essential oil emitters, antimicrobial films, nanopackaging, biodegradable and renewable packaging, and edible coatings [72].

2.3.7 Artisanal vs industrial breads

Typically, the surveyed flat breads showed an artisanal character (82%) (Figure 10). Those produced at industrial level accounted for 7%, and were: *Piadina* (Italy), *Kmaj* (Jordan), *Mlinci* and *Kukuruzna miješana ciabatta* (Croatia), *Souvlakopita* (*Pita* bread) (Greece), *Baladi* and *Shami* bread (Egypt), *Khobz* (Arabic bread) (Lebanon). Another 11% is produced either way.

The Egyptian *Bataw*, which was the most traditional farmer bread in Egypt, can be considered a case-study. It was not standardized, being made in a different way in different places. Farmers could prepare *Bataw* bread with wheat, corn, or mixes of these flours (the most common option), with or without fenugreek. Furthermore, this kind of bread was produced either in soft form and, to prolong shelf-life, in hard, dry form.

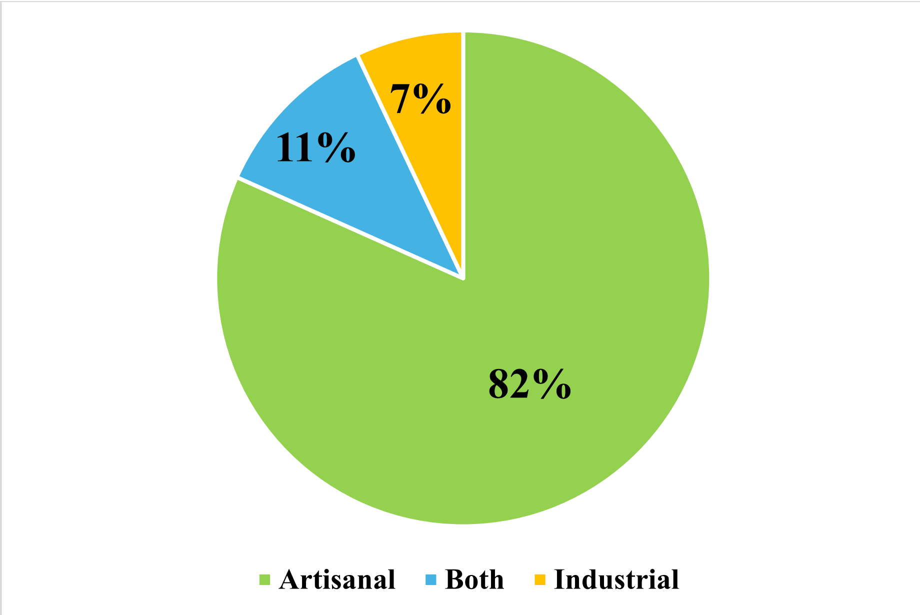

Figure 10. Percentage of flat breads produced in artisanal or industrial mode.

These findings demonstrate that the formulation of *Bataw* bread, a traditional product made mostly on a family basis, depends on the local availability of raw materials and personal preference of the family. However, this bread is currently not as widely consumed as in the past mostly due to the subsidization of another bread, namely *Baladi* bread. It should be considered that in Egypt bread consumption is one of the highest in the world, so bread subsidies have a strong influence on consumer choice [34]. Likely, changes in lifestyle and increasing urbanization may further enhance the abandonment of *Bataw* breadmaking. Therefore, there is a concrete risk of losing the memory and knowledge behind this kind of bread. This happens continuously to many food products, everywhere, but since bread is much more than simply a nutritious food, being linked to identity and local knowledge [3], abandoning a certain type of artisanal bread, in favor of a more industrialized one, is a phenomenon with important cultural repercussions.

Besides the *Bataw*, other breads which were found to be made on a family basis were the Egyptian *Zallut*, *Khobz min el dorra al rafi'ah, Farasheeh,* and *Merahrah*; the Jordan *Arbood*; the Lebanese *Mishtah el jreesh* and *Mullat al smeed*; the Italian *Carchiola*, *Puccia alla spasa* and *Pitt'ajima*; the Greek *Lambropsomo*, *Christopsomo*, *Spargana tou Christou* and *Vasilopita trifti* (whose cultural importance and ethnographical aspects are highlighted in the next paragraph).

2.3.8 Specific consumption patterns

The analysis of data also provided a cultural view of flat breads within each country. Preferences for food, including bread, are influenced by culture, family habits, traditions, religious believes and income [68]. One of the characteristics of contemporary consumer behavior and habits is that people often purchase products (including food products) not because they are "used for something", but because they symbolize something, as described by Solomon et al. [73] and Jones [74]. The role a product plays in people's lives, indeed, extends beyond the practical functions it fulfils. Bread is a "soul food" and is the symbolic food *par excellence*, to be treated with the respect due to what it represents. A strong link has been observed between the consumption of a specific flat bread type and certain periods of the year having a religious meaning, highlighting the iconic nature of cooking and eating [74]. The Greek *Lambropsomo* (Easter Bread) and *Lagana* are related to Easter [16]. The *Lambropsomo* is prepared by intertwining three long cylinders of dough, symbolizing the Holy Trinity, and on the surface of the dough four hard-boiled red-colored eggs are placed, which remind the blood of Jesus Christ. The *Lagana* is specifically consumed on Clean Monday (*Kathari Deutera*), i.e. on the first day of Lent of the Eastern Christianity, when sinful attitudes and non-fasting foods are left behind. That day is also named "Ash Monday", by analogy with Ash Wednesday (the day when the Western Churches begin Lent). Again in Greece, the *Christopsomo* is the typical Christmas Bread, eaten on Christmas' eve. The surface of this bread is decorated with a cross of dough and walnuts in shell [16]. The latter symbolize rebirth and are believed to bring prosperity to the family. Also the Greek *Spargana tou Christou* is related to Christmas. The name of these very thin pancakes, prepared during Christmas lent, literally means "The swaddling clothes of Jesus" [16]. The *Vasilopita trifti* or "New year's bread", instead, is a traditional Greek bread served at midnight on New Year's Eve to celebrate the life of Saint Basil of Caesarea (*Agios Vasilis*), who is Santa Claus according to Greek Christmas traditions [16]. After baking, a coin is inserted through the base of the bread, and whoever finds it is said to be granted luck for the rest of the year.

In Croatia, *Zagorski mlinci* are traditionally eaten with turkey at Christmas and New Year. Moreover, *Poljički soparnik* is known as the "poor man's dish" because it was always prepared on fasting days (All Saints' day or Good Friday) and holidays such as Christmas.

The Lebanese *Mishtah el jreesh*, now become a rare bread prepared only at home, is particularly associated with the Muslim month of Ramadan, for breaking the fast, while the *Mullat al smeed*, another rare Lebanese bread, was traditionally brought during the *Hajj* (pilgrimage to Mecca) because if its long shelf life (being dried) but, as travel times became shorter, *Mullat al smeed* began to disappear [75]. The meat variant of *Manouche*, instead, called *Lahem b aajin* (or "meat in dough"), is a "go to" meal for funerals and condolences in certain regions of Lebanon.

In Italy, the Sardinian double-layered flat bread *Spianata* was traditionally prepared in a decorated version for weddings, by using a special stamp named *pintadera*. The Italian *Borlengo* was typically consumed at Carnival, so its name is related to the Italian word "*burla*", meaning "joke" [16]. The *Pizza a scannatur di Carbone*, instead, was prepared on the day of the pig slaughter which, in the past, represented an important day, to be celebrated as a collective rite ending with a common lunch for those who helped in the slaughter [16].

2.4. Promoting the tradition

The application of quality schemes to flat breads deserves a specific discussion. As a way for promoting the most traditional and artisanal foods, several quality marks have been set up. This action is aimed at keeping their knowledge alive, reducing the erosion of food diversity, which is a modern problem induced by the globalization of food products.

The EU Regulation No 1151/2012 [20] provides the legal framework on quality schemes to protect the name of food and beverages having unique characteristics linked to their geographical origin as well as traditional know-how [20]. These quality schemes include the PDO, granted to products whose production, processing and preparation are entirely made within a particular geographical area; the Protected Geographical Indication (PGI), awarded to products for which at least one of the stages of production, processing or preparation takes place within a particular geographical area; the TSG, for products whose quality is not linked to a specific geographical area but is based on traditional processing methods or recipes. These quality marks are all recognizable by the presence of specific logos in the label of food and bring benefits to producers, who keep the exclusive right to use the protected name and usually get a premium price. At the same time, the consumers have a proof of heritage and tradition of food specialties they buy.

Many of the surveyed flat breads have been awarded of quality marks (Table 11). Five were PGI (namely the Italian *Piadina Romagnola*, *Focaccia di Recco* and *Schuttelbrot dell'Alto Adige*, as well as the Croatian *Poljički soparnik'* and *Zagorski mlinci*) and one was TSG (the *Pizza Napoletana*). Furthermore, being the "art" of the Neapolitan pizza-makers (*pizzaiuoli napoletani*) globally renowned, it has been inscribed on the Representative List of the Intangible Cultural Heritage of Humanity by the United Nations Educational, Scientific and Cultural Organization (UNESCO). Similarly, "the culinary art and culture of flattened sourdough flat bread *Ftira"*, was added by the UNESCO to the same list, being a key part of the cultural heritage of the inhabitants of the Maltese archipelago.

Table 11. Flat breads awarded by quality marks. PGI = Protected Geographical Indication; TSG = Traditional Specialty Guaranteed; PAT = *Prodotti agroalimentari tradizionali* (Traditional Agri-food Products); DeCO = *Denominazione Comunale di Origine* (Municipal Designation of Origin).

Quality mark		Releasing organism	Geographic validity	Number of breads	Bread names and country
Name	**Acronym**				
Protected Geographical Indication	PGI	European Commission	EU	5	*Piadina Romagnola* (Italy); *Schuttelbrot* (Italy); *Focaccia di Recco* (Italy); *Poljički soparnik'* (Croatia); *Zagorski mlinci* (Croatia)
Guaranteed Traditional Specialty	TSG	European Commission	EU	1	*Pizza Napoletana* (Italy)
Intangible cultural heritage of humanity	-	UNESCO	Global	2	The culinary art and culture of flattened sourdough flat bread *Ftira* (Malta); The Art of Neapolitan Pizza-makers (Italy)
Slow Food presidium	-	Slow Food Foundation for Biodiversity	Global	2	*Testarolo Pontremolese* (Italy); *Mungia Talo* (Spain)
Intangible cultural goods	-	Ministry of Culture, of the Republic of Croatia	Croatia	1	*Pogača z oreji* (Croatia)
Croatian Quality	-	Croatian Economy Chamber	Croatia	1	*Pogača Pogacha* (Croatia)
Municipal Designation of Origin	DeCO	Italian Municipalities	Italy	2	*Crostolo di Urbania* (Italy); *Farinata di Imperia* (Italy)
Traditional Agri-food Products	PAT	Italian Ministry of Agriculture, Food and Forestry	Italy	63	See detailed list in Table 12

Table 12. Italian flat breads awarded by the Italian quality mark "*Prodotti Agroalimentari Tradizionali*" (PAT, meaning "Agri-food Traditional Products"), geographically subdivided based on their origin in northern, central, southern Italy or its islands.

Region	No.	Bread names
Northern Italy		
Aosta Valley	-	-
Piedmont	2	*Farinata, Focaccia Novese*
Trentino-Alto Adige	-	-
Friuli-Venezia Giulia	-	-
Veneto	-	-
Lombardy	1	*Schiacciatina*
Emilia-Romagna	8	*Crostolo, Borlengo di Guiglia, Crescentina, Gnocco fritto, Crescenta fritta, Focaccia con ciccioli, Erbazzone, Crescenta*
Liguria	3	*Testarolo della Lunigiana, Farinata, Focaccia*
Total	*14*	-
Central Italy		
Tuscany	6	*Testaroli, Panigaccio, Neccio, Farinata, Schiaccia grossetana, Focaccia con i friccioli*
Umbria	2	*Torta al Testo, Schiacciata al formaggio*
Marches	5	*Crostolo, Cresciolina, Crescia sotto la cenere, Crescia d'la stacciola, Crescia brusca*
Abruzzo	2	*Pizza Scime (or Pizza scive, Pizza ascima, Pizza azzima), Pizza con le sfrigole*
Lazio	7	*Pizza bianca romana, Pizza con farina di mais, Pizza somma, Pizza rossa, Pizza fritta, Pizza sotto la brace, Pizza a fiamma*
Molise	3	*Pizza coi cicoli di maiale, Pizza di granone, Pizza scimia*
Total	*25*	-
Southern Italy		
Apulia	10	*Puccia salentina, Focaccia barese, Focaccia di S. Giuseppe, Calzone di Ischitella, Focaccia a libro di Sammichele di Bari, Paposcia, Pitilla, Pizza sfoglia, Scannatedda, Sceblasti*
Basilicata	4	*Carchiola, Pizza a scannatur di Carbone, Scarcedda, Pizza con i cingoli di maiale*
Calabria	1	*Pizza di maggio*
Campania	3	*Pizza, Pizza di farinella bacolese, Pizza di scarola*
Total	*18*	-
Islands		
Sardinia	6	*Carasau bread, Guttiau, Spianata, Pistoccu, Zichi, Focaccia Portoscusese*
Sicily	1	*Sfincione*
Total	*7*	-

According to the Slow Food Foundation for Biodiversity, Slow Food Presidia should be good tasting, sustainably produced, should have a local and social dimension, and represent a sense of place. This recognition requires that producers join a project to safeguard biodiversity and form a community. Two flat breads of this survey were Slow Food presidia, i.e. the Italian *Testarolo Pontremolese* and the Spanish *Talo*.

In Croatia, the quality schemes "Intangible cultural goods" (awarded by the Ministry of Culture, of the Republic of Croatia), and "Croatian quality" (by the Croatian Chamber of Commerce) applied to *Pogača z oreji* and *Pogača*, respectively.

The "*Denominazione Comunale di Origine*" (DeCO, meaning "Municipal Designation of Origin"), instead, is granted by the Italian Municipalities to recognize, promote and protect high quality artisanal agri-food products indigenous to their municipal territory. Two Italian breads were DeCO, while the remarkable number of 63 were *Prodotti Agroalimentari Tradizionali* (PAT, meaning "Traditional Agri-food Products"), which is another (more prestigious) Italian recognition awarded by the Italian Ministry of Agriculture to foods prepared according to traditional processing systems, homogeneous in the geographic area and consolidated through a period of time not inferior to 25 years (Tab. 12).

A list of PAT is released yearly, with new entries and eventually deletions for products which have been upgraded to PDO, PGI or TSG [76]. PAT flat breads were homogeneously distributed throughout the country.

Rare breads, instead, prepared only by the household cooks for family consumption and not for sale, were surveyed by the Slow Food Foundation for Biodiversity within the "Ark of Taste" project. The se breads were the Egyptian *Zallut*, *Khobz min el dorra al rafi'ah, Farasheeh,* and *Merahrah* [77], the Lebanese *Mishtah el jreesh* and *Mullat al smeed* [75], the Jordan *Arbood* [78] and the Italian breads reported in Table 13. The Ark of Taste project is aimed at keeping alive small-scale quality productions tightly linked to local culture, history and tradition [79,80].

Table 13. Breads surveyed by Slow Food Foundation for Biodiversity as "Ark of Taste".

Country	**Number**	**Bread names**
Egypt	*5*	*Khobz min el dorra al rafi'ah, Farasheeh, Zallut, Merahrah, Shamsi*
Italy	*7*	*Carchiola, Pizza scime (or Pizza scive, Pizza ascima, Pizza azzima), Pitt'ajima, Pizza a fiamma, Puccia alla spasa, Puccia ladina, Focaccia a libro di Sammichele*
Jordan	1	*Arbood*
Lebanon	2	*Mishtah, Mullat al smeed*
Total	15	-

It is indeed fundamental to draw attention to these local rare foods which are at risk of extinction, to prevent the reduction of food and cultural biodiversity. The Egyptian *Shamsi* bread, for example, which was another rare bread, has recently increased its popularity and has now gained certain market, as proved by the large number of webpages released by searching its name in Google. A possible means for keeping alive these food products is also to show their production to the tourists, who are attracted by local traditions, such as for the Jordan *Arbood* bread. This bread is typically baked for tourists enjoying the Wadi Rum tours. In this way, the promotion of traditional foods can generate an income to the local communities [81].

Another means to enhance the knowledge of rare flat breads is represented by the food blogs which attract the interest of many people prompt by the will of learning new recipes and rediscovering old ones. These tools, as well as Youtube videos, or even Wikipedia, easily carry the information also far from the area of origin. For example, the Italian breads inserted in the Ark of Taste (namely *Carchiola, Pizza scime - or Pizza scive, Pizza ascima, Pizza azzima - Pitt'ajima, Pizza a fiamma, Puccia alla spasa* and *Puccia ladina*) are all the object of several blogs and are mentioned in Wikipedia pages, with *Carchiola* having its own entry.

In Lebanon, instead, the popular garnished flat bread *Manouche* is the object of the "World *Manoucheh* Day", on November 2nd, though this bread has not an official quality label yet.

2.5. Conclusions

This survey gives an overview of the different recipe, process and product quality specifications of flat breads produced in the examined countries, as well as an insight on the related local culture. It appears that flat breads are really a large and diverse family of food products, with a rich history and ethnographical dimension.

Their main technical characteristics could be summarized as follows. Flours were from soft wheat (67.4%), durum wheat (13.7%), corn (8.6%), rye, sorghum, chickpea, and chestnut (together 5.2%). All garnished flat breads contained vegetable oils or lard, while 54% of plain flat breads did not contain fats. Leavening agents were: compressed yeast (55.8%), sourdough (16.7%), or baking powder (9.0%); 18.6% flat breads were unleavened. The baking temperatures were < 250 °C (50.3%), between 250 and 300 °C (7%), and > 300 °C (28.7%). Sixteen old-style baking systems were recorded, classified into baking plates and vertical ovens (*tannur* and *tabun*). Artisanal flat breads accounted for 82%, while the industrial for 7%. The diameter of breads was between 10 and 40 cm (39.9%), < 10 cm (1%), > 40 cm (4.9%). Not all flat breads were circular: 34.2% of them were oval or rectangular. The shelf life ranged from shorter than 3 days (58%), between 3 and 7 days (27.3%) and, in case of hard, dry flat breads, up to one year (9.1%). The main quality characteristics were golden color (45.4%), crunchiness (12.2%), and softness (14.1%) for hard and rollable flat breads, respectively. Quality schemes (national, European or global) applied to 91 flat breads.

In addition to the technical aspects, a clear social, ethnographical and cultural dimension was identified. Twelve flat breads were strongly associated to religious celebrations, and 15 flat breads were rare, prepared only by the household cooks in a rural environment, for family consumption.

The collected information, gathered in a publicly available database, will be fundamental for allowing further valorization and dissemination activities, and will be useful for the selection, within each one of the examined country, of the most suitable flat breads for nutritional fortification and technical innovation within the FlatBreadMine or any other research project.

Criteria for selecting breads can be proposed, as follows:

1) The type of bread chosen must be native to the country and widely consumed throughout the national territory. To maximize the nutritional impact on the general population, it would be of little usefulness to fortify a flat bread produced on a small-scale, consumed only in a restricted geographical area.

2) If different categories of flat bread are available in the considered country, all equally diffused, more than one bread should be selected, to represent the single-layered, double-layered, and garnished or fried products.

2.6. Future perspectives

Traditional and rare flat breads, prepared in a genuine and not globalized way using local raw materials, in addition to not risking the shortage of imported flours for geopolitical or climatic reasons, are well adapted to the territory and sustainable. However, these breads may progressively disappear due to changes in lifestyle and increasing urbanization, and their loss would lead to genetic erosion. Actions are therefore needed to prevent the reduction of cultural and biological diversity related to their disappearance. The strategy for such preventing actions, and directions for future research, should involve: 1) periodically surveying the existing flat bread diversity, with the same approach used in this article. It is worth noting that the FlatBreadMine database can be easily updated, as well as extended to include many other countries; 2) applying quality schemes to the most genuine and high quality products; 3) disseminating information and raising awareness by promoting the products both remotely (food blogs, Youtube video) and in person, also among tourists (because food has to be tasted, to see it is not enough).

Traditional flat breads produced on a small-scale and those, more industrialized, on a large-scale, have totally different features and should follow distinct paths, with the first as a specialty dedicated to a niche consumer, carrying a strong cultural message, and the latter for mass consumption, able to guarantee a real impact on the population if nutritionally improved.

2.7 Supplementary materials

The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3 390/foods11152326/s1, Table S1: List of questions for interviewing the bakers on flat bread production.

Supplementary Table S1. List of questions for interviewing the bakers on flat bread production.

Product
What is the most common name of this flat bread? Please specify if you know also other names.
Is the production artisanal or industrial? (This question aims to understand how important the contribution of practical experience and manual skills is in the production of flat bread)
Is this flat bread produced throughout the entire country or is typical of a single town or region? (In the latter case specify the name of town or region)
In case this flat bread is produced in a single geographic area, is it marketed also outside that area?
What is the thickness of the flat bread? What is its diameter? What is its weight?
What are the main quality features of the flat bread? (Flexibility, uniformity of color, regularity of shape…)
What is the shelf life of the product? Is the product packaged? How? (Which material? Under modified atmosphere? If yes which type?)*
What are the storage conditions?*
Process
What's the recipe? (Only flour, water, yeast and salt or also other ingredients? Possibly give the ratios)
What kind of flour is used (Whole grain or refined? From durum wheat, soft wheat, or other cereals or non-cereal grains?)
What are the quality criteria for selecting the raw material, if known? (Protein content, gluten content, gluten quality indices)
Is yeast used? What kind of yeast is used? (Commercial bakers' yeast, chemical yeast, sourdough?)
If fats are included in the dough, what type of fats are used? (For example lard or oil)
Which kneader is used? What is the time of kneading?
What are time and temperature of leavening?
How is shaping conducted? (Manually or mechanically?)
Is there a second leavening? (Time and temperature?)
What kind of oven is used? (Modern gas / electric oven or old-style wooden fueled oven? Is any traditional baking system used, such as "*testo*", "*tannur*" etc.?)
Could you specify time and temperature of cooking?
Is a second cooking performed to dry bread? What are time and temperature?
Cultural and ethnographic features
Is there any relationship between this bread and a specific religious celebration or an important time of the year for any rural tradition (harvest, sowing, etc.)?

**Questions removed after consulting with the President of a consortium of bakers specialized in the production of flat bread, because were considered too technical.*

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Chapter 3. Traditional Italian flatbreads: cultural diversity, processing technology and future perspectives

This chapter has been previously published as: Mefleh, M.; Vurro, F.; Summo, C.; Pasqualone, A. (2024). Traditional Italian flatbreads: cultural diversity, processing technology and future perspectives. *J. Ethn. Foods*, *11*(1), 24. **https://doi.org/10.1186/s42779-024-00238-2**

Traditional Italian flatbreads: cultural diversity, processing technology and future perspectives

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Graphical Abstract

Abstract

Flatbreads are particularly prevalent in the Mediterranean region, including Italy, where each community has its unique traditional recipe, preparation method, and baking system. This traditional narrative review provides an overview of the Italian flatbreads that have achieved national or international quality recognitions. The aims of this study are firstly, to scientifically evaluate these flatbreads and establishing a catalogue that includes both technical and cultural information, which are currently missing in the international scientific literature; secondly, to conduct a comparative analysis of the technical and cultural diversity of traditional Italian flatbreads, outlining areas for future research development. The examined flatbreads were found to be characterized by considerable diversity, reflecting the Italian region's diverse culinary heritage. The formulation is generally simple and includes flour, water, possibly yeast, and salt. Additional ingredients are region-dependent, reflecting local availability and include fats of animal origin, or ham, mostly found in flatbreads from Northern Italy; while olive oil or EVOO are common in flatbreads of Tuscany, Liguria, and Sardinia. The types of flour also differ regionally: besides soft wheat flour, durum wheat semolina is used in southern Italy and Sardinia, chestnut flour in Tuscany, rye flour in Alto Adige, chickpea flour in Liguria, and corn flour in central Italy. Historically, high-extraction flour and sourdough were largely used but have been replaced by refined flour and commercial baker's yeast over time. Flash baking (short time, high temperature) is generally adopted and some flatbreads, typical of Sardinia, are baked twice, resulting in complete dryness and long shelf-life. In contrast, quickly prepared unleavened bread, are a staple in the Tuscan-Emilian Apennines, Lunigiana, and the Po Valley. Overall, these results suggest encouraging the revival of the ancient baking tradition of using high-extraction flours and sourdough fermentation, which today are almost lost. Reintroducing these methods could increase the fiber, mineral and, vitamin content and ensure a rich sensory profile. Further research could focus on improving the nutritional quality, particularly, through salt reduction, acrylamide levels mitigation, and protein content increase. The lack of historical information highlights the need to perform historical research to gain a deeper understanding of origins, evolution and characteristics of Italian flatbreads.

Keywords: Italy, alternative flours, quality schemes, typicality, regional food, gastronomy, *piadina*, *pane carasau*, *testo*, *tigella*

3.1 Introduction

The earliest type of bread attested in history is unleavened and flat dating back to 14400 BCE [1], even before the Neolithic age and the advent of agriculture. It was not until 1300-1500 BCE that leavened bread was produced by Ancient Egyptians [2]. Flatbread remained in the traditional cuisine of many cultures around the world serving as a staple food [3-6], with a global market valued at $41.17 billion in 2019 and expected to reach $62.8 billion in 2026 [7].

Flatbread typically consists of a few basic ingredients, flour, water, salt, and, depending on whether it is leavened or unleavened, yeast. Optional ingredients can be added by incorporating them into the dough or as toppings, making the flatbread a perfect food carrier [8]. Flatbreads are the basis for the preparation of popular fast-food products around the world, including take-out *pizza*, *focaccia*, pancakes, and crepes [9]. In addition to being used as a spoon for grabbing food, they can be covered by food as an "edible plate" (*focaccia* and *pizza*), rolled around food as a "food wrap," or stuffed and folded as sandwiches [9,10].

Particularly prevalent in the Mediterranean region, flatbread accompanies almost every meal, and each community has its unique traditional recipe [11], preparation method, and baking system [12]. The latter is often a portable tool (such as a metal griddle or plate to be placed in the fireplace) rather than a regular oven, reflecting the restriction on the use of ovens in the past. In the Middle Ages, the construction and operation of ovens were restricted to the baker and upper-class families [13], while the lower classes had to bake bread in the communal oven, paying for its use. Clergymen could be exempt from paying the communal oven tax, as prescribed in the city of Altamura (Italy) by an edict of 1420 [14].

According to EU Regulation no. 1151/2012, foods that are part of the cultural heritage of the communities living in a specific geographical area are "typical" as their qualitative characteristics strictly depend on the local microflora, the composition of the soil and water, and the specific processing techniques [15]. The European recognitions granted to these foods are "Protected Designation of Origin" (PDO), "Protected Geographical Indication" (PGI), and "Traditional Specialties Guaranteed" (TSG) [15], depending on the strength of the link with the place where foods are made. Italy also established national quality schemes for protecting local specialties, such as the "*Prodotto Agroalimentare Tradizionale*" (PAT) or "Traditional Agri-food Product" [16], not to forget the "Slow Food Presidium" (SFP) recognized by the Slow Food Foundation for Biodiversity [17]. Italy is the European country with the highest number of foods recognized as typical, numbering 326 in 2023 excluding wines: 173 PDO, 149 PGI, and 4 TSG products [18]. Within this vast range of specialties, many are flatbreads.

The precious value of traditional Italian flatbread is due to the distinctive handcrafting techniques and methods of preparation and baking, that have been passed down through generations. The transmission of knowledge to master the art of breadmaking, partly oral and partly based on the replication of traditional gestural behaviors, was operated in the past by older women to girls of the same family [19]. This empirical knowledge, survived thanks to the efforts of passionate local scholars who recorded the testimonies of elders, is preserved thanks to protection consortia, by national and European legislation [15,16]. Italy is a long country with a very diverse orography and microclimate, historically composed of numerous duchies, small kingdoms, and states that merged into a national unity only in 1861. So, rather than a unified culture, Italy is characterized by great regional diversity and a strong local identity, called "*campanilismo*" (localism), which are most evidently manifested in the richness of the gastronomic landscape and culinary culture [20]. In addition, bread characteristics are primarily influenced by factors such as the grain used, flour type class, leavening and baking conditions, shape and size, along with the salt quantity and even the type of water used. Such diversification has given rise to what can be described as a "geography of bread", with more than 200 regional bread varieties having artisanal character [21,22]. In 2021, sales of artisanal bread totaled more than 1,400,000 tons, reaching 84.1% of the total bread sold in Italy, while industrial bread reached only 15.9% [23].

Traditional Italian flatbreads originated from a predominantly agricultural society that was very much alive until recent decades, but no longer exists today because of hard work in the fields. In the past, except for the nobility, most people could rarely afford "white bread," that is, bread made from refined flour [24], as well described by the Italian writer Giovanni Verga (1840-1922). In his 1882 novel "*Pane nero*" (literally "black bread," i.e., wholemeal bread, darker and denser than "white" bread), set in the Sicilian countryside of the late 19th century, Verga describes the constant struggle of peasants to obtain at least some black bread [25]. Also in the rest of the country, flatbreads prepared with little wholemeal flour mixed with chestnut, corn, or rye flour, or even, in times of famine, with ground tree bark, were a sign of food scarcity and poverty [26]. These flatbreads, prepared with inexpensive flours and mostly baked in the fireplace without the aid of an oven, were linked to everyday life. On the contrary, "white", thus "pure" and noble versions of the same flatbreads were prepared with refined wheat flour for festive and religious events. Decorated with flowers, hearts, crosses, doves, or solar symbols, these ritual breads were associated with weddings, Easter, All Saints' Day, New Years's Eve, and celebrations of patron saints (St. Rita, St. Restituta, St. Palmerio, St. Anthony) [19,27-29]. Ritual flatbreads were entrusted with the task of conveying auspicious or propitiatory messages for the new year or season, which could protect a couple (wedding breads) or a community (devotional breads) from the threats of natural events that could disrupt agricultural activities [27]. The preparation of breads for All Saints' Day in Nuoro, Sardinia, is described as a key event in the late 19th-century novel "*La Via del Male*" ("The Way of Evil") by the Italian writer Grazia Deledda (1871-1936) [30]. Nonetheless, especially when viewed from the perspective of today's economic well-being and under the leveling effect of globalization, also the "poor" flatbreads have a story to tell, and knowing it means recovering the memory of one's origins, not to mention their fundamental importance for the identity of Italian communities abroad [31]. The traditional food of a community is a cultural medium because it is a sign of continuity of ethnic identity between past and present, with no other food having acquired such an important and pervasive symbolic force over the millennia as bread [32].

Despite the cultural and economic importance of traditional Italian flatbreads, most of them have never been the subject of scientific studies published in international journals, so knowledge remains local and there is almost no international bibliography to refer to. Furthermore, a comprehensive compendium of Italian artisanal flatbreads is completely missing, which would instead represent a starting point for coordinated valorization actions and for improving the production technology, as well as for prompting scientific research.

Therefore, this review aimed to systematically evaluate traditional Italian flatbreads from a scientific perspective making a comparative analysis of their technical and cultural diversity and outlining areas for future research development. To the best of our knowledge, this is the first article on this subject, providing a complete overview and comparison of Italian flatbreads. To accomplish this objective, all available information, retrieved especially from literature in the Italian language, was gathered to create a proper synthetic catalog describing the technical characteristics, ingredients, and cultural aspects of each traditional Italian flatbread. Subsequently, the catalog, served as as the basis for a critical analysis presented in this article

3.2 Methodology and structure of the review

3.2.1 Databases and search terms

A traditional narrative review approach was adopted. The study was carried out in two primary phases: i) the identification of the Italian flatbreads officially awarded quality recognitions, and ii) a comprehensive literature search about the identified breads.

In the initial phase, a search was performed for the category "Bakery and pastry products" in the two institutional databases of the Italian Ministry of Agriculture, Food Sovereignty and Forestry: "List of Italian designations entered in the Register of Protected Designations of Origin, Protected Geographical Indications and Traditional Specialties Guaranteed – Updated November 2023" [18] and "National List of Traditional Food Products – 23rd Edition - 2023" [33], as well as by entering "Italy" as country and "Bread and baked goods" as category in the "Slow Food Presidia" global database [34]. The screening process included retaining only flatbreads, and led to the identification of a set of 23 items, namely two PGI, twenty PAT, and one SFP, reported in Table 1.

In the second phase, the selected flatbreads were the subject of a literature search, imposing no temporal restrictions. Each flatbread name was entered in Scopus and Google Scholar databases the latter provided a more helpful source due to the need to consider also books and articles in Italian language. The main difficulty encountered in this study lies in the fact that most Italian flatbreads had never been studied by the international scientific community, so information was local and very fragmented.

Finally, supplementary searches were carried out on specific themes that emerged in the literature, such as innovative techniques for quality control, safety issues, salt reduction, and the use of innovative ingredients.

3.2.2 Review structure

This review is structured to initially provide information on each individual flatbread in the same order as shown in Table 1 (that is PGI, then PAT and finally SFP flatbreads), allowing the reader to become acquainted with these food products, most of which remain largely unknown beyond their restricted area of origin despite being recognized by international institutions such as the European Commission. Successively, the review presents a critical comparative analysis of these flatbreads for their ingredients, processing steps (dough formation and shaping, leavening, and baking), and cultural aspects.

Table 1. Italian flatbreads with national or international quality recognitions. PGI = Protected Geographical Indication; PAT = *Prodotto Agroalimentare Tradizionale*; SFP = Slow Food *Presidium*.

Quality scheme	Region of origin	Flatbreads
PGI	Emilia Romagna	*Piadina romagnola*
	Trentino-Alto Adige	*Schüttelbrot*
PAT	Tuscany	*Panigaccio di Podenzana; Neccio*
	Tuscany and Liguria	*Farinata*
	Tuscany, Lombardia and Umbria	*Schiacciata*
	Umbria	*Torta al testo*
	Emilia Romagna	*Borlengo; Gnocco fritto; Gnocco ingrassato; Crescenta; Crescenta fritta; Crescentina*
	Emilia Romagna and Marche	*Crostolo*
	Marche	*Crescia sfogliata*
	Basilicata	*Carchiola*
	Puglia	*Puccia*
	Sardinia	*Pane carasau; Pane guttiau; Spianata; Pistoccu; Zichi*
SFP	Tuscany	*Testarolo pontremolese**

*The *Testarolo pontremolese* also holds the PAT designation.

3.3 PGI Italian flatbreads

3.3.1 Piadina romagnola

Piadina romagnola, or *Piada romagnola*, is a traditional bread of the Emilia Romagna region, recognized as PGI in 2014 [35]. It is prepared with refined wheat flour, water, *strutto*, olive oil or EVOO, and salt. Sodium bicarbonate may be added as a fermentation agent [36]. Regarding the ingredient "*strutto*", is important to clarify that in Italy, a country with a long pork butchery tradition, there is specific terminology for different types of pork fat. *Lardo* (lard) identifies the fat layer located just below the rind; *sugna* (suet) is the fat of the adrenal and interstitial area; while *strutto* is the product of the fusion of all the fatty parts of a pig. The fatty ingredient most commonly used in traditional Italian flatbreads is *strutto* [26].

Piadina romagnola is a circular flatbread with a diameter of 15-25 cm, 4-8 mm thick (Figure 1). A larger, thinner, and more flexible variant (23-30 cm in diameter, 3 mm thick) is prepared in the Rimini area, named "*Piadina romagnola alla Riminese*".

Figure 1. An example of unleavened flatbread: *Piadina romagnola*.

Today *Piadina romagnola* is made from wheat flour, while in the past it was commonly made from the cheaper cornmeal and was therefore identified as "the bread of poverty", as stated by the Italian writer Giovanni Pascoli (1855-1912) in the poem entitled "*La piada*". Photo credit: Pino Marzulli.

In the past, *Piadina romagnola* was cooked exclusively on stone slabs or on clay pans named "*testo*", "*tegghia*" or "*teglia*", produced only in the area of Montetiffi, a small village in the mountains of Romagna. Today, such pans are still produced in Montetiffi according to the same tradition and with the same clay, but in most cases *Piadina romagnola* is cooked on metal cooking plates or metal pans, at 200-250 °C for a few minutes [36]. In the past *Piadina romagnola* was commonly made of cornmeal and was thus identified as "the bread of poverty", as stated by the Italian writer Giovanni Pascoli (1855-1912) in the poem entitled "*La piada*", dedicated to this flatbread. Pascoli further defined *Piadina romagnola* as "the bread of freedom, which the venal oven disdains" because it was baked in the fireplace without paying the fee required for using an oven [37]. So, *Piadina romagnola* was a cheap alternative to regular loaf bread. Today, *Piadina romagnola*, perfect to be folded and eaten as a sandwich, is a typical street food sold in traditional kiosks, known as "*piadinerie*", widespread in the Emilia Romagna region. Tastings of *Piadina romagnola* stuffed with local soft cheeses and charcuterie are organized during the *Piadina* Days, within the Emilia Romagna Wine Food Festival [36]. One of the most traditional fillings is a combination of *Squacquerone di Romagna* PDO, *Prosciutto di Parma* PDO, and arugula salad, as reported by the Italian writer Mario Soldati (1906-1999) in the novel "*Viaggio in Emilia Romagna*". Considered "a secular bible of good eating and drinking", the novel describes the author's journey through Emilia Romagna in search of food with genuine flavors, from the late 1950s to the early 1970s [38].

Recognition of the PGI, along with consumers' considerable appreciation of the product, has increased the production volumes of *Piadina romagnola* and raised the need to improve its shelf-life [39,40]. Since it is forbidden to add preservatives [36], the shelf-life of *Piadina romagnola* is extended by packaging under a modified atmosphere. On a nutritional level, the variants where saturated fat from *strutto* is replaced by EVOO should be preferred [41]. *Piadina romagnola* enriched with selenium is effective in increasing dietary intake of this element [42].

3.3.2 Schüttelbrot

Schüttelbrot is a PGI from the Trentino-Alto Adige region [43]. It is a sourdough-leavened flatbread made by mixing rye and wheat flours (the latter <10%) with water, and salt [29]. This bread is flavored with fennel seeds and sometimes wild cumin, coriander, or aniseed [21]. After leavening, the dough is flattened by shaking with a circular motion (hence the name *Schüttelbrot*, which in German means "shaken bread") to obtain a thin disc (10-35 cm large, 0.3-1.5 cm thick), with a few central irregular holes [29]. *Schüttelbrot* is slightly sour and very dry, resulting in a long shelf-life [29,44].

3.4 PAT Italian flatbreads

3.4.1 Panigaccio di Podenzana

Panigaccio di Podenzana is a Tuscan PAT flatbread [33,45] made by mixing wheat flour with water and salt to obtain a dense batter [21,29], which is then baked on a red-hot small terracotta plate (around 15 cm diameter) named *testo* [12], homonymous but different from the metallic two-piece *testo* used for baking the *Testarolo pontremolese*. A small amount of the batter is poured onto the preheated terracotta *testo*, then another hot *testo* is placed on top and so on, resulting in a stack of about 10-12 plates [11]. The baking process is rapid, lasting only 1-2 min.

3.4.2 Neccio

Neccio, a PAT flatbread that originated in Tuscany [33,45], is a gluten-free crêpe-like bread with a diameter of 25 cm, produced from a dense batter of PDO *Garfagnana* chestnut flour [46], water and salt. Chestnut flour imparts dark brown color and marked flavor and its use in breadmaking has recently been reevaluated for its vitamin, mineral, and fiber content [47,48]. *Neccio* was traditionally baked in the fireplace between red hot refractory stone plates named "*testo*" [11], similar to the way also *Panigaccio di Pondenzana* is baked. Then, several *testo* were stacked and held together by a tool called *testaiola*. Chestnut leaves were used to line the *testo*, preventing the dough from sticking to it [21]. Today, *Neccio* is mostly cooked by pouring the batter onto the bottom of a two-piece cast-iron pan placed over the fire and pressing it down with the top.

3.4.3 Farinata

Farinata is a gluten-free pancake-like flatbread made of chickpea flour, water, EVOO, and salt, prepared in three neighboring regions (Liguria, Tuscany, and Piedmont) and recognized as PAT in each of them [33,45]. A wheat-based variant is *Farinata Bianca* or *Farinata Savonese*, named after the city where it originated, Savona. *Farinata* is a cousin bread of *Socca*, from Nice, in the neighboring French region of Provence. After mixing all the ingredients, the dough is poured into a large (about 1 m in diameter) circular pan made of tin-plated copper, left to rest for three hours, then baked in a wood-fired oven for about 15 min [29]. Chickpea flour is rich in proteins, total dietary fiber, unsaturated lipids, minerals, and bioactive compounds with antioxidant activity [49], so considerable interest has recently arisen in the use of this flour in breadmaking [50-52].

3.4.4 Schiacciata

Schiacciata, meaning "squashed", refers to the action of pressing the dough with the fingers to flatten it, without a rolling pin. This flatbread originated in Tuscany and Lombardia [33,45], with slightly different ingredients and names. In Tuscany, this flatbread is named *Schiacciata alla Fiorentina*, *Schiaccia Grossetana*, or *Schiaccia pizzicata di Montiano* and is prepared with wheat flour, water, and yeast, then seasoned with abundant EVOO [21]. In Lombardia, it is named *Schiacciatina* or *Chisolina* and incorporates *strutto* instead of EVOO, not typical of the area. Both are baked on the *testo* or under hot ash and charcoals [21].

3.4.5 Torta al testo

Torta al testo, a PAT from the Umbria region [33,45], is also called *Pizza sotto il fuoco* or *Torta sul panaro* [21, 53]. The dough is prepared by mixing wheat flour, water, lard or EVOO, baking soda, and salt [54]. Originally, farmers used to prepare the *Torta al testo* with cornmeal and bake it on a "*panaro*" or "*testo*". The latter evolved from being a large flat stone to a clay plate, and eventually to a cast iron plate [21]. After placing the dough on the preheated *testo*, it is pierced with a fork and, when a crust forms, it is turned over and covered with hot ash and charcoal until baked. This baking method contributes to the distinctive flavor of *Torta al testo* [53].

3.4.6 Borlengo

Borlengo (or *Burláng*, *Burleng*) is PAT from the Emilia Romagna region [33,45], specifically from the cities of Modena and Guiglia [55], made of wheat flour, water, salt, and occasionally eggs [22,56]. It is prepared from a very light batter called "*colla*" (5 liters of water per 1 kg of flour) [56]. The high degree of dilution allowed to save flour, as needed in times of scarcity. Furthermore, in case of wheat unavailability, chestnut or corn flours were used [22]. The main characteristic of *Borlengo* is its extreme thinness (0.5-0.7 mm), and crispiness. Since the Medieval period, *Borlengo* was associated with Carnival because it was so thin that it seemed made as a joke ("*burla*" in Italian), not like real bread [55].

Borlengo is baked on a large (45 cm diameter) tinned copper rimless pan with a very long handle, named "*sole*" (or *sôl*), greased with pork rinds [22,26]. It is traditionally seasoned with grated *Parmigiano Reggiano* PDO cheese and a typical sauce called "*Cunza*", prepared from bacon and/or lard, garlic, and rosemary [22,38,56].

3.4.7 Gnocco fritto

Gnocco fritto, named *pinzino* in the city of Ferrara [29], is a PAT from the Emilia Romagna region [33,45]. This fried flatbread is prepared with wheat flour, water, *strutto*, baking soda, and salt. The dough is finely sheeted, then cut into 5-15 cm long rectangles and fried in hot *strutto* [29]. During the frying process, the dough delaminates due to heat. Historically, *gnocco fritto* was prepared as a large pancake, 25 cm in diameter and 0.5 cm thick, featuring a central hole (referred to as "*umbréghel*" or belly button), made to allow perfect frying even in the center [26] (Figure 2). *Gnocco fritto* is a very fatty food, aligning with the lifestyle of the past where manual labor outdoors, especially in the cold season, was prevalent. Today, it is consumed occasionally as a snack [29].

Figure 2. An example of fried flatbread: *Gnocco fritto*.

This double-layer flatbread from the Emilia Romagna region of Italy is leavened with baking soda and is typically fried in hot *strutto* (pork fat). The picture shows the most traditional shape of *gnocco fritto*, that is a large circular pancake featuring a central hole. Photo credit: Pino Marzulli.

3.4.8 Gnocco ingrassato

Gnocco ingrassato or *Gnocc cott al fouren* (where "*cott al fouren*" means "baked in the oven" to distinguish it from the fried *gnocco*) is a PAT from the Emilia Romagna region [33,45] prepared by kneading wheat flour, water, lard, diced ham, yeast, and salt. The dough is flattened to 2-3 cm thickness, placed in a rectangular pan. and baked in the oven for 30-40 min at around 180 °C [21,57]. Originally from Reggio Emilia, it is known as *Spianata* in Modena and *Crescenta* in Ferrara [21]. A variant made with similar ingredients, but baked in a fireplace, by covering it with a sheet of damp paper and then embers, is known as *Gnocco sotto le braci*, or *Gnòch sott'al brâsi*.

3.4.9 Crescenta

Crescenta is traditionally prepared in the city of Bologna, in the Emilia Romagna region, as well as in Tuscany, where is named *Carscenta della Lunigiana* [33,45]. Instead, *Crescia*, *Crescia fogliata*, *Cresciolina* and *Crescia sotto la cenere* are all PAT of Marche [33,45]. These specialties share similarities and are closely related to *Gnocco ingrassato*. *Crescenta* is made with flour, water, lard, diced ham and bacon, yeast, and salt [21]. In Bologna *Crescenta* is an emblematic food that gives the colloquial name "*Crescentone*" to the raised pedestrian platform located in the city's main square, Piazza Maggiore [58].

3.4.10 Crescenta fritta

Crescenta fritta is a flatbread from the Emilia Romagna region [33,45]. In the city of Bologna, it is referred to as *Crescentina* or *Crescentina fritta* [21]. The method of preparation is similar to that of the *Gnocco fritto*, but the ingredients are slightly different: flour, milk, *strutto*, baking soda, and salt. The dough is shaped into diamond forms and then fried in *strutto*, resulting in high fat content [21,57].

3.4.11 Crescentina

Crescentina (or *Cherseinta sotto le braci*, *Chersèint ind-el tigèli, Crescentina di Modena*) is traditionally prepared in the Emilia Romagna [33,45] (Figure 3).

Figure 3. An example of leavened single-layer flatbread: *Crescentina*.

Always present at all local festivals and fairs in the Emilia Romagna region of Italy, *Crescentina* is generally stuffed with soft cheese and cured meats.

Originally from Modena, is also called *Tigella Modenese* or *Tigèla Modenese*. However, these latter names refer to the traditional baking tool used to bake it, named *tigella*, rather than the flatbread itself [26]. *Tigella* is a circular terracotta plate, 15 cm in diameter and 1.5 cm thick [59] named from the Latin word "*tegere*", meaning "to cover," because the dough was baked by placing it on a red-hot *tigella*, previously greased with *strutto*, then covering it with another *tigella*. A stack was then made in the fireplace [26]. *Tigella* usually has a decoration in relief, which remains imprinted on the dough's surface as it bakes. These decorations generally depict a star, a solar disc, or a six-petalled flower ("*rosa comacina*"), all emblems of life and regeneration, just as bread is a symbol of life and fertility, which is born from the earth, matures with the heat of the sun and then becomes nourishment through the heat of fire [26]. Today the traditional terracotta *tigella*, often displayed in traditional restaurants or ethnographical museums, is no longer used. Instead, cast-iron molds called *cottole*, decorated in the same way as the old ones, have become the commonly used baking tool [26].

Crescentina is made with wheat flour, water, salt, and occasionally lard and milk [59]. Originally unleavened, today *Crescentina* is generally leavened with either bakers' yeast or sodium bicarbonate [21,59]. Attention must be paid to homonyms because in the province of Bologna the name "*Crescentina*" identifies a different flatbread, which is fried [21].

3.4.12 Crostolo

Crostolo is a flatbread from the Montefeltro area, between the Emilia Romagna and Marche regions [33,45], prepared also in the city of Urbania, in the same region [60]. *Crostolo* is made by kneading wheat flour with eggs, milk, salt, and, as a key ingredient, black pepper, a valuable spice consumed in the past only by wealthy people. Dough sheets are then greased with *strutto* and rolled up into cylinders ("*bigoli*") used to make narrow spirals. The latter are flattened with a rolling pin to obtain thin discs of puff pastry, ready to be baked for a few minutes on a metal grill placed over an open fire. The grill forms dark parallel stripes on the surface of the *Crostolo*, which characterize its appearance [29]. The *Crostolo* is considered a very nutritive bread, owing to its eggs and milk content. This flatbread is a key product in any festival promoting the local traditional cuisine of the region, including the "*Sagra del Crostolo*", a yearly competition that awards the best *Crostolo* [60].

3.4.13 Crescia sfogliata

Originating from an area neighboring the one from which the *Crostolo* comes, hence often confused with the latter, the *Crescia sfogliata* is recognized as PAT in the Marche region [33,45], made of wheat flour, water, eggs, *strutto*, salt, and black pepper, without the addition of milk (used, instead, in the preparation of *Crostolo*) [22]. *Crescia sfogliata* is baked on a cast iron pan, named "*panaro*", so its surface is free of the dark streaks of the *Crostolo* [29]. Typically made in Urbino, the *Crescia sfogliata* is sometimes referred to as "*Piadina di Urbino*" because of its similar appearance to *Piadina romagnola*, but is a very different product, with much more nutritious ingredients.

3.4.14 Carchiola

Carchiola is a flatbread from the Basilicata region [33,45], also included in the Slow Food "Ark of Taste" [61]. Originating in the town of Avigliano, it was prepared with cornmeal by the poor, who could not afford wheat flour, and was typically baked in the fireplace, to avoid paying for the use of communal ovens. The preparation of *Carchiola* involves kneading corn flour, water, and salt, sheeting the dough to 2 cm thickness, and baking it on a metal grill equipped with a rotating mechanism, named *r'ticula* and unique to *Carchiola* bread, placed in the fireplace [11]. The rotating grill allows the *Carchiola* to be flipped over when the first side has been cooked. *Carchiola* has a hard consistency and is softened in legumes and vegetable soups [61].

3.4.15 Puccia

Puccia (or *Uliata,* or *Pane di semola,* or *Pane di orzo*) is a PAT of the Apulia region [33,45], made with durum wheat semolina (or sometimes barley), sourdough, water, EVOO, and salt [29,62]. After leavening, the dough discs (20 cm large) are baked on refractory stone at 350 °C for 1-2 min [62]. The addition of *L. plantarum* ITM21B fermentation product and 15% chickpea flour allowed the production of salt-reduced *Puccia* with improved nutritional and sensory properties [63]. Also, liquid sourdough made of selected strains of *Leuconostoc citreum* and *Weissella confusa* isolated from semolina was used for making *Puccia* without added salt, retaining taste and aroma. This approach was applied also at the industrial level [62,64]. *Puccia* is traditionally eaten by stuffing its "pocket" with meat and vegetables (Figure 4).

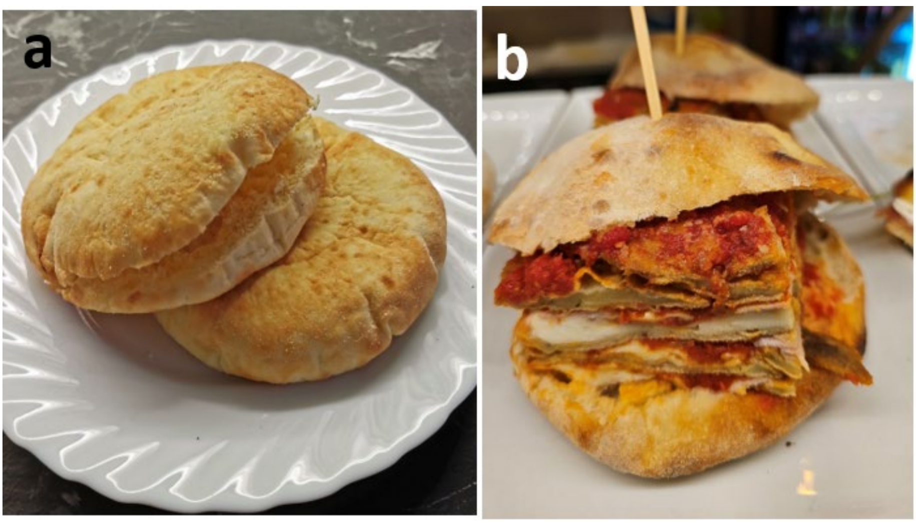

Figure 4. An example of leavened double-layer flatbread: *Puccia*.
A. Plain *Puccia*, a traditional flatbread from the Apulia region of Italy. This flatbread is made of durum wheat semolina and is leavened with sourdough. **B**. *Puccia* stuffed with grilled vegetables, meat and tomato sauce. Photo credit: Pino Marzulli.

3.4.16 Pane carasau

Pane carasau (or *Pana carasatu*, *Carta da musica*, *Pane de fresa*) is a Sardinian PAT [33,45]. The preparation of this sourdough-leavened flatbread, made of semolina (typically, in the past, a high-extraction rate one, named *semolato*), water, and salt, typically involves two baking rounds, the second of which (*carasatura*) after layer separation (*fresatura*), made to obtain a very thin (1-2 mm) and crisp product [65-67]. Its dryness allows for a long shelf-life and wide commercialization throughout Italy and abroad. The sensory and nutritional quality of *Pane carasau* improves by using liquid sourdough [65], able to enhance crispiness and lower the rapidly digestible starch while increasing the slow and inaccessible starch. The organic acids of sourdough, indeed, induce starch-gluten interactions making the starch less digestible and decreasing the postprandial glucose response [68]. Dough elasticity, instead, improves with leavening time [69]. Sourdough-leavened *Pane carasau* was found to positively influence gut microbiota in rats by inducing metabolic dormancy of *Clostridium* spp. [70].

3.4.17 Pane guttiau

Pane guttiau is a PAT from the Sardinia region [33,45] and is an EVOO-seasoned version of *Pane carasau*. The term "*guttiau*" in Sardinian means "dripped" or "sprinkled". This name originates from the practice of sprinkling EVOO over the two separate layers of *Pane carasau* after its first baking. The two layers, seasoned, are then briefly baked again to achieve dryness, and then salted [71]. *Pane guttiau* has a crunchy texture and is usually eaten as a snack, alone or paired with cold cuts or cheese [72].

3.4.18 Spianata

Spianata (or *Ispianada*, *Pane modde*, *Pane poddine*, *Pane fine*) is a traditional flatbread from the Sardinia region [33,45]. It is a sourdough-leavened flatbread made with durum wheat semolina (today refined, while in the past a high-extraction rate semolina was most used), water, and salt. After sheeting, the fermented dough is baked in a wood-fired oven. Circular in shape, 20-30 cm large, and one cm thick, *Spianata* has a minimal crumb [29].

Ritual variants of this flatbread are prepared to celebrate religious events, such as the festival in honour of Our Lady of Gonare, in the towns of Orani and Sarule, and other patronal festivals [19,28,29]. For this ritual variant only refined semolina is used. The ritual *Spianata* is stamped with hearts, crosses, and other symbols and, in the ultimate phase of baking, is sprinkled with water (*imbridadura*) to induce starch gelatinization and obtain a shiny surface [21,29]. On New Year's Eve (*Candelaira*) a special *Spianata* is prepared that, in addition to being decorated, is enriched with lard. The ritual breads are then given to children [28]. The custom of decorating bread in Sardinia dates back to antiquity. A circular terracotta stamp with geometric patterns in relief, named *pintadera* (Figure 5), has been interpreted by scholars as a tool for decorating bread in Nuragic times, being bronze figurines offering loaves decorated with similar motifs found in archaeological excavations [73].

Figure 5. Reproduction of an ancient *pintadera*. Scholars interpreted the circular terracotta stamp with geometric patterns in relief, named *pintadera*, as a tool for decorating bread in Sardinia during the Nuragic civilization, since bronze figurines offering loaves decorated with similar motifs have been found in archaeological excavations in the area. The Nuragic culture lasted in the Sardinia island, Italy, from the Middle Bronze Age up to the Roman colonization in 238 BC. Photo credit: Pino Marzulli.

3.4.19 Pistoccu

Pistoccu (meaning "baked twice") is traditionally prepared in Sardinia [33,45]. It is an elongated crisp bread slightly thicker than *Pane carasau* (3-4 mm) prepared with semolina (refined or, as in the past, high-extraction rate), water, bakers' yeast, and salt [28]. After kneading, the dough is left to rest for an hour, then rolled out and baked. Wood-fired ovens were used in the past, while today *Pistoccu* is mainly baked in steam ovens at 200 °C for 20 minutes. Once baked, each loaf is cut into two halves and baked again to achieve dryness in fan-assisted "rotor" ovens, to extend the shelf-life up to several months [21,29].

3.4.20 Zichi

Zichi is a Sardinian sourdough-leavened PAT flatbread [33,45] made from durum wheat semolina (refined or high-extraction rate, which was more widely used in the past), water, and salt. The dough is flattened using a wooden rolling pin to obtain 0.5 cm thick discs, 35-40 cm large, which were traditionally stamped with wooden stamps, named "*sa marca*", for identification [73]. *Zichi* is baked in a wood-fired oven at 400-500 °C for a few minutes. When, a few days after baking, the bread hardens, it is cut into small pieces and softened in sheep's broth [21]. Decorated *Zichi* breads are prepared for weddings ("*su pane 'e iscadda*") or for religious events (Figure 6), similarly to *Spianata*, using only refined semolina [73].

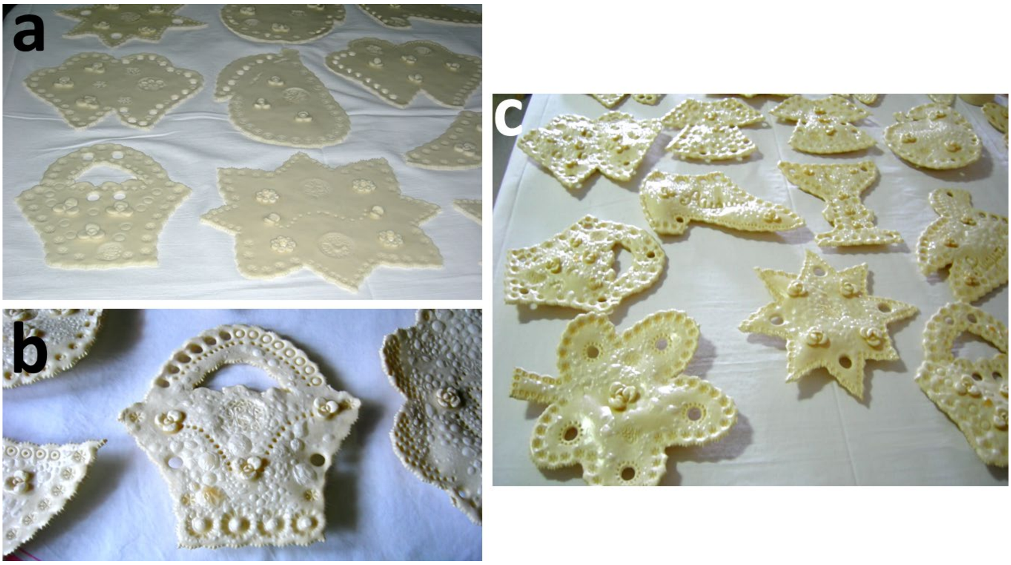

Figure 6. Ritual flatbread. **A**. Uncooked *Zichi* flatbread decorated for wedding (*su pane 'e iscadda*). These ritual flatbreads convey auspicious messages to protect the couple. **B.** Detail of a handbag-shaped decorated *Zichi* flatbread. C. Decorated *Zichi* flatbread after baking. It is visible the shiny surface, due to starch gelatinization. Photo credit: Angelo Morittu.

3.5 SFP Italian flatbread

3.5.1 Testarolo pontremolese

Testarolo pontremolese is a Tuscan SFP bread [33,45] also recognized as PAT [74]. It is a pancake-like bread round in shape, 40-45 cm in diameter, very thin and highly porous, prepared from a smooth batter of wheat flour, water, and salt [21]. Traditionally, *Testarolo pontremolese* is baked in the fireplace in a two-piece cast iron pan, named "*testo*" [21] (Figure 7). When the bottom piece of *testo*, a disc plate called "*sottano*", reaches the desired temperature, the batter is poured and covered with the top piece, a dome-shaped lid called "*soprano*". This ancient cooking method, native to the *Lunigiana* area, is unique. However, today *Testarolo pontremolese* is also produced industrially, cooking it on a hot steel plate without any cover. This flatbread is not eaten as it is. Cut into diamond shapes and soaked in hot water, is dressed with a fresh pesto made of basil, *Parmigiano Reggiano* PDO cheese, and EVOO [21].

Figure 7. The special baking system used to cook *Testarolo pontremolese*: *Testo.*

This baking system is made of cast iron and is composed of two pieces: a base, basically a pan, called *sottano*, and a lid, called *soprano*. Placed in the fireplace and made red-hot, this *testo* is exclusively used to bake *Testarolo pontremolese*. Photo credit: Lumachelli Pietro e Figli srl.

3.6 Comparative analysis of Italian flatbreads

Flatbreads can be broadly categorized as unleavened and leavened, the latter subdivided further into double-layer and single-layer varieties (Figure 8) [11,12].

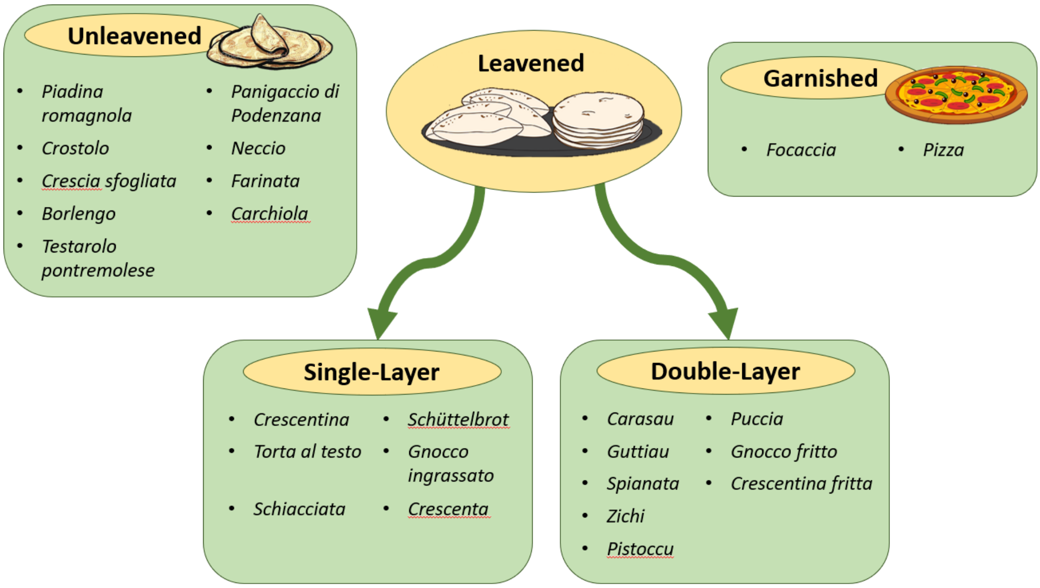

Figure 8. Classification of Italian flatbreads.

Flatbreads are broadly classified into three categories: unleavened; leavened (subdivided further into single-layer and double-layer varieties); garnished. From the nutritional point of view the latter represent a complete meal due to their rich garnishments, which may include cheese, meat or vegetables.

Unleavened flatbreads are: *Piadina romagnola*, *Crostolo*, *Crescia sfogliata*, *Borlengo*, *Testarolo pontremolese*, *Panigaccio di Podenzana*, *Neccio*, *Farinata,* and *Carchiola*. Leavened double-layered flatbreads are *Pane carasau*, *Pane guttiau*, *Spianata*, *Pistoccu*, *Zichi*, *Puccia*, *Gnocco fritto,* and *Crescenta fritta*. Leavened single-layered flatbreads are *Crescentina*, *Gnocco ingrassato*, *Crescenta*, *Torta al testo*, *Schiacciata* and *Schüttelbrot*. It is important to note the existence of another category - garnished flatbreads such as pizza and *focaccia*- which, although significant, are not considered in the present review.

The overall flowchart of the production process of unleavened and leavened, double- and single-layered, flatbreads is reported in Figs. 9 and 10.

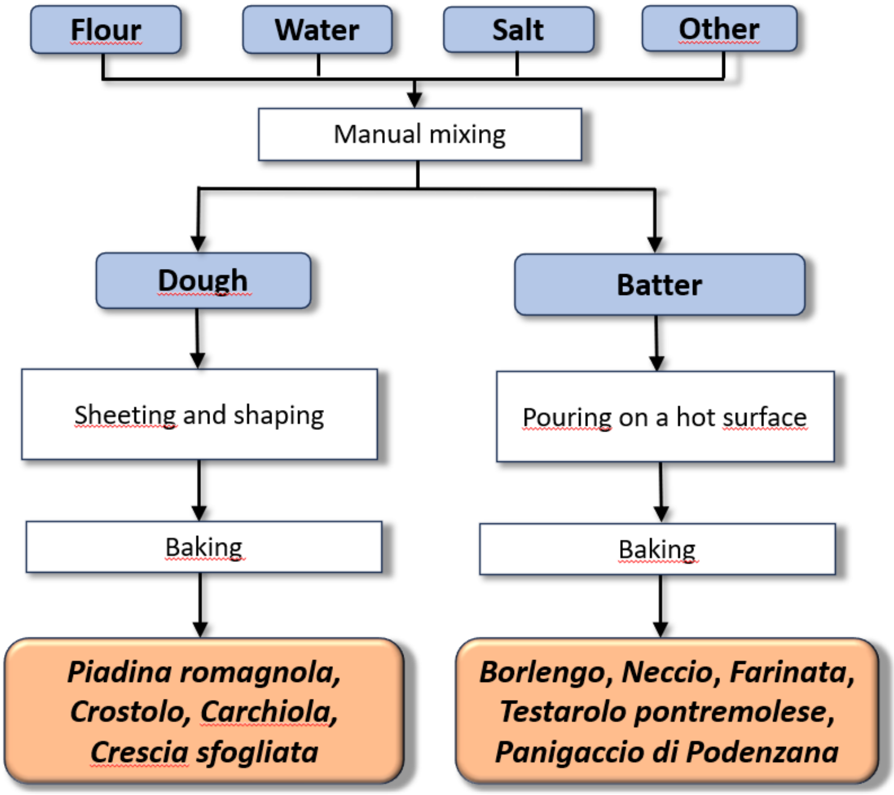

Figure 9. Flowchart of the production process of unleavened Italian flatbreads.

The flowchart details the procedure for preparing the unleavened flatbreads, which can be obtained either by preparing a batter or a dough. The specific names of breads are reported in the orange boxes.

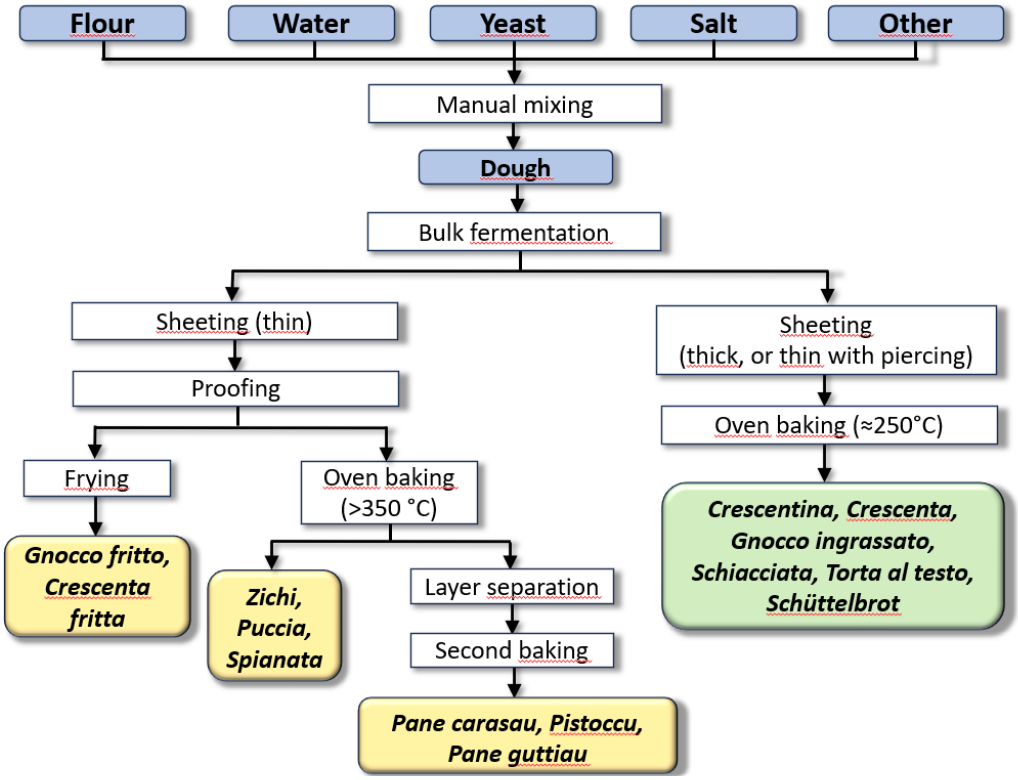

Figure 10. Flowchart of the production process of leavened double- and single-layered Italian flatbreads.

The flowchart highlights the main differences in the production process of double-layered flatbreads (reported in the yellow boxes) and single-layered ones (reported in the green box), consisting in a different thickness of the sheeted dough and different baking temperature. The flowchart also details the procedure for preparing the double baked flatbreads and the fried flatbreads.

Information on ingredients, dough consistency, leavening, and baking conditions, bread shape and size has been schematically summarised in three tables: unleavened flatbreads are summarized in Table 2, leavened double-layered flatbreads in Table 3, and leavened single-layered flatbreads in Table 4. A comparative analysis of the ingredients and main processing steps (dough formation and shaping, leavening, and baking) of the Italian flatbreads described so far is provided as follows, considering the entire group of flatbreads altogether to allow a complete discussion.

3.7 Ingredients

The formulation of Italian flatbreads is generally very simple, being the most common ingredients only flour, water, yeast (in the leavened ones), and salt. Additional ingredients can be oils and fats, and sometimes ham, eggs, and milk. The most used fatty ingredients are *strutto* (a name that stands for fat obtained by fusing all the fatty parts of a pig), and lard. These ingredients have a strong regional variation, being found only in flatbreads (*Piadina romagnola*, *Crostolo*, *Crescia sfogliata*, *Gnocco ingrassato*, *Crescenta*, *Torta al testo*, *Schiacciata*, *Gnocco fritto* and, occasionally, *Crescentina*) prepared in the regions of north-central Italy (Emilia Romagna, Marche, Umbria, and Lombardia), where animal farming is more common than in the south and pigs are usually reared.

Olive oil or EVOO is used less frequently. They are proposed today as a healthier alternative to *strutto* and lard in *Piadina* and *Torta al testo*, or are a typical ingredient of a few flatbreads originally from areas where olive cultivation is common, such as *Farinata* (from Tuscany and Liguria), *Guttiau* (from Sardinia), and the Tuscan version of *Schiacciata.*

Eggs and milk are found in a few flatbreads (*Crostolo*, *Crescia sfogliata*, *Crescenta*, *Crescenta fritta,* and, occasionally, *Borlengo*), usually in combination with *strutto*.

Added ingredients especially fat also have nutritional implications depending on whether are rich in unsaturated fatty acids and antioxidants, like EVOO, or whether are saturated, like *strutto* and lard [41]. Generally, flatbreads with added fats of animal origin, or egg and milk, were not intended for daily consumption but only for an occasional one. In the past, they were considered "rich" bread formulations, associated only with festive days, and today, similarly, they are mostly consumed in food festivals and convivial occasions.

Spices occur rarely in the preparation of Italian flatbreads, being black pepper used only in the preparation of *Crostolo* and *Crescia sfogliata*, and fennel seeds (or, occasionally wild cumin, coriander and aniseed) used for preparing the *Schüttelbrot*.

Regarding the type of flour, the history of cereal cultivation in Italy is ancient, dating back to 7000 BP, with the first flatbreads made with emmer (*Triticum turgidum* subsp. *dicoccum*) [75]. Today, Italian flatbreads are mostly made from bread wheat flour (*Triticum turgidum* subsp. *aestivum*) or durum wheat semolina (*Triticum turgidum* subsp. *durum*), reflecting wheat's status as the primary cereal crop. Local indigenous wheat cultivars are mostly used, specifically adapted to the area [73]. Durum wheat semolina is used in flatbreads prepared in southern Italy (*Puccia*) and especially in Sardinia (*Pane carasau, Pane guttiau, Spianata, Pistoccu, and Zichi*), being durum wheat largely cultivated in these regions and being less common in northern Italy. Carotenoid pigments in the semolina give the product its typical golden color [76].

Flatbreads produced using high-extraction (75–90%) or whole-grain flours are a good source of dietary fiber and minerals [10] and were largely used in the preparation of flatbreads in the past. Refined flour was used almost exclusively by the wealthier social classes or for the preparation of precious ritual breads, the latter being particularly typical in Sardinia (*Spianata* and *Zichi*) [19,28,29,73]. With the general improvement in economic conditions, refined flour has largely replaced high-extraction flour, being preferred to achieve lighter-colored bread [11]. Today the trend shows that people are returning to whole-grain bread for health reasons, but this type of bread is a niche product [77].

One advantage of flatbreads over loaf bread is that they can be easily made with non-wheat flours, also gluten-free, being high volume not required in this product, at least in the unleavened version. Alternative flours used in Italian flatbreads include chestnut flour, used for *Neccio* in Tuscany, rye flour, used for *Schüttelbrot* in Alto Adige, chickpea flour, used for *Farinata di ceci* in Liguria, and corn flour, for *Carchiola* [11].

In addition, the use of cornmeal has been recorded throughout history as a cheap alternative, in case of famine, for the preparation of some breads that, instead, today are made with wheat flour, such as *Piadina romagnola*, *Borlengo*, and *Torta al testo*. Being cheaper, cornbread was a staple food for the lower social classes, leading to the spread of pellagra among the poor in north-central Italy in the late 19th and early 20th centuries [78].

3.8 Dough formation and shaping

The mixing of ingredients, traditionally manual, can be done with automatic kneading machines, but the latter are used only for those breads that have a larger production such as *Piadina romagnola* and *Pane carasau*.

The consistency of dough varies from a semi-fluid batter to a thicker batter, to a consistent dough. In any case, regardless of dough consistency, and whether they are leavened or not, flatbreads are generally thin, varying in thickness from a few millimeters to a few centimeters. They are characterized by low specific volume, and high crust-to-crumb ratio [12].

Batters, ready to be poured onto a hot baking surface (Figure 9), are typically prepared for the majority of unleavened flatbreads (*Borlengo*, *Testarolo pontremolese*, *Panigaccio di Podenzana*, *Neccio*, and *Farinata*). The result is generally very thin (1-4 mm thickness). All the other Italian flatbreads require a consistent dough which is then sheeted and shaped as a disc of about 1-2 cm thickness. Usually, sheeting is made manually with the aid of a rolling pin. Alternatively, the dough can be flattened with the pressure of fingertips, as for the *Schiacciata*, or by vigorously shaking, as for the *Schüttelbrot*. At the industrial level, sheeting is done by laminating machines.

3.9 Leavening

The leavening phase is skipped for unleavened flatbreads (*Piadina romagnola*, *Crostolo*, *Crescia sfogliata*, *Borlengo*, *Testarolo pontremolese*, *Panigaccio di Podenzana*, *Neccio*, *Farinata* and *Carchiola*), resulting in a rapid procedure. In this case, the bread must be very thin to make it easier to chew. Unleavened flatbreads, commonly referred to as “wraps”, generally come from the Tuscan-Emilian Apennines, Lunigiana, and Po Valley, in north-central Italy (Figure 11).

Figure 11. Map of the Italian flatbreads produced in north-central Italy.

The map shows the geographic area of Tuscan-Emilian Apennines, Lunigiana and Po Valley. Unleavened flatbreads are shown in green, single-layer leavened flatbreads in red, and double-layer leavened flatbreads in black.

For leavened flatbreads, a bulk fermentation of the dough is carried out, followed by shaping. After shaping, a final proofing step is made only when an intense fermentation is crucial for achieving the desired bread structure, as in the case of double-layered flatbreads. Regarding the leavening agent, leavened flatbreads are often prepared with sourdough, a fermentation starter made at first from flour and water, and then from a portion of the previous breadmaking batch [6].

Table 2. Technical features of unleavened Italian flatbreads prepared according to the old tradition.

Bread name	Flour type§	Additional ingredients (other than flour, water, salt)	Dough consistency	Baking time (with old baking system)∞	Traditional (old) baking system	Bread shape and size
*Piadina romagnola**	Corn flour in the past; wheat flour today	In the past *strutto*,# today also olive oil, or extra virgin olive oil	Dough	3-5 min depending on thickness	On clay pans named "*testo*" or "*tegghia*" or "*teglia*" made red-hot by being placed in the fireplace	Circular; 4-8 mm thick; diameter 15-25 cm. The *Riminese* variant is 3 mm thick and has a diameter of 23-30 cm
Crostolo	Wheat flour	Egg, *strutto*,# milk, black pepper	Dough	3-4 min	On a hot metal grill placed over an open fire, which gives the surface of the bread its typical dark striped appearance	Circular; about 5 mm thick; about 25 cm diameter
Crescia sfogliata	Wheat flour	Egg, *strutto*,# black pepper	Dough	4-5 min	In a large (about 50 cm) cast iron pan named "*panaro*" placed in the fireplace	Circular; about 1 cm thick; diameter about 50 cm
Borlengo	Wheat flour or, in periods of famine, corn flour or chestnut flour in the past. Today only wheat flour	Occasionally egg	Thin batter	1-2 min	In a large (45-50 cm diameter) tinned copper rimless pan with a very long handle named "*sole*" placed in the fireplace	Circular; 1 mm thick; around 45 cm diameter
Testarolo pontremolese	Wheat flour	-	Thick batter	1-2 min	In a hot cast iron pan with a lid, named "*testo*", placed in the fireplace	Circular; 2-3 mm thick, 40-45 cm diameter
Panigaccio di Podenzana	Wheat flour	-	Thick batter	1-2 min	Between two small terracotta plates named "*testo*" (about 15 cm diameter) placed in the fireplace	Circular, 2-3 mm thick; 10-15 cm diameter
Neccio	Chesntnut flour	-	Thick batter	2-3 min	Between two medium sized terracotta plates named "*testo*" (about 25 cm diameter) placed in the fireplace	Circular; about 3-4 mm thick; 25 cm diameter
Farinata	Chickpea flour	Extra virgin olive oil or olive oil	Thick batter	5-6 min	In a very large pan (about 1 m diameter) of tin-plated copper placed in a wood-fired oven	Circular; 4-6 mm thick; up to 1 m diameter
Carchiola	Corn flour	-	Dough	3-4 min	On a metal grill with a rotating mechanism called "*r'ticula*" placed in the fireplace.	Circular; 4-5 mm thick; 25-30 cm diameter

*Originally unleavened, today chemical leavening agents may be added; §High extraction rate in the past, refined today; #*Strutto* is an Italian word indicating fat obtained by fusing all the fatty parts of a pig; ∞With the traditional old baking systems, in the fireplace or in wood-fired oven, temperature was between 300 and 400 °C.

Table 3. Technical features of leavened Italian flatbreads having a double-layered structure, prepared according to the old tradition.

Bread name	Flour type[§]	Additional ingredients (other than flour, water, salt)	Leavening agent	Leavening time and temperature	Baking time (with old baking system)[∞]	Traditional (old) baking system	Bread shape and size
Pane carasau	Durum wheat semolina	-	Sourdough or baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	2-3 min + 1 min; baking is done in two steps, the second step is done after the separation of the two layers and leads to complete dryness	On the slab of a wood-fired oven	Circular; 1-2 mm thick; about 30 cm diameter
Pane guttiau	Durum wheat semolina	Extra virgin olive oil	Sourdough or baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	2-3 min + 1 min; baking is done in two steps, the second step is done after the separation of the two layers and leads to complete dryness	On the slab of a wood-fired oven	Circular; 1-2 mm thick; about 30 cm diameter
Spianata	Durum wheat semolina	-	Sourdough	Several hours; RT	2-3 min	On the slab of a wood-fired oven	Circular; 1 cm thick; 10-20 cm diameter
Pistoccu	Durum wheat semolina	-	Baker's yeast	1 h; RT	3-4 min + 1 min; baking is done in two steps, the second step is done after the separation of the two layers and leads to complete dryness	On the slab of a wood-fired oven	Small rectangular; 3-4 mm thick
Zichi	Durum wheat semolina	-	Sourdough or baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	2-3 min	On the slab of a wood-fired oven	Circular; 0.5 mm thick; 30-40 cm diameter
Puccia	Durum wheat semolina or barley flour	Extra virgin olive oil	Sourdough	Several hours; RT	3-4 min	On the slab of a wood-fired oven	Circular; 2 cm thick; 20 cm diameter
Gnocco fritto	Wheat flour	*Strutto*[#]	Baking soda	15 min	Frying in hot *strutto* for 1-2 min at 250 °C	-	Circular (25 cm diameter) or diamond shaped (5 × 10 cm); 0.3 cm thick
Crescenta fritta	Wheat flour	Milk, *strutto*[#]	Baking soda	15 min	Frying in hot *strutto* for 1-2 min at 250 °C	-	Diamond shaped (5 × 10 cm)

[§]High extraction rate in the past, refined today; [#]*Strutto* is an Italian word indicating fat obtained by fusing all the fatty parts of a pig; [∞]With the traditional old baking systems, in the fireplace or in wood-fired oven, enough wood should be used to reach at least 350 °C, up to 500 °C.

Table 4. Technical features of leavened Italian flatbreads having a single-layered structure, prepared according to the old tradition.

Bread name	Flour type[§]	Additional ingredients (other than flour, water, salt)	Dough shaping modality	Leavening agent	Leavening time and temperature	Baking time (with old baking system)[∞]	Traditional (old) baking system	Bread shape and size
Crescentina	Wheat flour	Occasionally lard and milk	Sheeted using a rolling pin	In the past was unleavened, today baker's yeast or sodium bicarbonate are used	20 min, RT*	2-4 min	Between two small (15 cm diameter) circular terracotta plates called "*Tigella*" placed in the fireplace	Circular; 1.5 cm thick; 5-15 cm diameter
Gnocco ingrassato	Wheat flour	Lard, diced ham	Sheeted using a rolling pin	In the past sourdough, today baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	About 20 min	Rectangular pan in the fireplace	Rectangular (40×30 cm), 2.5-3 cm thick
Crescenta	Wheat flour	Lard, ham	Sheeted using a rolling pin	In the past sourdough, today baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	About 25 min	Rectangular pan in the fireplace	Rectangular (40×30 cm), 4-4.5 cm thick
Torta al testo	Corn flour in the past, today wheat flour	Lard or extra virgin olive oil	Sheeted using a rolling pin and pierced with a fork	Baking soda	15 min, RT	About 10 min	In a clay plate or eventually a cast iron plate called "*Panaro*" or "*Testo*" placed in the fireplace	Circular; 1 cm thick; 25-30 cm diameter
Schiacciata	Wheat flour	Extra virgin olive oil in Tuscany, *strutto*[#] in Lombardia	Flattened with the pressure of fingertips	In the past sourdough, today baker's yeast	Several hours for sourdough, 1 h for bakers' yeast; RT	About 15 min	On stone slabs or on clay pans named "*Testo*" placed in the fireplace	Circular; 2 cm thick; 25 cm diameter
Schüttelbrot	Mix of rye and wheat flours	Fennel seeds. Occasionally wild cumin, coriander or aniseed	Flattened by vigorously shaking	Sourdough	2 h; 25-40 °C	About 15 min, baked to dryness	On the slab of a wood-fired oven	Circular; 0.3-1.5 cm thick; 30-35 cm diameter

[§]High extraction rate in the past, refined today; *RT = Room Temperature (about 20 °C); [∞]With the traditional old baking systems, in the fireplace or in wood-fired oven, temperature was set around 250-300 °C; [#]*Strutto* is an Italian word indicating fat obtained by fusing all the fatty parts of a pig.

Flatbreads prepared with sourdough are *Gnocco ingrassato*, *Crescenta*, *Schiacciata*, *Schüttelbrot*, *Pane carasau*, *Pane guttiau*, *Spianata*, *Zichi*, and *Puccia*. The use of sourdough was predominant, especially in the past, but in the early 20th century this complex ecosystem composed of yeast and lactic acid bacteria [79] was gradually replaced by commercial baker's yeast (*Saccharomyces cerevisiae*). The latter accelerates fermentation time, but results in a shorter shelf life and a weaker sensory experience than sourdough [80]. Today, interest in sourdough is growing back again due to its ability to lower the glycemic index, improve protein digestibility, increase nutrient bioavailability [81,82], and reduce acrylamide content [83]. Liquid sourdoughs made of selected strains have been introduced in response to the need for a shorter and more controllable fermentation process [65,84].

An alternative to biological fermentation is to use a chemical leavening agent, a mix of sodium bicarbonate or "baking soda" and a weak acid able to release carbon dioxide. The latter then thermally expands with the heat of the oven causing the dough to swell [85]. Chemical leavening agents are used in *Crescentina*, *Torta al testo*, *Gnocco fritto*, and *Crescenta fritta*.

3.10 Baking and structuring the final bread

Flatbreads are generally characterized by "flash baking", that is they are baked at very high temperatures (up to 450-500 °C) for a very short time, a maximum of a few minutes [86]. While in the Middle East and Indian subcontinent baking is made by using a griddle or a hot plate, a *tannur*-style vertical oven [12], or even frying [87], the most traditional baking systems in Italy were terracotta plates placed in the fireplace [11,12]. The most common name for these old baking plates, made of terracotta or metal and having a diameter ranging from 15 to 25 cm, is *testo*, a name mainly used in Tuscany and Emilia Romagna. A special *testo* is the one used for *Testarolo pontremolese*, consisting of two pieces, a pan and a lid. Placed in the fireplace and made red-hot, it acts as a small portable oven in which only this specific type of flatbread is baked (Figure 7). Today, domed wood-fired ovens are the most popular baking systems among Italian consumers, complemented by modern electric ovens with refractory stone floors, or metal plates to be placed on the gas stove.

Baking causes the thermal dilation of gases, such as water vapor and eventually carbon dioxide generated by leavening agents, when used. Meanwhile, starch gelatinization and thermal denaturation of proteins confer the bread its final shape and structure. Depending on the presence or absence of gluten in the flour used, and according to the way the precedent processing steps, shaping and leavening, have been done, flatbreads can significantly inflate like a "balloon", with consequent "delamination," e.g., separation of the dough sheet into two distinct layers [9,12,88]. Also, baking temperature influences in determining delamination, being double-layered flatbreads are generally baked at higher temperatures than single-layered ones. Temperatures between 350 and 550 °C have been reported as required for perfect delamination [88]. After cooling, the bread loaf deflates but the two layers remain separated, resulting in a "pocket", suitable to be stuffed with meat or vegetable ingredients according to the personal taste.

In unleavened flatbreads, the absence of fermentation, often coupled with the use of gluten-free or poor gluten-containing flours, and the frequent preparation of batters instead of dough, does not allow thermal delamination, explaining why they remain flat and thin.

In the case of leavened flatbreads, fermentation induces significant inflation and a double-layered structure only if a finely sheeted dough has been prepared and wheat flour has been used. Only a properly formed gluten matrix can produce a dough having sufficient viscoelasticity to perfectly delaminate during baking, so all double-layer flatbreads (*Pane carasau*, *Pane guttiau*, *Spianata*, *Pistoccu*, *Zichi*, *Puccia*, *Gnocco fritto,* and *Crescenta fritta*) are made of wheat flour [89]. The majority of them are from Sardinia: *Pane carasau*, *Pane guttiau*, *Spianata*, *Pistoccu,* and *Zichi* [73,90,91], while another one, namely *Puccia*, is from Apulia. This category of flatbreads also includes two fried products, namely *Gnocco fritto* and *Crescenta fritta*, very different from others in the same category, being prepared with baking soda. They delaminate due to the thermal expansion of carbon dioxide and water vapor when immersed in hot frying oil.

Double-layer flatbreads can also be the subject of a double baking. Specifically, *Pane carasau*, *Pane guttiau*, and *Pistoccu* are baked in two steps, the first to achieve dough inflation and delamination, and the second to toast the bread. In detail, after the first baking step, the two layers of each loaf are separated by cutting them off at the edges, obtaining two extremely thin discs. The second baking is then done for a very short time (about 1 min) until each layer is toasted [66,67,92]. This double-baking procedure results therefore in a very thin, dry, and crispy flatbread with a long shelf-life [90]. In the past, dry flatbreads were the staple food of shepherds, who spent several months away from home, without the opportunity to bake fresh bread [12].

Single-layer flatbreads (*Crescentina*, *Gnocco ingrassato*, *Crescenta*, *Torta al testo*, *Schiacciata,* and *Schüttelbrot*) are fermented ones, but inflation during baking is undesirable. Therefore, the sheeted dough is pierced with a fork (such as in the case of *Torta al testo*) to eliminate most carbon dioxide, thus preventing thermal delamination in two layers. Alternatively, the dough disc is kept relatively thick (2 cm), which also helps prevent delamination. Most single-layer flatbreads come from the area of the Tusco-Emilian Apennines, Lunigiana, and Po Valley. In some cases (such as *Crescentina*, *Crescenta,* and *Gnocco ingrassato*) their names are characterized by the surprisingly high number of linguistic variants (confusing the non-locals) due to dialectal differences among the towns of production, even though these are not far apart.

3.11 Cultural diversity and social dimension

Italy is seen as a true “bread cornucopia” being one of the richest countries in terms of bread variety among the European panorama [93]. Some traditional Italian flatbreads are also found in classical Italian literature, such as Sardinian breads, recounted by Grazia Deledda [30] and the *Piadina romagnola*, which inspired Giovanni Pascoli [37] and Mario Soldati [38]. Besides the already discussed occurrence of ritual flatbreads (*Spianata* and *Zichi*) in ceremonial life, i.e., in patronal festivals and weddings, the cultural significance of flatbreads can be further explained in terms of the role they play in Italian cuisine, evidenced by the hundreds of websites and cookbooks that list recipes for various Italian flatbreads both traditional and reinvented, further garnished, filled or wrapped around classic and non-classic ingredients. In Italy, besides environmental and climatic regional differences, historical factors such as the past civilizations and successive occupations by other peoples, also varying among different parts of the country, strongly influenced gastronomy leading to well-differentiated regional culinary styles. Saying “traditional” in Italy means therefore taking into consideration all the 20 regions composing the country, generating a myriad of food cultures [94].

Similarly, the flatbread landscape is very rich in terms of regional and subregional diversity. This study identified and focused on 23 flatbreads in the Italian context that have received national and international quality awards, many other flatbreads in Italy do not have any quality label despite being equally important locally, not to mention that only plain flatbreads were considered, while there are also many garnished types [11]. The high degree of diversity observed in flatbreads is thus another expression of Italy’s diverse culinary heritage.

However, it is possible to identify some criteria for grouping these flatbreads. Although a limitation of the study is the lack of specific historical information available on Italian flatbreads, it was possible to highlight some regional variations probably rooted in local history, such as the contrast of double-layered flatbreads *versus* single-layered flatbreads. Double-layered flatbreads are prepared almost exclusively in Sardinia. Internationally, the double-layer structure is typical of flatbreads from the Middle East and North Africa [12], areas where first the Phoenicians and then the Carthaginians settled in antiquity and then spread to the coastal areas of the Mediterranean basin, including Sardinia. Therefore, the hypothesis of an ancient cultural influence cannot be ruled out. Similarly, a probable east-west cultural influence of *pide* on *pizza*, indeed, has been theorized by other authors [20]. On the contrary, the majority of single-layered flatbreads, typically prepared in the Tusco-Emilian Apennines, Lunigiana, and Po Valley and cooked on *testo* baking plates, hark back to the ancient Roman baking mode *sub testum* [11,95]. Bread is one of the most documented foods in primary sources about ancient Romans, and it was generally unleavened [93].

Other regional differences relate to texture and leavening conditions: crispy flatbreads with long shelf-life, fully leavened and double-baked to dryness, are typically made in Sardinia. Shepherding has always been widespread in this region, where shepherds spent months away from home overseeing sheep flocks in little inhabited rural areas and had to carry enough bread for the duration of the transhumance. In contrast, the production of unleavened breads, to be baked quickly as an evening meal upon returning home, consolidated in areas where agriculture was more developed and people worked in the fields all day, like in the Tusco-Emilian Apennines, Lunigiana, and Po Valley. These differences derive from the mutual interaction between peoples and their environments, already established as being the basis of the biocultural diversity in Italy [96].

Finally, most (16 out of 23) of the Italian flatbreads with national or international quality marks come from North-Central Italy, while only 7 are from the south and the islands.

These results reflect the gap in socio-economic conditions between the north and south still present in the country, where in the south there is still a lower degree of awareness of quality systems and their opportunities, and there are difficulties in managing bureaucratic procedures and in establishing producer consortia.

3.12 Prospect of Italian flatbread development

Traditional foods are generally not suitable for applying processing innovations as they are artisanal by nature, and are appreciated by consumers who are looking for a product that recalls the past. Nonetheless, innovation should not be ruled out. Interest in traditional and ethnic food has grown, and research projects, such as the "FlatBreadMine", funded within the PRIMA section 1 call [97], are focusing on balancing authenticity, taste, health benefits, and adaptability to new lifestyles, especially in terms of extended shelf-life. The World Health Organization (WHO) recommendations [98] encouraged the food industry to create innovative products with improved nutritional properties e.g., high in fiber and proteins, but low in fat, salt, and sugar while substituting baker's yeast with sourdough [99]. Given their daily consumption, flatbreads are ideal candidates for reformulation to enhance consumers' health.

Although the heritage of traditional Italian flatbreads is very rich, only a few of them have been the subject of scientific studies aimed at defining their nutritional properties or improving their quality. Most of the studies have focused on *Pane carasau* and *Piadina romagnola*. These flatbreads have moved out of a strictly local consumption sphere, being produced in large quantities by industrial companies that complement the small-sized artisanal ones, and being marketed throughout Italy and abroad. Indeed, the market for flatbreads has great growth potential, typicality being a recognized purchase driver [77]. However, at the industrial level, it is essential to overcome empirical process regulation and achieve quantitative standardization. To this end, quality monitoring by wireless and microwave-based sensors has been proposed to check the composition of *Pane* c*arasau* doughs through the dielectric signal [66,92]. These sensors are also able to monitor, during bread production, environmental parameters (temperature, relative humidity), conveyor belt speed, and, through image analysis, the morphological characteristics of the product before baking [66]. Similar monitoring systems could be applied to other flatbreads, with appropriate adaptations. The flatbread baking process in a home-scale electric oven has been also investigated. By monitoring the temperature distribution within the oven and the evolution of starch gelatinization during baking, a three-dimensional transient numerical model has been developed, helpful for electric oven designers [100].

Given the high temperature typically required for baking flatbreads, another key point is to increase safety by containing the level of acrylamide [101,102]. To this end, the addition of calcium carbonate is effective in mitigating this toxic compound [103]. Another strategy involved the use of pigmented cereals, rich in anthocyanins having antioxidant activity [104,105].

Salt reduction is another hot topic. The WHO and FAO have recommended a maximum daily salt intake of 5 g [106], highlighting the need to reduce salt content in staples such as flatbreads [107]. In addition to substitution with potassium chloride [108], other studies proposed a sourdough-based strategy due to the ability of lactic acid bacteria to mask salt reduction by acidification and proteolysis [63,64].

Some studies have addressed the incorporation of legume flours, either native or dry fractionated, to enrich the nutritional profile of Italian flatbreads, particularly to increase the protein content [50,109,110]. Innovative, nonconventional, ingredients have been also proposed to boost the nutritional value of international flatbreads [8]. Microalgae, added at ≤3% due to strong marine flavor, enhanced proteins, phenolics, carotenoids, and antioxidant activity of *tortilla* [111]. Whole hempseed flour increased the protein and fiber content of *chapati* [112]. House cricket powder, rich in high-value proteins and polyunsaturated fatty acids, resulted in darker products but with a similar texture to control when added to *chapati roti* at a 5% level [113,114]. Although these approaches have not yet been applied to Italian flatbreads, the use of non-conventional ingredients could be of interest to broaden commercial offerings. However, it is generally not easy for consumers to accept a new flavor, color, or texture in traditional food products. Increased awareness and communication are essential to facilitate the consumer's acceptance of these new, nutritionally enhanced flatbreads, mitigating food neophobia [115].

3.13 Conclusions

This study gives an overview of Italian flatbreads that have received national or international quality recognitions, establishing a catalog with technical and cultural information on each of them. The raw materials and main processing characteristics of flatbread in the Italian context have been critically compared. Key findings include:

- Twenty-three Italian flatbreads have national or international quality recognitions, predominantly originating from North-Central Italy.
- Flatbreads show a high degree of diversity, reflecting the Italian region's diverse culinary heritage.
- The formulation is generally simple and includes flour, water, possibly yeast, and salt. The region-specific ingredients include fats of animal origin, or ham, mostly in flatbreads from Northern Italy, and olive oil or EVOO in flatbreads of Tuscany, Liguria, and Sardinia.
- The type of flour is also region-dependent: besides soft wheat flour, durum wheat semolina is used in southern Italy and Sardinia, chestnut flour in Tuscany, rye flour in Alto Adige, chickpea flour in Liguria, and corn flour in central Italy. Historically used, high-extraction flour has been largely replaced by refined flour.
- The consistency of dough varies from a pourable batter for unleavened flatbreads, to a firm dough for rolling or hand-flattening.
- Nine flatbreads are unleavened. For the leavened ones, the use of sourdough, predominant in the past, has been replaced by commercial baker's yeast. Three flatbreads are prepared with baking soda.
- Ancient baking systems consisted of terracotta or metal plates to be placed in the fireplace, today replaced by metal plates used on the gas stove. Domed wood-fired ovens are also popular among Italian consumers, followed by modern electric ovens.
- Flash baking (short time, high temperature) is typical of all flatbreads.

- Flatbread baking leads to delamination and double-layered structure if the flour used has good gluten quality, the dough is fermented, finely sheeted, and correctly proofed, and the baking temperature is very high.
- Among leavened flatbreads, 8 have a double-layered structure and 6 have a single-layered structure.
- Three flatbreads are baked twice, resulting in complete dryness and long shelf-life. These crispy flatbreads are typical of Sardinia, where shepherding has always been widespread.
- Quickly prepared unleavened breads are well established in the Tuscan-Emilian Apennines, Lunigiana, and the Po Valley.
- Some traditional Italian flatbreads (namely *Spianata* and *Zichi*) are associated, as decorated variants, with weddings and patronal festivals, and some are found in classical Italian literature, such as the Sardinian breads and the *Piadina romagnola*.

Overall, these results point out that Italian flatbreads differ regionally in terms of ingredients and preparation methods, and suggest encouraging the revival of the ancient tradition of using high-extraction flours and sourdough fermentation, today almost lost, to increase the fiber, mineral, and vitamin content and to ensure a rich sensory profile. Flatbreads have a fundamental importance in the cultural heritage of Italian gastronomy, and their identity must be recorded and preserved. A limitation of this study was the lack of historical information, highlighting the need to perform historical research to gain a deeper understanding of Italian flatbreads. Furthermore, recommendations for future research and development in traditional Italian flatbreads can be pointed out in the technical field. Regional products, made by traditional processing methods and associated with a specific geographic origin, encompass a greater "emotional quality" than products of less defined origin. In turn, these emotional aspects positively influence consumers' food choices. In this light, Italian flatbreads are deeply rooted in their origin and linked to traditional practices. Nonetheless, there is room for technical improvement, which is not in contrast with their genuineness. Most of the Italian flatbreads have not been studied in depth from key perspectives, such as shelf-life extension and automatic quality control.

More attention and innovations are needed in this area. Future trends and prospects, to attract new market segments, could be the reformulation for nutritional improvement and diversification of sensory characteristics. Further research should also be conducted to provide health benefits, thorough the formulation of functional flatbreads, and to improve the sustainability of the production process.

3.14 References

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Part II

Chapter 1. Innovation of flat breads

1. Innovation of flat breads

Flat breads can be considered as a versatile base, similar to a blank canvas, open to a wide range of modifications to meet new consumer nutritional needs and new market demands [1].

In this context, efforts have been made to differentiate the basic ingredients, represented by flour, leavening agent and fats or oils, with the aim of exploring new formulations and improve these products, as described below.

1.1 Flours

The main ingredient of flat breads is flour. It can be obtained by milling cereals, pseudo-cereals, legumes and other vegetable matrix [1]. The main cereals and pseudocereals are considered as follows, with a special *focus* on the use of legumes to which, given their importance in the Mediterranean area and high protein content, one of the work packages of the Flat Bread Mine project is dedicated.

1.1.1 Cereal and pseudocereals

Although flat breads are mainly made from wheat, corn and rice flour, the use of “minor” cereals is not marginal. Many traditional bread recipes already include barley, oats, rye, millet and sorghum. For example, the first three are commonly used in the north of Europe, especially in Scandinavian countries, while the last two are used in Somalian flat breads [2,3].

Barley (*Hordeum vulgare*, family *Poaceae*) is a cereal crop used mainly for animal feed and for malting to produce beer and other alcoholic beverages. Whole barley is commonly used in soup, while flour is used in bakery products. It contains protein, minerals, and fiber [4]. The latter includes the β-glucan, a soluble fiber associated with lower cholesterol levels and a reduced risk of heart disease. In addition, the fiber in barley helps to regulate blood sugar levels, making it beneficial for individuals with diabetes [4]. Recently, several authors have experimented with the use of barley flour and barley bran in the formulation of flat breads [5-11]. Overall, all have observed a nutritional improvement in terms of increased fiber, particularly β-glucans, indicating their potential for use in flat breads, especially given their good sensory acceptability [7].

Similarly, oat (*Avena sativa* L.) is a cereal crop source of carbohydrates, fiber, protein, phenolic compounds, vitamins, and minerals [12]. Oat is also a source of β-glucan and is now widely used in the formulation of porridge, breakfast cereals and snack bars [12]. Like barley, oat has been widely used in the formulation of flat breads and are now a favourite ingredient in the formulation of protein-fortified products for athletes and vegans [8-10].

Rye (*Secale cereale* L.) is a cereal crop adapted to coldclimates, particularly common in northern and eastern Europe. Like other cereals, rye flour is the main ingredient in Italian *Schüttelbrot* and *Puccia Iadina* and Scandinavian *Flatbrød* [2].

Millet (*Panicum miliaceum* L.) and sorghum (*Sorghum bicolor* L.) are resilient crops used in Somali *Laxoox* and Yemeni *Lahoh* flat breads [3]. These cereals have been explored in innovative flat bread formulations to assess their nutritional and antioxidant benefits, particularly due to the presence of phenols and other bioactive compounds [13-18].

Finally, pseudocereals, such as quinoa (*Chenopodium quinoa* W.), amaranth (*Amaranthus* L. spp.), and buckwheat (*Fagopyrum esculentum* M.) offer valuable opportunities for flat bread formulation due to their richness in a variety of health-promoting compounds. These include protein, peptides, flavonoids, phenolic acids, vitamins, amino acids, dietary fiber, and unsaturated fatty acids, among others [19]. Their flours have shown promising results when used for fortification, with potential applications, including combinations with other cereals [18, 20-23].

1.1.2 Legumes

The Food and Agriculture Organization of the United Nations (FAO) [24] recognizes ten major types of legumes: dry beans, including soybeans, dry broad beans, dry peas, chickpeas, cowpeas, pigeon peas, lentils, Bambara beans, vetches, and lupins. In addition, there are "minor" legumes that are less widely used on an international level. The four most commonly utilized species for food purposes, available in both dry and hydrated forms, are beans, lentils, peas, and chickpeas. Legumes are a healthy and versatile food option, particularly suitable for people with celiac disease, due to their gluten-free nature, and for those following a plant-based diet. Leguminous crops are resilient to climate change and have an inherent ability to fix atmospheric nitrogen, improving soil fertility [24].

Legumes have been incorporated into some traditional flat bread recipes and have also been proposed for innovative formulations. There is a growing interest in fortifying flat breads with legumes, as indicated by the publishing trend shown in Figure 5.

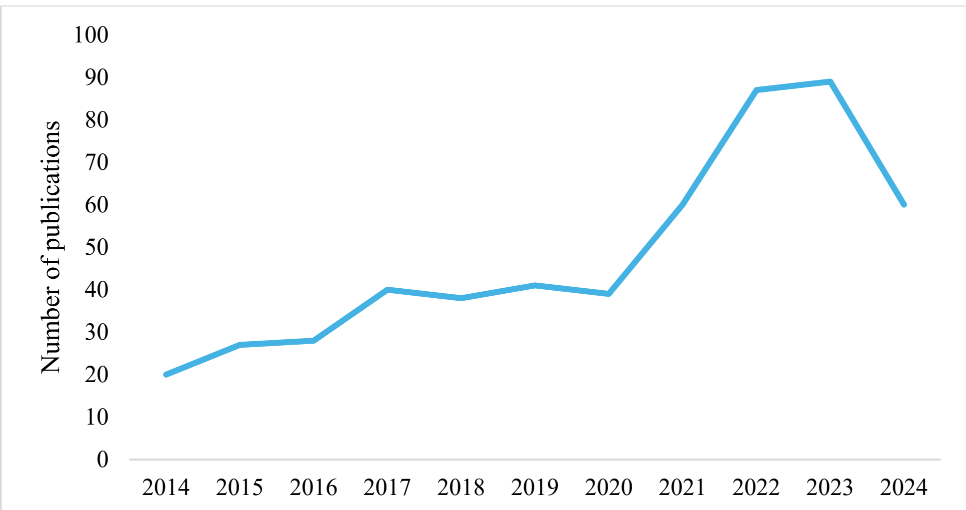

Figure 5. Number of published articles in the years 2014-2024 (Scopus search for “legume” and “flat bread” in all fields).

Considering the growing interest in legumes, it is necessary to study them further in terms of their benefits but also their possible nutritional drawbacks, looking for the best strategies to mitigate the latter.

1.1.2.1 Advantages of the legume inclusion

From a nutritional point of view, legumes are a source of vegetable proteins, generally ranging between 15 and 50%. The amino acid profile shows a significant content of lysine, an amino acid that is deficient in cereals. On the other hand, the presence of sulphur amino acids, such as cysteine and methionine, is limited. Therefore, the combination of cereals and legumes is recommended for a balanced meal, such as pasta, rice or other cooked cereals with cooked whole legumes or reduced in purée or flour, in the formulation of soups and bakery products [25,26].

Carbohydrates, mainly starch, accounted for 60 to 70% of the legume seed composition. The other non-digestible carbohydrates are fiber, in soluble and insoluble forms, which reduce the glycaemic impact of foods. Other carbohydrates are sugars, such as oligosaccharides, represented principally by raffinose, stachyose, and verbascose [25,26].

The lipid fraction of legumes is generally limited and is mainly represented by linoleic and linolenic acids. Soybeans and chickpeas are the richest compared to others [25,26].

In terms of micronutrients, B vitamins (folic acid, thiamine, and riboflavin) and minerals (magnesium, iron, manganese, zinc, copper, selenium, and calcium) predominate. Other minor components are the antioxidant compounds, mainly represented by phenolic compounds, carotenoids and tocopherols, which are active against oxidative stress and recognized for their health benefits [27].

1.1.2.2 Drawback of the legume inclusion

Legumes contain various compounds defined as “antinutrients” due to their ability to reduce the absorption of nutrients or the development of gastrointestinal disorders. Although some studies also suggest benefits related to the same compounds, they are generally considered a disadvantage of legume consumption, which must be overcome to take full advantage of the nutritional potential of legumes.

The raffinose family oligosaccharides (RFOs) is a group of soluble sugars which cannot be digested by the human body due to the absence of α-galactosidase, the enzyme responsible for their hydrolysis. Raffinose is the most important, hence the name, followed by stachyose and verbascose. The anaerobic fermentation of these sugars produces hydrogen, methane and CO_2, causing flatulence. This undesirable effect is often accompanied by nausea, cramps, diarrhoea and discomfort [28-30]. At the same time, the RFOs are now recognised for their prebiotic potential, considering the possibility of promoting the growth of *bifidobacteria* and *lactobacilli* in the cecum and reducing harmful bacteria [30].

Phytic acid, or myo-inositol hexaphosphate (IP6), reduces the availability of divalent and trivalent metal ions (e.g., Zn^{2+}, Fe^{2+}, Fe^{3+}, Ca^{2+}) and proteins by forming insoluble complexes that prevent their absorption. in the gastrointestinal tract and small intestine [30]. However, phytic acid is also recognised to have health benefits, such as antioxidant activity, prevention of type 2 diabetes, and anti-colon cancer activity [31].

Enzyme inhibitors, such as protease and α-amylase inhibitors, which are common in legume seeds, can reduce mineral bioaccessibility and decrease digestibility. These inhibitors target trypsin and chymotrypsin, inhibiting protein digestion. In addition, alpha-amylase inhibitors interfere with the breakdown of starch into oligosaccharides, slowing carbohydrate digestion and absorption [32].

Vicine and convicine, antinutritional compounds in faba beans, can cause haemolytic anemia, known as *favism.* This condition, which is common in the Middle East and the Mediterranean region (e.g. Sardinia, Italy), occurs in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In these subjects, red blood cells break down or die more quickly [33].

Finally, saponins, a class of compounds consisting of a lipophilic and a hydrophilic part, are responsible for emulsifying and foaming properties. These compounds are known to have adverse effects such as haemolytic, membranolytic and toxic activities [34].

Given the numerous nutritional benefits of legumes, researchers have investigated various techniques to reduce their anti-nutritional factor. Soaking, boiling, germination and fermentation are the traditional ones.

Soaking reduces the compounds characterized by good water-soluble, such as phytic acid, tannins, RFOs and saponins. Similarly, cooking reduces the thermolabile and water solubility compounds, also making the seeds edible and more pleasant. Germination and fermentation reduce or eliminate antinutrients in legumes, due to hydrolysis of compounds, structural changes or metabolic events. Close to these common treatments are emerging technologies, such as microwaving, extrusion and γ-irradiation [32].

Legume and legume-based foods are characterized by a sub-optimal texture, flavor, and color, which negatively affect their sensory properties. This may be an even more important issue than the anti-nutritional factors, because as the average consumer becomes more aware of the link between health and nutrition and a plant-based diet, their success in the marketplace may be limited by a poor sensory profile.

The volatile profile of legumes is the combination of aldehydes, alcohols, ketones, acids, pyrazines, furans, and sulfide compounds.

Legumes are generally associated with *bean*, *green*, *rancid* and *grassy* descriptors. These odour notes are mainly caused by compounds such as hexanal, heptanal, octanal, nonanal, (*E, E*)-2,4-decadienal, and (*E, E*)-2–4-nonadienal, which are the result of oxidative processes occurring in polyunsaturated fatty acids.

Other negatively reported compounds include dimethyl disulphide, a sulphur compound that contributes to a cabbage-like aroma. The volatile profile of legumes is influenced by intrinsic aspects of the seed, related to genetic aspects, and extrinsic aspects, related to cultivation, handling, drying and storage. Several studies have investigated these effects and potential solutions to improve the sensory acceptability of legumes [36-38]. These studies have mainly focused on peas, which are among the most commonly used in food formulations. However, more research has been suggested in this context on other widely used legumes, such as lentils.

When legume flours are mixed with wheat, their weak interactions reduce the elasticity and strength of the dough, due to the gluten dilution. Legume proteins cannot form strong gluten networks, which are essential for the viscoelastic properties of the dough.

This reduction in viscoelasticity negatively affects the dough's ability to incorporate air and retain gas during fermentation. As a result, breads made with legume proteins often have a poor crumb structure and suboptimal texture, lacking the desired lightness and chewiness [39].

In this context, the use of legume protein concentrates could allow a reduction in the amount needed to achieve a nutritional improvement, with less impact on the structural characteristics of the dough.

Alkaline and acid extraction are among the most popular conventional techniques for extracting proteins from legumes. This technique involves adjusting the pH of a solution of legume flour.

Alkaline substances, such as sodium hydroxide and potassium hydroxide, are used to reach a pH of 8–11, where proteins are solubilized. An acid, such as HCl, is then added to reduce the pH to around 4.0-4.8. This leads to precipitation and separation by centrifugation, filtration, or sieving [35]. However, wet fractionation is less environmentally friendly compared to dry fractionation, a sustainable technique based on physical separation. Dry fractionation works by efficiently separating protein bodies from other non-protein elements, such as starch granules and fiber, using appropriate milling techniques [40]. The milled flour is then separated into two fractions, one rich in starch and the other rich in protein, using a physical separation method. The most widely used technique for dry fractionation is air classification. In this method, air flows are introduced into a classifier chamber, creating centrifugal and gravitational forces that separate the flour into fine and coarse particles based on their size and density differences [40].

1.1.3 Vegetable powder and by-products

Several other plant ingredients, such as vegetables, fruits and various seeds, have been successfully tested in the formulation of flat breads.

Recently, Santamaria et al. (2024) [41] used plant powders (black and green olives, orange peel, lemon, tomato, beetroot, carrot, onion, artichoke, spinach, Swiss chard, kale and pak choi) at 2% in the formulation of flat bread and observed an increase in mineral content, fiber content and improved starch digestion *in vitro*, color and texture.

Similarly, Waseem et al. (2024) [42] used spinach powder in an unleavened flat bread to improve its sensory acceptability.

Pumpkin powder was used in different percentages, resulting in improved nutritional properties and antioxidant activity of the final bread 43].

Chapati bread, a typical Indian type, has been formulated with rice and wheat cereal by-products, to significantly increase fiber intake [44].

Grape pomace and grape seeds have been successfully used in the formulation of pizza, enriching this flat bread with phenolic compounds and antioxidants, significantly modifying the color and enhancing the volatile and sensory profile [45].

Another example is the use of hemp seeds in chapati, where the nutritional profile, rheological properties and digestibility were studied [46].

However, it should be emphasised that flat breads enriched with vegetables and seeds are already on the market and in demand by consumers.

Overall, the addition of plant-based ingredients has great potential, offering a diversification of colors, flavors and nutritional benefits. Nevertheless, it is important to consider the impact on texture and volatile profile, which are crucial for the acceptance of these innovative products.

1.2 Leavening agents

The flat breads can be classified into unleavened and leavened. The former are characterized by the absence of a fermentation phase. For the latter, this stage is crucial and is characterized by an increase in the volume of the dough, due to the production of CO_2 bubbles [47]. This event could be performed using different leavening agents, such as common baker's yeast, sourdough or baking powder [47]. The first has progressively replaced the traditional sourdough, due to the reduced time required and the lower effort. As a result, *Saccharomyces cerevisiae* is included in the list of ingredients of a large number of leavened flat breads [48].

The yeast cell metabolizes the carbohydrates, conducting the alcoholic fermentation and releasing energy. This event produces ethanol and CO_2 as principal metabolites and increases the volume of dough [47].

Sourdough is a dough made from flour and water and fermented by yeast and lactic acid bacteria (LAB). It is considered to be the oldest biotechnology used for souring and the production of cereal-based foods. There are four types of sourdough, depending on how the fermentation starts.

Type I is the traditional sourdough: it starts with a spontaneous fermentation carried out by the microbiota of the flour and water and is regularly propagated. The fermented dough is subjected daily to a process called "back-slopping", a cyclical reinoculation carried out with part of the fermenting dough, water and flour. Incubation is carried out at room temperature until the pH is stable and the dough can double in volume [49].

Type II sourdough is semi-liquid and is usually made with a selected starter. Incubation of the dough is carried out according to the starter chosen to achieve the fermentation.

Type III sourdough is the dried form of type II sourdough, using spray or drum drying technology. It is not a leavening agent, because it loses the leavening capacity with the drying treatment. It is used only as a nutritional and sensory improver. Finally, type IV sourdough is obtained using an initial starter, but the fermented dough is subjected to a back-slopping process [49].

The ratio of flour to water influences the consistency of the dough. An important parameter is the dough yield (DY), which is the ratio between the weight of dough and the weight of flour. The DY influences the LAB-yeast ratio. In general, liquid sourdough (DY >160) has a lower pH due to the acidification associated with the production of organic acids by the LAB [49].

Firm sourdoughs (DY < 160) have a higher yeast population. Recently, there has been a growing interest in sourdough, both for their nutritional benefits and for their positive organoleptic effects.

Fermentation can reduce anti-nutritional factors due to the acidic conditions and the microbial portfolio of enzymes (e.g. galactosidase, glucosidase and tannases) [49].

Another advantage of sourdough is the increase in resistant starch (RS), as the acidic environment can alter the structure of starch molecules, reducing their digestibility. This results in lower postprandial glycaemia. The inclusion of sourdough increases antioxidant activity, increasing free forms and total polyphenol content [49-52]. Finally, sourdough improves odor and taste, due to the production of acids, alcohols, aldehydes, esters and ketones. The proteolytic activity results in a higher concentration of free amino acids involved in the Maillard reaction [49-52].

Typically, sourdough is made from wheat flour, but recently other flours have been used, also taking into account the benefits of fermentation. The use of legumes, such as faba bean and chickpea flour and protein, has been experimented [53-55].

Many types of flat bread are made with traditional sourdough, but nowadays it has been replaced by compressed yeast in a large number of products, considering the lower labour and time required.

However, considering the interesting benefits, several authors have experimented with the use of sourdough in innovative flat breads. For example, Sanna et al. (2019) [56] used liquid sourdough in a semolina-based crispy flat bread and observed benefits in carbohydrate digestibility and an improvement in sensory acceptability. Similarly, the properties of *Sangak,* an Iranian flat bread, were improved by the use of sourdough, reducing the concentration of phytic acid and increasing the bioavailability of zinc [57]. In another study, Dastmalchi et al. (2016) noted a reduction in acrylamide in the same type of flat bread, using sourdough.

Finally, the last type of leavening agent is represented by baking powder, the combination of sodium or potassium bicarbonate and an acid compound, such as tartaric acid, whose interaction determines the production of CO_2 [47]. The baking powder is used, for example in the Italian *Piadina Romagnola, Crostolo, Torta al Testo, Pizza Scima* or in the Egyptian *Shamy* (Flat Bread Mine Database, 2022).

Zolfaghari et al. (2015) [58] studied the influence of the type of leavened agent on the volatile profile and sensory acceptability of *Barbari* bread, preferring the use of sourdough. A similar comparison and conclusions were performed by the same authors on *Lavash*, an Iranian single-layered flat bread [59].

1.3 Fats

In the formulation of many flat breads, the use of fats is required in the dough, during the mixing of ingredients. They lubricate the gluten proteins and limit their water absorption [60]. In some types of flat breads, the oil is added to the surface, after baking, to keep them soft and moist, enhance their flavor, and create a golden, slightly crispy crust (e.g. *Bazlama*). In the case of fried flat breads, fats are the medium in which the baking takes place, and this involves immersion in hot oil.

Fats can be classified into solid fats, such as butter, lard, margarine, and liquid fats, such as sunflower oil, olive oil or peanut oil [2]. The quantity and quality of fats could be considered relevant for the nutritional properties of the product, considering the formation of lipid oxidation products, with cytotoxic and genotoxic action [61]. In addition, the use of fats influences the dough structure and their machinability. Finally, they play a crucial role in the flavor and palatability of products.

Limited studies have been conducted on this the importance of fats in the formulation of some flat breads topic. For example, the type of oil has been evaluated in the formulation of pizza, comparing extra virgin olive oil and olive oil [62]. Similarly, the degree of oxidation of extra virgin olive oil in *focaccia* was evaluated [63]. Bavaro et al. (2021) [64] evaluated the use of liquid sourdough based on quinoa and amaranth as a fat substitute in the same flat bread, demonstrating a nutritional improvement represented by a 20% reduction in the amount of oil and a higher protein content than the traditional product.

In the light of the above and the nutritional and health criticism of fats, further studies could be carried out on this subject.

1.4 Commercial perspectives

In order to adapt to consumer habits and demand, the flat bread industries are proposing new versions of traditional products. In particular, flat breads are being enriched with protein to satisfy people following a protein-rich diet. Similarly, whole grains are being used to formulate natural and whole grain flat breads, including clean-label flat breads. Plant-based versions are available on the market, for example with seeds, vegetable powder, spices and herbs. Gluten-free versions are produced to meet the needs of people with celiac disease. The ready-to-eat options are the best solution for quick meal preparation and also offer significant diversification [65,66].

In this context, data collection was carried out between December 2021 and March 2022 in the countries involved in the Flat Bread Mine, in order to gather information on the types of flat breads available on the market, taking into account the nutritional data obtained from product labels [67]. A total of 232 flat breads were collected in the Mediterranean market, mainly with gluten. Single-layer were the most common with 127 products, while double-layer were 75 products. Wheat flour was the main ingredient in both types, but other flours such as spelt and durum wheat semolina were used. In the gluten-free versions, rice flour and starch are preferred. Overall, no additional fats were added to the single-layer products. In the products with additional oils, the rapeseed, olive, sunflower oils were preferred [67]

In terms of nutritional properties, the flat breads from Italy, Croatia and Spain were the richest and the most calorific, due to the fats. For example, *Piadina* is formulated with a relevant content of lard or oil, which is responsible for 9 kcal/g. On the other hand, the absence of fat reduces the energy [67]

To achieve a continuous nutritional and technological improvement of these products, the incorporation of higher amounts of legume flours and vegetable powders, the use of sourdough fermentation and the reduction of salt content could offer consumers even healthier alternatives.

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Chapter 2. Exploring the functional potential of pea-based sourdough in traditional durum wheat *focaccia*: Role in enhancing bioactive compounds, *in vitro* antioxidant activity, *in vitro* digestibility and aroma

This chapter has been submitted for publication as: **Vurro, F**.; Santamaria, M.; Summo, C.; Pasqualone, A.; Rosell, C. M.; Exploring the functional potential of pea-based sourdough in traditional durum wheat *focaccia*: Role in enhancing bioactive compounds, *in vitro* antioxidant activity, *in vitro* digestibility and aroma. *J. Funct. Food.* **2024** **(**Submitted, under review).

Exploring the functional potential of pea-based sourdough in traditional durum wheat *focaccia*: Role in enhancing bioactive compounds, *in vitro* antioxidant activity, *in vitro* digestibility and aroma

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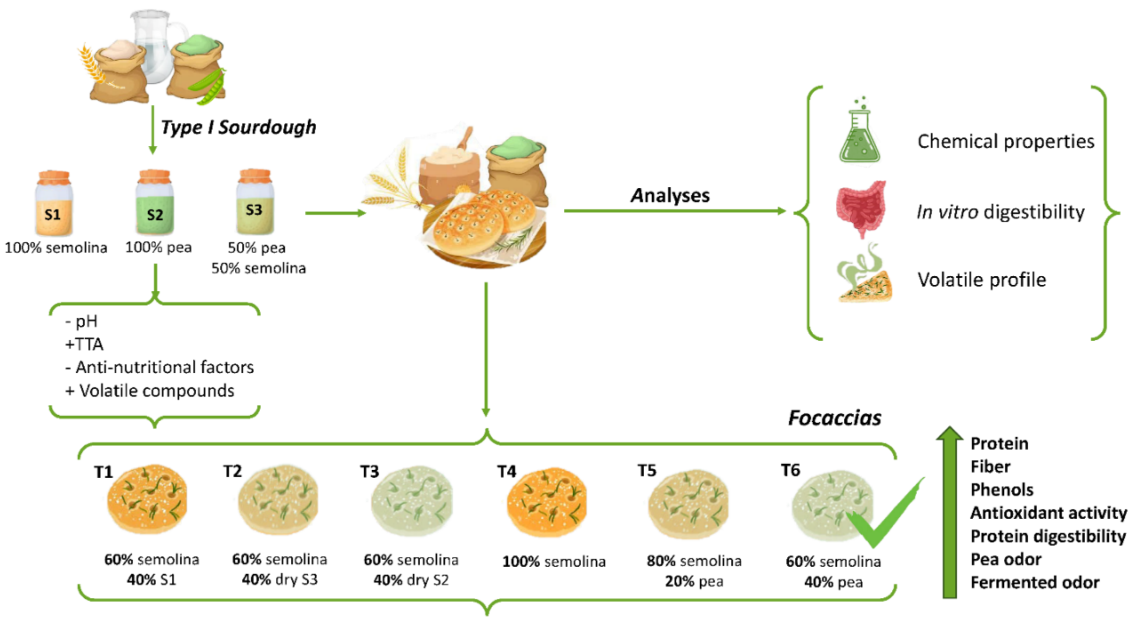
Graphical Abstract



Abstract

Legume-based sourdough is gaining momentum. This study aimed to compare the effectiveness of type I sourdough prepared with durum wheat semolina (S1), pea flour (S2) or 50:50 semolina/pea flour (S3) in improving the nutritional quality, antioxidant compounds, *in vitro* digestibility and aroma of traditional durum wheat *focaccia*. Six *focaccias* were prepared: three with 40% of S1, S2 and S3, and three with the corresponding amount of unfermented flours. Pea sourdough increased the content of phenolic compounds (8.82±0.12 mg GAE/g d.m. in *focaccia* with 40% pea flour and 4.92±0.41 mg GAE/g d.m. in unfermented semolina *focaccia*), and consequently increased the antioxidant activity. *Focaccias* with pea flour or pea sourdough were “source of protein” and “high fiber”, according to UE Reg. 1924/2006. Pea sourdough slowed down starch *in vitro* digestibility while enhancing protein digestibility and leading to a more complex volatile profile, with increased content of aldehydes, alcohols and Maillard reaction compounds.

Keywords: Flat bread; fermentation; legume flour; antioxidants; *in vitro* digestion; odorants

2.1 Introduction

Bread is a staple food with a rich history and a prominent cultural significance, especially when considering the gastronomic culture of Mediterranean populations. Leavening, the cornerstone of breadmaking, can be achieved using baking soda (“soda bread”), baker's yeast (composed mainly of *Saccharomyces cerevisiae*) or sourdough, a microbial consortium of bacteria and yeasts [1]. Sourdough represents the oldest application of biotechnology in the production of cereal-based foods and is classified into three types, depending on how the process of fermentation starts and its degree of hydration. Type I is obtained by spontaneous fermentation of a dough made of flour and water in a ratio of about 2:1; in type II, fermentation of a more hydrated dough is driven by the addition of selected microorganisms; type III is the dry form of type II, obtained by spray-drying, drum-drying or freeze-drying [2]. Over the years, sourdough, especially spontaneous type I, has gradually been replaced by baker's yeast to reduce fermentation times and overcome the difficulties associated with the daily back-slopping of sourdough. On the other hand, the use of sourdough is increasingly recommended due to health benefits related to protein and carbohydrate improved digestibility, reduction of antinutrients (e.g. phytates, tannins and enzyme inhibitors), increased antioxidant activity and enhanced aroma of the end product [3]. In recent years there has been a revival of fermentation with sourdough, linked to the general rediscovery of the traditions of the past. This trend was particularly evident during the COVID-19 crisis, which led many consumers to make their own bread at home with sourdough, either by choice or because baker's yeast supplies were depleted [4].

Focaccia is an Italian traditional oil-seasoned flatbread, commonly eaten as a street food throughout the country [6]. This flatbread is made by flattening a dough in a baking pan with fingertips, then fermenting, seasoning it generously with vegetable oil and various toppings, and finally baking [6]. In southern Italy, *focaccia* is traditionally prepared with durum wheat semolina (*Triticum turgidum* var. *durum*) [7], a raw material that has recently proven to be a viable alternative to wheat flour in a time of climatic and socio-political changes [8]. *Focaccia* is very appreciated for its sensory properties but is known to be rich in fat and poor in protein and fiber [6,9].

The addition of legume flours, such as chickpea and pea flours [7,10], has been proposed to improve the protein and fiber content of *focaccia*, as well as the content of bioactive compounds, known to have beneficial effects on human health (Padhi et al., 2017)[11]. However, to reduce antinutrients, legume flours should be fermented, producing a sourdough [3], as shown in recent studies that proposed using pea-based sourdough in conventional and gluten-free bread [12,13]. These studies observed a reduction in bread volume, a less important parameter in a product such as *focaccia*, which is typically characterized by reduced thickness. On the other hand, sourdough fermentation achieved a decrease in pea odor, generally poorly accepted by consumers [12,13]. In addition, peas, primarily appreciated for protein content, contain also antioxidant compounds, namely phenolics, able to effectively inhibit free radicals and to prevent oxidative reactions at cellular level. Therefore, the consumption of peas goes beyond a merely nutritional function, offering potential health benefits [14]. Pea is the second legume cultivated in Italy after faba bean, with an increasing production in 2023 [15].

The use of sourdough from any type of legume flour is still unexplored in *focaccia*. Therefore, the aim of this study was to improve the nutritional features, *in vitro* antioxidant activity, *in vitro* digestibility and aroma of traditional Italian durum wheat *focaccia* by using pea-based sourdough.

2.2 Materials and methods

2.2.1 Materials

Durum wheat semolina (De Cecco, Fara S. Martino, Italy) (carbohydrates 68 g/100 g; protein 14 g/100 g; fat 1.5 g/100 g; fiber 2.9 g/100 g; ash 0.87 g/100 g), baker's yeast (Mulino Caputo, Naples, Italy), olive oil (Olearia De Santis, Bitonto, Italy) and sea salt (Com-Sal Srl, Pesaro, Italy) were purchased from local retailers. Pea flour (carbohydrates 56 g/100 g; protein 24 g/100 g; fat 1.1 g/100 g; fiber 8.8 g/100 g; ash 3.01 g/100 g) was kindly provided by Andriani Spa (Gravina in Puglia, Italy).

2.2.2 Preparation of sourdough

Three type I sourdoughs were prepared according to Eraslan et al. (2023)[16], with few modifications, from: i) durum wheat semolina 100% (S1); ii) pea flour 100% (S2); iii) semolina and pea flour 50:50 (w/w) (S3). In detail, flour and tap water were mixed manually to obtain a homogeneous dough, with a dough yield (DY) = 200 (DY = dough weight/flour weight ×100). The dough was incubated in a sealed jar at 30 °C for 16 h (Memmert proofer, EN.CO., Spinea, Italy) to achieve a spontaneous fermentation. Then, 50 g of fermented dough were mixed with 50 g of semolina/pea/semolina-pea flour and 50 g of water, incubating again for 16 h at 30 °C, followed by 8 h storage at 4 °C. Flour and water addition and mixing, fermentation, and cold storage were repeated daily for 15 days (back-slopping). The resulting sourdoughs were freeze-dried (LyovaporTM L-200 Lyophilizer, Buchi, Switzerland), milled (ETA-Vercella, Turin, Italy), and sieved at a particle size of 212 µm (Giuliani Tecnologie, Turin, Italy). Freeze-dried, powdered sourdoughs were packed in plastic bags, and stored at 4 °C.

2.2.3 Microbial counts in the sourdough

For each sample, 10 g of freeze-dried sourdough was mixed with 9 mL of sterile peptone water (0.1%) (w/v) (Scharlab Chemine S.A., Barcelona, Spain) and homogenized in the stomacher for 3 minutes. The method UNE-EN 15787 [17] was used to determine *Lactobacillus* spp., with some modifications. The serial dilutions were prepared and then plated on Man, Rogosa and Sharpe agar (MRS) (Scharlab Chemine S.A., Barcelona, Spain). The plates were incubated under microaerophilia conditions at 37 ºC for 48 h. The yeasts and molds count (ISO 21527-2:2008(E)) [18] were incubated in dichloran glycerin selective agar (DG18 agar) (Scharlab Chemine S.A., Barcelona, Spain) at 25 ºC for 5 days under aerobic conditions. The analyses were performed in triplicate.

2.2.4 Preparation of focaccia

Six types of *focaccia* were prepared (Tab. 1), according to the production process described by Vurro et al. (2022) [6], with few modifications. Flour, yeast, freeze-dried sourdough and water were mixed with a spiral kneader (Bosh MFQ40304, München, Germany) for 6 min. Then, salt and oil (50% of the total amount) were added, continuing to knead for 6 min. The dough, manually flattened (about 1.5 cm thick) and circularly shaped with a pastry ring (diameter of 10.8 cm) (Tescoma, Cazzago San Martino, Italy), was placed into metal pans previously greased with oil (30% of the total amount) and left to rise for 90 min at 35 °C, RH = 33.5% (Memmert proofer, EN.CO., Spinea, Italy). A piece of dough was sampled at the end of leavening for analyses. Finally, the remaining oil (20% of the total amount) was evenly poured on the surface of *focaccia*, which was baked in an electric oven (Oem Ali Group, Bozzolo, Italy) at 200 °C for 25 min. Three different batches were made for each *focaccia* sample. The baked *focaccias* and the *focaccia* dough samples were freeze-dried (LyovaporTM L-200 Lyophilizer, Buchi, Switzerland), powdered, packed in plastic bags, and stored at 4 °C.

Table 1. Formulation of *focaccia* samples (S1 = 100% wheat sourdough; S2 = 100% pea sourdough; S3 = 50:50 wheat-pea sourdough).

Ingredients (g)	**Type of *focaccia***					
	With sourdough			**Without sourdough**		
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Durum wheat semolina	60	60	60	100	80	60
S1	40	0	0	0	0	0
S2	0	0	40	0	0	0
S3	0	40	0	0	0	0
Pea flour	0	0	0	0	20	40
Olive oil	10	10	10	10	10	10
Salt	2	2	2	2	2	2
Baker’s yeast	1	1	1	1	1	1
Water	70	70	70	70	70	70

2.2.5 pH and total titratable acidity

The pH and total titratable acidity (TTA) were determined in sourdough and *focaccia*, as well as in *focaccia* dough sampled before baking. The pH was determined by a pHmeter with a food penetration probe (HANNA instruments, Woonsocket, RI, USA). The TTA was measured as described in the American Association of Cereal Chemists (AACC) method 02–31.01 (AACC, 2010)[19]. The analyses were performed in triplicate.

2.2.6 Antinutritional factors

The antinutritional factors were determined in the flours, sourdough, and *focaccia*. Stachyose, raffinose and sucrose were determined by high-performance liquid chromatography (HPLC) (Agilent Technologies, Santa Clara, USA), equipped with Refractive Index Detector (RID 1260), as described by De Angelis et al. (2021) [20], while the content of phytic acid was measured with the assay kit (Megazyme

International, Bray, Ireland), following the manufacturer's procedure. Three replicates were carried out.

2.2.7 Bioactive compounds and in vitro antioxidant activity of focaccia

The total carotenoid pigments were determined according to the AACC method 14–60.01 (AACC, 2010)[19], measuring the absorbance at 435.8 nm with a Cary 60 UV-Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). The total carotenoid content was expressed as mg β-carotene/kg d.m. The phenolic compounds were extracted and quantified as described in Pasqualone et al. (2023) [21], expressing the results as mg of gallic acid (GAE)/g d.m. The antioxidant activity *in vitro* was determined by 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazylcrylhydrazyl (DPPH) assays as reported by Vurro et al. (2022) [6]. The results were expressed as μmol Trolox equivalents (TE)/g d.m. Three replicates were carried out for all the analyses.

2.2.8 Nutritional composition of focaccia

The moisture content was determined by a moisture analyzer at 105 °C (Radwag Wagi Elektroniczne, Radom, Poland) according to the AACC method 44-01.01 (AACC, 2010)[19]. The protein content (total nitrogen × 6.25) and the ashes were determined as described in the AACC methods 46-11.02 and 08-01.01, respectively (AACC, 2010) [19]. The lipid fraction of *focaccia* was extracted and quantified as described by the AOAC 945.38 F (AOAC, 2006)[22]. The total dietary fiber was determined by the enzymatic–gravimetric procedure, according to the AOAC method 991.43 (AOAC, 2006)[22]. Total carbohydrates were calculated by difference, subtracting to 100 the sum of moisture, lipids, ashes and protein. Results were expressed as g/100 g of fresh matter. The energy value (kcal) was calculated considering the Atwater general conversion factors and the contribution of 2 kcal/g from the total dietary fiber, according to Annex XIV of Regulation (EU) No 1169/2011 [23]. Total starch was quantified with a commercial kit (Megazyme International, Bray, Ireland) following the manufacturer's procedure. Results were expressed as g/100 g d.m. The analyses were performed in triplicate.

2.2.9 In vitro protein digestibility of focaccia

In vitro protein digestibility (IVPD) was determined as described in Espinosa-Ramírez et al. (2018) [24]. An aliquot of sample containing 6.25 mg of protein was suspended in 1 mL of water and placed in a water bath at 37 °C. The pH was adjusted to 8.00. Trypsin solution (Trypsin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, USA) was prepared at a concentration of 1.6 mg/mL, with an activity of 13,766 BAEE units/mg protein, adjusting the pH to 8.00. An aliquot of 0.1 mL of enzyme solution was added to the sample suspension and the drop of pH was recorded, starting from 5 s after the addition, at 1 min intervals for 10 min. IVPD was calculated according to the equation: $IVPD = 210.464 - 18.1x$, where x = pH after 10 min. Three replicates were carried out.

2.2.10 In vitro carbohydrate digestibility of focaccia

In vitro digestibility of carbohydrates was determined by measuring the release of glucose from the samples incubated first with porcine pancreatic α-amylase (Sigma-Aldrich, St. Louis, MO, USA) and then with amyloglucosidase (AMG) (Novozymes, Bagsværd, Denmark), followed by colorimetric reaction catalyzed by glucose oxidase–peroxidase (GOPOD) (Megazyme International, Bray, Ireland) as described by Santamaria et al. (2022) [25] and absorbance measure at 510 nm with a microplate reader (Epoch, BioTek, Winooski, VT, USA). Starch was calculated as glucose (mg) × 0.9. Rapidly digestible starch (RDS) was assessed after 20 min of hydrolysis, slowly digestible starch (SDS) between 20 and 120 min, while resistant starch (RS) was the unhydrolyzed fraction after 24 h. The digestion kinetics was calculated according to the equation: $C = C\infty\ (1 - e^{-kt})$ where C = percentage of starch hydrolyzed at t time, $C\infty$ = equilibrium concentration or maximum hydrolysis and k = kinetic constant. The analyses were performed in triplicate.

2.2.11 Volatile profile of sourdough and focaccia

The volatile compounds (VOCs) of freeze-dried sourdough and *focaccia* were evaluated by gas chromatography/mass spectrometry analysis (GC-MS), after an extraction phase with headspace solid-phase microextraction (HS-SPME), as reported in Vurro et al. (2022) [6]. The identification of VOCs was performed with the reference mass spectra of the National Institute of Standards and Technology (NIST) and Wiley libraries. Their quantification was carried out using 1-propanol as the internal standard. The concentrations of VOCs were expressed as μg/g of sample. The analyses were carried out in triplicate.

2.2.12 Statistical analysis

Statistical analysis was carried out by Minitab Statistical Software 21 (Minitab Inc., State College, PA, USA). The results were all expressed as mean ± standard deviation (SD) of replicates. The significant differences (α = 0.05) were verified through the application of parametric one and two-way analysis of variance (ANOVA), followed by the Tukey HSD test. The two variables considered in the two-way analysis of variance were the inclusion of sourdough (*S*) and the inclusion of pea flour (*P*).

2.3 Results and discussion

2.3.1 Sourdough properties

Table 2 shows pH, TTA and microbial count of sourdoughs. After 15 days, the lowest pH value was reached in the wheat-only sourdough (S1), while the addition of 50% pea flour (S3) increased the pH, reaching the maximum value of 4.15 in pea-only sourdough (S2). The highest ΔpH was observed in S1, while no significant differences were assessed between S2 and S3. The nutritional composition of pea and wheat flours and the environmental microbiota were responsible for the differences observed [26], being the incubation conditions kept constant. The pH of sourdoughs was in line with the most common range of 3.5-4.3, reported in literature [2]. The spontaneous microbiota fermented the carbohydrates and produced organic acids, influencing both pH and TTA of sourdoughs. TTA was higher in S2 and S3, suggesting that more organic acids were produced when pea flour was included in sourdough [27], but without affecting the final pH. The discrepancy observed between pH and TTA was probably related to the buffering capacity of pea flour, due to its higher mineral content than wheat flour [26], in agreement with the findings of other authors in buckwheat, quinoa, and teff sourdoughs [28].

Table 2. pH, total titratable acidity (TTA), and microbial counts (LAB, yeasts and molds) of sourdoughs (S1 = 100% wheat sourdough; S2 = 100% pea sourdough; S3 = 50:50 wheat-pea sourdough).

Sample	Initial pH	Final pH (after 15 d)	ΔpH	TTA (mL NaOH 0.1M/ 10 g)	LAB (Log CFU/g)	Yeasts and molds (Log CFU/g)
S1	5.75 ± 0.05^{b}	3.4 ± 0.00^{c}	2.35 ± 0.05^{a}	16.12 ± 0.88^{b}	6.34 ± 0.05^{b}	2.28 ± 0.09^{c}
S2	6.10 ± 0.00^{a}	4.15 ± 0.05^{a}	1.95 ± 0.05^{b}	22.93 ± 1.01^{a}	8.88 ± 0.06^{a}	5.30 ± 0.05^{a}
S3	5.85 ± 0.05^{b}	3.75 ± 0.05^{b}	2.1 ± 0.00^{b}	21.42 ± 0.52^{a}	5.03 ± 0.05^{c}	3.98 ± 0.05^{b}

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$.

LAB counts were higher than yeast and mold ones (Tab. 2), which suggests their better adaption to the fermenting conditions. Significant differences were found in the microbial composition of the tested sourdoughs. Both LAB and yeasts and molds were higher in the 100% pea sourdough (S2). Lazo-Vélez et al. (2021) [29] explained high levels of LAB and yeasts with high amounts of mono- and disaccharides, which contribute to their metabolism. However, those values decreased in S3, probably due to the coexistence of species through either mutualism or antagonism. The flour type influences the development of bacteria and yeast species, which affects the digestion of different carbohydrates [2]. Furthermore, yeast and mold counts were lower in wheat containing sourdoughs (S1 and S3). It has been reported that wheat sourdough does not exceed 5 log CFU/g in fungal counts (yeasts and molds) [30].

In legumes and whole cereals, fermentation is typically adopted to reduce the antinutritional compounds [30]. The latter belong to different classes of metabolites, among which phytates and oligosaccharides of the raffinose family (RFOs) are the main ones in legumes [31].

A significant reduction of phytates was observed in the three sourdoughs compared to the starting flours (Tab. 3), indicating that the acidic conditions enhanced the endogenous phytase activity of the flours, probably reinforced also by the enzymatic activity of microorganisms [32]. The phytates were reduced from 0.19 g/100 g d.m. in semolina to 0.04 g/100 g d.m. in S1 (100% wheat sourdough). The initial concentration detected in semolina was lower than data reported by Millar et al. (2019) [26] but in line with Hager et al. (2012) [33]. The concentration of phytates of pea flour, accounting for 0.82 g/100 g d.m. and intermediate to the values reported by Millar et al. (2019) [26] and Pedrosa et al. (2020) [34], lowered to 0.57 g/100 g d.m. in S2 (100% pea sourdough).

By reducing phytates, known to chelate calcium, iron, copper and zinc, sourdough could increase the availability of minerals.

Table 3. Antinutritional factors of flours and sourdoughs (S1 = 100% wheat sourdough; S2 = 100% pea sourdough; S3 = 50:50 wheat-pea sourdough).

Sample	Phytates g/ 100 g d.m.	Stachyose (mg/g d.m.)	Raffinose (mg/g d.m.)	Sucrose (mg/g d.m.)
Durum wheat semolina	0.19±0.01[a]	4.68±0.36[a]	2.35±0.11[a]	11.02±0.29[a]
S1	0.04±0.00[b]	0.84±0.04[b]	n.d.	0.57±0.03[b]
Pea flour	0.82±0.01[a]	57.27±0.76 [a]	11.57±1.27[a]	31.56±0.19[a]
S2	0.57±0.01[b]	14.50±4.93[b]	0.32±0.02[b]	0.78±0.02[b]
Mix semolina-pea flour (50:50)	0.52±0.05[a]	26.95±2.07[a]	6.40±0.21	26.14±3.17[a]
S3	0.11±0.00[b]	9.79±2.13[b]	n.d.	0.61±0.02[b]

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. The comparison has been performed comparing the sourdough with the corresponding flour. n.d.= not detected

Similarly, microbial enzymes, such as α-galactosidase, catalyzed the hydrolysis of RFOs [32], which significantly decreased with fermentation in all three sourdoughs considered, compared to the starting flours. The main gastrointestinal disorders associated with the consumption of legume-based foods are attributed to these carbohydrates. However, researchers have recognized and reassessed the prebiotic action exerted on the genera *Bifidobacteria* and *Lactobacillus* that populate the large intestine, which may provide human health benefits [32].

2.3.2 Focaccia physic-chemical and nutritional properties

Both the variables "inclusion of sourdough" and "inclusion of pea flour" had a significant effect ($p \leq 0.05$) on the pH and TTA of the doughs and *focaccia* samples, confirmed by a significant interaction of the two variables (Tab. 4). The use of sourdough resulted in a significant decrease in pH and an increase in TTA of dough and *focaccia* (T1, T2 and T3 samples) compared to non-fermented flours (T4, T5 and T6 samples). Furthermore, T3 *focaccia*, containing 100% pea sourdough, showed the highest TTA, in agreement with the results observed in the starting sourdoughs (Tab. 2). Among the *focaccia* samples without sourdough, T5 and T6, which contained pea flour, had higher TTA than T4, prepared without pea flour. This result could be due to the higher fiber content of pea flour. A study conducted by Al Khatib et al. (2020) [35] showed a strong positive correlation between TTA and fiber content in pita bread.

Table 4. pH and total titratable acidity (TTA) of dough and *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Formulation	**pH**		**TTA (mL NaOH 0.1M/ 10 g)**	
	Dough	***Focaccia***	**Dough**	***Focaccia***
T1	4.49 ± 0.04^{e}	4.50 ± 0.00^{c}	7.15 ± 0.25^{b}	4.50 ± 0.50^{c}
T2	4.53 ± 0.03^{e}	4.54 ± 0.07^{c}	11.95 ± 1.25^{a}	7.60 ± 0.10^{b}
T3	4.73 ± 0.01^{d}	4.64 ± 0.00^{c}	13.18 ± 0.73^{a}	9.03 ± 0.48^{a}
T4	5.99 ± 0.01^{c}	6.29 ± 0.09^{b}	4.18 ± 0.18^{c}	1.23 ± 0.03^{e}
T5	6.17 ± 0.00^{b}	6.46 ± 0.12^{ab}	5.18 ± 0.18^{c}	2.73 ± 0.47^{d}
T6	6.29 ± 0.02^{a}	6.50 ± 0.04^{a}	5.60 ± 0.10^{bc}	2.85 ± 0.35^{d}
***p-value* (P*S)**	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$
***p-value* (S)**	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
***p-value* (P)**	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant

The phenolic compounds significantly increased with the inclusion of pea flour or pea sourdough in *focaccia,* and the interaction of the two variables was significant ($p \leq 0.05$) (Tab. 5). The observed contents of phenolic compounds in the samples with unfermented pea flour (T5 and T6) agreed with Davies-Hoes et al. (2017) [36], who fortified bread with pea flour at different particle sizes. The acidification process and the microbial enzymatic activity, in fact, promoted the bioconversion of phenolic compounds, concentrated in the pea cotyledon [11] and bound to the cell walls, into more available forms [16,32]. The increase in phenolic compounds observed with the inclusion of sourdough, compared to the non-fermented versions of *focaccias*, amounted to 32%.

Table 5. Bioactive compounds and antioxidant activity of *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	Carotenoids (mg β-carotene/kg d.m.)	Phenolic compounds (mg GAE/g d.m.)	DPPH (µmol TE/g d.m.)	ABTS (µmol TE/g d.m)
T1	4.42±0.34^{f}	7.15±0.11^{b}	0.24±0.03^{c}	0.02±0.00^{d}
T2	13.14±0.53^{d}	8.07±0.20^{a}	0.52±0.03^{b}	0.48±0.05^{c}
T3	18.96±0.31^{b}	8.82±0.12^{a}	0.90±0.05^{a}	1.14±0.04^{b}
T4	8.13±0.56^{e}	4.92±0.41^{d}	0.35±0.02^{c}	0.53±0.04^{c}
T5	15.24±0.52^{c}	5.33±0.34^{d}	0.54±0.05^{b}	1.00±0.05^{b}
T6	26.39±0.85^{a}	6.13±0.58^{c}	1.01±0.10^{a}	1.83±0.19^{a}
p-value (P*S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazylcrylhydrazyl; ABTS = 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid. Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant

A significant effect ($p \leq 0.05$) of both "inclusion of sourdough" and "inclusion of pea flour" was observed for the content of carotenoids, with a significant effect of the interaction ($p \leq 0.05$) (Tab. 5). Their concentration, ranging from 4.42 to 26.39 mg β-carotene/kg d.m., decreased with the addition of sourdough, as observed when comparing T5 with T2 (-14%) and T6 with T3 (-16%). This reduction was likely ascribed to oxidation events related to the incorporation of oxygen and the production of hydrogen peroxide by microbial metabolism during sourdough fermentation [37]. Carotenoids, characterized by pro-vitamin A and antioxidant activity, are the main pigments of durum wheat, and are responsible for the typical golden color of semolina, pasta and baked goods [7]. Likewise, together with tocopherols, carotenoids are the main lipophilic antioxidants of pea cotyledons [11], with a content varying according to the varieties (10.1-40.21 mg β-carotene /kg) [11,38]. The *in vitro* antioxidant activity, evaluated by the DPPH and ABTS assays, highlighted a positive influence exerted by pea flour, both unfermented and fermented ($p \leq 0.05$). Therefore, this ingredient shows functional potential in terms of antioxidant properties, in addition to other health benefits reported in the literature, such as hepatoprotective, anti-hyperglycaemic and anti-tumour effects [11].

Table 6 shows the proximate composition of the *focaccias.* Both "inclusion of sourdough" and "inclusion of pea" had a significant effect on the moisture and fiber content ($p \leq 0.05$), while for lipids and protein, only "inclusion of pea" had a significant effect ($p \leq 0.05$) and the interaction between the two variables was not significant. The moisture content varied from 22.28 to 28.22 g/100 g, with the lowest value in wheat-only *focaccia* prepared with sourdough (T1). The different fiber content (higher in pea flour) could corroborate the different capacities of the flours blends to retain water, influencing therefore the moisture content of *focaccia*. Lipids were in the range 8.12-8.84 g/100 g. This limited range of variation was due to the use of the same amount of olive oil in all the formulations and to the minimal contribution of lipids by the other ingredients used. The ash content was directly related to the minerals of the flours used. Indeed, the highest concentration of ash was observed in all the samples containing pea flour or pea sourdough (T2, T3, T5 and T6), which were richer in minerals than wheat flour, as also observed by Millar et al. (2019) [26]. All the samples with pea flour had a higher protein content than those containing only wheat flour (T1 and T4), and were able to provide more than 12% of the energy value of the product. Therefore, the "source of protein" nutritional claim applied, according to Regulation (EC) No 1924/2006 [39]. Contrarily to protein, total carbohydrates were lower in all pea-fortified samples. The substitution of semolina with pea flour diluted the total starch content, considering the direct correlation with the chemical composition of the starting flours, while the fermentation did not interfere ($p \geq 0.05$). Similar results were found recently by Moreno-Araiza et al. (2023) [40], when comparing pea fortified bread with bread prepared with wheat flour only. Also, the fiber content of *focaccias* containing pea flour and pea sourdough (T2, T3, T5 and T6) was markedly higher than 6 g/100 g, meeting the requirements for the "high fiber" nutritional claim (Regulation (EC) No 1924/2006)[39]. A significant ($p \leq 0.05$) effect of "inclusion of sourdough", "inclusion of pea" and their interaction was observed for the antinutritional compounds (namely raffinose family oligosaccharides and phytates) (Tab. 6), suggesting that the inclusion of legumes increases their concentration, but fermentation reduces it. Indeed, the concentration of antinutritional compounds was higher in T5 and T6, with pea flour, than in the wheat-only *focaccias*, but significantly decreased when pea was fermented into sourdough. Baik et al. (2012) [41] and Coda et al. (2017) [3] highlighted the ability of fermentation to reduce the antinutrients in bread enriched with chickpeas, lentils and faba beans.

Overall, the obtained results highlight that the nutritional profile of the fortified *focaccias* significantly improved by adding pea sourdough due to the increase in protein, fiber and bioactive compounds, without markedly raising the antinutrients. This improvement limits the typical negative nutritional features of *focaccia*, particularly its richness in fat and poverty in protein and fiber [7,8].

The addition of pea sourdough is therefore a good strategy to improve the nutritional features of this traditional product, with potential health benefits, linked to the reduction of the risk of chronic and inflammatory diseases [42]. Similar nutritional improvements have been previously observed in bakery products enriched with flours or sourdough prepared from legumes other than peas, such as black chickpea flour used in the formulation of *focaccia* [7], or type I sourdough prepared from chickpea or chickpea-bean-lentil flour blends, used in breadmaking [16,43].

Table 6. Nutritional composition and antinutritional compounds of *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	Moisture (g/100 g)	Lipids (g/100 g)	Protein (g/100 g)	Carbohydrates (g/100 g)	Fiber (g/100 g)	Ash (g/100 g)	Energy value (kcal/100 g)	Total starch (g/100 g d.m.)	Stachyose (mg/g d.m.)	Raffinose (mg/g d.m.)	Sucrose (mg/g d.m.)	Phytates (g/100 g d.m.)
T1	22.28±1.93^{c}	8.84±0.18^{a}	9.08±0.52^{c}	57.95±2.22^{a}	4.87±0.21^{c}	1.85±0.04^{c}	335.64±6.98^{a}	52.94±4.38^{ab}	0.57±0.01^{d}	0.1±0.01^{d}	5.75±0.56^{d}	0.13±0.00^{d}
T2	27.51±0.39^{ab}	8.17±0.17^{ab}	10.64±0.33^{b}	51.68±0.38^{bc}	7.50±0.36^{b}	2.01±0.09^{bc}	307.80±1.79^{cd}	47.07±1.43^{bc}	4.59±0.7^{c}	0.01±0^{d}	6.68±0.67^{d}	0.20±0.01^{c}
T3	28.22±0.26^{a}	8.12±0.56^{b}	12.49±0.04^{a}	48.89±0.78^{c}	8.93±0.25^{a}	2.29±0.06^{a}	300.70±2.36^{d}	40.72±1.70^{cd}	8.87±0.7^{b}	0.46±0^{c}	5.22±0.47^{d}	0.37±0.01^{a}
T4	26.38±0.27^{ab}	8.73±0.07^{ab}	8.75±0.40^{c}	54.56±0.71^{b}	5.37±0.60^{c}	1.59±0.07^{d}	321.03±0.92^{b}	57.74±1.45^{ab}	3.22±0.05^{cd}	0.01±0^{d}	15.19±0.4^{c}	0.15±0.01^{d}
T5	25.74±0.12^{b}	8.72±0.01^{ab}	10.93±0.86^{b}	52.67±0.86^{b}	8.10±0.40^{ab}	1.95±0.09^{bc}	316.64±1.08^{bc}	49.27±6.40^{abc}	10.81±1.58^{b}	2.14±0.12^{b}	21.79±0.33^{b}	0.29±0.03^{b}
T6	27.43±0.16^{ab}	8.40±0.18^{ab}	13.31±0.45^{a}	48.75±0.26^{c}	9.07±0.21^{a}	2.11±0.01^{ab}	305.70±0.51^{d}	36.76±0.42^{d}	26.88±1.52^{a}	7.8±0.02^{a}	27.69±2.85^{a}	0.39±0.01^{a}
p-value (P*S)	$p \leq 0.05$	ns	ns	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	ns	ns	ns	$p \leq 0.05$	$p \leq 0.05$	ns	ns	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns not significant. * "Source of protein" according to Regulation (EC) No 1924/2006.

2.3.3 *In vitro* digestibility of proteins and carbohydrates

Both the variables "inclusion of sourdough" and "inclusion of pea flour" had a significant effect ($p \leq 0.05$) on the *in vitro* digestibility of proteins and carbohydrates of *focaccia* samples (Tab. 7). *In vitro* protein digestibility (IVPD) was determined by hydrolysis with trypsin, recording the subsequent drop in pH [3,24]. This analysis gives an indication of the potential behaviour of protein during the digestion process, related to the nutritional quality in terms of availability of amino acids [3,24]. IVPD values above 78% were observed in all the *focaccia* samples (Tab. 7). The addition of unfermented pea flour (T5 and T6) resulted in lower IVPD compared to *focaccia* prepared with semolina alone (T4), probably due to the fiber and antinutrients contributed by the pea. The decrease of IVPD, indeed, progressed as the amount of pea flour increased. The fortification with sourdough prepared with pea flour alone (T3) overcame this drawback as it led to significantly higher IVPD than *focaccias* with the highest amount of unfermented pea flour (T6). This improvement was due to both the proteolytic activity of the LAB and the inactivation of the protease inhibitors occurred during sourdough fermentation [3,24]. However, conflicting results were observed for the samples containing semolina and semolina-pea sourdough (T1 and T2), which presented an inferior IVPD with respect to the unfermented ones (T4 and T5). A similar situation was reported by other authors [44], who explained it with either the possibility that higher hydrolysis has already occurred in these samples during the fermentation, prior the analysis, or that protein aggregated or complexed the starch, with a reduction of activity of the enzyme added during the analysis.

The *in vitro* digestibility of starch was evaluated by measuring the release of glucose during the incubation with hydrolytic enzymes. Overall, a decrease of RDS and an increase of SDS was observed in the *focaccia* samples containing unfermented pea flour or pea-based sourdough compared to samples prepared with unfermented semolina or semolina sourdough. In detail, sample T1, prepared with semolina sourdough, was characterized by the highest content of RDS and the lowest content of SDS (Tab. 7). The addition of increasing amounts of pea sourdough (samples T2 and T3) progressively and significantly lowered the content of RDS compared to T1, while the amount of SDS increased significantly. Among the samples prepared without sourdough, T6, prepared with the greatest addition of pea unfermented flour, showed the lowest amount of RDS and the highest amount of RS. The levels of RS in the other samples did not show significant differences among them. Accordingly with these data, the maximum hydrolysis ($C\infty$) and the kinetic constant (k) were the highest in *focaccia* prepared with semolina sourdough (T1), and significantly decreased when pea flour was added, especially unfermented (T6). Therefore, while *focaccia* prepared with semolina sourdough could have a greater impact on glycaemic levels, the addition of peas could slow down glucose release. The slower rate of starch degradation after the addition of pea flour or pea sourdough was probably related to the fiber and protein content of pea flour, which created a physical barrier limiting the activity of the enzymes [45], as well as to the effect of acidification, similarly inhibiting the activity of α-amylase and α-glucosides [46].

Table 7. *In vitro* protein digestibility (IVPD), content of different starch fractions (RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch), and kinetic parameters of starch hydrolysis ($C\infty$ = equilibrium concentration or maximum hydrolysis, k = kinetic constant) assessed in *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	IVPD (g/100 g protein)	RDS (g /100 g starch)	SDS (g /100 g starch)	RS (g /100 g starch)	$C\infty$	k
T1	78.61±0.09^{de}	62.44±3.26^{a}	25.53±2.16^{d}	12.02±1.1^{b}	91.82±0.74^{a}	0.062±0.005^{a}
T2	78.79±0.27^{d}	53.42±1.04^{b}	36.85±1.06^{c}	9.73±2.1^{b}	78±0.54^{b}	0.044±0.000^{b}
T3	81.32±0.09^{a}	43.36±0.03^{c}	43.70±1.38^{a}	12.94±1.42^{b}	72.61±0.2^{c}	0.034±0.001^{cd}
T4	79.96±0.18^{b}	43.08±3.43^{c}	44.69±2.51^{a}	12.23±0.92^{b}	79.36±0^{b}	0.029±0.000^{d}
T5	79.33±0.09^{c}	46.95±1.08^{c}	42.22±0.97^{ab}	10.82±2.06^{b}	68.02±1.62^{d}	0.041±0.004^{bc}
T6	78.15±0.18^{e}	41.41±2.61^{c}	39.35±0.08^{bc}	19.23±2.7^{a}	50.13±2.29^{e}	0.035±0.002^{cd}
p-value (P*S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant.

2.3.4 Volatile profile of dry sourdoughs and focaccias

The olfactory impact of foods contributes significantly to consumer acceptance and choice, requiring special attention in the development of new products, especially with legume flours, to which unpleasant odors are generally attributed [47]. On the other hand, sourdough has a positive effect on the aroma of bakery products, which is influenced by the complexity of the microbiota, time and temperature of fermentation, and number of back-sloppings [2,48].

In order to quali-quantitatively analyse the main volatile compounds of sourdoughs and *focaccias*, they were extracted by headspace solid-phase microextraction, then a gas-chromatographic analysis was carried out, coupled to mass spectrometry. The content of aldehydes differed among sourdoughs (Tab. 8), with higher concentrations in S2 and S3, both containing pea flour. The most abundant aldehyde was hexanal, correlated with the linolenic acid oxidation and with the fermentation process [47,48]. This aldehyde is principally associated with beany, fatty, rancid and green notes, which are generally negatively considered [47,49]. A similar trend was observed for nonanal, originated by the oxidation of oleic acid, as well as for heptanal and octanal, from oleic acid and/or linoleic acid, equally responsible for grassy and vegetal notes [49]. Only one ketone, the 6-methyl-5-heptene, was identified and quantified. It was present only in pea-containing sourdough, although in little amounts.

Lipid oxidation also generated alcohols, such as 1-hexanol and 2-heptanol, while the fermentation of carbohydrates produced ethyl alcohol [49]. Of particular relevance, in S2 and S3 (containing pea), were 1-octen-3-ol, 2-methyl-1-butanol, and 3-methyl-1-butanol (the latter quantitatively relevant), all typically detected in the volatile profile of peas [47,49]. The first two compounds could derive from leucine and isoleucine, involved in the Ehrlich pathway in yeast cells [50].

2-Pentylfuran, another compound associated with the typical pea odor [47,49], was significantly higher in S2 and S3 (2.34 and 1.67 μg/g respectively), than in S1.

The LAB fermentation produces abundant acetic acid, which is responsible for the sour aroma of bread prepared with sourdough [48]. The concentration of acetic acid was higher in the sourdough formulated with semolina only, than in the others. Numerous esters were also detected, mostly acetates and lactates. Esters arise from fermentation and can enhance the complexity of the volatile profile, contributing with several sensory notes. For example, ethyl lactate, higher in S1, is associated with caramel and butter notes, whereas octyl acetate, higher in S2 and S3, confers green and mushroom notes. Finally, ethyl acetate, one of the most abundant volatile compounds reported in sourdough, characterized by a fruity odor [48], was more concentrated in S1. These differences were related to the raw material and wild microflora composition, inducing different metabolic pathways [48].

Table 8 reports also the volatile compounds of *focaccia* samples. The volatile compounds of sourdough were generated mainly by enzymatic and microbial processes during fermentation, while thermal reactions caused the formation of new compounds in the baked *focaccia*, such as the Maillard reaction products.

An overall comparative evaluation shows that the amounts of volatile compounds decreased significantly from sourdough to *focaccias*. Previous studies carried out on bread reported that this decrease is principally due to the evaporation during baking but also to the “dilution” of sourdough in the final product. For example, from sourdough to bread the majority of acids and esters, which are among the main fermentation compounds and are very volatile, tend to disappear [48], as it was the case also in the examined *focaccias*. On the contrary, 2-methylbutanal and 3-methylbutanal increase from sourdough to bread due to the free amino acids provided by sourdough, similarly to the findings observed in *focaccia* samples.

The use of pea flour and pea sourdough had a significant ($p \leq 0.05$) impact also on the quali-quantitative profile of the volatile compounds of *focaccia*. Aldehydes were the predominant class, followed by alcohols. The concentration of hexanal, octanal, nonanal, 2-heptenal and 2-octenal was higher in the *focaccias* with pea flour and pea-based sourdough, in line with their recognized role in the perception of typical pea odor. 2,4-Heptadienal was detected only in T2 and T3 *focaccias*, in agreement with its presence in the volatile profile of the two pea-based sourdoughs used (S2 and S3). Lipid oxidation, fermentation and Maillard reaction produced benzaldehyde, with almond and sweet flavors ([48], significantly higher in the T1, T2 and T3 *focaccias,* containing sourdough. This compound is typical of the volatile profile of bread and baked goods, resulting influenced by the type of yeast, the amount added, and the temperature of fermentation [50]. The 2- and 3-methylbutanal, oxidized metabolites of 2- and 3-methylbutanol, were markedly higher when sourdough was used, with significantly lower concentration in the case of pea-based sourdough (T2 and T3). The content of these Strecker aldehydes, originated from leucine and isoleucine, presented a wide range of variation, between 0.82 and 15.49 μg/g for 2-methylbutanal and 1.67-57.67 μg/g for 3-methylbutanal.

Ethyl alcohol, coming from the fermentative process, largely evaporated during baking and ranged from 0.91 to 3.42 μg/g. 3-Methyl-1-butanol, known to play a role in pea odor perception, was more abundant in pea-containing *focaccias* than in those prepared with semolina only. It ranged between 1.72 and 4.15 µg/g. Similarly, the 1-octen-3-ol was higher when pea flour, and especially pea-based sourdough, were used.

A significant reduction of esters, attributable to the baking process, was observed by comparing the volatile profiles of sourdoughs and *focaccias*, as previously reported by other authors [48]. As far as acids are concerned, the only one detected was acetic acid, which was only present in the samples with sourdough (about 0.17-0.21µg/g). Acetic acid, with sour notes, could positively enrich the aroma of the *focaccias,* representing an added value.

The Maillard reaction between sugars and reducing amino acids generated new compounds known to impact on the aroma of bread and bakery goods [48]. The sugar to amino acids ratio influences the intensity of the reaction and, therefore, the concentration of the end products of the reaction, such as furans and pyrazines [50]. The concentration of furans and pyrazines was generally higher in *focaccias* with sourdough and in those with pea flour, due to higher lysine content of pea flour, further enhanced by the proteolytic process occurred during sourdough fermentation, that released small peptides and free amino acids [26,51].

In general, a positive effect of sourdough fermentation is recognized [51], although the comparison among studies is limited by the difference in microbial composition and fermentation conditions, both strongly influencing the final odor properties. Overall, the use of pea sourdough in *focaccia* increased the levels of some typical odorants of legumes, but also led to higher concentrations of acetic acid, esters, and Maillard reaction compounds which could mask the legume-related ones, generally considered unpleasant. Therefore, the use of pea-sourdough in the production of baked goods should be encouraged.

Table 8. Volatile profile of sourdough samples (S1 = 100% semolina sourdough; S2 = 100% pea sourdough; S3 = 50:50 semolina-pea sourdough) and focaccia samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1.

Compounds (µg/g)	S1	S2	S3	T1	T2	T3	T4	T5	T6
Aldehydes									
2-Furancarboxaldheyde	-	-	-	2.03±0.11[a]	0.52±0.02[b]	0.57±0.08[b]	0.27±0.04[c]	0.54±0.02[b]	0.52±0.05[b]
2-Heptenal	-	-	-	0.33±0.03[d]	1.34±0.05[b]	2.67±0.03[a]	0.00±0.00[e]	0.90±0.05[c]	0.91±0.04[c]
2-Octenal	-	-	-	0.00±0.00[b]	0.22±0.38[b]	1.69±0.19[a]	0.00±0.00[b]	0.07±0.01[b]	0.10±0.01[b]
2,4-Heptadienal	0.00±0.00[c]	7.64±0.53[a]	3.45±0.97[b]	0.00±0.00[b]	0.08±0.02[a]	0.10±0.01[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
2,4-Hexadienal	0.20±0.00[c]	3.68±0.28[a]	2.50±0.10[b]	-	-	-	-	-	-
2-Methylbutanal	-	-	-	15.49±1.54[a]	7.46±0.59[b]	3.17±0.31[c]	0.82±0.10[e]	1.66±0.01[d]	1.16±0.02[d]
3-Methylbutanal	-	-	-	57.67±4.33[a]	26.43±2.16[b]	10.36±0.19[c]	1.67±0.14[e]	3.09±0.26[d]	2.47±0.18[d]
Benzaldehyde	-	-	-	3.67±0.33[a]	2.88±0.15[b]	3.56±0.25[a]	0.63±0.08[d]	1.23±0.20[c]	1.51±0.06[c]
Heptanal	2.02±0.17[c]	7.84±0.38[b]	8.83±0.06[a]	-	-	-	-	-	-
Hexanal	24.73±1.75[c]	47.82±0.65[a]	28.2±0.02[b]	4.65±0.56[d]	14.13±0.78[b]	17.69±1.08[a]	1.40±0.11[e]	4.84±0.28[d]	6.93±0.41[c]
Hexenal	1.08±0.05[c]	4.01±0.05[a]	1.95±0.12[b]	-	-	-	-	-	-
Nonanal	8.38±0.95[b]	10.37±0.20[a]	10.16±0.29[a]	1.84±0.30[a]	1.57±0.03[a]	1.76±0.09[a]	0.22±0.02[d]	0.33±0.01[c]	0.53±0.01[b]
Octanal	0.81±0.04[c]	1.31±0.01[a]	1.18±0.03[b]	1.54±0.04[b]	1.51±0.11[b]	2.82±0.12[a]	0.25±0.01[e]	0.49±0.05[d]	0.78±0.04[c]
Alcohols									
1-Heptanol	-	-	-	0.27±0.07[c]	0.78±0.05[b]	1.46±0.05[a]	0.24±0.02[c]	0.78±0.12[b]	0.96±0.03[b]
2-Heptanol	1.36±0.08[c]	2.77±0.16[a]	2.34±0.01[b]	-	-	-	-	-	-
1-Hexanol	16.02±0.18[c]	26.23±0.86[b]	31.46±0.88[a]	-	-	-	-	-	-
1-Octen-3-ol	2.64±1.29[c]	11.15±1.46[a]	5.30±0.73[b]	0.22±0.12[cd]	0.80±0.04[ab]	1.11±0.26[a]	0.06±0.01[d]	0.23±0.03[cd]	0.51±0.07[bc]
2-Ethyl-1-hexanol	2.97±0.08[a]	2.69±0.20[a]	3.20±0.31[a]	0.00±0.00[b]	0.21±0.01[a]	0.00±0.00[b]	0.21±0.02[a]	0.19±0.03[a]	0.19±0.01[a]
2-Methyl-1-butanol	0.00±0.00[c]	3.90±0.10[a]	0.47±0.03[b]	-	-	-	-	-	-
3-Methyl-1-butanol	72.56±1.25[b]	102.31±0.76[a]	100.56±7.39[a]	2.31±0.14[c]	2.74±0.08[b]	4.15±0.12[a]	1.72±0.02[e]	2.00±0.15[de]	2.09±0.06[cd]
Ethyl alcohol	11.52±0.75[b]	15.00±1.00[a]	13.89±0.07[a]	0.91±0.08[e]	2.00±0.08[bc]	3.42±0.11[a]	1.52±0.05[d]	1.89±0.13[c]	2.30±0.22[b]
Nonanol	-	-	-	0.11±0.01[b]	0.72±0.02[a]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
Esters									
1-Butanol-3-methyl acetate	18.75±1.02[a]	7.79±0.50[b]	0.00±0.00[c]	-	-	-	-	-	-
Decanoic acid ethyl ester	2.13±0.03[a]	1.90±0.08[b]	1.54±0.01[c]	0.00±0.00[d]	0.00±0.00[d]	0.00±0.00[d]	0.52±0.09[a]	0.11±0.04[b]	0.12±0.00[c]
Ethyl acetate	66.61±2.13[a]	7.59±0.73[c]	30.87±0.96[b]	-	-	-	-	-	-
Ethyl heptanoate	0.00±0.00[c]	0.62±0.07[b]	2.30±0.26[a]	-	-	-	-	-	-
Ethyl lactate	15.89±0.19[a]	2.11±0.16[c]	11.67±0.75[b]	0.45±0.01[a]	0.40±0.02[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
Hexanoic acid ethyl ester	1.67±0.03[b]	4.90±0.22[a]	1.13±0.08[b]	-	-	-	-	-	-

Octanoic acid ethyl ester	1.52±0.06[b]	0.00±0.00[c]	8.47±0.31[a]	-	-	-	-	-	-
Octyl acetate	0.00±0.00[c]	8.42±0.18[a]	4.20±0.07[b]	-	-	-	-	-	-
Acids									
Acetic acid	37.58±2.65[a]	26.63±0.91[b]	27.11±0.72[b]	3.18±0.03[a]	3.17±0.05[a]	3.21±0.01[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Ketones									
6-Methyl-5-heptene	0.00±0.00[c]	1.20±0.10[a]	0.97±0.07[b]	-	-	-	-	-	-
Furans									
2-Furanmethanol	-	-	-	0.16±0.03[d]	0.23±0.02[c]	0.25±0.02[b]	0.18±0.00[c]	0.31±0.04[a]	0.25±0.01[b]
2-Pentylfuran	1.59±0.05[c]	2.34±0.03[a]	1.67±0.05[b]	0.35±0.04[a]	0.18±0.01[c]	0.26±0.02[b]	0.07±0.00[d]	0.09±0.01[d]	0.10±0.01[d]
Pyrazines									
Ethyl-pyrazine	-	-	-	0.11±0.00[c]	0.13±0.00[b]	0.15±0.00[a]	0.07±0.01[e]	0.07±0.01[e]	0.09±0.00[d]
Methyl-pyrazine	-	-	-	0.27±0.01[c]	0.31±0.01[b]	0.42±0.01[a]	0.12±0.01[e]	0.12±0.01[e]	0.17±0.03[d]

Data are presented as means ± SD of replicates. The statistical analysis was performed by comparing the three sourdough and the six *focaccia* samples separately. Different letters in the same row indicate significant differences at $p \leq 0.05$.

2.4 Conclusions

This study showed that the addition of pea sourdough improves the nutritional profile of *focaccia*, rich in lipids and carbohydrates and relatively poor in protein and fiber. *Focaccia* enriched of pea sourdough could be labelled as “source of protein” and “high fiber”. Furthermore, pea sourdough improved the characteristics of this traditional street food not only in terms of nutritional profile but also by expressing its functional potential. Indeed, the fermentation of pea contributed to the increase in total phenolic content, resulting in higher *in vitro* antioxidant activity. The antinutritional factors identified in pea flour, such as phytates and oligosaccharides of the raffinose family, were reduced by fermentation, thanks to the microbial activity and acidification occurring in the preparation of sourdough. In addition, a positive effect on the *in vitro* digestibility was observed. Pea sourdough slowed down the starch digestibility, while enhancing the digestibility of protein. Finally, pea sourdough led to a more complex volatile profile, by increasing the content of aldehydes, alcohols and Maillard reaction compounds, which positively reflect on the aroma of *focaccia*.

These findings show that the use of pea sourdough is a simple and effective way to improve the quality of bakery products while modulating digestibility, and suggest promoting the use of pea sourdough on a larger scale. This simple reformulation is a response to evolving consumer needs for traditional, nutritionally balanced foods prepared using minimally processed and locally available ingredients. Reformulated bakery products, such as *focaccia*, with pea sourdough, could prompt an increase in the consumption of legumes, as suggested by WHO, fitting well into the direction of a protein transition to more sustainable sources. Further research is ongoing regarding the effects on the sensory acceptability and shelf life of *focaccia*, and to explore the benefits of antifungal activity and potential anti-staling effects of pea sourdough.

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Chapter 3. Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free *focaccia* flat bread

This chapter has been previously published as: De Angelis, D.; **Vurro, F**.; Santamaria, M.; Garzon, R.; Rosell, C. M.; Summo, C.; Pasqualone, A. Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free *focaccia* flat bread. *LWT* **2023**, *182*, 114873. **https://doi.org/10.1016/j.lwt.2023.114873**

Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free *focaccia* flat bread

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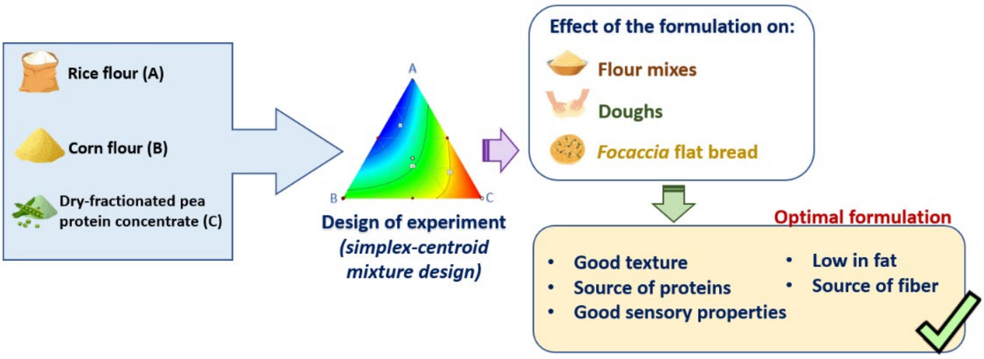
Graphical Abstract



Abstract

The aim of this work was to formulate a gluten-free *focaccia* flat bread based on rice and corn flour fortified with dry-fractionated pea protein concentrate (55 g/100 g protein content). A simplex-centroid mixture design with ten formulations helped to study how the flour ratios influenced the physical and sensory properties of dough and breads. The special cubic model significantly described all the responses determined in the dough and flour mixes, and most of those determined in the *focaccia*. The pea protein concentrate influenced the pasting properties of the flour mixes resulting in a decrease of viscosity. The midpoint of the experimental domain (*focaccia* containing 5 g/100 g of pea protein concentrate and 20 g/100 g of rice flour and corn flour each) was optimal, being not affected by the discolorations typical of pea (a* = 11.97, b* = 31.86, corresponding to an orange hue), having crumb hardness and chewiness of 9.11 N and 4.83 N, respectively, and moderate legume odor and flavor (5.6 and 5.3 c.u. in a 0–9 scale, respectively). The selected formulation could be labelled as “source of protein” (energy value provided by proteins >12%), “source of fiber” (fiber >3 g/100 g), and “low-fat” (fat<3 g/100 g).

Keywords: Air classification, Legume protein, Pasting properties, Mixture design, Bakery products.

3.1 Introduction

Flat breads are among the most ancient processed foods [1], but are still very popular, being easily adaptable to different consumer needs. Depending on their thickness and on their mono- or bi-layered structure, flat breads indeed can be either rolled around, or stuffed in their "pocket", or topped with a variety of ingredients to give palatable street foods, which fit well the modern pace of life [2]. A recent survey showed the existence of a multitude of traditional flat breads throughout the Mediterranean basin, with Italy specialized in the production of the mono-layer topped ones, garnished with several ingredients which vary according to local availability and taste [3].

Among these Italian garnished flat breads, *focaccia* (related to the French fougasse and to the Spanish coca de recapte) is particularly popular, after pizza, and is consumed throughout the entire country under various regional names reflecting the seasoning type or the geographical origin. With a simple recipe typically based on wheat flour, vegetable oil, yeast and salt, *focaccia* has been the object of previous investigations substantially aimed at improving its nutritional features by raising the content of proteins and fibers and reducing lipids or improving their quality [4,5]. However, such a widely appreciated bakery product has not been formulated in a gluten-free version yet, despite the increased request of gluten-free foods.

Gluten-free breads are often characterized by a poorer nutritional composition compared to the conventional counterparts, due to higher lipids, lower proteins, and unbalanced amino acid composition [6]. The list of ingredients is usually long due to the additives used to mimic the gluten behavior [7].

Consumers, instead, prefer low-processed products with few ingredients and are attracted by clean labels [8]. This is a challenging aspect since consumers also require products with sensory and nutritional properties similar to the gluten-containing reference foods [9].

Among the available gluten-free flours, corn and rice flours are the most used [10]. Nevertheless, incorporating pulse flours can improve the nutritional value in terms of protein, dietary fiber and mineral content [6,11,12]. However, the addition of pulse flours to bread, especially at the doses required for reaching a high protein content, negatively impacts on the textural and sensory features [11].

To overcome these issues, protein concentrates or isolates from pulses can be used, generally in a relatively low amount, comprised between 2% and 10% [6]. However, most of the pulse proteins proposed so far were obtained through a resource-intensive process, i.e., wet fractionation, in terms of consumption of water and chemicals [13], while more sustainable and less processed ingredients should be used [14].

From this perspective, the pulse protein concentrates obtained by dry-fractionation are a promising alternative, being obtained by solely physical methods, usually an air fractionation, and reaching protein contents of about 55 g/100 g [13]. Compared to wet-extracted protein, dry-fractionated protein concentrates have a different functionality in terms of water absorption and solubility, which are important properties for the breadmaking process.

However, the application of pulse protein concentrates obtained by dry-fractionation is still poorly investigated. In particular, there are some studies concerning the use of chickpea [15] and faba-bean proteins [16] for preparing wheat-based bread, and pea proteins for wheat-based cakes [17], pointing out the need to better study the effect of dry-fractionated protein in gluten-free breadmaking. The formulation of a food product is a complex and challenging task that should be supported by rational tools, such as the design of experiments, to reach the best compromise between the efforts employed during product development and the result acquired [18].

Therefore, the aim of this study was to formulate a gluten-free *focaccia* enriched of dry-fractionated pea protein concentrate.

The optimal formulation was selected through a simplex-centroid mixture design, to point out the effect of the three main ingredients, namely dry-fractionated pea protein concentrate, rice and corn flours, on the physicochemical and sensory properties of the final product.

3.2 Materials and methods

3.2.1. Raw materials

Rice flour (Lo Conte, Rome, Italy) (fat 0.5 g/100 g; carbohydrates 82 g/100 g; fiber 0.5 g/100 g; protein 7 g/100 g), corn flour (Mulino Rossetto, Portelongo, Italy) (fat 1.0 g/100 g; carbohydrates 75 g/100 g; fiber 2.9 g/100 g; protein 7.5 g/100 g), psyllium husk powder (Plantago ovata Forsk, Biotiva, Straßlach-Dingharting, Germany), yeast (*Saccharomyces cerevisiae,* Mulino Caputo, Naples, Italy), sea salt (Com-Sal Srl, Pesaro, Italy) were purchased from local retailers. Dry-fractionated pea protein concentrate (55 g/100g of protein) was kindly provided by Innovaprot srl (Gravina, in Puglia, Italy).

3.2.2. Experimental design and focaccia preparation

A simplex-centroid mixture design was planned to study the effects of three components, namely the main ingredients of *focaccia* (rice and corn flours, pea protein concentrate), with the following constrains (g/ 100 g): rice flour ($15 \leq x^1 \leq 30$); corn flour ($15 \leq x^2 \leq 30$); pea protein concentrate ($0 \leq x^3 \leq 15$). The sum of the components was 45 g/100 g, whereas the other 55 g/100 g were constituted by the other ingredients and kept constant (Table 1).

Table 1. Formulation of the *focaccia* samples according to the simplex-centroid mixture design.

Trial	Rice flour (x^1) (g/100 g)	Corn flour (x^2) (g/100 g)	Pea protein concentrate (x^3) (g/100 g)	Other ingredients** (g/100 g)	Baking time (min)
1	30	15	0	55	20
2	20	20	5	55	15
3	15	15	15	55	11
4	22.5	15	7.5	55	13
5*	15	15	15	55	11
6*	30	15	0	55	20
7	22.5	22.5	0	55	20
8	15	30	0	55	20
9	15	22.5	7.5	55	13
10*	15	30	0	55	20

*Replication. ** Other ingredients: water (50 g), yeast (1 g), salt (1.5 g), and psyllium husk powder (2.5 g).

The experimental points were chosen according to the D-optimality criterion and a special cubic model, which require seven experiments for three variables [18]. Moreover, three replicate points were also included in the model to consider the variability related to the preparation process. In fact, three replicates, for a total of ten experiments are often used in research activities based on three-components mixture design [18]. The *focaccia* samples were prepared according to the following procedure. Firstly, the flours (rice, corn, dry-fractionated pea protein concentrate), psyllium husk powder (2.5 g) and yeast (1 g), were mixed with 25 mL of water at low speed for 1 min with a spiral kneader (G3 Ferrari, Rimini, Italy). Secondly, salt (1.5 g) was added, dissolved in additional 25 mL of water, and kneading continued for 5 min. The dough was flattened manually, using pastry rings having a diameter of 10.8 cm (Tescoma, Cazzago San Martino, Italy), then left to rise for 1 h and 30 min at 35 °C, RH = 33.5% (Memmert proofer, EN.CO. Srl, Spinea, Italy), and baked in an electric oven (Oem Ali Group Srl, Bozzolo, Italy) at 220 ◦C for the min reported in Table 1. Owing to the different physi-cochemical properties of the doughs it was not possible to keep the baking time constant. By contrast, after preliminary trials it was decided to keep constant the hydration level, by varying the baking time, and guaranteeing the optimal cooking of all the 10 formulations. Further explanations about the baking time are discussed in paragraph 3.3.

3.2.3. Analyses of the flour mixes and dough

The water absorption index and the water solubility index were determined on the ten flour mixes, according to the procedures reported in Du, Jiang, Yu, and Jane (2014)[19]. The analysis was carried out in triplicate. The pasting behavior of flour mixes was analyzed by Rapid Visco Analyzer (RVA 4500; Perten Instruments, Hagersten, ¨ Sweden). The flours were blended in a roller mixer (Fisher Scientific, Massachusetts, USA) for 24 h. Flour blends (3.5 g, 14% moisture basis) were suspended into 25 mL of distilled water. Slurries were stirred at 960 rpm for 10 s to complete dispersion and then kept at 160 rpm during the assay. Heating started at 50 °C for 1 min, followed by a temperature increase up to 95 °C in 3 min 42 s, held for 2 min 30 s at 95 °C, and then cooling down to 50 °C in 3 min 48 s, which was kept for 2 min. Pasting parameters included: onset (time at which viscosity starts to increase), viscosity at 95 °C, setback rate (slope during cooling phase), viscosity at 50 °C and final viscosity [20]. The analysis was carried out in triplicate. The color coordinates (L^*, a^*, b^*) of the doughs were determined by means of the CM-600d spectrophotometer (Konica Minolta, Tokyo, Japan). Five replicates were carried out.

3.2.4. Analyses of focaccia samples

The textural properties were evaluated by a texture profile analysis (TPA), according to Pasqualone et al. (2019) [4] with few modifications, using a ZI.0 TN texture analyzer (ZwickRoell GmbH & Co. KG, Ulm, Germany), equipped with 50 N load-cell and a compression probe having a 36 mm diameter. The sample was cut in uniform pieces of 30 mm side and compressed twice at 1 mm/s with 5 s of pause within the two compressions, reaching 40% sample deformation in both the compressions. Four replicates were carried out.

The image analysis was carried out as in De Angelis et al. (2020)[21]. Briefly, the images of the crumb were acquired with a Sony α-6100 mirrorless camera, equipped with a Sony 16–50 mm f/3.5-5-6 lens (Sony Corporation, Tokyo, Japan), and processed by the ImageJ software (National Institutes of Health, Bethesda, USA), after being converted into 8-bit grayscale. An image section of 65 × 12 mm was cropped from the center, filtered by thresholding function to obtain the best cell resolution. The number of cells and the percentage of cells with an area higher than 5 mm^2 were determined [22]. Four replicates were carried out.

The variations of weight, thickness and diameter induced by baking were determined as percentage with a technical balance (Mettler Toledo, Columbus, Ohio, USA) and a caliper, respectively. Four replicates were carried out.

Color (L^*, a^*, b^*) of crust and crumb was determined by means of the CM-600d spectrophotometer (Konica Minolta, Tokyo, Japan). Five replicates were carried out.

3.2.5. Quantitative descriptive analysis of focaccia samples

The sensory evaluation was carried out according to the Quantitative Descriptive Analysis (QDA) methodology by a trained panel of eleven people (5 male, 6 female, age 23–55 y), following the ethical guidelines of the laboratory of Food Science and Technology of the Department of Soil, Plant and Food Science of the University of Bari, Italy, and the standard procedures described in Vurro et al. (2022). The panelists were regular consumers of bakery products and legumes, and did not suffer any food intolerances or allergies. They were informed about the study aims and signed an individual written informed consent. The typical odor associated with legume/pea and the typical odor of corn were scored for their intensity on an anchored 9-points scale, using the following contractual units: 0 contractual units (not perceived), 3 (mildly perceived), 6 (distinctively perceived) to 9 (highly perceived). The evaluation was carried out in triplicate.

3.2.6. Selection of the optimal formulation and nutritional evaluation

The optimal formulation of the gluten-free *focaccia* was selected by the overall observation of the contour plots representing the variation of the analytical characteristics of the flour mixes, doughs and *focaccia* samples in the experimental domain, as well as considering the calculated nutritional values, with particular attention to the content of proteins and fibers. The selected *focaccia* (the one with 5 g/100 g pea protein concentrate) was subjected to the analysis of proximate composition as follows. Protein content (total nitrogen × 6.25) was determined with the AACC method 46–11.02 (AACC International, 2009)[23]; the lipid content was determined by a Soxhlet apparatus (Velp Scientifica srl, Usmate, Italy), using diethyl ether as solvent, according to the AOAC method 945.38 F (AOAC, 2006)[24]. The total dietary fiber was determined by the enzymatic-gravimetric AOAC method 991.43 (AOAC, 2006)[24]. The moisture content was determined at 105 °C by an automatic moisture analyzer (Radwag Wagi Elektroniczne, Radom, Poland). The carbohydrate content was determined as the difference by subtracting the protein, moisture, and lipid contents from 100. The energy value (kcal) was calculated considering the contribution of 4 kcal/g from proteins and carbohydrates, 9 kcal/g from lipids and 2 kcal/g from total dietary fibers, according to the Annex XIV of Regulation (EC) No. 1169/2011 [25]. Three replicates were carried out.

3.2.7. Statistical analysis

The responses of the experimental design were modeled according to the postulated special cubic model and the regression coefficients (R^2), the adjusted coefficients of determination (R^2 adj), as well as their significance ($p \leq 0.05$) were calculated by the software Design-Expert 11 (StatEase Inc., Minneapolis, USA). Data were subjected to one-way analysis of variance ANOVA followed by Tukey's HSD (Honestly Significant Differences) test for multiple comparisons at a significance level $\alpha = 0.05$ by using the Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA).

3.3 Results and discussion

3.3.1. Model evaluation

The regression models calculated for the responses of the flour mixes, dough, and *focaccia* samples together with their significance, are shown in Table 2.

The responses determined on flour mixes and dough were all highly significant ($R^2 \geq 0.98$ and R^2 adj ≥ 0.93, respectively), meaning that the selected models adequately describe the relationship between the experimental factors and the response variables. Instead, not all the responses calculated on *focaccia* were significant, but the values of R^2 and R^2 adj of the significant ones were very high (≥ 0.95 and ≥ 0.86, respectively). The processing steps of *focaccia*-making, i.e., kneading, leavening, and baking, probably resulted in a higher variability of the responses, reducing their significance. However, it should be considered that the interpretation of the model coefficients of mixture designs is not so clear as it happens for the designs for independent variables, because the coefficients are not directly related to the effects [18]. Therefore, perusal of the contour plots depicting the variations of each parameter is fundamental to easily have an immediate and comprehensive overview of the phenomena occurring in the experimental domain.

3.3.2. Characteristics of flours and dough

The water absorption index (WAI) and water solubility index (WSI) of flour mixes were significantly affected by the ingredient ratios (Table 3). The addition of pea protein concentrate led to a decrease of WAI and an increase of WSI (Figure 1a and b). Rice flour, instead, was responsible of the highest WAI value, and corn flour had an intermediate effect. WAI is mostly due to hydrated and swollen starch after the hydrothermal treatment of flour, while WSI measures the amount of soluble solids after the same treatment. Both WAI and WSI are related to the chemical composition of flours, in terms of content and properties of starch and proteins therein [26]. Generally, the dry-fractionated pulse proteins are characterized by low WAI and high WSI [13] because of their low starch content and the presence of soluble [27]. Also the pasting behaviors of flour mixes varied according to the different ingredient ratios (Figure 1c–g). The onset was lower in mixes with high amount of rice flour, indicating an earlier swelling of the rice starch, which was delayed in the presence of the other flours (Figure 1c). Indeed, the well-shaped peak of viscosity during heating was only visible when high amount of rice flour, and low amount of pea protein concentrate, were present (Figure 2), probably due to the starch dilution when the level of pea protein increased.

Table 2. Regression coefficients of the model and their significance for all the responses determined on the flour mixes produced by the simplex-centroid mixture design. (A: rice flour; B: corn flour; C: pea protein concentrate).

	A	B	C	AB	AC	BC	ABC	R^2	Adjusted R^2	*p-value*
Flour mixes										
WAI	**4.55**	**3.90**	**3.46**	-0.35	-0.31	-0.64	0.20	**0.99**	**0.98**	**< 0.001**
WSI	**0.03**	**0.03**	**0.13**	0.01	-0.01	0.01	-0.06	**1.00**	**0.99**	**< 0.001**
Onset (min)	**3.35**	**4.15**	**4.13**	0.00	0.05	0.05	1.73	**0.98**	**0.93**	**0.014**
Viscosity at 95ºC (cP)	**2288**	**1072**	**296**	**602**	**-1249**	**-933**	**-4409**	**1.00**	**1.00**	**< 0.001**
Viscosity at 50ºC (cP)	**3213**	**2728**	**1089**	67	**-753**	**-850**	-1949	**1.00**	**1.00**	**0.001**
Final viscosity (cP)	**3564**	**3127**	**1262**	89	**-722**	**-973**	-1698	**1.00**	**1.00**	**< 0.001**
Setback rate	**754**	**648**	**380**	**-87**	**110**	**202**	309	**1.00**	**1.00**	**< 0.001**
*L**	**72.12**	**69.27**	**56.62**	-2.49	-1.57	-4.98	0.44	**0.99**	**0.98**	**< 0.001**
*a**	**2.94**	**5.21**	**-1.26**	-0.92	-4.25	-4.43	**37.98**	**0.99**	**0.98**	**< 0.001**
*b**	**19.56**	**30.13**	**33.12**	-8.15	-1.63	-6.33	**145.28**	**0.99**	**0.98**	**< 0.001**
Gluten-free Focaccia flat bread										
N. cells	**108.00**	**137.00**	**145.00**	**-98.00**	**63.33**	**-34.67**	703.00	**0.99**	**0.97**	**0.00**
Cells > 5 mm^2 (%)	**13.65**	**10.01**	**9.26**	5.82	-7.86	0.03	-38.48	**0.95**	**0.86**	**0.04**
Firmness (N)	**4.37**	**11.71**	**18.27**	**-19.03**	**14.27**	-5.02	-33.72	**1.00**	**0.99**	**0.00**
Springiness	0.82	0.89	0.87	-0.15	-0.27	-0.06	1.09	0.92	0.75	0.10
Chewiness (N)	**2.49**	**7.03**	**8.94**	**-11.67**	6.05	-6.36	0.26	**0.98**	**0.93**	**0.02**
Cohesion	0.70	0.67	0.56	0.04	-0.03	-0.31	0.49	0.87	0.62	0.17
Weight loss (%)	23.79	22.92	14.47	-2.98	-16.08	-18.11	-68.07	0.93	0.80	0.07
Variation in diameter (%)	-14.64	-9.00	-4.84	-4.26	2.07	-6.64	33.10	0.94	0.82	0.06
Variation in thickness (%)	68.26	55.73	80.31	**312.02**	45.57	**118.58**	-319.74	**0.99**	**0.97**	**0.00**
*L** crumb	72.12	70.67	67.16	-0.38	-22.04	-24.74	-40.93	0.85	0.55	0.21
*a** crumb	**5.38**	**6.92**	**2.96**	**-4.53**	2.91	1.05	26.93	**0.98**	**0.95**	**0.01**
*b** crumb	**29.51**	**39.67**	**34.85**	**-25.06**	**-8.33**	**-13.33**	**63.84**	**1.00**	**0.99**	**0.00**
*L** crust	67.04	61.59	58.65	17.61	-2.92	-3.67	-108.66	0.88	0.63	0.16
*a** crust	8.98	11.61	9.59	-7.66	1.97	-1.49	84.51	0.81	0.44	0.28
*b** crust	**26.23**	**38.36**	**38.69**	-2.71	**16.25**	3.38	**-106.77**	**0.99**	**0.97**	**0.00**
Corn odor	**5.72**	**5.78**	**1.85**	1.99	-8.48	-6.87	0.87	**0.97**	**0.90**	**0.03**
Legume odor	**0.10**	**0.22**	**6.89**	-0.24	**11.36**	**12.18**	16.43	**0.99**	**0.97**	**0.00**

WAI = water absorption index; WSI = water solubility index; bold font indicates significant terms ($p \leq 0.05$).

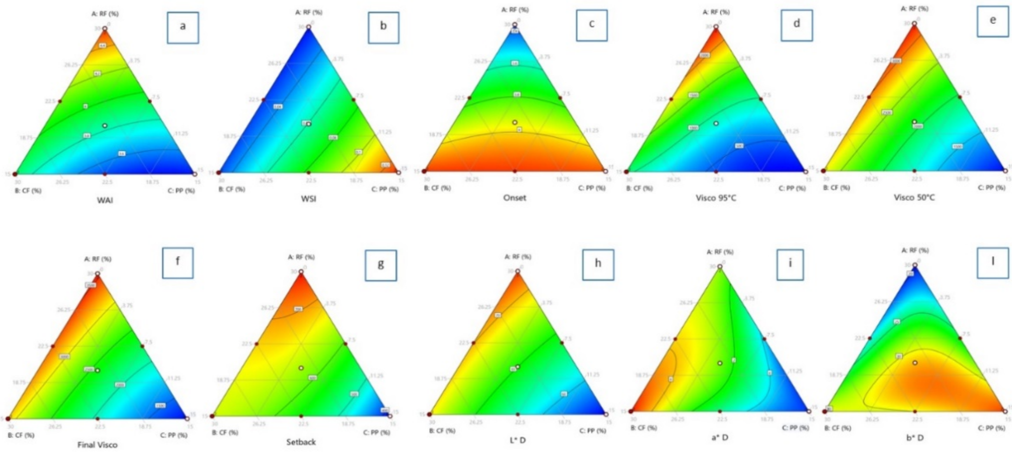


Figure 1. Contour plots depicting the variations of the physic-chemical parameters and pasting properties of flour mixes and dough at different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). For flour mixes: WAI = water absorption index (a); WSI = water solubility index (b); onset of gelatinization (c); viscosity at 95 ◦C (d); viscosity at 50 ◦C (e); final viscosity (f); setback (g). For dough: $L*$ index (h); $a*$ index (i); $b*$ index (l). Color variation from blue to red indicates an increase of the considered parameter.

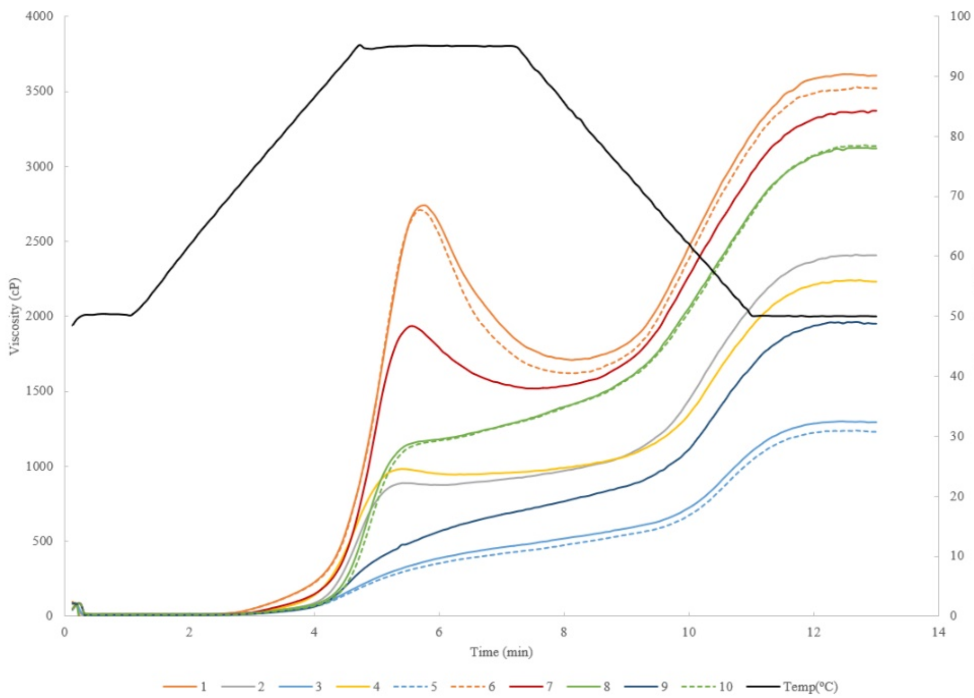


Figure 2. Viscoamylogram, based on temperature changes due to heating and cooling phases, of flour mixes at different ratios of corn flour, rice flour, and pea protein concentrate. Flour mix compositions, coded from 1 to 10, are reported in Table 1.

These findings confirmed the observed values of WAI. Particularly, the flour mixes with the highest amount of rice had the highest viscosities at 95 °C (2274, 2303 mPa s), at 50 °C (3256, 3171 mPa s), and final viscosity (3608, 3521 mPa s) (Table 3), evidencing the formation of a firmer gel able to resist thermal and mechanical stress. Furthermore, these blends also displayed higher slope during cooling, which indicates a faster retrogradation rate of amylose. The addition of pea flour modified these parameters.

Again, by diluting starch as pea flour increased, there was limited starch swelling and amylose leaching which, in turn, corresponded to weaker gel. The mixture with 15 g/100 g pea flour, indeed, presented lower viscosities at 95 °C (309, 284 mPa s), 50 °C (1117, 1062 mPa s) and final viscosity (1291, 1233 mPa s), as well as lower setback rate during cooling. The latter could be a positive feature of the fortified *focaccia,* indicating that high protein pea flour limits starch retrogradation affecting the realignment of the amylose chains.

The color of dough was significantly influenced by varying the ratio of the three ingredients in the flour mixes (Figure 1h–l). The highest lightness (L^*) was observed when rice flour was more abundant, while the addition of pea protein concentrate led to a decrease of L^* and of the green/red coordinate (a^*). The latter reached negative values, corresponding to green, when the pea protein concentration was ≥7.5 g/ 100g, unless relevant quantities of corn flour (22.5 g/100 g) were also present, as in trial 9 ($a^* = 0.87$) (Table 3). A green hue is particularly critical, being unusual in bakery products and capable of negatively influencing consumer perception [28]. The midpoint of the experimental domain, corresponding to 5 g/100 g of pea protein concentrate, was not affected by this unwanted discoloration ($a^* = 2.64$). The highest value of the blue/yellow coordinate (b^*) was observed in the lowest part of the contour plot, where rice flour was in the lowest concentration, substituted by pea protein and corn flour (Figure 1l).

3.3.3. Crumb structure, texture and baking-induced variations of focaccia

The results of the image analysis performed on the crumb of *focaccia*, reported in Table 4, were highly significantly modelled (Table 2). Overall, the samples showed a crumb characterized by a dense network of fine cells. Indeed, the percentage of cells with an area greater than 5 mm^2 was less than 14% of the total cells detected.

The contour plots (Figure 3a and 2b) highlight that the addition of pea protein concentrate increased the number of cells and decreased their size. By contrast, when rice flour prevailed, less pores with larger dimensions were observed. The lower gelling properties observed in the flour mixes added of pea protein concentrate (Table 3) probably had an impact on the features of cell crumb network, reducing the viscosity of the dough matrix and, in turn, its ability to retain fermentation gas. However, the differences detected were significant for the number of cells observed but not for the percentage of cells >5 mm^2 (Table 4).

The textural properties of the *focaccia* samples were influenced by the ratio of the mix ingredients, and significantly varied among the ten trials (Table 4).

In particular, *focaccia* firmness varied between 3.28 and 18.52 N, covering a wide range of values typical of both gluten-free [29] and wheat-based breads (García-Segovia et al., 2020; Pasqualone et al., 2019). The special cubic model significantly fitted the responses of firmness and chewiness, whereas it was not significant for springiness (p = 0.096) and cohesion (p = 0.166) (Table 2).

Table 3. Physicochemical properties of the flour mixes and doughs prepared according to the mixture design.

Trial	WAI	WSI	Pasting properties					Dough color		
			Onset (min)	Viscosity at 95ºC (cP)	Viscosity at 50ºC (cP)	Final viscosity (cP)	Setback rate	*L**	*a**	*b**
1	4.50±0.02^{a}	2.76±0.29^{a}	3.4±0.0^{c}	2274±136^{a}	3256±30^{a}	3608±33^{a}	758±46^{a}	73.01±0.20^{a}	2.94±0.23^{c}	19.86±0.80^{f}
2	3.83±0.04^{d}	6.06±0.07^{d}	4±0.1^{ab}	880±34^{c}	2101±46^{d}	2410±35^{d}	630±3^{bc}	65.02±0.16^{d}	2.64±0.21^{c}	31.19±0.71^{bc}
3	3.43±0.01^{e}	13.34±0.11^{e}	4.1±0.1^{a}	309±40^{d}	1117±83^{f}	1291±96^{f}	381±0^{d}	56.61±0.22^{g}	-1.63±0.23^{g}	32.31±0.31^{ab}
4	3.93±0.01^{cd}	7.48±0.24^{cd}	3.8±0.1^{b}	980±10^{c}	1963±49^{d}	2233±37^{d}	595±13^{bc}	63.98±0.14^{e}	-0.22±0.08^{e}	25.93±0.43^{d}
5	3.49±0.01^{e}	12.48±0.15^{e}	4.2±0.0^{a}	284±11^{d}	1062±18^{f}	1233±23^{f}	379±16^{d}	56.63±0.11^{g}	-0.88±0.10^{f}	33.92±0.54^{a}
6	4.60±0.00^{a}	2.41±0.06^{a}	3.3±0.1^{a}	2303±250^{a}	3171±5^{a}	3521±60^{ab}	750±66^{a}	71.23±1.12^{b}	2.93±0.39^{c}	19.26±1.07^{f}
7	4.14±0.07^{b}	3.05±0.06^{b}	3.8±0.1^{b}	1831±30^{b}	2987±28^{b}	3368±29^{b}	679±5^{ab}	70.07±0.41^{c}	3.84±0.21^{b}	22.81±1.38^{e}
8	3.86±0.05^{cd}	3.19±0.06^{cd}	4.1±0.0^{a}	1090±53^{c}	2733±20^{c}	3117±21^{c}	642±2^{bc}	69.11±0.58^{c}	5.09±0.30^{a}	29.67±1.32^{c}
9	3.52±0.00^{e}	8.26±0.09^{e}	4.2±0.1^{a}	451±16^{d}	1696±44^{e}	1951±44^{e}	565±3^{c}	61.70±0.53^{f}	0.87±0.15^{d}	30.04±0.26^{c}
10	3.94±0.05^{c}	3.25±0.00^{c}	4.2±0.00^{a}	1055±45^{c}	2722±31^{c}	3137±39^{c}	653±10^{abc}	69.43±0.19^{c}	5.34±0.3^{a}	30.59±0.62^{bc}

WAI = water absorption index; WSI = water solubility index; Data are presented as means ± SD of the replicates; Different letters in the same column indicate significant differences at $p \leq 0.05$.

Table 4. Crumb structure, texture baking-induced variations, color and odor of the *focaccia* samples prepared according to the mixture design.

Trials	1	2	3	4	5	6	7	8	9	10
Number of cells	108.33±6.24^{bc}	143.75±9.54^{a}	135.75±19.19^{a} b	147.00±10.23^{a}	94.00±11.22^{c}	129.50±9.04^{ab}	140.50±7.42^{a}	108.33±6.24^{bc}	143.75±9.54^{a}	135.75±19.19^{a} b
Cells > 5 mm2 (%)	13.64±5.13^{a}	9.32±2.20^{a}	9.49±4.47^{a}	8.47±2.06^{a}	13.28±3.52^{a}	9.64±1.89^{a}	9.57±1.53^{a}	13.64±5.13^{a}	9.32±2.20^{a}	9.49±4.47^{a}
Hardness (N)	4.80±0.72^{e}	9.11±2.45^{d}	14.89±0.70^{b}	18.03±1.27^{a}	3.28±0.62^{e}	13.73±1.78^{bc}	11.90±1.03^{cd}	4.80±0.72^{e}	9.11±2.45^{d}	14.89±0.70^{b}
Springiness	0.81±0.03^{d}e	0.85±0.02^{bcd}	0.77±0.01^{e}	0.89±0.02^{ab}	0.82±0.04^{cde}	0.87±0.01^{abc}	0.90±0.03^{a}	0.81±0.03d^{e}	0.85±0.02^{bcd}	0.77±0.01^{e}
Chewiness (N)	2.69±0.42^{cd}	4.83±1.28^{bc}	7.23±1.51^{b}	9.81±1.88^{a}	1.84±0.19^{d}	6.39±0.57^{b}	7.11±0.89^{b}	2.69±0.42c^{d}	4.83±1.28^{bc}	7.23±1.51^{b}
Cohesion	0.70±0.02^{a}	0.63±0.05^{ab}	0.62±0.10^{ab}	0.61±0.10^{ab}	0.70±0.06^{a}	0.54±0.02^{b}	0.66±0.07^{ab}	0.70±0.02^{a}	0.63±0.05^{ab}	0.62±0.10^{ab}
Weight loss (%)	25.79±2.41^{a}	13.74±3.49^{b}	15.11±2.46^{b}	12.88±1.68^{b}	22.61±2.32^{a}	14.17±1.37^{b}	23.51±2.27^{a}	25.79±2.41^{a}	13.74±3.49^{b}	15.11±2.46^{b}
Variation in diameter (%)	-13.92±3.56^{c}	-9.25±0.29a^{bc}	-9.23±1.27^{abc}	-6.56±1.61^{a}	-12.89±2.85^{bc}	-8.58±2.91^{ab}	-9.16±0.87^{abc}	-13.92±3.56^{c}	-9.25±0.29^{abc}	-9.23±1.27^{abc}
Variation in thickness (%)	65.57±9.88^{bc}	109.20±25.00^{a} b	85.68±11.89^{bc}	75.60±25.00^{bc}	140.00±11.55^{a}	97.70±24.50^{b}	54.51±7.47^{c}	65.57±9.88^{bc}	109.20±25.00^{a} b	85.68±11.89^{bc}
***L** crumb**	75.18±0.89^{a}	63.23±0.66^{d}	65.62±0.72^{c}	66.96±1.66^{c}	71.30±1.28^{b}	62.73±1.17^{d}	71.57±1.01^{b}	75.18±0.89^{a}	63.23±0.66^{d}	65.62±0.72^{c}
***a** crumb**	5.03±0.25^{c}	6.02±0.34^{b}	4.02±0.31^{d}	2.98±0.35^{e}	4.80±0.43^{c}	5.20±0.32^{c}	6.98±0.36^{a}	5.03±0.25^{c}	6.02±0.34^{b}	4.02±0.31d
***b** crumb**	29.68±1.62^{cd}	31.85±1.53^{bc}	30.10±1.39^{c}	33.90±1.25^{b}	27.40±1.13^{d}	33.93±1.24^{b}	39.82±0.91^{a}	29.68±1.62^{cd}	31.85±1.53^{bc}	30.10±1.39^{c}
***L** crust**	72.34±0.92^{a}	59.63±2.31^{c}	60.00±0.43^{c}	56.79±1.34^{d}	68.72±1.64^{b}	59.20±0.78^{cd}	61.28±0.90^{c}	72.34±0.92^{a}	59.63±2.31^{c}	60.00±0.43^{c}
***a** crust**	7.79±0.78^{e}	11.97±1.00^{ab}	9.78±1.00^{cd}	10.27±1.03^{bc}	8.38±0.91^{de}	10.23±0.88^{bc}	12.12±0.48^{a}	7.79±0.78^{e}	11.97±1.00^{ab}	9.78±1.00c^{d}
***b** crust**	26.17±1.34^{d}	31.86±1.30^{c}	37.47±0.71^{b}	39.51±0.57^{ab}	31.78±1.24^{c}	39.88±1.05^{a}	37.98±0.92^{ab}	26.17±1.34^{d}	31.86±1.30^{c}	37.47±0.71^{b}
Corn odor (c.u)^{a}	6.00±2.18^{a*}	3.00±1.51^{b}	1.67±1.66^{b}	1.50±1.08^{b}	6.25±1.98^{a}	2.10±2.38b	6.44±1.33^{a}	6.00±2.18^{a*}	3.00±1.51^{b}	1.67±1.66^{b}
Legume odor (c. u.)	0.20±0.42b	5.60±1.96a	6.33±1.73a	7.56±1.24a	0.10±0.32b	6.60±2.59a	0.00±0.00^{b}	0.20±0.42^{b}	5.60±1.96^{a}	6.33±1.73^{a}

^{a} C.u. = contractual units. Data are presented as means ± SD of the replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$.

The contour plots of the textural properties of *focaccia* samples are reported in Figure 3c–f. The addition of pea protein concentrate led to an increase of firmness, as shown by the red areas in the right angle of the contour plot (Figure 3c). By contrast, higher concentrations of rice flour led to softer texture, as displayed by the blue area in the top left. A similar behavior was shown by chewiness (Figure 3f), highlighting that the firmness was directly related to the effort needed to masticate the sample. The cohesion and springiness of *focaccia* samples were significantly affected by the ratio of ingredients, but with little variations (Table 4). The addition of high doses (15 g/100 g) of pea protein concentrate led to a less cohesive product, meaning that a lower effort was required to compress twice the samples, compared to the products prepared without the pea protein (Figure 3e and f). Generally, baked goods with a high cohesion remain compact during the mastication and this is a preferred quality feature [29].

The springiness showed the highest value when the maximum concentration of corn flour was used (trial 10), indicating that this flour gave a better ability to withstand a second deformation compared to the others. The increase of hardness and other textural parameters after the addition of pea protein was previously recorded [30]. This behavior agreed with the results of the image analysis on the crumb structure, which indicates the presence of a more compact network in the *focaccia* obtained by the addition of pea protein.

The baking-induced variations in weight and size of *focaccia* samples are reported in Table 4. Despite not being significantly described by the special cubic model ($p = 0.069$), weight loss was significantly affected by the addition of pea protein concentrate, regardless of its amount. In particular, all the samples containing pea protein showed a weight loss below 16%, which is significantly lower compared to the products containing only rice and corn flour, having a weight loss >21%. This trend is evident by observing the contour plot shown in Figure 3g. These results, usually due to higher water binding capacity of pulse proteins, in our case could be explained by the lower baking time necessary for the products containing the pea protein, which was 5–9 min lower compared to the other flat breads (Table 1).

The cooking time, in turn, can be explained by the different physicochemical properties of the doughs, being positively correlated ($p < 0.05$, R from 0.82 to 0.97) with all viscosity parameters during cooking and cooling. Indeed, the doughs prepared with the pea protein concentrate had a lower ability to bind water (Table 3) compared to the other formulations, which means that during the baking process, the water easily evaporated from the dough, leading to a higher heat transfer and temperature rice [31]. Although several commercial pea protein isolates have high water binding capacity, lower values for this parameter have been reported for dry-fractionated pulse protein [13]. This can be related to the presence of protein in the native state which tends to absorb less water compared to the isolates, in which protein denaturation may have occurred during the extraction procedures [27].

The variations in thickness and diameter were not significantly described by the model. Slight but significant differences indeed were recorded (Table 4). The decrease in diameter varied from − 3.13%, at the highest level of pea protein, to − 15.36%, when pea protein was absent. Also in this case, it is reasonable to hypothesize that the different heat transfer during baking influenced the size changes occurring in *focaccia*. Indeed, it was previously reported that a more drastic heating, i.e. happening in the *focaccia* with pea proteins, leads to an early protein denaturation and/or starch gelatinization, hardening the dough matrix and making the expansion phenomena difficult [31]. This also corroborates with the results of the image analysis which demonstrated the presence of a crumb network constituted of small and numerous pores.

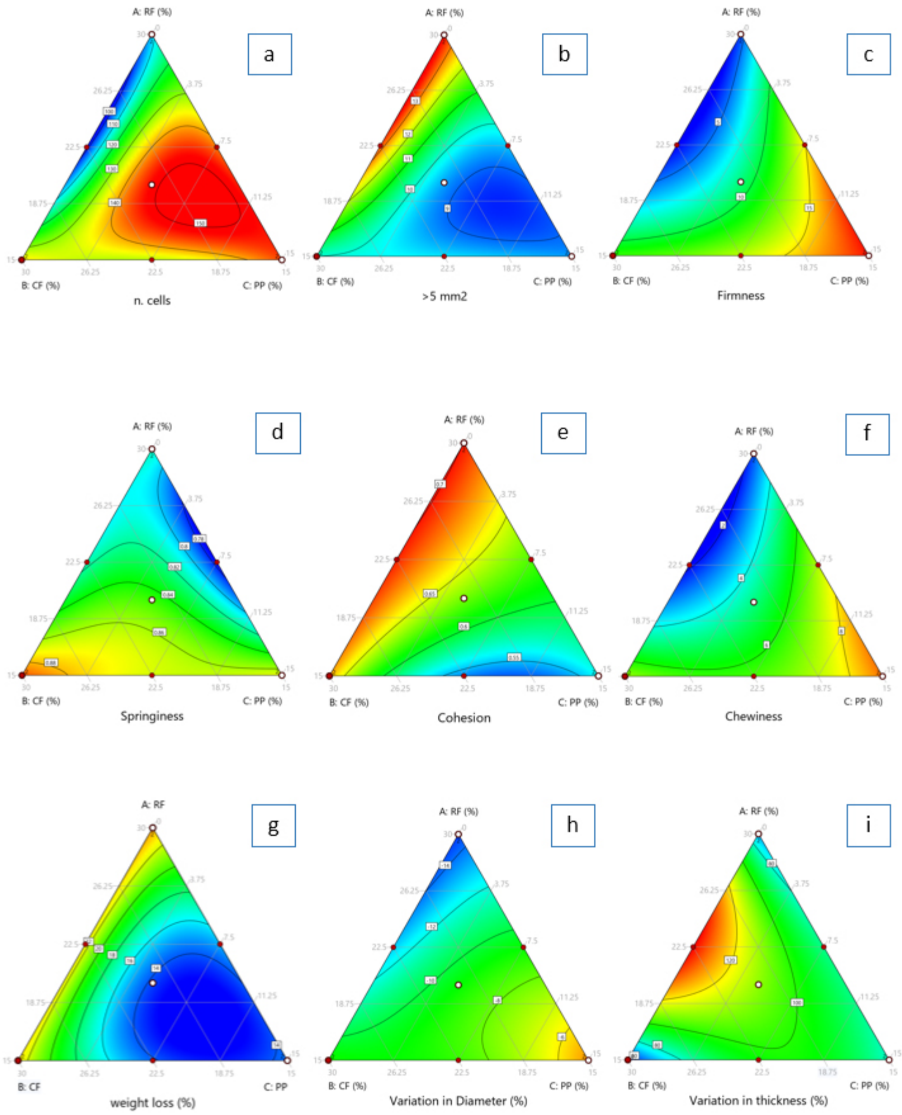


Figure 3. Contour plots depicting the variations of the textural properties and crumb cell characteristics of *focaccia* samples prepared with different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). Number of cells (a); percentage of cells >5 mm^2 (b); firmness (c); springiness (d); cohesion (e); chewiness (f). Color variation from blue to red indicates an increase of the considered parameter.

3.3.4. Color and odor of focaccia

The ratio of the ingredients significantly influenced the color of crust and crumb. Similar trends were observed for the color indices of crumb and crust, except for *b**, which in the crumb was positively related only to the level of corn flour, while in the crust was positively influenced also by the addition of pea protein concentrate (Table 4, Figure 4a–f). L^* decreased with the addition of the dull, greenish, pea protein concentrate because of its intrinsic color contribution, as already observed in the dough (Table 3), and due to the higher concentration of substrates of the Maillard reaction which, with baking, resulted in a darker crust [32]. Elevated additions of pea protein concentrate determined the lowest values of a^*, especially in *focaccia* crumb (Figure 4b), while corn flour increased the same parameter, in agreement with the results observed in the dough. However, a^* values were always positive, indicating that the Maillard reaction and sugar caramelization induced by baking were sufficient to turn the color towards an orange hue, eliminating the greenish one which affected the appearance of unbaked dough. Similar results were observed by Pico et al. (2019)[32] and Ziobro et al. (2016)[30], both in the crumb and in the crust of gluten-free bread fortified with pea protein, as well as by Matos, Sanz, and Rosell (2014)[33] in gluten-free muffins added of pea protein isolate, and by Mancebo, Rodriguez, P., & Gomez (2016)[34] in gluten-free cookies enriched of pea protein.

The typical sensory notes of the main odorous ingredients were perceived by the panelists. The odor of legumes was perceivable in all the samples containing pea protein concentrate, with an intensity scored 5.60 c.u. out of a 9 point scale when 5 g/100 g of pea protein were added, up to 7.56 c.u. at the level of 15 g/100 g (Table 4). The contour plot (Figure 4g) shows that the perception of this sensory descriptor becomes negligible at a level of about 2 g/100 g of pea protein concentrate.

The odor of corn was perceivable but partly masked by the legume odor contributed by the pea protein concentrate. In absence of the latter, corn odor was scored >6 (Figure 4h). It was previously reported that the presence of chickpea flour in bakery products led to a distinct perception of the typical legume odor [4]. However, this is one of the critical aspects to consider when developing formulations containing legumes, because the overall acceptability of the products is negatively influenced by the legume odor and flavor [11]. In our case, the use of a protein concentrate implies that to reach certain nutritional goals, such as a relevant protein content, the addition of a relatively low quantity of legume would be sufficient, mitigating the impact on the sensory properties.

3.3.5. Selection of the optimal formulation and definition of the nutritional properties of the fortified focaccia

Based on the observed impact of pea protein concentrate on the main physic-chemical properties of dough and final product (gelling ability, texture, color and odor) it is appropriate to keep relatively low this ingredient in a fortified gluten-free *focaccia*.

The formulation containing 5 g/100 g of pea protein concentrate and 20 g/100 of corn flour and rice flour each (trial 2, Table 1), which represents the midpoint of the experimental domain, was selected because it best balanced the nutritional aims of the fortification study with the textural and sensory features. This level of addition was esteemed to allow an increase of protein content of about 75–78% compared to the formulations without pea flour.

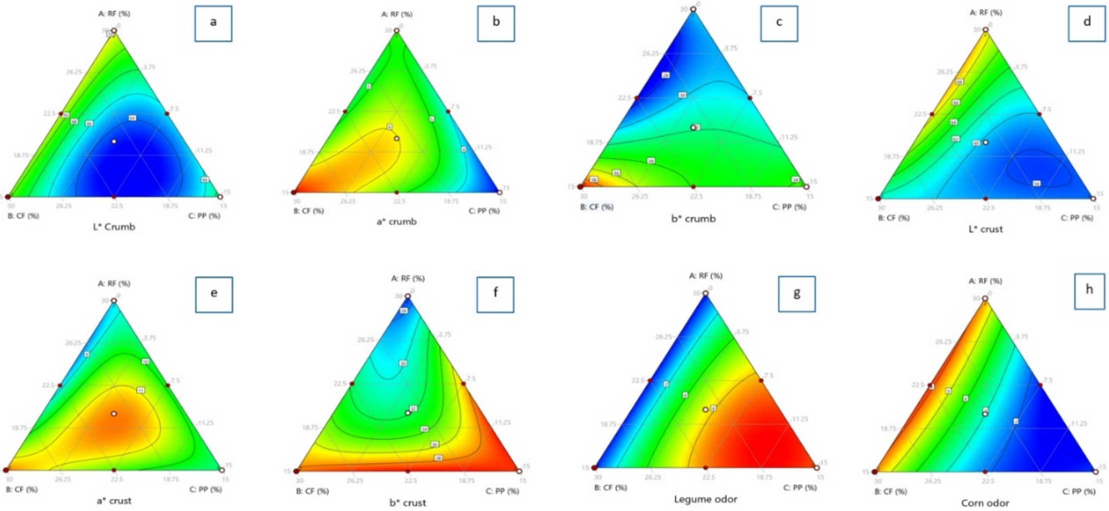


Figure 4. Contour plots depicting the color and odor of *focaccia* samples prepared with different ratios of corn flour (CF, from 15 to 30 g/100g), rice flour (RF, from 15 to 30 g/100g), and pea protein concentrate (PP, from 0 to 15 g/100 g). *L** index of crumb (a); *a** index of crumb (b); *b** index of crumb (c); *L** index of crust (d); *a** index of crust (e); *b** index of crust (f); legume odor (g); corn odor (h). Color variation from blue to red indicates an increase of the considered parameter.

The nutritional composition of the selected *focaccia* was then experimentally determined (Supplementary Table 1). As intended, the protein content of the selected *focaccia* was high enough to reach the conditions required to label it with the "source of protein" nutritional claim (European Parliament and Council, 2006), i.e., more than 12% of the energy value of the final product was supplied by proteins (30 kcal out of a total energy value of 234 kcal). This result is interesting compared to previous trials which required a double concentration of legume flour to achieve a similar or even lower protein content [35], resulting in a higher alteration of the sensory characteristics [11]. Moreover, to further improve its nutritional features, the formulation of the experimental gluten-free *focaccia* did not include oils (which, instead, are abundant in the conventional *focaccia*).

The lipid content, therefore, derived exclusively from the flours used and was therefore below the maximum limit of 3 g/100 g imposed by the Regulation (EC) No. 1924/06[36] (European Parliament and Council, 2006) to label "low-fat" the final product. The fortified *focaccia* fulfilled the EC Reg. 1924/06 also for another nutritional claim. Fibers, indeed, important to reduce the risk of chronic and metabolic diseases [37], reached the amount (>3 g/100 g) needed for the "source of fiber" claim [36].

The moisture content of fortified *focaccia* was slightly higher than that reported by other authors in gluten-free flat breads fortified with legumes or other cereals different from rice and corn [38]. This result was probably due to the higher thickness of *focaccia* (approximately 2 cm, compared to the few millimeters of the other flat breads considered in these studies) or to the effect of psyllium husk powder, known to limit the evaporation of water during baking [39].

3.4. Conclusion

The sector of gluten-free baked goods is increasingly attracting the interest of food companies and researchers, particularly concerned by the need to formulate clean label and sustainable foods, with good nutritional and sensory characteristics. The dry-fractionated protein concentrates are low-processed ingredients with a low environmental impact, which could fulfill the consumer expectations for simple, genuine, and transparent food products. This study explored the effect of different ratios of rice, corn and a dry-fractionated pea protein concentrate used in the formulation of a gluten-free *focaccia* flat bread, to evaluate how they influenced the properties of dough and final product. The simplex-centroid mixture design was effective in helping the evaluation.

The results have shown that the addition of pea protein concentrate influenced the pasting properties of the flour mixes, also leading to differences in crumb porosity, color, texture and sensory characteristics. Balancing the physical and sensory properties with the nutritional value, the trial containing 5 g/100 g of pea protein, 20 g/100 g of corn flour and 20 g/ 100 g of rice flour was chosen as the optimal. Despite this low addition level, the fortified *focaccia* could be labelled as a "source of protein", "source of fiber" and "low-fat" according to the EC Regulation No. 1924/ 06 [34], showing 8.27, 0.73 and 3.92 g/100 g of protein, lipids and fiber, respectively.

Overall, considering the need to propose new gluten-free flat breads, having high nutritional value and sensorially acceptable, the use of dry-fractionated pea protein concentrate has proved to be an effective and sustainable strategy. Moreover, starting from our findings, further studies could investigate the use of other pulse species with different protein composition, and consequently, with different functional properties, in order to expand the knowledge about the effect of dry-fractionated protein on the physicochemical and sensory quality of the products.

3.5 Supplementary material

Nutritional composition of the optimized *focaccia*.

Parameter	Amount
Moisture (g/100 g)	40.53±1.21
Carbohydrates (g/100 g)	50.48±1.37
Protein (g/100 g)	8.27±0.34
Lipids (g/100 g)	0.73±0.01
Fibers (g/100 g)	3.92±0.22
Energy value (kcal/100 g)	234±5

Data are presented as means ± SD of three replicates.

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Chapter 4. Exploring volatile profiles and de-flavoring strategies for enhanced acceptance of lentil-based foods: A review

This chapter has been previously published as: **Vurro, F.**; De Angelis, D.; Squeo, G.; Caponio, F.; Summo, C.; Pasqualone, A. Exploring volatile profiles and de-flavoring strategies for enhanced acceptance of lentil-based foods: a review. *Foods*, **2024**, *13*(16), 2608. **https://doi.org/10.3390/foods13162608**

Exploring volatile profiles and de-flavoring strategies for enhanced acceptance of lentil-based foods: A review

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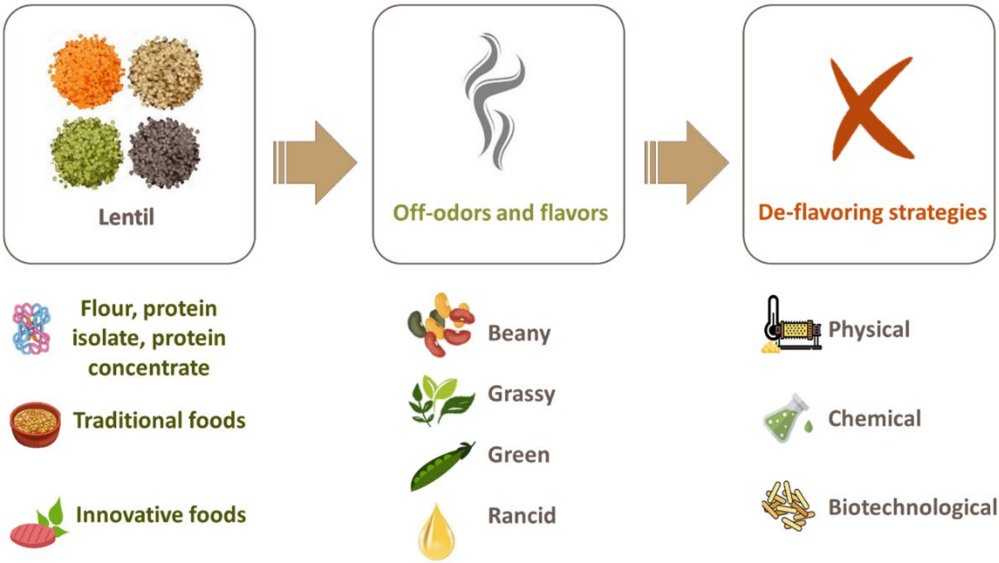
Graphical abstract



Abstract

Lentils are marketed as dry seeds, fresh sprouts, flours, protein isolates, and concentrates used as ingredients in many traditional and innovative food products, including dairy and meat analogs. Appreciated for their nutritional and health benefits, lentil ingredients and food products may be affected by off-flavor notes described as "beany", "green", and "grassy", which can limit consumer acceptance. This narrative review delves into the volatile profiles of lentil ingredients and possible de-flavoring strategies, focusing on their effectiveness. Assuming that appropriate storage and processing are conducted, so as to prevent or limit undesired oxidative phenomena, several treatments are available: thermal (pre-cooking, roasting, and drying), non-thermal (high pressure processing, alcohol washing, pH variation, and addition of adsorbents), and biotechnological (germination and fermentation), all of which are able to reduce the beany flavor. It appears that lentil is less studied than other legumes and more research should be conducted. Innovative technologies with great potential, such as high-pressure

processing or the use of adsorbents, have been not explored in detail or are still totally unexplored for lentil. In parallel, the development of lentil varieties with a low LOX and lipid content, as is currently in progress for soybean and pea, would significantly reduce off-flavor notes.

Keywords: legume; *Lens culinaris* Medik.; volatile compounds; off-flavor; food processing; aroma; odor; plant breeding.

4.1 Introduction

Since ancient times, legumes, also known as “the poor man’s meat”, have been the main source of protein in the Mediterranean diet. Among them, chickpeas (*Cicer arietinum* L.), fava beans (*Vicia faba* L.), lentils (*Lens culinaris* Medik.), peas (*Pisum sativum* L.), and lupins (*Lupin* spp.) are widely cultivated and consumed [1–3]. Due to the promotion of new dietary lifestyles (e.g., flexitarian, vegetarian, and vegan diets) and the increasing awareness of reducing meat consumption for environmental and health reasons, the demand for legumes and legume-based products is expected to grow significantly, with a compound annual growth rate (CAGR) of 5.3% over the period of 2023–2032 [4].

A comprehensive analysis of global lentil production from 1961 to 2022 reveals a steady upward trend, surging up to eightfold and reaching 6.66 million tons in 2022, according to FAOSTAT (2023) [5]. Lentils have gained prominence in the marketplace, being recognized by consumers for their healthy nutritional profile and versatility of use, making them an economical meal solution that is widely consumed around the world [6]. In fact, lentils can be consumed as dried seeds, characterized by the shortest cooking time among legumes, or as fresh sprouts [7]. Dried seeds are boiled to prepare soups, curries, or salads, such as *masoor* in Asian countries, *mujaddara* in Arab countries, *cotechino con lenticchie* in Italy, and *koshari* and *hlalem* in the Mediterranean region. They can be used as a base for vegetarian balls, *hummus*, and pancakes [8,9]. In addition, lentils can be made into flour, which can be used to prepare popular dishes such as *papad* (or *papadam*), a South Asian fried cracker [8,10]. Lentil flour and lentil protein can be used as ingredients in many other food products, including baked goods [11–13], pasta [14], extruded snacks [15,16], protein drinks [17], plant-based meat analogs [18], and dairy alternatives [19,20].

Compared to cereals, lentils are higher in protein and lower in carbohydrates, with variations in composition due to genetic differences, environmental factors, and agronomic practices [6]. Their carbohydrate content ranges from 43% to 75% and has a limited impact on the glycemic index due to the presence of slowly digestible starch and fibers (5–27%). Their protein content ranges from 16% to 31%, with the amino acid profile dominated by glutamic acid, aspartic acid, arginine, leucine, and lysine. Conversely, cystine, tryptophan, and methionine are present in lower proportions [6]. Therefore, the common practice of combining lentils with cereal-based foods (rice, bread, or pasta) balances the amino acid profile and is nutritionally optimal. In addition, lentils are low in fat (1.0–3.5%) and high in minerals (2–6.5%) [6,21], contributing to the dietary intake of iron (Fe), zinc (Zn), and selenium (Se) [10].

Lentil cotyledons are also rich in bioactive compounds such as flavonoids, carotenoids, and tocopherols, recognized for playing a role in preventing oxidative stress, cellular damage, and several pathological conditions [6].

However, some negative implications associated with lentil consumption should also be considered. Lentils contain antinutritional compounds (e.g., trypsin inhibitors, phytates, raffinose-family oligosaccharides, and tannins) that reduce the digestibility of nutrients or cause gastrointestinal discomfort and flatulence [6]. These antinutritional compounds may be reduced by cooking or fermentation [22]. In addition, lentils have high lipoxygenase activity (LOX, E.C. 1.13.11.12), which is responsible for the oxidation of polyunsaturated fatty acids [23–25]. As a result, off-flavors may arise, adversely affecting the sensory quality of lentil derivatives [7].

Perceptible flavor notes in lentils can be intrinsic, such as “beany” (reminiscent of all boiled legume seeds), “green” (reminiscent of green pea), and “grassy” (reminiscent of cut grass) notes, or they can be developed during processing and storage, such as the typical rancid note of aged oil [7] caused by lipid oxidation [26]. Off-flavors in legumes and legume-based foods are a well-documented phenomenon [27–29]. The beany off-flavor is particularly noted by consumers as an unfamiliar flavor that limits the acceptability of foods containing legume flours or legume proteins. Reported as early as the late 1970s [30], the problem of the beany off-flavor is still a hot topic today [31] because of the global shift towards legume-based foods. Han et al. [32] developed a gluten-free cracker snack using green and red lentils mixed with other flours and proteins. A strong beany flavor was reported by many consumers. Ryland et al. [33] formulated a snack bar containing micronized flaked lentils, but the lentil flavor, among other attributes, had a great influence on consumer acceptability.

Strategies for reducing the off-flavors of pea and soybean have been studied in order to provide the food industry with sensory-neutral ingredients from these legumes [34–37]. However, there is a significant gap in the scientific literature regarding lentil de-flavoring. Despite the popularity of this legume, studies on its volatile profile and on techniques for reducing off-flavors remain fragmented. The reviews published on lentils so far have focused only on their nutritional, techno-functional, and health properties [38–42]. In this context, this review delves into the origins of the volatile compounds of lentils, examining the current state of research.

De-flavoring strategies are described, focusing on their effectiveness and potential applicability in the food industry context.

4.2. Methodology of the review and state of the art

A traditional narrative review approach was adopted to critically summarize studies on the volatile compounds responsible for the off-flavors in lentils and the main strategies for achieving their reduction. A literature search and data collection were carried out by accessing scientific databases (Scopus, Web of Science, PubMed, and Google Scholar) and considering all articles that contained the keyword combinations “lentil” and “flavor”/“off-flavor”/“volatile compounds” in the article title, abstract, or keywords, published from January 2016 to June 2024. Supplementary searches were then carried out on specific themes that emerged in the literature, such as innovative de-flavoring techniques proposed for other pulses that could also be useful for lentils.

Figure 1 shows the trend of articles published between 2016, declared the “International Year of Pulses” by the Food and Agriculture Organization of the United Nations (FAO), and June 2024. Over recent years, there has been a significant increase in the number of articles regarding “lentil” and “volatile compounds”. A particularly high number of articles with the words “lentil” and “flavor” can be observed in 2022. Instead, the words “odor” and “aroma”, which refer to orthonasal olfaction (while flavor is retronasal olfaction), were less present in the scientific literature.

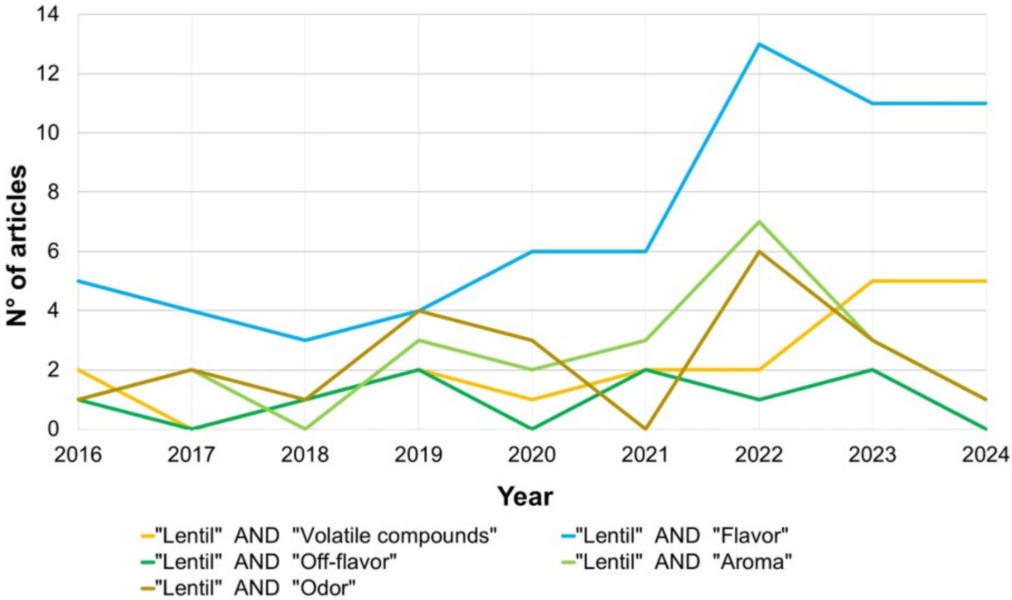


Figure 1. Number of published articles in the years 2016–2024 showing “lentil” and “flavor”/“off-flavor”/“volatile compounds/“aroma”/“odor” in the field “article, abstract, and keywords” (Own elaboration on data retrieved from the Scopus database).

4.3. Flavor/aroma notes typically present in lentils and their origins

Table 1 lists the principal flavor/aroma notes and their associated volatile compounds identified in lentil flours, protein isolates, concentrates, and derived foods. All these flavor/aroma notes are undesirable and limit acceptability by consumers, who generally prefer more sensory-neutral lentil-derived products [33].

The main off-flavor of lentils, named “beany” (or named “legumy” in early papers), is an unpleasant note described as a combination of multiple attributes, including musty, earthy, dusty, sour, green, sweaty, nutty, and brown [44]. This sensory note, inherent in fresh, raw, legumes, is due to plant metabolism and, later, to enzymatic degradation during harvesting, storage, and processing. According to Troszyńska et al. (2011) [7], the reference for this sensory note is a suspension of lentil seeds in hot water 1:3 (*w*/*v*). The beany flavor results from the combination of aldehydes, alcohols, ketones, and furans naturally present in the seeds or formed through chemical reactions occurring during processing. The presence of a single molecule alone does not generally explain this unpleasant beany flavor. The list of compounds considered to be responsible for this broad and complex flavor note may vary among studies, also because every volatile compound can change its sensory effect depending on its concentration.

Table 1. Principal undesirable flavor/aroma notes, descriptions, and associated volatile compounds identified in lentil flours, protein isolates, concentrates, and derived foods.

Undesirable Flavor/Aroma Note *	Description	Volatile Compounds	Origin	References
Beany	Pea-like, musty/earthy, musty/dusty, sour, nutty, brown	(*E*,*E*)-2,4-heptadienal, 1-octen-3-ol, 1-octen-3-one, 3-octen-2-one, 2-pentyl furan, 3-methyl-1-butanol, 2-butanone, 2-pentanone, 2-hexanone, 3-isobutyl-2-methoxypyrazine, and 2-isopropyl-3-methoxypyrazine	Inherent in legume seeds, protein and amino acid degradation, oxidation of fatty acids during storage and processing	[26,28,43,44]
Grassy	Cut grass off-flavor, greasy and fatty	Hexanal, heptanal, nonanal, (*Z*)-3-hexen-1-ol	Inherent in legume seeds, oxidation of fatty acids during storage and processing	[26,43]
Green	Fresh green pea	Hexanal, heptanal, nonanal, 2-pentyl furan	Inherent in legume seeds, oxidation of fatty acids during storage and processing	[26,43]
Rancid	Aged oil and fat	Hexanal, heptanal, octanal, (*E*)-2-heptenal (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-hexenal, (*E*,*E*)-2,4-decadienal, and pentanal **	Oxidation of fatty acids during storage and processing	[35]
Brothy	Boiled beef, cooked potatoes, cooked vegetables	Methional	Degradation of methionine during processing	[26]
Sulfur	Metallic, cabbage, egg, onion	Dimethyl sulfide and dimethyl trisulfide	Degradation of methionine and cysteine during processing	[26]

* According to orthonasal (aroma and odor) or retronasal (flavor) olfaction; ** Combinations of these compounds may result in a beany flavor.

Vara-Ubol et al. (2004) [44] observed that 3-methyl-1-butanol, 1-pentanol, (*E*,*E*)-2,4-heptadienal, acetophenone, and 1-octen-3-one are responsible for a beany note when their concentration is ≤ 10 ppm, changing to "sweaty" (for 3-methyl-1-butanol and 1-pentanol), "barnyard, manure" (for 1-pentanol), "rancid, fishy" (for (*E*,*E*)-2,4-heptadienal), or "musty/earthy, floral, sweet" (for acetophenone and 1-octen-3-one) at higher concentrations, while 1-octen-3-ol provides a beany flavor at concentrations between 100 and 1000 ppm. Xu et al. (2019) [45] defined hexanal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal, 3-methyl-1-butanol, 1-hexanol, and 2-pentyl-furan as beany flavor markers. Further studies by other authors [35] pointed out that some compounds, such as hexanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-hexenal, (*E*,*E*)-2,4-decadienal, and pentanal, are responsible for off-flavors (namely "rancid"), but do not have a beany flavor, *per se*, and should be considered as "non beany". However, these compounds are able to enhance the intensity of the beany note. The situation is further complicated by the fact that the same authors observed that combinations of these "non-beany" volatiles may ultimately result in a beany flavor [35].

The aforementioned volatiles, including aldehydes, alcohols, ketones, and furans, linked to the "beany" flavor/aroma note (or, according to Trindler et al. [35], to a "non-beany" off-flavor, that is, "rancid") result from the degradation and oxidation of lipids. The main event they arise from is the physical breakdown of cell tissues, causing the release and activation of lipase and LOX. Lipase releases free fatty acids from triglycerides, then LOX prompts the oxidation of linoleic and linolenic acids with the production of C6 and C9 aldehydes and alcohols [23,46], with the latter coming mainly from aldehyde reduction by the activity of alcohol dehydrogenase (ADH) [28]. The enzymatic activity of LOX also alters pigments (carotenoids and chlorophyll), resulting in off-flavors and discoloration [23]. Alternatively, the autooxidation of linoleic and linolenic acids may occur [23,46].

Methyl ketones (such as 2-butanone, 2-pentanone, and 2-hexanone) are generated through the decarboxylation of 3-oxo free fatty acids or via the aldol condensation of an aldehyde and an aldol intermediate [29]. 2-Pentyl furan, the most common representative lipid-derived furan, arises from the autoxidation and cyclization of linoleic acid or from the thermal interaction of (*E*,*E*)-2,4-decadienal with cysteine [28,29].

A key compound formed by linoleic acid is hexanal, an aldehyde considered to be a marker of oxidation in food matrices [26,47]. Hexanal is associated with notes described as "green" (sensory reference = green seeds of peas [7]), "grassy" (sensory reference = 1% (*Z*)-3-hexen-1-ol, [7]), and "rancid", together with other aldehydes such as heptanal and octanal [26,48]. Green and grassy notes are typical of lentil sprouts [7].

Other studies, despite not being carried out specifically on lentils, also assessed that some pyrazine derivatives, which can be the products of protein and amino acid degradation, are responsible for "beany" and/or "green" flavors [35,37,49], such as 3-isobutyl-2-methoxypyrazine and 2-isopropyl-3-methoxypyrazine. In contrast, pyrazines such as 2,5-dimethy-lpyrazine and 3-ethyl-2,5-dimethylpyrazine have a pleasant "nutty" or "roasted" flavor [27,34,50].

The "brothy" flavor (sensory reference: one stock cube in 2 L of boiling water [51]) is another note identified in the lentil flavor profile, related to the presence of methional derived from methionine by Strecker degradation, which imparts an off-flavor reminiscent of cooked potatoes and cabbage [52]. Dimethyl sulfide and dimethyl trisulfide recall metallic, cabbage, egg, and onion off-flavors, are highly disliked by consumers, and can be defined as a "sulfur" note [26].

Over the years, researchers have often reported the negative sensory effects of fortifying food products with legumes, suggesting the need to find viable solutions to overcome these problems. Unsurprisingly, both traditional and innovative lentil-based recipes often include herbs and spices such as turmeric, ginger, oregano, bay leaf, and rosemary, which are rich in palatable essential oils, to mask the beany off-flavor [53–55]. However, with a thorough understanding of the effects of basic processing steps and storage on the volatile profile of lentil flours, actions should be taken to select the appropriate conditions for seed treatment and storage, flour production, and any other applied processes, especially to avoid or mitigate off-flavors [35].

4.4. Effect of storage and basic processing on the volatile profile

4.4.1. Storage

Storage may greatly affect the volatile profile of lentils. Factors such as temperature, exposure to oxygen and light, and moisture content influence the kinetics of chemical reactions. Low temperatures and/or a low moisture content are essential for the storage of any bulk grain, preventing mold and oxidation, and mitigating the formation of off-flavors. Red lentils stored at 10 °C and 20 °C for 16 weeks showed no significant increase in free fatty acids (ready to be oxidized), even having a moisture content of 17.5%. If the moisture content decreased to 10%, the lentils showed a limited increase in free fatty acids during storage at 40 °C for up to 10 weeks, while samples with a 12.5% moisture content tripled their free fatty acids in the same storage time [56]. This study did not evaluate volatile compounds, but in another legume, peas, a moisture content of less than 10% prevented the onset of unpleasant odors for up to one year of storage at 30 °C [35]. For red lentil, buyers and processors prefer a seed moisture content of 13% to facilitate dehulling and splitting, but for "safe" storage (absence of discoloration, off-flavors, mold, and insect proliferation) for up to 28 weeks, the temperature must not exceed 20 °C or, to reach 50 weeks, 15 °C [57].

Low temperatures can further extend storage time. Lentils with a moisture content of 13% can be stored for up to 175 weeks at 5 °C [58], but no details were given by the authors on the effect of such prolonged storage on flavor. Raw peas stored at 4 °C for 12 months showed lower contents of aldehydes and sulfur compounds than those stored at 22 °C due to reduced LOX activity at low temperature [59]. More specific studies would be needed on lentil to assess the effect of storage conditions on the evolution of the volatile compounds responsible for the onset of off-flavor.

4.4.2. Dehulling and milling

Lentils are first subjected to conditioning, which is a moistening treatment to soften their hulls and facilitate their subsequent removal. Dehulling and milling are then carried out to separate the outer layers of the seed, known as the hull, from the inner edible portion (cotyledon), subsequently reducing the latter to smaller particles or flour [60].

This series of mechanical operations, leading to certain temperature increases due to friction occurring during and to relevant exposure to oxygen, can affect the flavor profile of lentils [35]. The removal of the hulls of brown and green lentils resulted in variations in their volatile profiles, with a reduction in non-terpene volatiles and the formation of phenylpropanoids and apocarotenoids [43]. The dehulling of lentils has been reported to improve their flavor [58] and reduce the content of tannins, mainly responsible for a bitter sensory note [6]. Therefore, today, lentils are commercialized mainly in dehulled form.

Oxidation compounds such as nonanal and 2-methyl-1-hexanol increase with milling [35]. Rajhi et al. [43] confirmed that milling enhances exposure to oxygen and increases the intensity of the beany flavor of lentils. Tissue damage and enzyme release, causing volatile production by enzymatic mechanisms, are unavoidable during processing, but can be minimized. For instance, peas milled to a 500 μm average particle size showed lower levels of volatile compounds than those milled to 250 μm [61]. Similar studies should be conducted on lentils to define the optimal conditions to reduce the off-flavors related to milling.

4.5. De-flavoring strategies

The treatments that can be undertaken to reduce the off-flavors in lentil flours and derived food ingredients and products are schematized in Figure 2, while their main mechanisms of action, advantages, and disadvantages are summarized in Table 2.

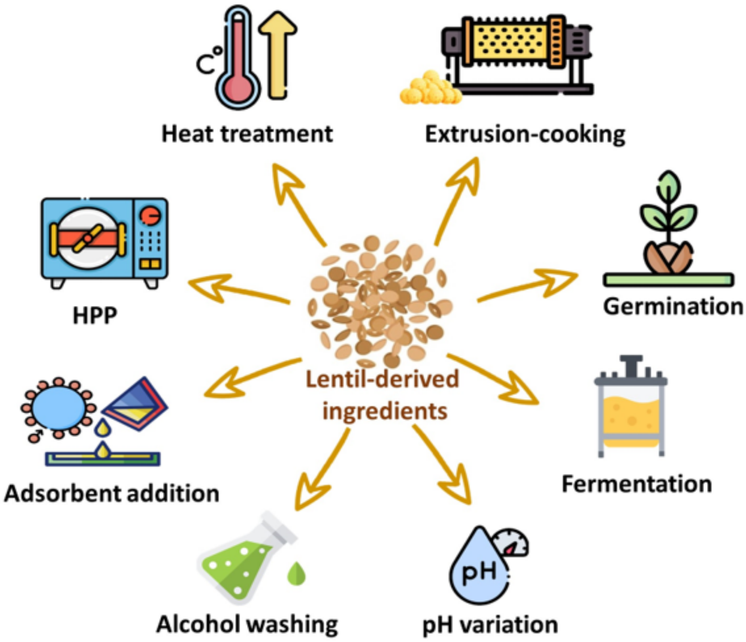


Figure 2. De-flavoring strategies applicable to lentil seeds, flours, or protein isolates and concentrates. HPP = high-pressure processing.

Depending on the type of treatment, there may be changes in the volatile profile due to physical–chemical modifications of components (e.g., protein and enzyme—including LOX—denaturation); chemical interactions between molecules (e.g., Maillard reaction leading to new flavors); and the removal or adsorption of the compounds responsible for undesirable flavors. Some treatments may lead to a decrease in LOX activity, resulting in a reduction in oxidation compounds (e.g., heat treatments, high pressures, use of solvents, or pH changes), while others may lead to the formation of new chemical compounds that enrich the volatile profile and may mask the perception of off-flavors (e.g., fermentation, germination, or extrusion cooking).

However, economic and environmental issues must be considered in the view of practical applications, because some treatments (such as HHP and extrusion cooking) require significant investments in equipment, energy, and/or water, while others are easily replicable without relevant additional costs. Finally, some treatments, such as alcohol washing, pH variations, and adsorbent addition, require special attention to avoid the possible presence of chemical residues in food, by adopting purification steps and exclusively using food-grade chemicals.

Table 2. Treatments applicable to lentil flours and lentil-based ingredients to mitigate flavor.

Type of Treatment	Mechanism of Action	Advantages	Disadvantages	References
Heat treatment (pre-cooking, roasting, and drying)	Reduction in LOX activity; volatilization of off-flavors; new flavors	Low investment cost; operational ease and simplicity	Energy consumption; possible color alterations	[62,63]
Extrusion cooking	Volatilization of off-flavors; new flavors	Short processing time	Energy consumption; investment cost	[64,65]
High-pressure processing	Reduction in LOX activity	Short processing time	Investment cost; energy consumption; water consumption	[66,67]
Alcohol washing	Reduction in LOX activity; solubilization of off-flavors	Low investment cost; operational ease and simplicity	Chemical residues; reduction of protein solubility	[68]
Variation in pH	Reduction in LOX activity	Low investment cost; operational ease and simplicity	Chemical residues	[28]
Adsorbents	Physical removal of off-flavors	Low investment cost; operational ease and simplicity	Chemical residues, energy consumption; water consumption	[69]
Germination	New flavors	Low investment cost; operational ease and simplicity; minimal or no chemicals needed	Possible oxidations	[7,43,70–73]
Fermentation	New flavors	Low investment cost; operational ease and simplicity; minimal or no chemicals needed	Long processing time	[74]

4.5.1. Heat Treatments

4.5.1.1. Pre-cooking, roasting, and drying

The most common heat treatments for lentils are pre-cooking (boiling or steaming), roasting, and drying. These treatments can involve the application of microwave and radio frequencies to achieve the desired outcomes, such as specific changes in physical–chemical properties, making food more digestible and palatable. The heat treatment of lentil seeds and flour denatures LOX and possibly peroxidase, reducing their activity. However, the thermal process may promote the autoxidation of unsaturated free fatty acids [62]. Another effect of thermal treatments, such as roasting, is that they produce new volatile compounds derived from the Maillard reaction, such as furans and pyrazines, that can mask the beany flavor.

Shariati-Ievari et al. [75] investigated the effect of infrared heat treatment on LOX activity and, consequently, on the volatile compounds of green lentils while being micronized. A significant reduction in LOX activity was observed after treatment at 130 °C, and a further decrease at 150 °C, compared to untreated flour, with a reduction in the hexanal and 2-hexenal levels. Burgers prepared with heat-treated lentil flour had a good overall acceptability and flavor, while those prepared with untreated flour were characterized by a beany off-flavor. Also, blanching and steaming are effective in deactivating LOX and decreasing the off-flavors in pulses, such as peas and beans [76], although there have been no studies carried out on lentil.

Different heat treatments, such as roasting, pre-cooking, and drying, have different effects on the flavor profile: roasting increases and complexifies the volatile profile, while pre-cooking and drying decrease the flavor intensity. In detail, the roasting of lentil seeds for 20 min at about 100 °C reduced the total alcohols, did not change the total aldehydes, and increased the pyrazines compared to raw lentils, with an overall increase in the total volatile compounds. In contrast, lentil pre-cooking (by boiling for 20 min) markedly reduced the total volatiles compared to raw lentils by lowering the level of total alcohols, without affecting the total aldehydes [46].

Drying is the final stage in the preparation of pre-cooked lentil flours or wet-fractionated lentil proteins, and is required after the fermentation of lentils and before their grinding when preparing fermented flours [77]. For preparing pre-cooked lentil flours, cooked seeds must be mashed in a blender with water and finally spray-dried. Drying (by setting the inlet and outlet temperature of the spray-drier at 130 and 80 °C, respectively) was found to slightly increase the total volatiles compared to pre-cooked seeds, but these still remained below the concentration found in raw lentils. Alcohols were reduced by drying, but aldehydes (especially hexanal) strongly increased compared to the raw lentils [46].

Drying also reduced the total volatiles of fermented lentils. Fermentation tends to enrich the volatile profile (see Section 5.7), but the total sum of volatiles decreased after hot-air drying. Alcohols and aldehydes (particularly 1-octene-3-ol, hexanal, benzaldehyde, and 3-methoxybenzaldehyde) were the chemical compounds that varied the most. These compounds decreased progressively with an increasing temperature (from 50 to 70 °C) [77]. The loss of some volatile compounds at higher temperatures is due to thermal degradation and volatilization [78]. In contrast, pyrazines were generated by the Strecker degradation and Maillard reactions, resulting in pleasant caramel-like, burnt, and cooked notes [79]. As a result, combined with fermentation, hot-air drying improved the flavor of lentils.

Innovative techniques should be also considered, such as microwave and radio frequency heating, which were found to effectively inhibit the LOX activity of soybean in a very short time, de-flavoring the soybean protein [80,81]. Microwaves were also used to set up a dehydration system working in vacuum, which was able to remove flavors from plant-based non-dairy beverages [82]. These techniques have not been applied to lentil ingredients so far.

4.5.1.2. Extrusion cooking

Extrusion cooking is a versatile thermo-mechanical process widely used in the lentil value chain to produce snacks, textured proteins, and meat analogs [65,83,84]. The deactivation of LOX occurs at temperatures above 100 °C, like those adopted during extrusion cooking, but requires sufficient time. During extrusion cooking, which is usually a relatively fast process (with the residence time in the extruder cooker being between 2 and 10 min) [64], residual LOX activity and high exposure to oxygen may significantly affect the lipid fraction generating the compounds responsible for off-flavors [23]. The chemical composition of the starting flour also has an influence. In fact, during the production of legume-based extruded snacks, some off-flavors, such as "fatty/cardboard-like" and "fatty/deep-fried", were rated lower in lentil snacks than in blue lupin snacks, opposite to a "popcorn-like" flavor, which was scored higher in the lentil snacks [83].

Temperature and screw speed, the two main parameters set during the process, were found to affect the volatile compounds. During the production of lentil-based high-moisture meat analogs, nonanal and benzaldehyde increased by raising the temperature to 150 °C, but decreased at values of > 160 °C. Accordingly, the odor concentration decreased as the temperature increased [65]. Similar results were found when studying the effect of the extrusion temperature on the flavor of texturized soybean proteins. The authors hypothesized that increased flash evaporation at the exit of the die, occurring at higher temperatures, caused a loss of volatile compounds [85]. In detail, the levels of 2-pentyl-furan, 2-nonanone, 1-octen-3-ol, nonanal, hexanal, benzaldehyde, and 2-heptanone decreased at temperatures of >155 °C [85].

Screw speed showed a similar effect to temperature. The total flavor compounds of lentil-based meat analogs were more concentrated when the screw speed was raised from 800 to 1000 rpm, but decreased at values of > 1000 rpm [65]. Therefore, at certain settings of temperature and screw speed, extrusion cooking can contribute to the reduction in flavors.

4.5.2. High-pressure processing (HPP)

High-pressure processing (HPP) is a non-thermal technology that relies on applying a pressure between 300 and 600 MPa to food for a few minutes. The treatment is performed in a vessel where the product is immersed in water, responsible for the instantaneous transmission of pressure throughout the product [66]. HPP offers the possibility of extending the shelf lives of food products by reducing microbial populations, and has been evaluated as a method for modifying the techno-functional properties of plant proteins [67], including dry fractionated lentil protein concentrate [86] and yellow lentil protein concentrate [87]. The application of HPP can alter the gelling and swelling ability, solubility, viscosity, and foam/emulsion stability of legume proteins by modifying their structure via pressure-induced denaturation [88]. Therefore, considering that proteins are known to bind flavor compounds by trapping them physically or via chemical interactions [67], some research studies have evaluated the effect of HPP on flavor. The ability of HPP to reduce the interactions between volatile compounds due to structural modifications has been observed in plant proteins by Bi et al. [89] and Houde et al. [90]. Moreover, HPP is able to inactivate LOX [91].

The effect of different values of pressure has been studied in pea milk, pointing out that treatments at 550 MPa led to retaining a greater amount of hexanal than those at 200 MPa [89]. However, no study to date has specifically evaluated the effect of HPP on the volatile profiles of lentil proteins or lentil-based foods, despite the fact that this technology has much potential to be explored as a de-flavoring strategy.

4.5.3. Variation in pH

The principal method for obtaining legume protein isolates is wet extraction, based on the dispersion of flour in water at pH 9 to solubilize proteins, followed by isoelectric precipitation at pH 4.5–4.8 [92]. Similar to other enzymes, LOX has a different pH optimum based on different isoforms [93]. The wet extraction of protein from legume flour can, therefore, impact the LOX activity and, consequently, influence some key components of the beany flavor. In addition, changing the pH or adding salt during protein extraction can affect the protein structure, hydrophobicity, and surface charge, altering flavor–protein bonds and promoting decreased off-flavors [28].

Few studies have investigated the influence of pH on the volatile profile of legumes, being primarily focused on soy and peas. Gao et al. [94] compared pea proteins obtained by alkaline extraction at pHs of 8.5, 9.0, and 9.5. They found that the activity of LOX was reduced with an increase in the pH, lowering the concentrations of the volatile compounds typically associated with the beany flavor. Similarly, Iassonova et al. [95] compared different pH treatments and observed the highest levels of hexanal, 1-octen-3-ol, and nonenal in soybeans at pH 6.8 and lower concentrations of these volatiles at higher or lower pH values.

In view of the growing interest in protein foods enriched with legume isolates and concentrates, obtained by wet methods, the influence of pH also needs to be studied in depth in lentil proteins, as it may have an impact on the overall characteristics of the final product. Interestingly, pH shifting has been investigated as a strategy to modulate the quality of meat analogues [96], highlighting the needs of examining, in depth, the possible influence on the formation of volatile compounds.

4.5.4. Alcohol washing

The washing of legume flours or protein concentrates using alcoholic solutions (ethanol at 20, 50, and 80% *v*/*v*, with water) improves their flavor profiles due to the alcohol solubility of several volatile compounds (e.g., hexanal, nonanal, heptanal, 1-penten-3-ol, 2-penten1-ol, and butanone), reducing beany and green off-flavors. Ethanol also alters the enzymatic activity of LOX. The best ethanol concentration for this purpose was found to be 80% [68]. Additionally, the washing process removes impurities, including simple sugars, oligosaccharides, fats, and ash, contributing to achieving cleaner and more refined legume flours.

Tan et al. [97] suggested that washing with ethanol reduced the 1-hexanol in pea proteins when using appropriate concentrations. However, at 15% and 25% *v*/*v*, the LOX activity was not reduced, with increases in hexanal, 1-octanol, 1-nonanol, 1-octen-3-ol, trans-2-hexenal, and nonanal, which are associated with beany, green, and earthy off-flavors. Instead, lentil protein isolate washed with ethanol 75% (*v*/*v*) at a ratio of 1:10, *w*/*v*, then centrifuged and vacuum-dried, was effectively stripped of off-flavors [98]. An influence of vacuum-drying could not be excluded, with this technique being known to allow for de-flavoring [82]. Isopropanol was also proposed, but it is not a viable option, as it is not food grade.

Ethanol extraction can also be combined with the supercritical CO_2 extraction of flavor compounds, which was used to de-flavor pea flour. This process must be studied to identify the optimum conditions (in this case, 22% ethanol, 86 °C, and 42.71 MPa) in order to maximize the de-flavoring effect [99]. Also, the particle size of flour has to be considered [100], because it can influence the diffusion of the solvent within particles and, consequently, the extraction rate.

These results show that washing with alcohol can be effective, but highlight that preliminary tests are needed to define the optimal alcohol concentration, even when used in combination with other extraction systems. Moreover, the physical-chemical properties may change after alcohol washing, with a reduction in protein solubility often being observed [25,35]; therefore, the suitability of this treatment must be verified case by case, also considering the economic and environmental sustainability of the process.

4.5.5. Addition of adsorbents

The use of solid adsorbents and membrane filtration is a promising nonthermal physical treatment that can de-flavor lentil ingredients. This process consists of the use of adsorbent material, generally resins, to de-flavor the product. This approach was tested on lentil protein isolates by Guldiken et al. [69], who used synthetic resins (Amberlite XAD16N, Amberlite XAD7HP, Amberlite XAD4, Sepabeads SP207, and Diaion HP20) constituted by polystyrene/divinylbenzene or acrylic ester, with varying pore sizes ranging from 50 to 580 Å. The lentil protein isolate was solubilized in water at 5%, adjusting the pH at 9.5, then the resins were added, keeping a 1:0.5 *w*/*w* ratio between the protein and the adsorbent. This was followed a filtration step, pH correction, and lyophilization. Differences in the effectiveness of de-flavoring were observed, depending highly on the properties of the absorbent material, such as its polarity, surface area, matrix, and pore diameter. In particular, Amberlite XAD16N, Sepabeads SP207, and Diaion HP20 reduced the major aldehydes by 32–41% and, specifically, hexanal by 39–52%, in the following ascending order of effectiveness: Sepabeads-SP207 < Amberlite-XAD16N < Diaion-HP20. 3,5-Octadien-2-one was markedly reduced by all adsorbents (by 61–92%), as well as alcohols (by 80–84%) and 2-pentylfuran (by about 50%).

The addition of β-cyclodextrin is another possible way to reduce the flavor of lentil ingredients, as this molecule is able to remove volatile compounds by trapping them in its inner core (complex formation). Treatment with β-cyclodextrin is similar to that of treatment with adsorbents. It requires first solubilizing the lentil ingredient, then adding the β-cyclodextrin. Finally, the suspension must be filtered to remove the complexes, and the protein must be dried. The de-flavoring effectiveness of β-cyclodextrin has been demonstrated in soybean and pea proteins [50,101], but has not yet been tested in lentil.

Further investigations should also be carried out to explore the potential application of adsorbent-based methodologies on a large scale, by integrating an adsorption step in the regular productive process of protein isolates by wet extraction. In addition, water and energy consumption could be a limitation, since dry fractionation has been recently suggested as a more sustainable procedure compared to wet extraction for obtaining pulse protein concentrates [102,103].

4.5.6. Germination

Germination is the process in which a dormant seed begins to sprout, and a radicle (the embryonic root) and shoot emerge and start to grow into a young plant. This economical and traditional process involves benefits associated with a reduction in antinutrients and increases in the B vitamins and antioxidant activity of lentils [6,73]. Moreover, germinated lentil flours have an increased water absorption and foaming activity compared to regular lentil flours [70,71,104]. Compared with other de-flavoring techniques, which, to date, have generally not been extensively studied on lentils, numerous studies have instead evaluated the effect of germination on this legume, driven by the nutritional interest of this technique. Some of these have also examined the evolution of flavor.

During germination, the structure of the endosperm is disrupted with germination, which could allow for an easier removal of protein-bound flavors. However, the activation of enzymes, including LOX, may induce biochemical changes such as lipid and protein degradation and oxidation [72]. The overall volatile profiles of lentils can be complexified with germination, so this process cannot always be properly termed a de-flavoring technique. Moreover, conflicting results are sometimes reported among studies (see Table 3), resulting either in a desirable or non-desirable change in flavor, probably attributable to the different lentil varieties considered (with different chemical compositions), the use of dehulled or non-dehulled lentils, and different conditions for both germination and post-germination treatments. However, it appears that, by controlling the germination conditions, particularly the germination time (keeping it short) and post-germination treatment (preferring freeze-drying over oven-drying at a medium temperature), it is possible to achieve a decrease in beany flavor [72].

No post-germination treatment was conducted in a study focusing on assessing the sensory properties of fresh sprouts prepared by germinating (20 °C, 99% RH, in the dark) green lentils for seven days, with sensory checks performed every 24 h [7]. This study highlights the pure effects of germination without the influence of subsequent treatments. The panelists assessed a significant decrease in beany flavor after five days of germination. Further prolonging the germination for six and seven days did not induce further significant reductions. However, other undesirable flavors behaved differently. In detail, the grassy flavor increased after three days, then remained constant; the green flavor did not change over the entire period of germination; and off-flavors increased from the sixth day of germination onward. Although this study did not evaluate volatile compounds, so could not give information from the chemical point of view, it is undoubtably valuable due to the importance of sensory features for any food product. Indeed, it must be emphasized that germination may lead to inherent beany flavor decreases, but some new flavors that could also be undesirable could increase. Overall, it seems that keeping germination times below three days prevents the occurrence of other unpleasant flavors.

The negative effect of a prolonged germination time was also confirmed by Rajhi et al. [43], who found that germinated lentil flours (at 20 °C and 99% RH in the dark for five days, followed by drying at 45 °C for 6 h and grinding) showed an increase in 2-pentyl furan, nonanal, and decanal compared with raw seeds. Interestingly, pentyl furan appeared after the grinding of germinated seeds due to exposure to oxygen during this process, highlighting that all necessary steps after germination should be carefully considered (grinding and drying), as they have an influence on volatiles.

Flours prepared from green lentils that were germinated for two days (22–24 °C under a wet cloth, in the dark, after overnight soaking, followed by oven drying at 60 °C for 17–18 h) [70,71] showed a decrease in beany flavor after both 1 and 2 days of germination, but with better results after 1 day [71]. An increase in aldehydes compared to non-germinated flour (with a greater increase after 24 h and a lower increase after 48 h), as well as an increase in alcohols (more elevated after 48 h than 24 h), was ascertained. In detail, strong decreases in nonanal and 2-pentyl furan (both without significant differences between 24 and 48 h) and slight increases in the concentrations of hexanal (more present after 24 h than after 48) and heptanal were observed (more abundant after 48 h than 24 h) compared to the non-germinated flour. Among alcohols, 1-hexanol and 1-octen-3-ol increased, with the latter being not detected in non-germinated flour and progressively increasing with a longer germination time.

In addition to flour, lentil protein isolate is another semi-processed ingredient widely marketed today. The olfactometric profiles of protein isolates extracted from lentil seeds germinated for only one day (25 °C, 99% RH in the dark, followed by freeze drying and grinding to flour), compared with protein isolate obtained from non-germinated seeds, showed reductions in some volatile compounds associated with a beany flavor, while others very slightly increased. All the beany volatiles saw marked increases in their concentrations with longer germination times (3–5 days) [72]. In detail, nonanal, 3-octen-2-one, heptanal, and (*E*, *Z*)-2,6-nonadienal were reduced after 1 day of germination and, with the exception of nonanal, which remained at a low concentration, all increased with longer germination times. Hexanal and (*E*)-2-octen-1-ol increased very slightly with one day of germination, but their level increased much more at longer germination times [72]. Therefore, since the beany flavor of protein isolates improved with short germination (24 h) but intensified significantly if germination continued, care must be taken in timing.

Germinated lentils have been also proposed for preparing a plant-based beverage. In this case, no significant differences were found in the legume odor and flavor and in the overall sensory quality of the beverage compared to a control prepared with non-germinated lentils. In detail, the nonanal content decreased and (*E*, *E*)-2,4-nonadienal content increased, while hexanal and pentanal remained unchanged with the germination (25 °C for three days) and preparation of the beverage (sterilizing at 121 °C for 15 min, mixing with water at a ratio of 1:9, blending, and filtering) [73]. The addition of raspberry–cranberry pulp was proposed as an easy and effective way to improve the sensory features of this beverage.

A recently published study outlined the possibility of extrusion cooking germinated lentils to prepare meat analogs [65], achieving a reduction in volatile compounds. Germination and extrusion had a synergistic effect, reducing some volatile compounds responsible for the beany flavor, such as hexanal, 1-hexanol, 2-nonenal, (*E*)-2-octen-1-ol, and (*E,E*)-2,4-nonadienal. In parallel, other volatile compounds could be formed under these conditions, such as the products of the Maillard reaction, typically characterized by pleasant nutty and roasted flavors.

Table 3. Effect of germination on the volatiles of lentil flours, protein isolates, and lentil-based beverage.

Lentil Product	Type of Lentil	Germination Days	Germination Conditions	Post-Germination Treatments	Beany Volatile Compounds Involved		Effect on Beany Flavor	Reference
					Increasing	Decreasing		
Lentil sprouts	Green lentil, Aldona cultivar	7	Soaked 3.5 h at RT, then kept at 20 °C, 99% RH, in the dark	None	n.d.	n.d.	Decreasing	[7]
Lentil flour	Brown and green lentil	5	Dehulled, then sown in Petri dishes at 20 °C, 99% RH, in the dark	Drying at 45 °C for 6 h and grinding	2-Pentyl furan, nonanal, and decanal	-	Increasing	[43]
Lentil flour	Altamura lentil, not dehulled	1	Soaked overnight, then kept at 22–24 °C, under a wet cloth, in the dark	Drying at 60 °C for 17–18 h and grinding	Aldehydes and alcohols	2-Pentylfuran	Decreasing	[70]
Lentil flour	Altamura lentils, not dehulled	2	Soaked overnight, then kept at 22–24 °C, under a wet cloth, in the dark	Drying at 60 °C for 17–18 h and grinding	Aldehydes and alcohols	2-Pentylfuran	Decreasing	[70]
Lentil flour	Green Altamura lentil	1	Soaked overnight, then kept at 22–24 °C, under a wet cloth, in the dark	Drying at 60 °C for 17–18 h and grinding	Hexanal, heptanal, and 1-octen-3-ol	2-Pentylfuran and nonanal	Decreasing	[71]
Lentil flour	Green Altamura lentil	2	Soaked overnight, then kept at 22–24 °C, under a wet cloth, in the dark	Drying at 60 °C for 17–18 h and grinding	Hexanal, heptanal, 1-octen-3-ol, and 1-hexanol	2-Pentylfuran and nonanal	Decreasing	[71]
Protein isolate	Commercial, not specified	1	Soaked 5.5 h, then kept at 25 °C, 99% RH, in the dark	Freeze-drying and grinding, then wet extracting the protein	Hexanal and (*E*)-2-octen-1-ol	Nonanal, 3-octen-2-one, heptanal, and (*E*, *Z*)-2,6-nonadienal	Decreasing	[72]
Protein isolate	Commercial, not specified	3–5	Soaked 5.5 h, then kept at 25 °C, 99% RH, in the dark	Freeze-drying and grinding, then wet extracting the protein	Hexanal, (*E*)-2-octen-1-ol, nonanal, 3-octen-2-one, heptanal, and (*E*, *Z*)-2,6-nonadienal	-	Increasing	[72]
Lentil-based beverage	Brown lentils	3	At 25 °C in a commercial sprouter	Sterilizing at 121 °C for 15 min, mixing with water at a ratio of 1:9, blending, and filtering	(*E*, *E*)-2,4-nonadienal	Nonanal	Unchanged	[73]

RH = relative humidity; RT = room temperature; and n.d. = not determined.

4.5.7. Fermentation

Fermentation is the oldest biotechnological approach applied to food and beverages, involving the use of spontaneous or selected microorganisms, typically bacteria and/or yeasts, which metabolize carbohydrates and other compounds present in the matrix. Fermentation enhances nutrient bioavailability and protein digestibility, reduces antinutrients, such as phytic acid and protease inhibitors, promotes antioxidant activity, and reduces carbohydrate content. Probiotic lentil-based fermented beverages prepared using *Lactobacillus* strains were proposed by Verni et al. [17]. Boeck et al. [105] fermented a yogurt alternative using lentil protein isolate and selected strains of lactic acid bacteria. A sensory evaluation of the final product scored the "beany flavor" from 4.4 to 6.1, depending on the strain, on a 0–10 scale. Canonico et al. [106] used wild yeasts to produce a functional craft beer with reduced alcohol content, fortified with hydrolyzed red lentils as a protein source. They found that specific yeast strains resulted in increased aroma compounds compared to traditional *Lactobacillus thermotolerans*/*S. cerevisiae* cultures, influencing the sensory profile by imparting a fruity aroma. Perri et al. [107] carried out the fermentation of whole and sprouted lentils using selected lactic acid bacteria (LAB), resulting in sourdoughs that were later incorporated into bread formulations. The inclusion of sourdough had a significant effect on the volatile profile of the bread, improving its flavor.

Fermentation can impart unique flavors and aromas. Through their enzymes, microorganisms degrade proteins, lipids, and complex carbohydrates, releasing their basic components (peptides, amino acids, fatty acids, and simple sugars), which can be precursors to flavors and aroma compounds as fermentation proceeds, modifying the volatile profiles of the starting lentils [74]. The effect on the volatile profile is strongly related to the microbial composition, the compositional characteristics of the lentils used, and the fermentation parameters. Therefore, optimization is always needed to ensure that the variations in volatiles induced by fermentation mitigate the beany and grassy notes associated with hexanal and other products of lipid and protein degradation, contributing to the overall sensory quality of fermented lentil products. Fermentation is relatively inexpensive and requires minimal or no chemicals, but can be time-consuming.

Solid-state fungal fermentation with *Pleurotus ostreatus* (28 °C for 14 days) improved the volatile profile of lentil flour of the cultivar Pardina [77] by markedly reducing 1-hexanol and hexanal compared with non-fermented lentils, while benzaldehyde and 3-octanone (absent in the starting lentils) strongly increased. These compounds varied with successive oven-drying or freeze-drying, with 3-octanone decreasing and benzaldehyde (giving a pleasant almond flavor) further increasing, while hexanal and 1-hexanol remained unchanged. In lentils of the Castellana cultivar, instead, hexanal increased with fermentation, but then decreased with drying, while benzaldehyde strongly increased but then decreased with drying. Nonanal, not detected in Pardina, increased with the fermentation of Castellana lentils but decreased with successive drying. The volatiles were, therefore, significantly influenced by both the lentil cultivar and the post-fermentation operations.

Several volatiles, such as hexanal, 1-hexanol, hexanoic acid, 2-pentyl furan, and (*E*,*E*)-2,4-nonadienal, monitored during the solid-state fermentation of green and red lentils, were found to be significantly correlated with microbial counts and pH [47]. Lentils subjected to solid-state fermentation with *Pediococcus acidilactici* showed a more complex volatile compound profile than lentils subjected to submerged fermentation with the same strain [108]. Compared to non-fermented lentils, hexanal was markedly reduced by submerged fermentation, while it remained almost unchanged with solid-state fermentation. Nonanal did not change with either fermentation type. Benzaldehyde increased more with solid-state than with submerged fermentation.

Kryachko et al. [109] focused on microorganisms able to produce cyclodextrins (described in Section 5.5). The authors proposed a two-stage process starting with acid treatment to allow for volatile compounds to be released by legume proteins, followed by washing and redissolving the protein precipitate, fermentation with cyclodextrin-producing microorganism(s), and the removal of the precipitated cyclodextrin complexes with flavor compounds. Alternatively, enzymes such as cyclodextrin glycosyl transferases (CGTases) can be used to remove off-flavors [110]. These treatments have not been applied to lentil ingredients; therefore, studies should be conducted with this purpose.

4.5.8. Breeding for low LOX and lipids

Possible strategies to mitigate the beany flavor of lentil and lentil-based products could involve the genetic selection and utilization of varieties with low contents of the volatile compound precursors responsible for off-flavors and/or low enzymatic activity. In fact, the lipid content and fatty acid composition are significantly influenced by the variety [111]. Furthermore, lentil is known to have high LOX activity, which is quite variable among different cultivars [112]. It has also been observed that tannin extract from the lentil seed coat counteracts the lipid peroxidation catalyzed by lipoxygenase and β-carotene, suggesting an increase in the phenolic compounds in lentil [113]. However, the antinutritional effect of tannins is a major drawback.

Genome editing with Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9 (CRISPR-Cas9) technology has been recently applied to edit LOX-related genes in soybean [114] and pea [115]. The developed soybean lines were LOX-free and still await further evaluation. The pea lines showed a significant reduction in LOX activity and, in turn, in the concentrations of key volatiles, such as hexanal, 2-hexenal, heptanal, (*E*)-2-heptenal, 1-octen-3-ol, octanal, (*E*)-2-octenal (*E*,*E*)-2,4-nonadienal, and 2-pentyl furan. Modulating enzyme activity may be a better strategy, since LOX plays a role in plant physiology; hence, its absence may affect agronomic characteristics.

No studies aimed at lowering the LOX activity of lentil or its lipid content have been conducted so far. Future research activities could more thoroughly study the effect of lentil composition on the development of volatile compounds in food and, consequently, set up specific molecular breeding programs for lentils for improving their traits, such as beany flavors.

4.6. Improvement of communication strategies

Lentils are popular in Asia, the Middle East, and in Mediterranean countries, but are much less commonly consumed in Western countries, especially the US and Canada

[116,117]. Barriers to the consumption of lentils in Canada have been attributed, in most cases, to a lack of knowledge on recipes to make them palatable, ultimately resulting in cultural non-acceptance [117]. It should be noted that "beany" is considered as an "atypical" and unusual flavor note by consumers unfamiliar with lentils, but is less disliked when people are more familiar with pulses. In addition, millennials, more sensitive to environmental issues and climate change, may be more open to appreciate legume-based foods that are sustainable compared to those of animal origins. Lentils snacks were found to be appreciated by Spanish millennials, with an liking score of 7.0 over a 9-point hedonic scale [118].

Although plant-based foods (including lentil-based foods) specifically designed to mimic their conventional counterparts (milk and meat analogs) are an emerging trend, the sensory perception of these products remains a major challenge to be addressed. In fact, while consumers may expect these products to lack the "foreign" sensory notes associated with legumes and to perfectly mimic other sensory characteristics, achieving this perfect imitation often requires the addition of various additives and ingredients, potentially reducing acceptance by consumers seeking "clean label" foods. However, new horizons for the food industry are opening up thanks to the application of modern tools such as artificial intelligence, which is useful for formulating original food products suited to individual tastes and discovering the best flavor combinations while optimizing consumer acceptance and taking into account nutritional goals and economic aspects [119].

In addition, as we are experiencing the rapid development of these product categories, perhaps the time is ripe for plant-based foods to be communicated for their identity and sensory characteristics, without presenting them only as an imitation of other foods. The promotion of nutritional education, incentive strategies such as free tasting, and appropriate communication when launching new food products containing legumes, highlighting their nutritional benefits [120], could positively influence consumer acceptability and, at the same time, make their sensory properties more familiar. Therefore, it can be hypothesized that future consumer trends may focus more on the specific identity and sensory characteristics of lentil-based foods, broadening consumer awareness, promoting consumption, and depowering the importance of de-flavoring.

4.7. Conclusions and future perspectives

Lentil-based products are already being marketed and consumed, and food companies are investing in the development of new fortified formulations for market launch. However, the sensory impact of such foods should be carefully evaluated and modulated, as it is responsible for the success of these products.

This review provided a detailed analysis of the volatile compounds of lentil-based ingredients and strategies for mitigating their associated off-flavors. Assuming that the appropriate storage and processing are conducted, so as to prevent or limit undesired oxidative phenomena, several treatments are available: thermal, non-thermal, and biotechnological, all able to reduce the beany flavor. However, the literature review showed that lentil is less studied than other legumes, so more research should be conducted, because varietal differences in responses to various de-flavoring treatments occur, so it is not possible to directly translate the results obtained with other legumes to lentil. Indeed, innovative technologies with great potential, such as high-pressure processing (HPP) and the use of adsorbents, have not been explored in detail or are still totally unexplored in lentil.

Further research should also be carried out to better understand the economic implications of these various de-flavoring treatments. Conducting comprehensive assessments on the costs and benefits of different techniques is essential, considering their impacts on final pricing and industrial scalability.

In parallel, to prevent the formation of off-flavors, instead of eliminating them, the development of lentil varieties with a low LOX and lipid content, as is currently underway for soybean and pea, would significantly reduce the volatile compounds forming from lipid oxidation.

Finally, as the field of innovative lentil-based foods is growing rapidly, a question arises: will increased consumer awareness of the nutritional benefits and greater knowledge of the inherent sensory characteristics of lentil-based foods reduce the importance of de-flavoring in the future?

4.8 References

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Chapter 5. The use of durum wheat oil in the preparation of *focaccia*: Effects on the oxidative stability, physical and sensorial properties

This chapter has been previously published as: **Vurro, F.**; Summo, C.; Squeo, G.; Caponio, F.; Pasqualone, A. The use of durum wheat oil in the preparation of *Focaccia*: effects on the oxidative stability and physical and sensorial properties. *Foods* **2022**, *11*(17), 2679. **https://doi.org/10.3390/foods11172679**

The use of durum wheat oil in the preparation of *focaccia*: Effects on the oxidative stability, physical and sensorial properties

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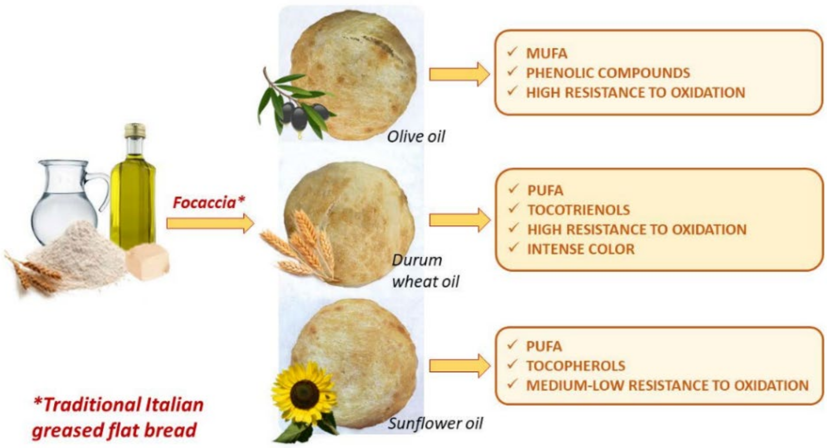
Graphical Abstract



Abstract

Durum wheat oil is an innovative oil which could be considered the "second life" of durum wheat milling by-products. This study proposed to use this oil in the reformulation of a traditional Italian greased flat bread, namely *focaccia*, whose typical sensorial features are due to the presence of relevant amounts of oil in its formulation. The chemical, physical and sensorial features of *focaccia* with durum wheat oil (DWO) were compared with those of *focaccia* prepared with olive oil (OO) and sunflower oil (SO). The results showed the prevalence of polyunsaturated fatty acids in DWO, followed by SO. DWO was more resistant to oxidation than SO (induction time 86.2 and 66.3 min, respectively), due to its higher content of tocotrienols (1020 and 70.2 mg/kg in DWO and SO, respectively), but was less resistant than OO, richer in monounsaturated fatty acids, and containing phenolic compounds. The volatile oxidation markers, namely hexanal and nonanal, were less present in OO and DWO than in SO. Texture and color were positively influenced by the use of durum wheat oil, allowing the nutritional improvement of this flat bread in a sustainable and circular key.

Keywords: flat bread; durum wheat oil; acidic composition; tocotrienols; tocopherols; volatile compounds; texture profile analysis; sensorial properties

5.1 Introduction

Bread is a traditional staple food consumed by people worldwide, with hundreds of different examples [1]. Among them, the flat breads are the oldest, still very popular due to their high versatility, organoleptic properties, and convenience [2,3]. These reasons justify the relevant growth of their market, especially in relation to the modern style of life and the new eating preferences [4].

Italy has a long tradition of garnished flat breads, some of which are renowned also abroad, such as pizza. *Focaccia* is another typical garnished flat bread widely consumed in several Italian regions, where it originated [5]. *Focaccia* is oven-baked into a low pan and, prior to cooking, is topped with fresh tomato and oregano, or onions and potatoes, or cheese, or salt and rosemary, etc. giving a myriad of nuanced different types [6]. This old and traditional food product, which has been included in the list of Italian Traditional Agri-Food Products (TAP) [7] is similar to pizza, but has some distinct characteristics [2,5]. The consumption patterns of *focaccia* and pizza are different: the first is a quick snack at any time [5], while the second is usually preferred for dinner or lunch (except for the "*pizza a portafoglio*", the typical street food of Naples) [8]. The difference between pizza and *focaccia*, however, is not limited to the consumption pattern, because also the formulation, appearance, and sensory characteristics are different. The preparation of pizza and *focaccia* starts in the same way, by kneading flour, water, yeast and salt. Then, only in the preparation of *focaccia*, abundant oil is incorporated into the dough and poured on its surface to confer the typical greasiness [2,5]. Instead, in the preparation of the "Traditional Specialty Guaranteed" (TSG) Neapolitan pizza, only a very little amount of oil is used [9]. Specifically, only extra virgin olive oil can be used in the TAP labeled *focaccia* and TSG labeled pizza, which are high quality niche products. However, most of the commonly marketed *focaccia* and pizza, which are not labeled TAP or TSG, contain olive oil or sunflower oil.

Pizza has been the object of several investigations aimed at improving its nutritional features [10-13], without neglecting the gluten-free versions [14,15], but very few studies are available on *focaccia*. The reformulation of *focaccia* using *Apulian black chickpea* flour, which provides anthocyanins and increases the antioxidant activity, was investigated by Pasqualone et al. [12], while Delcuratolo et al. [16,17] evaluated the role of *focaccia* topping on the oxidation stability and content of polar compounds arising from triacylglycerol oxidation and hydrolysis, responsible for negative implications for health [18]. However, a single study investigated the use of fat replacers to reduce the oil content of *focaccia* [19], and no studies compared the effect of different vegetable oils on its nutritional and sensory characteristics.

Italy is not only famous for pizza and *focaccia*, but also for pasta and special baked goods made of durum wheat semolina [20-22]. However, the milling process to obtain semolina involves the production of by-products (bran, germ and various middlings) [23], which should be upcycled and reintroduced into the food system to comply with the principles of circular economy [24,25]. These by-products have a proven potential for oil extraction [25]. A previous study has evaluated the effect of using durum wheat oil in the preparation of biscuits [26], whose long shelf life can be affected by rancidity onset. The substitution of sunflower oil with durum wheat oil significantly increased the resistance of biscuits to oxidation due to the richness of durum wheat oil in tocols, especially tocotrienols [26].

In this historical moment, the war in Ukraine is causing problems for the supply of sunflower oil [27], the fourth most consumed oil in the world [28]. Moreover, since 2013, the "silent war" of *Xylella fastidiosa* has been changing the Italian landscape, causing a decrease in the production of olive oil [29]. Therefore, alternatives to these largely consumed oils should be considered. The use of durum wheat oil in a traditional product as *focaccia* could valorize the entire durum wheat supply chain and, at the same time, could offer producers and consumers an alternative to the currently used oils.

Therefore, the aim of this study was to evaluate the effect of durum wheat oil on the oxidation stability and physical-sensory characteristics of *focaccia*, in comparison with olive oil and sunflower oil.

5.2 Materials and Methods

5.2.1. Materials

Durum wheat oil, prepared as reported in Squeo et al. [25], was provided by Casillo Next Food Srl (Corato, Italy). Wheat flour type 0 (Casillo Spa, Corato, Italy) (carbohydrate 72 g/100 g; proteins 11 g/100 g; fat 2 g/100 g; fiber 2 g/100 g), sunflower oil (Olearia De Santis, Bitonto, Italy), olive oil (Olearia De Santis, Bitonto, Italy), yeast (*Saccharomyces cerevisiae,* Mulino Caputo, Naples, Italy), and sea salt (Atisale Spa, Margherita di Savoia, Italy) were purchased from local retailers.

5.2.2. Sample preparation

Three different types of *focaccias* were prepared: i) *focaccia* with sunflower oil (SO); ii) *focaccia* with olive oil (OO); iii) *focaccia* with durum wheat oil (DWO), according to the formulation reported in Table 1. The *focaccia* samples were prepared as described in Pasqualone et al. [12]. Flour, water, and yeast were kneaded for 6 min by using a spiral kneader (Bosh MFQ40304, München, Germany). Then, salt and oil (70% of the total oil amount) were added, and kneading was continued for 6 min. The first fermentation was carried out for 1 h and 30 min in controlled conditions at 35 °C, RH = 20% (Memmert proofer, EN.CO. Srl, Spinea, Italy). The leavened dough was divided into portions, which were manually shaped as discs having 1.5 cm thickness and about 30 cm diameter. The discs were put into metal pans, previously greased with oil (10% of the total oil amount), and left to rise again in the same conditions. *Focaccia* surface was then greased by pouring oil (20% of the total oil amount), followed baking in an electric oven (Oem Ali Group Srl, Bozzolo, Italy) at 200 °C for 25 min.

Table 1. Formulation of the experimental *focaccia* samples. SO = *focaccia* with sunflower oil; OO = *focaccia* with olive oil; DWO = *focaccia* with durum wheat oil.

Ingredients	SO (g)	OO (g)	DWO (g)
Wheat flour type 0	600	600	600
Water	420	420	420
Sunflower oil	85	-	-
Olive oil	-	85	-
Durum wheat oil	-	-	85
Salt	15	15	15
Yeast	5	5	5

5.2.3 Determination of the resistance to oxidation

RapidOxy oxidation stability tester (Anton Paar, Blankenfelde-Mahlow, Germany) was used, as described in AOCS Method Cd 12c-16 [30]. Two g of sample (*focaccia,* finely crushed, or oil) were oxidized at a temperature of 140 °C with an oxygen pressure of 700 kPa until pressure decreased by 10%. The samples were tested in triplicate.

5.2.4 Determination of fatty acids composition

The fatty acid composition of oils used in the *focaccia* preparation was analyzed as described by Squeo et al. [25]. A gas-chromatograph (mod. 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with an FID detector (set at 220 ◦C) and an SP2340 capillary column of 60 m × 0.25 mm × 0.2 mm film thickness (Supelco Park, Bellefonte, PA, USA) was used to separate the fatty acid methyl esters. The comparison with the retention time of the standard mixture (C_4-C_{24}) (Sigma-Aldrich, St. Louis, MO, USA) was used for the identification of each fatty acid present in the sample. The analyses were carried out in triplicate.

5.2.5 Lipid extraction

The lipid fraction of *focaccia* was extracted by the Soxhlet apparatus (SER 148 extraction system, Velp Scientifica Srl, Usmate, Italy). The solvent used for the extraction was diethyl ether (Carlo Erba, Milan, Italy).

5.2.6 Determination of tocopherols and tocotrienols

The tocopherols and tocotrienols of oils and of the lipid fraction extracted were determined by HPLC (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA. USA). Primarily, 0.02-0.03 g of sample were dissolved in 1 mL of 2-propanol. The samples were filtered by a 0.45 µm polytetrafluoroethylene (PTFE) filter and injected into an HPLC system consisting of a Waters 600E quaternary pump (Milford, MA, USA), a 7725i Rheodyne injector (20-µL sample loop) and a fluorescent detector (excitation wavelength 292 nm, emission wavelength 330 nm). The stationary phase was an Acclaim™ 120 Å C18 column, with a particle size of 3 µm, and 3×150 mm in length (Thermo Fisher Scientific, Waltham, MA, USA); the mobile phase was 96:4 (v/v) methanol and water at a flow rate of 1 mL/min. The software used was Chromeleon (Thermo Fisher Scientific, Waltham, MA, USA). The single tocol was determined by the external standard method based on a previously set calibration curve. The content of tocopherols and tocotrienols was expressed as mg/kg of the total weight of oil. The analyses were carried out in triplicate.

5.2.7 Determination of polar compounds of the lipid fraction of focaccia

The polar compounds of the oil extracted by *focaccia* samples were separated by silica gel column chromatography and quantified using high-performance size-exclusion chromatography (HPSEC) according to Difonzo et al. [31]. The content of polar compounds was expressed as g/100 g of oil extracted. The analyses were carried out in triplicate.

5.2.8 Determination of antioxidant activity

The extraction of antioxidant compounds was conducted as described by Troilo et al. [32], with the only changes in the ratio of sample and extraction solvent and the number of washes, from 1:5 and two respectively.
The sample extracts were submitted to the radical scavenging assays using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to Difonzo et al. [33]. The absorbance was read using a Cary 60 spectrophotometer (Cernusco, Milan, Italy). The results were expressed as μmol Trolox equivalents (TE)/g. The determinations were carried out in triplicate.

5.2.9 Determination of volatile compounds

Volatile compounds of the *focaccias* were analyzed by headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography/mass spectrometry (GC–MS) as described by Difonzo et al. [31]. The identification of the volatile compounds was carried out using an LRI and by computer matching with the reference mass spectra of the National Institute of Standards and Technology (NIST) and Wiley libraries. The quantification of the volatile compounds was done considering the standardization of the respective peak areas with the peak area of the 1-propanol, used as internal standard. The results were expressed as μg/g of sample. The analyses were carried out in triplicate.

5.2.10 Texture profile analysis

Texture profile analysis (TPA) was executed as described in Pasqualone et al. (2019) [12], with the only modification of the use of a cylindrical probe (36 mm diameter). The following parameters were calculated from the TPA graphic: hardness (N), chewiness (N), cohesiveness, and springiness. Six replications were carried out.

5.2.11 Color determination

The color of *focaccia* (crumb and crust) was measured using the CM-600d colorimeter (Konica Minolta, Tokyo, Japan) supported by SpectraMagic NX software (Konica Minolta, Tokyo, Japan). The color properties were determined in the CIE (International Commission on Illumination) color space. Lightness (L^*, from black to white), red index (a^*, from green to red) and yellow index (b^*, from blue to yellow) were determined**.** The total color difference (ΔE) was calculated as follows:

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

where L^*_0, a^*_0, and b^*_0 were the color coordinates of *focaccia* with olive oil (OO), while $L*$, $b*$, and $a*$ were the color coordinates of the other *focaccia* samples. The calculation considered the mean values. The following ΔE scale was considered for the evaluation of the results: 0–0.5 = not relevant difference; 0.5–1.5 = a slight difference 1.5–3.0 = difference recognizable by an experienced observer; 3.0–6.0 = an appreciable difference; 6.0–12.0 = a large difference; and >12.0 = very evident difference [14]

Nine replications were carried out.

5.2.12 Determination of dimensional parameters

The diameter (D) and thickness (T) of *focaccia* before and after baking were determined as described in Pasqualone et al. [12]. A caliper was used. The percentage variation due to baking was calculated as follows:

$$\text{\% of variation of D (or T)} = \frac{[\text{D (or T) after baking} - \text{D (or T) before baking}]}{\text{D (or T) before baking}} \times 100$$

The analyses were carried out in triplicate.

5.2.13 Determination of sensory properties

The quantitative descriptive analysis (QDA) of *focaccia* was made according to the International Standardization Organization (ISO) standard 13299 [34] by a trained panel of eight members, previously selected for their reliability, consistency, and discriminating ability. The panel was composed of four men and four women, ranging in age from 23 to 55 years who expressed written consent, according to the ethical guidelines of the laboratory of Food Science and Technology of the Department of Soil, Plant and Food Science of the University of Bari (Italy). The panelists were regular consumers of baked products and had no food allergies or intolerances. A pre-test session was made, as outlined in the ISO Standard 11132 [35]. A total of 12 descriptors were selected considering the *focaccia* samples with different oil: 3 descriptors for visual appearance (surface color intensity, inner color intensity, crumb porosity); 3 descriptors for the odor (*focaccia* odor, roasted odor, oxidized odor); 3 for texture perceived during the tasting (crumb elasticity, softness, crumb moisture); 3 for taste (saltiness, sweetness, greasiness). The intensity of every attribute was expressed on a 10 cm unstructured linear scale (contractual units - c.u.). The scale anchors for *focaccia* odor, roasted odor, oxidized odor, saltiness, sweetness, and greasiness were: 0 c.u. = minimum intensity; 10 c.u. = maximum intensity. The scale anchors for surface and inner color intensity were: 0 c.u. = ivory; 10 c.u. = brown. The scale anchors for the crumb porosity were: 0 c.u. = dense structure with very few pores; 10 c.u. = open structure, very porous. The scale anchors for crumb moisture were: 0 c.u. = dry; 10 c.u. = wet. The scale anchors for crumb softness were: 0 c.u. = very hard; 10 c.u. = very soft. The scale anchors for crumb elasticity were: 0 c.u. rigid =; 10 c.u. = very elastic.

The samples were randomized and presented to the panelists in white dishes marked with alphanumeric codes. The testing was performed at ambient room temperature (20 ± 2°C). In accordance with the ISO 8589 [36] standard, the sensory analysis was carried out by physically separating panelists during analysis. Three replicates were carried out.

5.2.14 Statistical Analysis

Statistical analysis was carried out by Minitab Statistical Software (Minitab Inc., State College, PA, USA). The results were all expressed as mean ± standard deviation (SD). The Anderson-Darling test was applied to evaluate the normal distribution of the data and the Levene test was used to evaluate the homoscedasticity of variances. The significant differences (α= 0.05) were verified through the application of the parametric one-way analysis of variance (ANOVA), followed by the Tukey HSD test, considering the type of oil as independent variable.

5.3 Results and Discussion

5.3.1. Oxidation stability, fatty acid composition and tocols content

Lipid oxidation is a negative event affecting many food products, particularly when their fat content is relevant and the processing or storage conditions are favourable to degradative reactions. Oxidative events cause a change in taste, texture and appearance, as well as the production of toxic compounds and the loss of nutritional value [26].

RapidOxy oxidation stability tester was used to evaluate the effect of varying the oil type on the oxidative stability of *focaccia* samples. This instrument, which does not need solvents, forces the pro-oxidising conditions and measures the induction time (IT) of the lipid fraction, which is known to be positively related to the resistance to oxidation [37]. OO *focaccia* showed the highest IT, followed by DWO and SO (Table 2).

Table 2. Resistance to forced oxidation of the oils and *focaccia*. SO = *focaccia* with sunflower oil; OO = *focaccia* with olive oil; DWO = *focaccia* with durum wheat oil.

Sample	Induction Time (min)
Focaccia	
OO	134 ± 2.06^{a}
SO	66.3 ± 4.81^{c}
DWO	86.2 ± 2.53^{b}
Oils	
Olive oil	59.5 ± 0.07^{a}
Sunflower oil	30.7 ± 0.64^{c}
Durum wheat oil	39.8 ± 0.26^{b}

Data are presented as means ± SD of three replicates. Different letters for the same sample type indicate significant differences at $p < 0.05$.

This trend mirrored the differences observed for the oils which, in turn, could be explained in terms of different fatty acid composition and content of antioxidant compounds.

Polyunsaturated fatty acids (PUFAs) were the most abundant class in durum wheat oil and sunflower oil (Table 3). Particularly notable was the content of linolenic acid (n-3 PUFA) observed in durum wheat oil, accounting for 5.06%±0.03, while in sunflower oil the content of linolenic acid was significantly lower (0.90%±0.01). This difference is relevant because studies suggest that n-3 PUFAs reduce the risk of inflammatory and cardiovascular diseases, steatohepatitis, obesity and diabetes [38]. Although the concentration of linolenic acid in durum wheat oil was lower than in the typical sources, such as flaxseeds, it was higher than the values reported for the majority of commonly used oils, such as, besides sunflower oil, olive oil, corn oil and palm oil [39-41].

Monounsaturated fatty acids (MUFAs) were the most represented fatty acids in olive oil, which contained an amount of oleic acid accounting for 71.0%±0.11. The saturated fatty acids (SFAs) were significantly more abundant in durum wheat oil and olive oil than in sunflower oil.

Being PUFAs the most susceptible to oxidation, the observed fatty acid composition easily explains the highest resistance to oxidation of olive oil, which had the lowest content of PUFAs. Moreover, the higher resistance to oxidation observed in durum wheat oil compared to sunflower oil, though the former had a slightly higher PUFA content, could be attributable to its higher content of SFAs, as well as to the greater presence of antioxidant compounds, primarily tocols [25].

Table 3. Percentage of the main fatty acids in the olive oil, sunflower oil and durum wheat oil used in the preparation of *focaccia* samples.

Fatty Acids (%)	Olive oil	Durum wheat oil	Sunflower oil
$C_{14:0}$	0.02±0.00^{c}	0.07±0.00^{b}	0.08±0.00^{a}
$C_{16:0}$	12.9±0.06^{b}	14.84±0.04^{a}	6.48±0.01^{c}
$C_{18:0}$	3.24±0.04^{b}	1.38±0.01^{c}	3.82±0.01^{a}
$C_{18:1}$	71.0±0.11^{a}	22.2±0.05^{c}	30.3±0.00^{b}
$C_{18:2}$	9.42±0.08^{c}	55.1±0.05^{b}	57.5±0.01^{a}
$C_{18:3}$	0.90±0.01^{b}	5.06±0.03^{a}	0.23±0.01^{c}
∑SFA	16.9±0.03^{b}	17.0±0.00^{a}	10.8±0.01^{c}
∑MUFA	72.5±0.11^{a}	22.6±0.06^{c}	30.5±0.00^{b}
∑PUFA	10.6±0.08^{c}	60.5±0.06^{a}	58.7±0.01^{b}

∑SFA, Sum of saturated fatty acids; ∑MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids. Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

Tocotrienols and tocopherols are recognized as natural antioxidants typical of vegetable oils and are used as additives by the food industries to cope with the low oxidative stability of PUFAs [38]. The concentrations of tocols ascertained in the oils are shown in Figure 1A, while those of the lipid fraction extracted from the *focaccia* samples are reported in Figure 1B. Durum wheat oil and DWO *focaccia* were the richest in tocotrienols, while tocopherols were more present in sunflower oil and in the corresponding *focaccia* (SO). This difference was interesting because studies have suggested that tocotrienols exert a greater antioxidant action than tocopherols and have more relevant health benefits [42,43]. Durum wheat oil had 1094 mg/kg of tocotrienols, significantly higher than the amount determined in sunflower oil and olive oil, where these antioxidants were very low or absent. Other authors reported similar findings in sunflower and olive oil [44] and observed that wheat flour gives only a minimal contribution to the content of tocotrienols of baked goods [45].

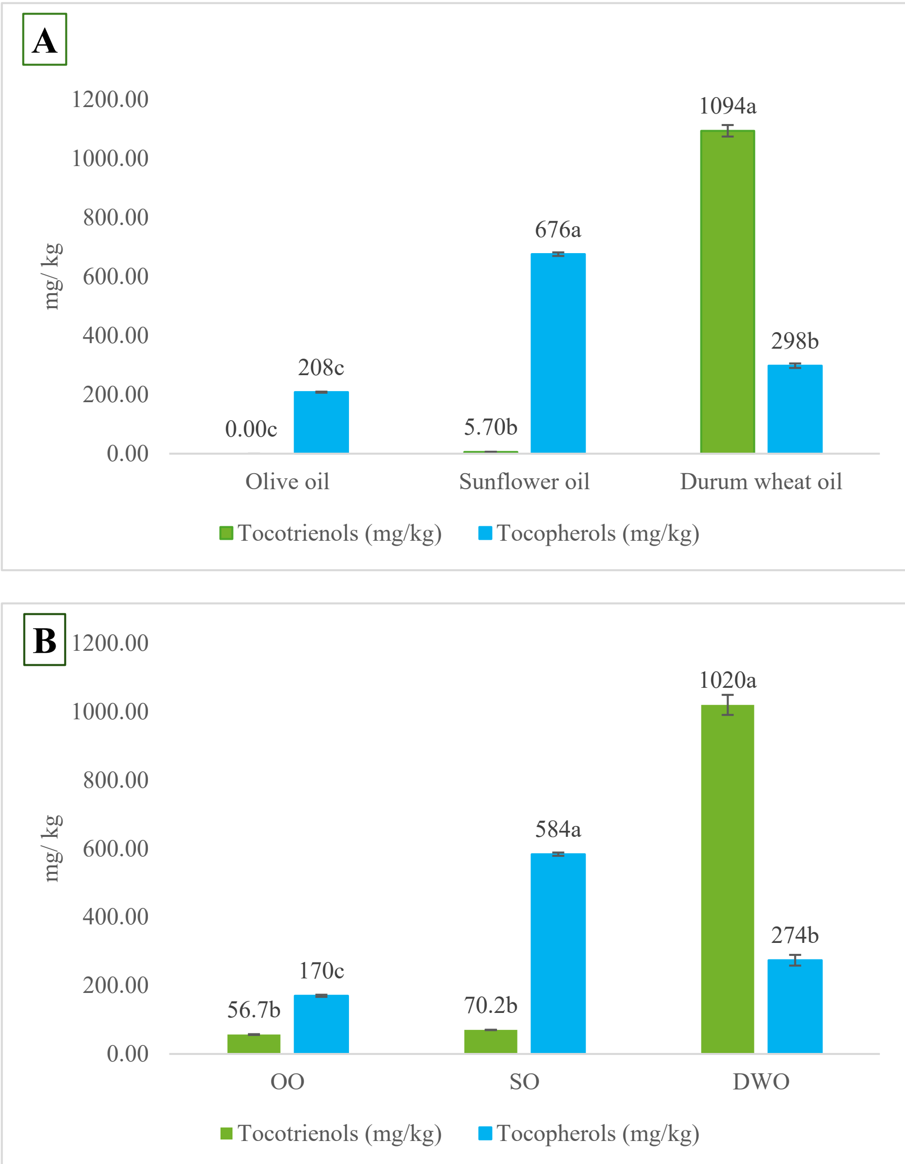


Figure 1. Tocopherols and tocotrienols content (mg/kg) of: oil (**A**) and *focaccia* SO = focaccia with sunflower oil; OO = *focaccia* with olive oil; DWO = *focaccia* with durum wheat oil (**B**). Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

5.3.2 Polar compounds content

The oxidative reactions that affect the lipid fraction of food determine the formation of compounds characterized by higher polarity than the unaltered triacylglycerols. In particular, the oxidized triacylglycerols (ox-TAG) are composed of triacylglycerols with an oxidized fatty acyl group, while the triacylglycerol oligopolymers (TAGP) are obtained from the latter with bonds that generate complex molecules, such as dimers and polymers. Finally, diacylglycerols (DAG), monoacylglycerols (MAG), and free fatty acids (FFA) arise from the hydrolysis of the triacylglycerols, as a result of lipolytic enzyme activity and moisture [18,46].

Recently, Chen et al. [18] compared the polar compounds of peanut, rapeseed, soybean, and linseed oils in different cooking conditions, observing that the unsaturated fatty acids can lead to a high level of polar compounds. SO *focaccia*, indeed, was richer in TAGP and ox-TAG (Figure 2) than OO and DWO *focaccia*. These results were strongly associated with the oxidation stability, therefore the MUFA-rich oils and/or the antioxidant-rich oils are able to limit the production of potentially adverse compounds [46].

DWO *focaccia* had a significantly higher content of DAG than SO and OO, probably due to lipolytic events affecting the raw materials used for the extraction of oil. The milling industry should consider specific containment measures for these events [25], in spite of the fact that authors have shown the health benefits of DAG, especially on body weight [47]. DAG also have a function in the food industry due to their emulsifying properties [26,48,49].

The presence of polar compounds in *focaccia* was previously reported. Delcuratolo et al. [16] studied the role of different toppings on the content of polar compounds, considering only the use of extra virgin olive oil. The type of *focaccia* topping influenced the exposition to thermal stress: potato-topped *focaccia*, which was moister than *focaccia* topped with onion and rosemary, was characterized by a less intense lipid degradation. Our study, on the other hand, highlights that the type of oil influences the concentration of polar compounds in the final products. Our trials were made in the absence of toppings to avoid many interferences. However, the optimal combination of topping and oil type could allow to dramatically limit the content of polar compounds in *focaccia*, thus avoiding consumer exposure to them.

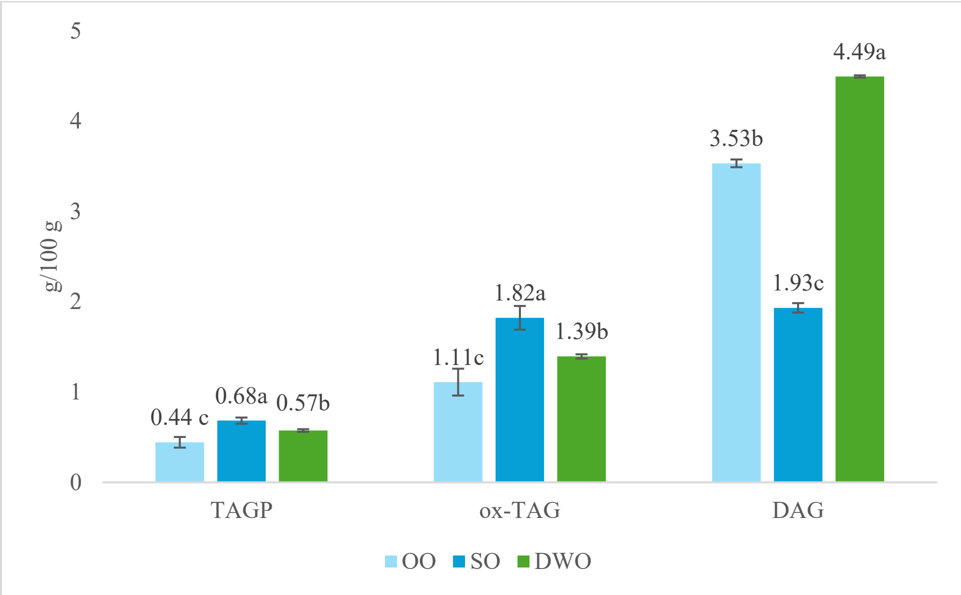


Figure 2. Polar compounds content (g/100 g of extracted fat) of *focaccia*. SO = *focaccia* with sunflower oil; OO = *focaccia* with olive oil; DWO = *focaccia* with durum wheat oil; TAGP = triacylglycerol oligopolymers; ox-TAG = oxidized triacylglycerols; DAG = diacylglycerols. Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

5.3.3 Antioxidant activity

ABTS and DPPH assays are based on the change of color of the sample extract, in connection with the capacity of an antioxidant to reduce a colored oxidant. The antioxidants derive from the sample, while the oxidants are in the solution that is prepared for the assay [50]. With both these assays, DWO and OO *focaccia* showed a higher antioxidant activity than SO (Table 4). These findings reflected the high content of bioactive compounds of the durum wheat and olive oils, namely tocols in durum wheat oil and phenolic compounds in olive oil (accounting for 81.5±3.15 mg GAE/kg oil – data not shown). Also, other studies [51] compared wheat germ oil (from *T. aestivum*), sunflower oil and olive oil. The latter, however, was selected to have a high-phenolic olive oil, accounting for 320 mg GAE/kg oil, which is remarkably high considered that the refining process reduces these compounds [52]. The authors detected the highest antioxidant activity in the high-phenolic olive oil, followed by wheat germ oil and, then, sunflower oil [51].

Table 4. Total antioxidant capacity of experimental *focaccia*. OO =*focaccia* prepared with olive oil; DWO = *focaccia* prepared with durum wheat oil; SO = *focaccia* prepared with sunflower oil.

Focaccia type	ABTS (µmol TE/g)	DPPH (µmol TE/g)
OO	0.64 ± 0.03^{ab}	0.46 ± 0.01^{ab}
DWO	0.70 ± 0.04^{a}	0.55 ± 0.02^{a}
SO	0.56 ± 0.04^{b}	0.38 ± 0.03^{b}

Expressed as µmol/g Trolox equivalent. Data are presented as means ± SD of three replicates. Different letters in the same column indicate significant differences at $p < 0.05$.

5.3.4 Volatile Compounds

Bread is characterized by over 540 volatile compounds [53]: alcohols, aldehydes, esters, ketones, acids, pyrazines, furans, and sulfur compounds [54], although only a small part of them really influences the flavor profile [53]. Different volatile compounds may have a different origin. Microorganisms ferment the sugars and produce ethanol, which is partly lost during baking, while some of them take part in secondary fermentation events, which lead to short-chain alcohols and fatty acids, esters and carbonyl compounds [53,54]. The oxidation of lipids causes the production of aldehydes, such as hexanal, nonanal, octanal, heptanal, and 2-heptenal. The typical baking flavor is due to the Maillard reaction involving amino acids and sugars. The caramelization of sugars and thermal degradations of sugars and amino acids form furans, acetic acid, acetaldehyde and other compounds [54].

In the current study, the type of oil used in the preparation of *focaccia* significantly influenced the volatile profile (Table 5). Hexanal and nonanal, markers of lipid oxidation, were significantly higher in SO, followed by DW and OO, mirroring the other chemical determinations. Also, 2-methylbutanal was different among the different oils: the SO and DWO were richer than OO, while the content of 3-methylbutanal was higher in DWO, followed by SO and OO. These compounds, due to the Maillard reaction [55], positively influence the aroma of crust, conferring a malty and roast odor [54]. Several authors described the effect of the fatty acid composition on the intensity of the Maillard reaction and have found that its development is favored by higher unsaturation level [56].

The Maillard reaction generated also benzaldehyde and furans; the content of the first was significantly lower in OO, while the latter were significantly higher in DWO. This result could be attributed to the simultaneous presence, in DWO, of high concentrations of PUFA and diglycerides, which positively influence the presence of furans, as observed by Emektar et al. [57]. Pyrazines, with their olfactory properties, confer a pleasant roasted odor to the baked goods [54,58], and are therefore used as additives to improve the organoleptic properties of bread and other bakery products [58]. DWO was significantly richer of pyrazines than SO and OO. These findings could be connected to the different acidic composition of the oils used, in particular to the PUFA content, which was the highest in durum wheat oil, followed by sunflower oil, then olive oil. In support of this, Negroni et al. [59], studying the formation of pyrazines in glucose-lysine or xylose-lysine model systems added of olive oil, canola oil and sunflower oil, suggested that higher unsaturation levels could lead to a higher presence of pyrazines.

The fermentation of *focaccia*, conducted by compressed yeast (*Saccharomyces cerevisiae*), produced ethyl alcohol. Despite its partial evaporation during baking, ethyl alcohol contributes to the aroma of baked goods [60], but its concentration was not influenced by the type of oil.

Table 5. Volatile compounds of experimental *focaccia*. OO = *focaccia* prepared with olive oil; DWO = *focaccia* prepared with durum wheat oil; SO = *focaccia* prepared with sunflower oil.

Volatile compounds (μg/g)	***Focaccia* type**		
	OO	**DWO**	**SO**
Aldehydes			
Hexanal	15.7±0.01^{c}	22.2±0.0^{b}	25.8±1.07^{a}
Heptanal	1.00±0.08^{b}	1.85±0.02^{a}	2.01±0.24^{a}
Nonanal	4.91±0.33^{b}	4.83±0.15^{b}	7.20±0.01^{a}
2-Methylbutanal	12.0±0.45^{c}	17.7±0.64^{b}	20.5±0.31^{a}
3-Methylbutanal	16.3±0.63^{c}	25.5±0.66^{a}	22.7±0.08^{b}
Octanal	1.35±0.02^{ab}	0.87±0.16^{b}	1.71±0.12^{a}
2-Heptenal	5.00±0.01^{b}	4.63±0.11^{c}	9.75±0.15^{a}
2,4-Heptadienal	0.78±0.06^{c}	1.56±0.08^{b}	3.28±0.11^{a}
Benzacetaldheyde	2.57±0.04^{b}	4.58±0.09^{a}	1.83±0.08^{c}
Benzaldehyde	6.18±0.55^{b}	7.55±0.36^{a}	7.28±0.18^{a}
Alcohols			
Ethyl alcohol	2.29±0.32^{a}	2.10±0.46^{a}	2.67±0.22^{a}
2-Phenylethanol	8.54±0.16^{a}	4.73±0.12^{c}	7.57±0.23^{b}
1-Hexanol	6.14±0.00^{b}	2.82±0.13^{c}	10.8±0.08^{a}
Carboxylic acid			
Acetic acid	2.95±0.00^{a}	1.41±0.09^{c}	1.73±0.05^{b}
Ketones			
Methyl ethyl ketone	1.72±0.28^{a}	1.73±0.17^{a}	1.48±0.04^{a}
Furan compounds			
2-Furanmethanol	1.28±0.25^{c}	9.67±0.90^{a}	6.59±0.27^{b}
Furan-2-pentyl	2.25±0.26^{c}	4.80±0.35^{a}	3.83±0.09^{b}
2-Furancarboxaldehyde, 5-methyl-	0.50±0.10^{c}	1.44±0.07^{a}	0.63±0.04^{b}
2-Furancarboxaldehyde (furfural)	1.46±0.01^{c}	5.32±0.06^{a}	5.15±0.06^{b}
Pyrazines			
Methyl-pyrazine	2.72±0.11^{c}	10.4±0.82^{a}	8.20±0.85^{b}
Ethyl-pyrazine	1.42±0.12^{c}	2.66±0.07^{a}	2.42±0.02^{b}

Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

5.3.5 Physical determinations

Ingredients and processing, especially baking, are the principal responsible for the color of baked products: the golden-brown color of the crust is considered an important quality parameter [61]. Table 6 reports the colorimetric indices of *focaccia* prepared with different oils, shown in Figure 3. DWO crumb and crust were significantly less luminous (lower $L*$) and showed higher $a*$ (more intense red tone) than SO and OO, while no significant differences were observed for $b*$ (yellow index). These results agreed with data of volatile compounds. In fact, higher levels of pyrazines and furans, both arising from thermal reactions which cause browning, were observed in DWO than in the other two *focaccia* types. These observations were reinforced by the calculation of total color differences (ΔE) of crust and crumb, with OO taken as reference. The color differences of the crumb were lower than those observed in the crust, being the latter more exposed to heat and, then, more interested by non-enzymatic browning. In particular, the difference in color between OO and DWO crumb were considered recognizable only by an experienced observer ($1.5<\Delta E<3.0$). Instead, the difference in crust color was considered clearly recognizable ($3.0<\Delta E<6.0$). Other authors, studying bread, have reported an effect of the type of oil on the color of crumb and crust [62,63].

Table 6. Physical determinations (color, texture and dimensional variations during baking) of the experimental *focaccia* samples. OO = *focaccia* prepared with olive oil; DWO = *focaccia* prepared with durum wheat oil; SO = *focaccia* prepared with sunflower oil.

	***Focaccia* type**		
	OO	**DWO**	**SO**
Color			
Crumb			
$a*$	$0.40±0.08^b$	$0.87±0.11^a$	$0.67±0.08^a$
$b*$	$18.2±0.31^a$	$18.99±0.46^a$	$21.0±0.38^a$
$L*$	$72.8±1.37^{ab}$	$71.15±1.58^b$	$74.5±0.13^a$
ΔE	-	1.88	3.28
Crust			
$a*$	$7.15±0.34^b$	$10.1±1.77^a$	$9.31±0.66^{ab}$
$b*$	$32.0±1.14^a$	$33.4±2.02^a$	$33.2±2.39^a$
$L*$	$67.5±0.82^a$	$62.7±1.89^b$	$64.7±1.99^{ab}$
ΔE	-	5.82	3.71
Texture			
Hardness (N)	$7.69±1.06^b$	$8.67±1.13^b$	$12.1±1.13^a$
Springiness	$0.94±0.01^a$	$0.94±0.02^a$	$0.95±0.01^a$
Chewiness (N)	$5.73±0.84^b$	$6.39± 0.44^b$	$9.80±0.48^a$
Cohesiveness	$0.79±0.01^a$	$0.82±0.07^a$	$0.82±0.01^a$
Dimensional variations during baking			
Diameter variation (%)	$-0.73±0.01^a$	$-0.73±0.01^a$	$-0.75±0.01^a$
Thickness variation (%)	$117±9.91^a$	$110±11.7^a$	$118±8.08^a$

Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

Texture profile analysis (TPA) consisted of compressing a food sample twice, in a reciprocating motion that mimics the action of the jaw [64]. Four parameters were measured: hardness, springiness, chewiness and cohesiveness. The springiness and cohesiveness were very similar in all *focaccia* types, while hardness and chewiness showed significant differences among samples (Table 6). In particular, the use of durum wheat oil and olive oil were related to a lower hardness and chewiness than sunflower oil. This result could be related to the content of DAG, which was higher in these two oils than in the sunflower one. DAG, indeed, together with monoglycerides, are extensively used in breadmaking as emulsifiers, to improve crumb softness. In addition, their presence can delay the firming process, due to the ability to form complexes with amylose and amylopectin [49,65].

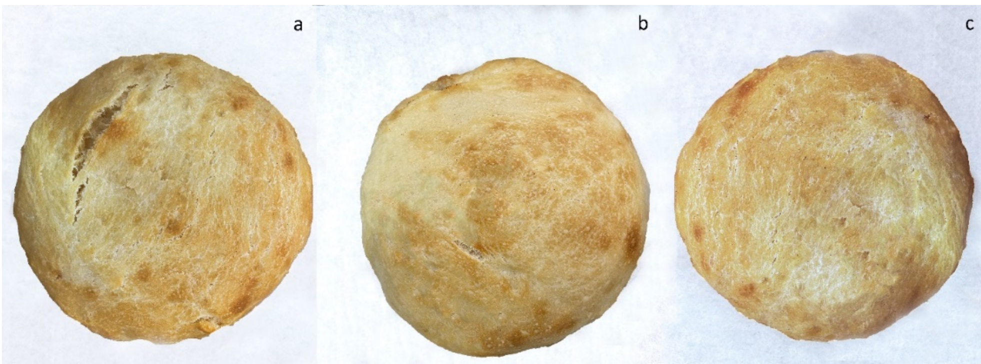


Figure 3. (a) *Focaccia* with olive oil; **(b)** *focaccia* with durum wheat oil; **(c)** *focaccia* with sunflower oil.

During baking, an increase in volume occurs due to the thermal expansion of gases [12]. As a consequence, the thickness of *focaccia* increased with baking, at a similar extent in all samples (Table 6). Meanwhile, the diameter decreased, but without an influence exerted by the type of oil. The effect of oil on the variation of dimensional parameters during baking, therefore, was secondary, while other works reported a significant effect by the type of flour, due to different fiber and gluten content [12].

5.3.6 The sensory profile of focaccia

The type of oil significantly influenced also the sensory properties of *focaccia* (Table 7).

Table 7. Sensory profile of experimental *focaccia*. OO = *focaccia* prepared with olive oil; DWO = *focaccia* prepared with durum wheat oil; SO = *focaccia* prepared with sunflower oil.

	***Focaccia* type**		
Sensory descriptor	**OO**	**DWO**	**SO**
Surface color	3.80±0.35 [b]	5.55±0.28 [a]	4.22±0.30 [b]
Inner color	0.58±0.12 [b]	0.85±0.05 [a]	0.75±0.00 [a]
Crumb porosity	4.27±0.35 [b]	5.53±0.12 [a]	3.57±0.25 [b]
Focaccia odor	6.50±0.05 [b]	7.67±0.25 [a]	6.75±0.05 [b]
Oxidized odor	0.00±0.00 [b]	0.00±0.00 [b]	0.63±0.10 [a]
Roasted odor	1.15±0.15 [b]	1.67±0.22 [a]	1.13±0.15 [b]
Crumb elasticity	5.38±0.06 [a]	5.17±0.38 [a]	5.60±0.22 [a]
Softness	6.18±0.29 [b]	7.08±0.08 [a]	5.77±0.08 [b]
Crumb moisture	5.55±0.05 [a]	5.55±0.79 [a]	5.48±0.08 [a]
Greasiness	6.07±0.19 [a]	5.12±0.43 [b]	5.93±0.19 [a]
Sweetness	1.15±0.13 [a]	1.22±0.28 [a]	1.42±0.08 [a]
Saltiness	5.03±0.20 [a]	4.37±0.25 [b]	4.90±0.09 [a]

Data are presented as means ± SD of three replicates. Different letters in the same row indicate significant differences at $p < 0.05$.

The perception of crumb and crust color varied with the type of oil, with DWO darker than the others. The sensory evaluation of color agreed with the instrumental determination. Moreover, DWO was perceived as softer and more porous than the other *focaccia* types, while the elasticity of crumb was similar in all samples.

The type of oil did not affect the perception of sweetness and crumb moisture, while the panelists perceived DWO to be less salty and oily, which is interesting, considering the preference of consumers for a *focacci*a not excessively greased [12]. A hint of oxidized odor was detected only in SO, while it was not observed in DWO and OO. Roasted odor, and the typical odor of *focaccia*, were perceived significantly more intense in DWO, due to its higher content of pyrazines.

5.4 Conclusions

Considering the significant nutritional, sensory, and health importance of the lipid fraction of *focaccia,* this work has suggested that the choice of oil to be used is not trivial. Although olive oil, rich in MUFAs, has proved to be the most resistant to oxidation, durum wheat oil, rich in PUFAs and tocols, was more stable than sunflower oil thanks to the greater presence of antioxidants. Moreover, the use of durum wheat oil determined a positive effect on the physical and sensory characteristics of the end-product. Therefore, the reformulation of bakery products with this oil will increase the value of the by-products generated by the durum wheat milling industries, while respecting the principles of the circular economy. This oil could offer a healthier alternative to the consumers, while combining the tradition of *focaccia*-making with a viable strategy for product innovation, and at the same time increasing the sustainability of durum wheat chain.

Durum wheat oil could also respond to the need of finding new alternatives to sunflower oil, the supply of which is facing considerable difficulties due to the war in Ukraine. It should be considered, however, that durum wheat oil is a high quality niche product with a relatively high price (5.00 €/kg, compared to 2.50-3.00 €/kg for olive oil and 1.50-2.00 €/kg for sunflower oil). Its price is justified by the high nutritional value related to the remarkably high concentration of tocols, especially tocotrienols, and interesting levels of n-3 PUFAs. Currently, there is a single producer of durum wheat oil, with a productive capacity of 4000 tons/year. Therefore, there is still not enough durum wheat oil to make up for potential losses in olive and sunflower oil, but there is good development potential because other companies will probably start producing it in the future.

Future investigations, however, are needed to deepen the knowledge of the effect of this oil during shelf-life and to widen its application in the food sector and beyond. In particular, the performance of durum wheat oil during *focaccia* storage should be studied, setting up shelf-life studies in comparison with other refined oils. Furthermore, durum wheat oil could also find interesting applications in pharmaceuticals, nutraceuticals, and in the cosmetic sector, which could represent the main destinations, alongside the food one, to valorize the cereal by-products.

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Part III

Conclusions

This doctoral dissertation focused on the oldest known type of bread and, arguably, one of the oldest foods produced in the world, flat breads. Accordingly, the project is embedded within the broader Flat Bread Mine international project, which involves a partnership of nine countries in the Mediterranean basin. The two principal aims of the project were 1) the valorization and 2) the innovation of flat breads, to preserve and enhance these traditional and current food products, which are a staple food for many countries, focusing on innovative reinterpretations, to improve their nutritional profile.

First of all, a “mine” of information has been established, collecting data on ingredients, recipes, and traditional production methods of the flat breads produced and consumed in the Mediterranean area and collecting them in an online available database. This approach has made it possible to acquire a detailed knowledge of the organoleptic, nutritional and technological characteristics of flat breads. In addition, the Flatbread Mine database is “open”, so it can be updated and extended to other countries. Afterwards, the analysis focused on Italian flat breads through a comprehensive review of the existing literature. This review made it possible to study regional types, their specific characteristics and traditional production methods, highlighting the cultural and technical diversity of the Italian flat bread heritage. This approach made it possible to identify the innovation potential that could be applied to the Italian traditional products while respecting their local characteristics.

In order to fully valorize flat breads, it is essential to regularly evaluate their diversity, using the approach adopted in this study.

The activities related to the valorization of Mediterranean and Italian flat breads have highlighted the opportunities for improvement and innovation, optimising formulations by introducing alternative ingredients. In this context, among the different types of flat bread identified, *focaccia* was chosen as a reference product for innovation. This choice was motivated by its sensory characteristics, its versatility in formulation and its roots in Italian tradition. In addition, *focaccia* lends itself particularly well to formulation changes.

Thanks to this analysis, it was possible to identify new ingredients including legume flours, additional proteins and innovative oils. They not only improved the overall quality of the breads, but also contributed to a better understanding of the production processes and application potential.

The addition of legume flours to the formulations was chosen for their consistent protein and fiber content, which significantly improved the nutritional profile of cereal-based foods. The addition of legume flours also allows the enrichment of the *focaccia* with essential amino acids and micronutrients, offering an innovative solution to increase the nutritional value compared to common cereal flours.

Fortifying *focaccia* with pea flour and pea sourdough has shown an improvement of the nutritional profile. The typical composition is high in lipids and carbohydrates, but low in protein and fiber. *Focaccias* fortified with pea flour in native and fermented versions could be labelled as "source of protein" and "high fiber" according to EC Regulation No. 1924/06. Fermentation contributed to an increase in total phenolic content, which increased *in vitro* antioxidant activity. Fermentation also reduced anti-nutritional factors and affected the digestibility of proteins and starch. The pea flour and the pea sourdough enriched the volatile profile of the *focaccias*, increasing the content of some compounds, such as aldehydes, alcohols and Maillard reaction compounds. Pea flour is a minimally processed and locally available ingredient that can be used either as is or after spontaneous fermentation.

The pea proteins obtained by dry-fractionation were included in the formulation of a gluten-free *focaccia*, in combination with rice and corn flour, and studied how they influenced the properties of the dough and the final product, using the simplex-centroid mixture design for evaluation. Overall, pea proteins influenced the dough properties, crumb porosity, color, texture and sensory characteristics. The optimal formulation was selected, by observing the contour plots, resulting in "source of protein", "source of fiber" and "low-fat" according to EC Regulation No. 1924/06. It was formulated with 5 g/100 g pea protein, 20 g/100 g corn flour and 20 g/ 100 g of rice flour.

Both studies showed the influence of legumes on the sensory characteristics and volatile profile of the enriched *focaccias*. However, legume odor perception can alter the acceptability of products, reducing also the potential intent of purchase.

Given the identified impact on odor resulting from the use of legume flours, a specific review of literature was carried out on the volatile compounds associated with lentils, selecting this legume due to its appreciation and the limited number of studies on this topic. This in-depth study provided a comprehensive analysis of the volatile compounds in lentil-based ingredients and, above all, underlined the main strategies to mitigate their associated off-flavors. Provided proper storage and processing practices are followed to prevent or limit undesirable oxidative phenomena, several treatments are available - thermal, non-thermal and biotechnological - all of which are effective in reducing beany flavors.

A significant ingredient in the formulation of *focaccia* is the oil, unlike other types of flat breads. It significantly impacts the nutritional properties of the final product. Therefore, the study of the oil fraction can improve the lipid profile of *focaccia*, increasing the thermal stability and contributing to a more nutritionally balanced product.

The choice of oil is a critical factor in the recipes. While olive oil, rich in MUFAs, showed the highest resistance to oxidation, durum wheat oil, rich in PUFAs and tocols, proved to be more stable than sunflower oil due to its higher antioxidant content. In addition, the use of durum wheat oil had a positive effect on the physical and sensory properties. Reformulating bakery products with this oil not only improves the product but also adds value to the by-products of durum wheat milling, in line with the principles of the circular economy.

Moreover, starting from the present PhD project further studies could investigate the use of other legumes with different compositions, physicochemical and sensory quality, evaluating the effects on the nutritional benefits, volatile profile, effects on digestibility and sensory acceptability.

In this perspective, also other types of lipid fractions could be tested, assessing their impact in the *focaccia* formulation, to identify further alternatives that could improve the quality of the product and promote the sustainability of the ingredients used.

In the wide panorama of flat breads, other types could be considered, constituting a tool for knowledge, valorization and innovatively proposing them. Thus, flat breads once again prove to be ancient foods but also foods of the future.

Acknowledgments

Al termine di questo percorso, il mio dottorato può essere paragonato alla crescita di una pianta: le radici hanno conferito stabilità e assorbito preziose conoscenze, consentendone lo sviluppo; le foglie, catturando la luce, hanno sostenuto la fotosintesi e favorito la mia crescita professionale e personale in questi tre anni.

Esprimo la mia sincera gratitudine ai miei tutor, che con umanità e competenza scientifica sono stati per me una guida preziosa e un costante esempio.

Un ringraziamento particolare alla professoressa Antonella Pasqualone e al professor Carmine Summo, per la fiducia, il supporto e i numerosi insegnamenti che hanno arricchito la mia esperienza: la loro passione ha rappresentato per me uno stimolo continuo a crescere, imparare e migliorare sotto ogni aspetto.

Riconosco con gratitudine il contributo dei docenti e ricercatori della sezione di Tecnologie Alimentari, per il loro continuo supporto, la condivisione di esperienze e l'impegno verso una formazione di qualità.

Un pensiero speciale va alla professoressa Cristina Molina Rosell, tutor durante il mio periodo di ricerca presso l'Instituto de Agroquimica y Tecnologia de Alimentos (IATA) a Valencia, per l'attenta supervisione e la disponibilità, e al team, in particolare Raquel Garzon e Maria Santamaria, che mi hanno accolta e accompagnata durante i mesi trascorsi nel laboratorio 109.

Ringrazio, inoltre, tutti i professori, ricercatori e dottorandi conosciuti nell'ambito del progetto Flat Bread Mine, con i quali, giorno dopo giorno, abbiamo costruito e visto crescere questo progetto.

Ringrazio profondamente i miei colleghi e amici per il tempo condiviso tra studio e ricerca e per i tanti momenti di supporto reciproco, confronto e crescita.

Un ringraziamento speciale va alla mia famiglia: ai miei genitori e a mio fratello, per la pazienza, l'amore incondizionato e il sostegno instancabile nei momenti di maggiore impegno.

Infine, grazie a tutte le persone che hanno attraversato questi anni contribuendo, con la loro presenza, alla mia crescita e alla costruzione di conoscenze. Grazie a chi, parente o amico, è stato parte integrante e insostituibile di questo percorso, essenziale in questo prezioso tratto di vita.

This thesis is supported by the PRIMA program under grant agreement No. 2031, project Flat Bread of Mediterranean area: INnovation & Emerging process & technology (Flat Bread Mine). The PRIMA program is an Art.185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation. The results and content found on this paper reflects only the author's view. The PRIMA Foundation is not responsible for any use that may be made of the information it contains.