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Valorization of agri-food wastes for feed and food production

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Abstract

Increasing attention to food safety and sustainability issues created significant challenges for the food industry. Consumer tastes, which are increasingly aware of the link between diet, health, and the environment, now significantly impact the quality of meat and meat products, which is no longer determined only by objective standards that attest to their edibility. It therefore becomes imperative to create new strategies to enhance the health benefits of meat and related products while maintaining a high level of environmental compliance. This Ph.D. project focused on optimizing pork meat's nutritional profile, oxidative stability, and technological properties while promoting sustainable farming through dietary modifications. The first strategy addressed was to confirm that dietary intervention is a strong tool for obtaining healthier meat in terms of fatty acid composition. The study confirmed that including extruded flaxseed, with a favorable n-6/n-3 polyunsaturated fatty acid (PUFA) ratio, in pig diets enhanced the meat's lipid profile by increasing n-3 PUFAs and optimizing the n-6/n-3 ratio without compromising technological properties or oxidative stability. Since pork is particularly susceptible to lipid oxidation, additional strategies were developed to stabilize meat products replacing the most widely used synthetic antioxidants, enhancing their nutritional and technological qualities, and improving sustainability. For this reason, the research approach focused on integrating agro-industrial by-products, rich in phenolic and bioactive compounds, into meat products and animal feed. For the first approach, two trials were conducted using by-products as food ingredients. The first trial evaluated the effects of hazelnut skin and dry tomato peel in pork burgers over seven days of refrigerated storage. Three formulations were analyzed: a control and two experimental versions with 2.5% hazelnut skin or dry tomato peel. Hazelnut skin demonstrated strong antioxidant properties, reducing oxidation during storage and cooking while preserving color. Though lacking antioxidant power, dry tomato peel contributed to nutritional enrichment, particularly in fiber content and fatty acid composition. Encouraged by the outcomes of the first trial, which underscored the strong antioxidant capabilities of hazelnut skin, a subsequent study was conducted to assess the potential of incorporating a green polyphenolic extract from hazelnut skin, developed by the University of Turin. Two experimental formulations were tested: one with 2.5% hazelnut skin and another with 1% polyphenolic extract, alongside a control. Both the hazelnut skin and extract exhibited antioxidant properties, improving lipid oxidation stability and color preservation confirming that the phenolic compounds in this matrix are bioactive. However, some aspects, such as color acceptability and tenderness, require further optimization. The second approach involved incorporating hazelnut skin and its extract into pig feed to assess their effects on live performance and meat quality. An in vivo trial was conducted with three diets: a control, one supplemented with 0.6% hazelnut skin, and another with 0.1% polyphenolic extract. The *longissimus thoracis* muscle and subcutaneous fat were analyzed for chemical composition, physical properties, and oxidative stability. A refrigerated storage test was also performed. While dietary interventions did not yield significant overall changes, subtle benefits were observed. These included increased thigh yield and reduced cooking losses in the extract-fed group, as well as improved oxidative stability and a lower

n-6/n-3 ratio in the skin-fed group. Importantly, no negative effects on growth performance, carcass traits, or meat quality were detected, highlighting the commercial potential of hazelnut by-products. This Ph.D. research underscores the potential of hazelnut by-products as innovative tools to improve the nutritional and technological quality of pork meat, as well as the sustainability of the entire pork supply chain. Their use represents a real opportunity to enhance agro-industrial resources, reduce waste, and meet the growing demand for healthier and more sustainable products.

Chapter 1. Introduction

1. Overview

Food industry waste and meat quality represent two critical issues in today's context, marked by a growing focus on sustainability and food safety. On the one hand, managing agro-food by-products and waste poses significant challenges in reducing environmental impact and promoting a circular economy. On the other hand, ensuring or improving the quality of meat and processed products is essential to meet consumer demands and comply with increasingly stringent standards for health and nutritional value.

1.1. Meat quality

It is not easy to find a definition of meat quality in the literature. Certainly, this is because this definition depends on numerous intrinsic and extrinsic factors that can be objective when we consider quality from a 'suitability for storage and consumption' point of view (Taheri-Garavand et al., 2019) and subjective if we instead talk about meat quality from the point of view of the consumer making ethical and economic nutritional choices (Grunert et al., 2004). In general, meat has physical, biological, and chemical characteristics (Huang et al., 2014). Specifically, the main quality characteristics assessed from a technological perspective include color, texture, pH, tenderness, and freshness (Nache et al., 2016; Sun et al., 2016; Tao et al., 2012; Wei et al., 2015). As just stated, however, the consumer is now an active participant in the decision-making process on what the defining characteristics of meat quality should be, and in recent years, the most important aspect on which their views and concerns are focusing is the nutritional characteristics of meat (Teixeira & Rodrigues, 2021).

Meat and meat derivatives are foods of high nutritional value due to their considerable protein content (20-35%), which includes all essential amino acids in balanced proportions. In addition, they are rich in fat-soluble vitamins (A, D, E, K), B vitamins such as B6 and B12, n-3 series polyunsaturated fatty acids (PUFA), as well as important minerals such as highly bioavailable iron, selenium and zinc (Mourouti et al., 2015) and, especially in ruminant meat, an appreciable level of conjugated linolenic acid (CLA) (Chin et al., 1992). Meat consumption has traditionally been linked to good health, but it has been linked to a negative health image in recent years. Consuming less meat, especially red and processed meat, is advised to lower the risk of metabolic syndrome and cancer (Biesalski, 2005; WCRF/AIRC, 2009).

However, due to rapid economic development, meat consumption in Europe remained relatively constant over the past decade (Figure 1).

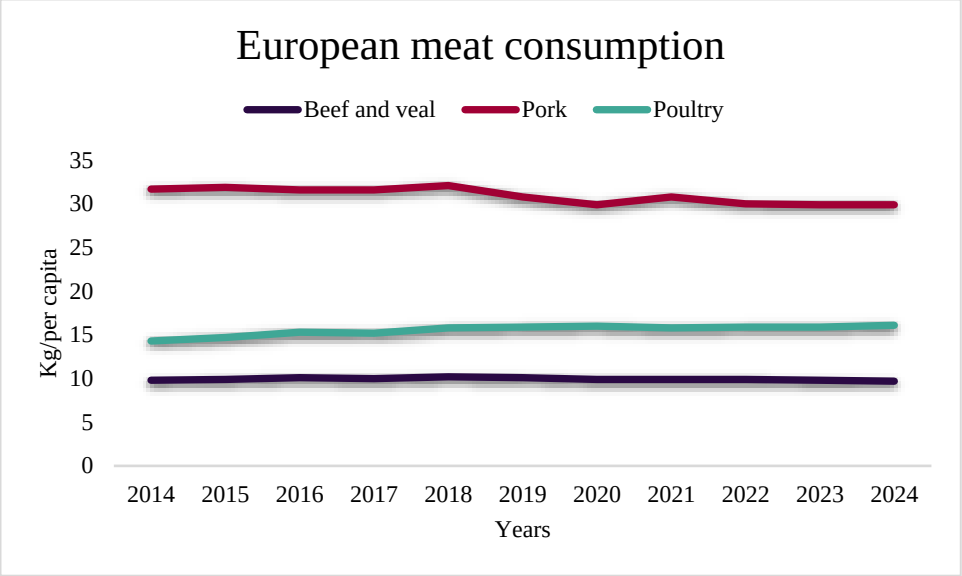

Figure 1. European meat consumption between 2014 and 2024 (Organisation for Economic Co-operation and Development, OECD, 2023).

Therefore, strategies are needed to improve the quality of meat and meat products by making them healthier for consumers. It is precisely these consumers who are at the center of numerous and ongoing questions about their perception of the healthiness of meat (Di Vita et al., 2019; Plasek et al., 2020; Shan et al., 2017a; Shan et al., 2017b). It is, therefore, essential to develop new production and processing strategies that can improve the health properties of meat and its derivatives.

Concerning the latter, the strategies used to improve the quality of meat products are mainly based on improving their composition by incorporating certain bioactive components, reducing the amounts of exogenous additives and harmful indigenous compounds formed. In particular, nutritional enrichment with natural bioactive plant compounds such as antioxidants and dietary fibres (Hu & Yu, 2015; Montalvo-González et al., 2018; Pateiro et al., 2018) or probiotics (Cavalheiro et al., 2015) and the reduction of harmful components such as salt, nitrates/nitrites, and N-nitrosamines (Fellendorf et al., 2018; Jeong et al., 2020; Zhou et al., 2020).

As regards unprocessed meat, traditional methods most commonly used include genetic and nutritional interventions aimed at modifying carcass and tissue composition, for example by reducing fat deposits. A significant case in point is the pig sector, where techniques have been implemented to reduce the fat content of carcasses.

In Italian heavy pig production, in particular, backfat thickness has been reduced by about 50% over the years (Figure 2).

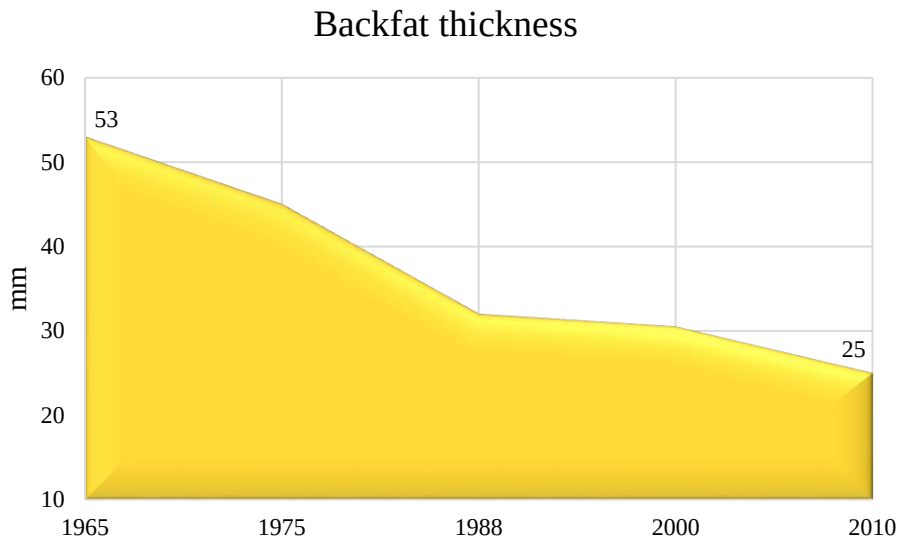

Figure 2. Backfat thickness variation in Italian heavy pigs between 1965 and 2010 (Lo Fiego et al., 2018).

Particularly concerning fat, the fatty acid composition of the fatty deposits of meat is of crucial importance, as the ratio of n-3 to n-6 polyunsaturated fatty acids (PUFAs) appears to significantly influence human health. Numerous studies show that a diet rich in n-3 PUFAs and characterized by a low n-6/n-3 PUFA ratio offers greater health benefits (O'Connell et al., 2017; Lee et al., 2018). However, the typical Western diet has a PUFA n-6/n-3 ratio of between 15:1 and 20:1 (Simopoulos, 2006; Kobayashi et al., 2006), compared to a recommended value of between 1:1 and 4:1 (Simopoulos, 2002). In this context, the content of specific fatty acids and the ratio between different fatty acid classes are used to calculate lipid nutritional indices, tools commonly used to assess the nutritional quality and healthiness of the lipid profile of meat and other foods. Therefore, monitoring, analyzing, and optimizing the fatty acid profile in meat and meat products is essential to ensure quality control and promote positive effects on human health. In pigs, as in other monogastric animals, it is possible to change the composition of lipid fatty acids by changing the dietary source of lipids (Belmonte et al., 2021; Minelli et al., 2020).

1.2. Food waste

Food waste is a topic of growing global concern, as a significant amount of food, potentially suitable for human consumption, is wasted along the food supply chain. This phenomenon is not only a resource management issue but also has economic, environmental, and moral implications. The recognition of food waste as a critical issue emerged in the 1990s, as highlighted by studies like Kroyer (1995). In recent years, various definitions of food waste have been adopted to better describe the phenomenon and its implications. The Food and Agriculture Organization (FAO), for example, distinguishes between food losses, that occur involuntarily during the stages of harvesting, post-

harvest handling, processing, and distribution, and food waste, which mainly occurs at the retail and consumption stages (FAO, 2011).

Global production capacity has seen a significant increase in recent decades, surpassing the growth of the human population. Currently, enough food is produced to feed 10 billion people, a figure that corresponds to the projected global population for 2050 (Holt-Giménez et al., 2012). However, it is estimated that about one-third of the food produced is lost or wasted along supply chains (FAO, 2011). The food sector encompasses numerous processing industries that transform raw materials, primarily agricultural, into value-added products. However, the fruit and vegetable industry, which is the most productive globally, generates significant losses of biomass suitable for human and animal consumption, partly due to the high volumes of production. According to the FAO, food losses and waste in this sector account for about 60% of total production (Sagar et al., 2018), with losses ranging from 3% to 50% of the total raw materials processed. This inefficiency has dramatic consequences: economic losses estimated at approximately one trillion dollars per year; consumption of one-quarter of the world's agricultural water resources; and use of an agricultural area equivalent to the size of China (Santagata et al., 2021). Considering the increasingly widespread global environmental concerns and resource scarcity, the food industry is becoming increasingly aware of the importance of sustainable practices and waste reduction. Despite the warnings scientists have been issuing for years about the effects of our lifestyles on the environment and climate, we have so far been unable to plan and implement the necessary changes. This is primarily because it requires a comprehensive rethinking of our food production-distribution-consumption model. This is why, in recent years, attention to food waste has increased, as demonstrated by the rise in scientific publications on the topic. Figure 3, based on data from the Scopus databases, shows a growing trend since 2014, with a significant increase in studies related to food waste compared to previous years.

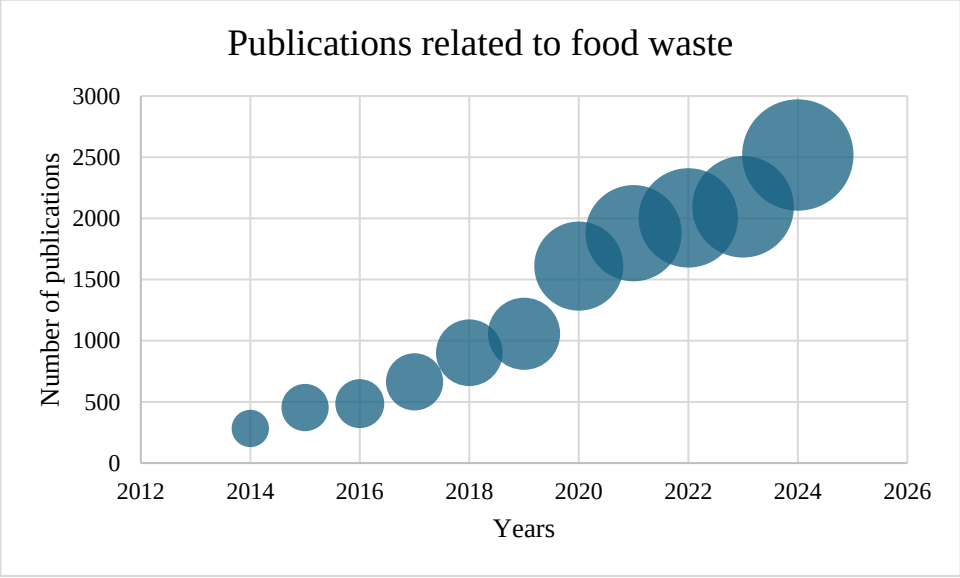

Figure 3. Number of publications related to food waste between 2014 and 2024 (Scopus databases, 2025).

In Europe, the reduction of food waste is at the core of numerous political and regulatory initiatives. These efforts are guided by an integrated approach to promote environmental and economic sustainability by preventing food losses at all stages of the product life cycle. This focus aligns with global commitments made under the United Nations' 2030 Agenda for Sustainable Development, which includes Goal 12.3: to halve per capita food waste and reduce food losses along production and supply chains.
Over the years, several policy actions have been adopted, including:

- Waste Framework Directive (2008/98/EC) (DIRECTIVE, 2008): Established a hierarchy of priority actions for waste management, placing prevention at the top, followed by reuse, recycling, and, as a last resort, disposal.
- Integrated Product Policy (EC, 2003): Promoted more efficient resource management throughout the entire life cycle of products.
- Resource Efficiency Flagship Initiative (EC, 2011): Emphasized the importance of optimizing the use of natural resources, including waste prevention.
- Bioeconomy Strategy Communication (EC, 2012): Highlighted the role of the bioeconomy in addressing challenges related to food waste, fostering innovation, and sustainable development.
- European Green Deal (EC, 2019): A reform program spanning various areas, including agriculture, with proposed changes and support mechanisms to enable the ecological transition. The main strategy suggested is "Farm-to-Fork," which includes specific measures to monitor and reduce waste along the entire food supply chain; encourage the adoption of sustainable practices in the agricultural, industrial, and distribution sectors; and promote consumer awareness and engagement in reducing waste.
- Proposal for Revision of the Waste Directive (EC, 2023): The European Commission has proposed binding national targets for reducing food waste by 2030, including a 10% reduction in food production and processing, and a 30% reduction in retail, food service, and households.

These policies are complemented by principles of industrial ecology, such as the cradle-to-cradle concept and the circular economy model, which aim for a "zero waste" society. In this context, waste is no longer considered a discard but a resource for new products and applications. A practical example is industrial symbiosis, where residues from one sector become productive inputs for another, creating closed and sustainable systems (Mirabella et al., 2014). The environmental impact of the food production system has been at the center of public debate for several years, highlighting the urgency of making this food supply chain more environmentally sustainable. However, despite the growing concern, a survey of Norwegian consumers by Austgulen et al. (2018) concluded that consumers may not yet be ready to make food choices based on what is best for the climate or the

environment. Therefore, greater sustainability must come primarily from food production systems and their design.

Current approaches within the circular economy primarily rely on the butterfly diagram developed by the Ellen MacArthur Foundation. However, achieving a fully closed and continuous cycle presents a complex economic challenge (Brown & Buranakarn, 2003). To address this difficulty, Velenturf et al. (2019) propose an integrated model that links the production and consumption system with the biophysical environment and the fundamental principles of the circular economy. This model emphasizes the importance of incorporating avoidable waste and industrial materials into the production system.

Industrial materials, at the end of their useful life, are redesigned to ensure they do not negatively impact the biophysical environment, while those that do not require modification are directly reintegrated into the cycle. The goal is to create a system where products are designed to minimize or eliminate waste generation. This approach fosters a production culture in which products are conceived to have a second useful life, with added value and a significant reduction in energy consumption and the use of natural resources (Jurgilevich et al., 2016).

Countries such as Germany, France, and Italy, in the face of established regulations by Europe, have promoted government initiatives for the use of food waste deemed unfit for human consumption in feed production and composting (Teigiserova et al., 2020). Considering the goal to be achieved and the approach to be used, in the field of food production, the recovery, valorization, and utilization of agro-food by-products for several large and diverse uses appears to be an appropriate and successful strategy, as well as necessary.

1.3. Green strategy

The conception and development of strategies defined as green to meet the technological challenges facing the food industry and at the same time consider the exploitation of the world's resources and the waste that accumulates along the food production and processing chain is a necessary action. Agro-food by-products present an effective solution to the challenges outlined above. In particular, they appear to fulfill well the task of implementing the quality of meat and its processed products or to keep it unchanged by replacing components with a greater environmental and anti-nutritional impact while managing to make the food chain more sustainable by complying with the directives and indications given on the ecological transition and the bioeconomy.

1.3.1. By-products

Every year, the global agro-food industry generates over 190 million tons of by-products (Kumari et al., 2018). These by-products are produced at every stage of the food production chain, from agricultural cultivation to processing and distribution. They mainly consist of waste materials such as seeds, peels, stems, leaves, and residual pulp from fruits and other plants. Managing, recycling, or disposing of these materials is of critical importance. However, many of these by-products can be economically valorized depending on their characteristics and quantities (Cecilia et al., 2019). This,

in turn, creates new economic opportunities, helping to enhance environmental and social benefits for all stakeholders, from producers to supply chain operators and consumers (Salim et al., 2017).
So far, most agro-food waste has been used as a source of fuel, livestock feed, or organic fertilizer. Today, with the availability of modern technologies and the principles of "green chemistry," new concepts have been developed that lead to the effective use of agro-food wastes and by-products to produce value-added products (Ben-Othman et al., 2020).
This study focuses on plant-based by-products. In general, this type of by-product includes a significant percentage of inedible parts, such as seeds, shells, flowers, and roots in some cases, which can account for 10-50% of the total weight on average (Calderón-Oliver & López-Hernández, 2022).

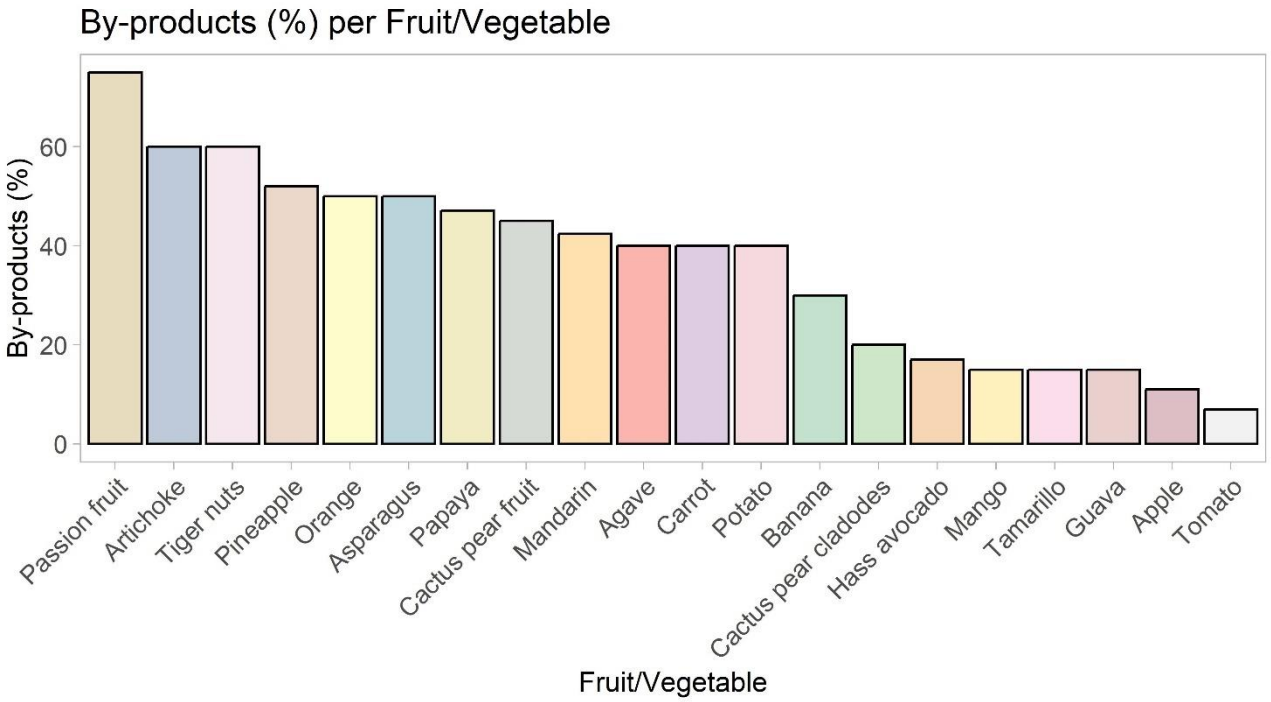

Figure 4. The average percentage of by-products generated during the processing of different fruits and vegetables. Left to right: Schieber et al. (2001), Llorach et al. (2002), Sánchez-Zapata et al. (2009), Ayala-Zavala et al. (2010), Raj et al. (2016), Rodríguez et al. (2006), Ayala-Zavala et al. (2010), Bensadon et al. (2010), Ayala-Zavala et al. (2010), Iñiguez-Covarrubiaset al. (2001), Schieber et al. (2001), Schieber et al. (2001), Schieber et al. (2001), Bensadon et al. (2010), Calderón-Oliver et al. (2016), Ayala-Zavala et al. (2010), Suárez-Montenegro et al. (2021), Schieber et al. (2001), Ayala-Zavala et al. (2010), Schieber et al. (2001).

Beyond the ecological and economic advantages, recovering by-products is crucial because they are a source of compounds with high technological and nutritional value, such as fibers, proteins, fatty acids, natural pigments, and phenolic compounds, essential in the food industry (Table 1).

Table 1. Examples of the main biomolecules contained in agri-food by-products

Biomolecules	Matrix	References
Total dietary fiber	Apple pomace	Dhillon et al. (2013)
	Tomato pomace	Navarro-Gonzálezet al. (2011)
	Citrus peel	Pacheco et al. (2019)
	Mango peel	Baddi et al. (2018)
	Legume seed coatings	Sęczyk et al. (2017)
Cellulose	Banana stems Maize straw Onion and oat peel	Ahmad Khorairi et al. (2023)
Hemicellulose	Grapefruit waste Chokeberry waste Cherry waste Citrus peel Blackcurrant pomace	Nawirska & Kwaśniewska (2005)
Pectin	Orange and lemon peel Grape skin	Venkatanagaraju et al. (2020)
Phenolic Compounds	Blackcurrant Cranberry, cactus and grape peel	Marcillo-Parra et al. (2021)
	Cherry Apple by-products	Rojas-García et al. (2022)
	Mango peel and seed	Ayala-Zavala et al. (2010)
	Carrot pomace	Omre et al. (2018)
	Pomegranate leaves	Yu et al. (2021)
	Winery wastes	Teixeira et al. (2014)
	Grape stems	Nieto et al. (2020)
	Citrus fruits peel	Goula & Lazarides (2015)
	Banana peel	González-Montelongo et al. (2010)
	Hazelnut skin	Capaldi et al. (2025)
Carotenoids and Tocopherols	Green pea pods	Rudra et al. (2020)
	Tomato skin and seeds	Nour et al. (2015)
	Carrots by-products	Tiwari et al. (2019)
Proteins	Apple and carrot pomace Mango, banana, and orange peel Potato and tomato solid waste Cabbage leaves Pea pods, shell, and peel Citrus peel	Sharma et al. (2016)
MUFA	Grape bunches Red lees	Ferreira et al. (2021)
	Carrot peel Cereals bran oil	Otero et al. (2021)
	Olive seed	Alves et al. (2018)
	Hazelnut skin and oil	Rondanelli et al. (2023)
PUFA	Grape pomace, stems and bunches Brewer's spent grain	Ferreira et al. (2021)
	Tomato seed	Cantarelli et al. (1993)
	Quince seed oil	Matthäus & Özcan (2015)

Because of these characteristics, food waste is considered a nutritionally, functionally, and nutraceutically valuable raw material. Several studies have recently highlighted the fact that agricultural food by-products have enormous potential to be used in a variety of food applications, such as their use to obtain bioactive compounds (Yadav et al., 2024), the development of alternatives for livestock feed (Westendorf, 2000), and their use in new food formulations (Rațu et al., 2023). Moreover, recycling these coproducts is also very much in line with current consumer trends toward "clean-label" foods (Santiesteban-López et al., 2022). While additives identified with E numbers in the EU are safe and some of them are indeed of "natural" (~ plant) origin such as vitamins E and C (E307 and E300, respectively), consumers are wary of such food components. Instead, they focus their attention on the "free-from" version of foods, which are perceived as more "natural" and "healthier" (Saulais et al., 2023). In this doctoral work, the use of these by-products to solve three important problems and challenges in animal production and meat products was considered: i) lipid oxidation in meat and meat products and the replacement of the currently most widely used synthetic antioxidants; ii) nutritional enrichment and improvement of the technological characteristics of meat products; iii) increasing the sustainability of livestock farming and meat quality.

1.4. Lipid oxidation in meat

Meat is a diverse mixture of water, protein, fats, inorganic compounds, and a small amount of carbohydrates. Due to their characteristic composition, meat and meat-based products are prone to deterioration, making them susceptible to quality degradation throughout storage and processing (Devatkal et al., 2014; Fernandes et al., 2017; Gómez & Lorenzo, 2012). Lipid oxidation is the main process responsible for the deterioration of the quality of meat and meat products (Min & Ahn, 2005). The process of lipid oxidation is extremely complex and involves numerous interacting mechanisms. The unsaturated fatty acids in meat react with molecular oxygen, this reaction results in hydroperoxides, which are considered the first oxidation products. Unlike other fat-derived products, hydroperoxides are odorless and do not give off any scent. However, as these compounds are highly unstable, they decompose rapidly, giving rise to a large number of secondary compounds, such as hydrocarbons, aldehydes, ketones, alcohols, esters, and acids (Ross & Smith, 2006), which cause unpleasant tastes and odors in meat. These reactions are influenced by several factors: intrinsic ones (meat composition) and extrinsic ones (processing and storage conditions) that can promote or inhibit them (Wąsowicz et al., 2004). Therefore, the oxidative stability of meat depends on the balance of anti- and pro-oxidant compounds (Pereira & Abreu, 2018). Beginning with the animal's slaughter, the degradation continues until the finished product is consumed (Chaijan & Panpipat, 2017). Therefore, to avoid these responses and reduce the financial losses of the meat business (Králová, 2015), all intermediate processes (handling, processing, and storage) need to be closely controlled. Oxidation reactions not only reduce the nutritional value of meat by depleting essential fatty acids and vitamins, but they also lead to a gradual decline in sensory quality. This deterioration is typically first observed as changes in color, texture, and the development of rancid odors and flavors, which adversely impact consumer acceptance (Purrinos et al., 2011) and shorten the shelf life of the product (Devatkal et al., 2014; Fernandes et al., 2016; Lorenzo et al., 2014; Rodríguez-Carpena et al., 2011). Since 'quality'

and 'health' are among the main factors driving food choices, and considering that appearance, color, texture, flavor and aroma are key attributes for meat acceptance, controlling or, at least, reducing the process of lipid oxidation is of crucial importance for the food industry (Brøndum et al., 2000). The meat industry primarily relies on the addition of antioxidants as a strategy to counteract lipid oxidation in meat and meat products (Domínguez et al., 2019; Gómez et al., 2018; Lorenzo et al., 2018). These compounds play a crucial role in preventing and reducing the formation of free radicals and other reactive oxygen species responsible for the oxidation of sensitive molecules, such as lipids. Currently, the most commonly used synthetic antioxidants in food are phenolic antioxidants (Rodil et al., 2012), including butylated hydroxyanisole (BHA, identified as food additive E320), butylated hydroxytoluene (BHT, E321), tertiary butylhydroquinone (TBHQ, E319), and propyl gallate (PG, E310). The toxicity of BHA, BHT, and TBHQ has been extensively studied under various experimental conditions, and findings indicate that excessive addition or improper use of synthetic phenolic antioxidants can lead to carcinogenicity, cytotoxicity, oxidative stress induction, and endocrine-disrupting effects, which warrant attention (Carocho et al., 2018; Kim et al., 2016; Xu et al., 2021). This issue, along with changing consumer preferences, has driven a surge of research focused on the development and application of natural antioxidants in food production systems (López-Pedrouso et al., 2022; Novais et al., 2022; Pateiro et al., 2021). Notably, several studies have reported that various plant extracts, that support consumer health, may even exhibit stronger antioxidant capacity than synthetic antioxidants (Jayawardana et al., 2019; Zhang et al., 2018). Natural antioxidants can be found in all the various parts of plants such as cereals, fruits, nuts, seeds, leaves, roots, skins, and barks. This wide range of substances with different chemical characteristics has an antioxidant capacity that is closely linked to the presence of compounds such as vitamins A, C, and E, flavonoids, and other phenolic compounds. Among natural antioxidants, phenolic compounds are predominant due to their H• donating capacity and their high efficiency in absorbing free radicals (Ding et al., 2015; Kumar et al., 2015; Velasco & Williams, 2011). This diverse group of compounds includes phenolic acids, considered promising agents for the development of new products (Fang et al., 2002) and categorized into various subgroups. Among these are derivatives of benzoic acids, such as gallic acid, and cinnamic acids, such as coumaric, caffeic, and ferulic acids. Additionally, the group comprises tannins, both hydrolyzable and condensed, flavonoids (including flavonols, flavones, flavanones, flavanonols, flavanols, anthocyanins, isoflavonoids, chalcones, flavones, and isoflavones), and other polyphenols, such as resveratrol, curcumin, and ellagic acid (Barba et al., 2014; Dai & Mumper, 2010; Tsao, 2010). In this context, plants and plant-based products, including by-products derived from fruit and vegetable processing industries, represent a rich source of antioxidants (Table 2) with protective and preventive properties (Yang & Suh, 2013). It is well established that key compounds in pomegranate peel include gallic acid and hydrolyzable tannins, while grape seeds are rich in caffeine, catechins, and proanthocyanidins (Rockenbach et al., 2011; Shan et al., 2009; Singh et al., 2018). Extracts from the peel of Jaboticaba fruit, native to Brazil, are a source of flavonoids and anthocyanins (Leite-Legatti et al., 2012). Potato peel is particularly rich in chlorogenic acid, with a concentration of 27.56 mg per 100 g (Kanatt et al., 2005), whereas

pineapple peel contains myricetin and other polyphenols (Díaz-Vela et al., 2015). It has also been demonstrated that peels and seeds have significantly higher polyphenol content and greater antioxidant capacity compared to the edible parts of the fruit. For instance, citrus residues such as kinnow peels and seeds and pomegranate seeds contain polyphenols at concentrations ranging from 3000 to 6000 and 2000 micrograms per gram of extract, respectively (Devatkal et al., 2010). Similarly, various parts of citrus peel, including the flavedo and albedo of oranges, lemons, limes, and grapefruits, exhibit significant antioxidant capacity due to the presence of flavonoids, ascorbic acid, reducing sugars, and carotenoids (Guimarães et al., 2010).

Table 2. Agro-industrial by-products used as antioxidants in meat products.

Meat product	By-product	Concentration	Reference
Meat patties	Kinnow rind (*Citrus reticulate*) powder	2%	(Devatkal & Naveena, 2010)
Meat patties	Pomegranate (*Punica granatum*) rind and seed powder	2%	(Devatkal & Naveena, 2010)
Low-fat pork sausages	Tomato powder	0, 0.8, 1.2, 1.5%	(Kim et al., 2011)
Pork burgers	Blueberry pomace	1-2%	(Peiretti et al., 2020)
Beef loin, Pork loin	Citrus (*Citrus unshiu*) peel extract	0.1%	(Jo et al., 2004)
Raw pork	Grape seed (*Vitis vinifera* L.) extract	100 mL/20 g meat	(Shan et al., 2009)
Raw pork	Pomegranate peel (*Punica granatum*) extract	100 mL/20 g meat	(Shan et al., 2009)
Bologna type sausages	Jaboticaba peel (*Plinia jaboticaba*)	0.25, 0.5, 0.75, 1%	(de Almeida et al., 2015)
Cooked goat meat nuggets	Pomegranate peel (*Punica granatum*)	1%	(Devatkal et al., 2014)
Porcine patties	Avocado seed	5%	(Rodríguez-Carpena et al., 2011)
Raw minced pork	Onion peel extract	0.05%	(Shim et al., 2012)
Raw chicken breast patty	Japanese plum peel (*Prunus salicina*) extract	1.6%	(Basanta et al., 2018)
Processed lamb meat	Potato peel extract	0.04%	(Kanatt et al., 2005)
Porcine and bovine ground meat	Lotus rhizome knot and lotus leaf extract	3%	(Huang et al., 2011)
Cooked turkey breast meat	Grape seed extract	0.0, 0.4, 0.8, 1.6 g/kg	(Mielnik et al., 2006)
Ground pork patties	Wild thyme byproduct extract	0.075-0.150 μL/g	(Šojić et al., 2020)
Pork burgers	Rice bran extract	0.5, 1, 2%	(Martillanes et al., 2020)
Lamb meat patties	Olive waste extract	100-400 mg GAE/kg	(Muíño et al., 2017)
Beef burgers	Pistachio green hull water extract	250–1000 mg/kg	(Sadeghinejad et al., 2019)
Chicken nuggets	Moringa flower extract	1–2%	(Madane et al., 2019)
Raw beef meatballs	Pomegranate peel nanoparticles (*Punica granatum*)	1, 1.5%	(Morsy et al., 2018)
Beef patties	Chestnut bur, hull, leaf	250, 500, 1000 ppm	Zamuz et al. (2018)

1.5. Nutritional and technological characteristics

The characteristics of by-products previously discussed make them interesting not only for stabilizing the product from an oxidative point of view but also for improving it nutritionally by going on to modify the macronutrient intake of the final product and improve its technological performance.

1.5.1. Nutritional composition

The incorporation of plant by-products improves the nutritional characteristics of meat products by increasing the protein, vitamin and mineral content but mainly by improving the lipid profile and especially by providing fiber that is absent in meat. This is essential because while high consumption of processed meats has been associated with cancer (World Cancer Research Fund, 2007), high dietary fiber intake is associated with reduced risk of several types of cancer (Hu et al., 2023). Several by-products when substituted for parts of ingredients in meat foods have contributed to improved fiber content. Specifically: replacing pork fat with peach palm flour (0,3,6, and 9%) in lamb burgers (Echeverria et al., 2020), replacing butyl hydroxytoluene (BHT) in beef burgers with grape skin flour (0, 0.5, 1.0, 1.5, and 2%) (de Alencar et al., 2022), the replacement of wheat flour or pork fat with banana peel flour in beef and pork frankfurters (Salazar et al., 2021) or the replacement of sodium tripolyphosphate in Irish breakfast sausages with apple pomace and silver coffee skin (Thangavelu et al., 2022). Due to their higher unsaturated fatty acid content, by-products when added to meat foods can change their fatty acid composition as in the case of seed pomace and tomato peels that when included in raw fermented sausages (0.5 and 1%) increased monounsaturated (MUFA) and PUFA content compared to the control (Skwarek & Karwowska, 2022). Or as in the case of Cinta Senese Frankfurter-type sausages in which nitrites and nitrates were replaced with olive oil pomace, grape seed and chestnut tannin mixture and had higher PUFA omega 6, PUFA omega 3 and PUFA content (Parrini et al., 2019).

1.5.2. Color

During storage, the color of meat products undergoes degradation. Instrumental evaluation of meat color considers redness (a*) as the most important parameter because it is associated with consumer acceptance (Faustman et al., 2010). Lorenzo et al. (2013) showed that grape seed and chestnut leaf extracts can affect the color of cured “chorizo” salami by significantly increasing the value of a*. Likewise, the addition of kiwi pulp powder resulted in a significant effect on the color parameters of low-fat pork patties by showing a decrease in brightness and an increase in redness and yellow (Zhao et al., 2021). This effect was much more noticeable when larger doses of kiwi pulp powder were used. It should be emphasized that altering the color of meat products with plant powders or extracts is almost always easy as plant by-products are rich in natural pigments, but it is essential to study their preservation over days. Sayas-Barberá et al. (2020), for example, observed that adding different levels of date pit powder (*Phoenix dactylifera* L.) to beef burgers had a protective effect on redness in a dose-dependent manner and that samples with the highest dose of the plant by-product showed higher a* values after 10 days of storage. Skwarek & Karwowska (2022) saw, on the other hand, that by

adding tomato pomace at concentrations of 0.5 to 1.5% as sodium nitrite substitutes in fermented raw sausages, the latter had higher a* values during 60 days of refrigerated storage.

1.5.3. Cooking and texture parameters

Cooking yield is also a very important parameter for the quality of meat products, as it impacts quality and consumer acceptance, particularly texture parameters such as tenderness and juiciness (Zhao et al., 2021). Dried apple pomace powder was characterized to determine several functional properties, including a high-water retention capacity, which then resulted in buffalo meat sausages with high cooking performance at 6 % inclusion concentrations (Younis & Ahmad, 2015). The addition of date nut (*Phoenix dactylifera* L.) powder to beef burgers prevented loss of diameter probably due to the higher retention of water and fat in the matrix related to the higher fiber content of this plant by-product (Sayas-Barberá et al., 2020). This resulted in a higher cooking yield, which increased as the level of date kernel utilization increased. The same result was obtained with kiwi pomace in pork patties whose water retention properties increased as the concentration of powder increased (0, 0.5, and 7%) (Zhao et al., 2021). In contrast, the inclusion of olive leaf extract in cured sausages improved texture parameters in all levels tested (Difonzo et al., 2022) probably due to the polysaccharide content of the extract and its ability to form gels (Khemakhem et al., 2018).

1.5.4. Antimicrobial activity

The composition of meat products and the technological procedures applied during processing make them susceptible to microbiological spoilage that causes undesirable changes in appearance and texture and imparts unpleasant tastes and unacceptable odors to the product, reducing its quality, flavor, and shelf life. The main species responsible for microbial spoilage in raw meat products include bacteria such as *Pseudomonas*, *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Salmonella*, *Escherichia*, *Clostridium*, *Bacillus*, and *Brochothrix*. Extracts obtained from plant by-products also have significant antimicrobial properties and may be better accepted by consumers than artificial antimicrobial additives such as nitrites/nitrates that are traditionally used. Olive leaf extract has been shown to be effective in counteracting sulfite-reducing *Clostridia*, their spores, coliforms, *Escherichia coli*, and coagulase-positive *Staphylococcus* in cured sausages, even in the absence of nitrates and nitrites (Difonzo et al., 2022). Similarly, Skwarek & Karwowska (2022) highlighted the antimicrobial properties of tomato pomace in dry fermented sausages. Results showed a reduction in *Enterobacteriaceae* counts with the addition of different amounts of tomato pomace, with the lowest levels observed in samples with a higher percentage of pomace (1.5%). These antimicrobial characteristics are attributed to the phenolic compounds in tomato pomace, which contribute to the improved microbiological quality of the finished product. Black chokeberry (*Aronia melanocarpa* L.) pomace extracts (2%) also have great potential as a natural antimicrobial that has been tested in pork burgers and cooked ham. The extract has indeed shown excellent efficacy against the growth of *Listeria monocytogenes*, *Brochothrix thermosphacta*, and *Pseudomonas putida* (Tamkute et al., 2021).

1.6. Sustainability of livestock farming and meat quality

Consumers are increasingly attentive to the quality of foods, particularly those of animal origin, and are aware of the deep connection between nutrition, wellness, and environmental protection. This issue assumes great importance, considering the growing concerns about the sustainability of production methods. Indeed, intensive livestock farms have long been at the center of public debate for their environmental impact, underscoring the need to make this food chain more ecosystem-friendly. Several studies confirmed that consumer perceptions toward healthier meat products are now mainly associated with the way meat and meat products are produced (García-Torres et al., 2016), animal welfare concerns (Akaichi et al., 2019), sustainability, and respect for the environment (Li et al., 2016; Petrescu et al., 2020; Sanchez-Sabate & Sabaté, 2019).

However, since consumers are only to a small extent ready to make food choices based on what is best for the environment greater sustainability must develop directly within production systems. In the context of animal production in Europe, feed production, in addition to being the highest economic burden, in the case of pigs amounting to 55% (FEFAC, 2023), is one of the main environmental burdens coming to account for 70% of the environmental impact, again in the case of pig production (Andretta et al., 2021; Pomar et al., 2021) and that is why much of the energy invested on the issue of increasing the sustainability of livestock production has focused on the diets of farm animals. Agrifood wastes and by-products have been considered to solve this problem for many years, partly because the possibility of converting this type of waste into feed is much more advantageous than other forms of disposal considered for food waste (Shurson, 2020).

The significant unevenness in government policies globally regarding the recycling of food waste into animal feed severely hampers the possibility of reusing valuable nutrients. However, with increasing social pressure and consumer demands for food production with a reduced carbon footprint and more careful resource management (Gandenberger et al., 2011; Gardner et al., 2004; Grunert et al., 2014), recycling food waste into animal feed needs to be reevaluated as a viable solution on a global scale, provided strict biosafety processes are adopted and regulated.

Some countries, such as Japan (since 2001), South Korea (since 1997), and Taiwan (since 2003), introduced stringent regulations and invested significantly in infrastructure using appropriate heat treatments, allowing between 35% and 43% of food waste to be converted into animal feed (Zu Ermgassen et al., 2016). Such strategies are essential to reduce the global dependence of crop production on feed. Also, in the study by Zu Ermgassen et al. (2016), it is estimated that if the European Union implemented a regulated and centralized system similar to those already successfully adopted in Japan and South Korea, land use for pork production in the EU could be reduced by 21.5%, corresponding to 1.8 million hectares.

This figure is particularly relevant considering that monogastric, such as pigs and poultry, are highly dependent on crops that compete directly with human food. In fact, in Europe, 65% of the 1.57 billion metric tons of cereals, cereal by-products, and oilseed meal consumed, or about 1 billion metric tons, go to feeding pigs and poultry (Mottet et al., 2017).

As a result, there is a huge opportunity to recover energy and nutrients from different sources of food waste and convert them into feed, especially for pigs and poultry, which are unable to use forage fiber efficiently and require diets with higher energy and nutrient density than ruminants.

However, these alternative feed options are environmentally sustainable only if they do not compromise growth performance (Monteiro et al., 2017). Agroindustry by-products as feed can often be limited by the presence of antinutritional factors for a given category of animals, such as high fiber content in the case of monogastric (Yang et al., 2021).

Despite this, several studies showed that the use of by-products in diets not only helps with sustainability but can also help improve growth performance and meat quality, particularly by modifying the lipid profile of adipose depots and oxidative stability. Indeed, it is known that the animal lipid profile varies depending on several factors including diet (Min & Ahn, 2005) and that the presence of exogenous antioxidants in feed can increase the stability of meat lipids (Li & Liu, 2012) by going on to reduce some sources of oxidative stress, such as heating, and thus inhibit their negative effect on muscle tissue (Ismail et al., 2013). In swine, such theories have been pursued in different research.

A study on dietary supplementation of grape pomace verified that it could have a beneficial impact on piglet welfare and improve productivity and meat quality (Kafantaris et al., 2018). Indeed, the authors verified that piglets fed grape pomace had significantly increased antioxidant mechanisms in many of the tissues studied, that oxidative stress-induced damage was decreased, that the average daily gain was increased, and that the omega-3 fatty acids content was significantly increased in the quadriceps muscle examined.

Still, ensiled bergamot pulp fed at 15% to heavy pigs also increased α-linolenic acid, docosapentaenoic acid, docosahexaenoic acid, and consequently n-3 PUFAs without altering oxidative stability and meat colorimetric parameters (Scerra et al., 2022).

Pieszka et al. (2017), in a study conducted on fattening pigs, on the other hand, determined that the use of black currant pomace improved meat oxidative status and vitamin E content, dried carrot pulp positively affected the thick of back fat, dried apple and strawberry pulp improved the fatty acid composition of *longissimus lumborum* muscle especially concerning omega-3 series fatty acids, and that aronia pomace led to more favorable pig production parameters, including daily intake and gain.

1.7. By-products under study

The choice of raw materials to be used in this doctoral project focused on which by-products characterized the agri-food industries in Italy since the success of this type of green strategy, namely the inclusion of by-products within existing food supply chains, is closely related to distance (Tanguy et al., 2017) since the choice of new products must be untethered from a major environmental impact related to transportation (De Quelen et al., 2021). For this reason, two extremely widespread and developed food realities in Italy were considered for the choice of by-products to be tested: the hazelnut processing industry and the tomato processing industry.

1.7.1. Hazelnut skin

The hazelnut (*Corylus avellana* L.) belongs to the Betulaceae family and is one of the most widely consumed nuts in the world so much so that it ranks second in nut production after almonds. Italy ranks second among world producers of hazelnuts, contributing nearly 20% to global production and 15% to international exports. It also has the highest per capita consumption among producers, at 0.520 kg of hazelnuts per person per year (Forte et al., 2022; Misachi, 2018). It is generally consumed whole, raw or roasted, or used as an ingredient in processed product industries, mainly in baked goods or confectionery. During its processing, it undergoes a series of manipulations that produce various wastes, including the hazelnut skin that represents the perisperm of the fruit. This skin is the by-product of the roasting process and accounts for about 2.5% of the total weight of the hazelnut because it absorbs oil during the roasting itself (Alasalvar et al., 2009a). It is estimated that about 90% of domestic production goes to the processing industry, while the remaining 10% is reserved for fresh consumption (Forte et al., 2022; Misachi, 2018). This implies that just under 3,000 tons of hazelnut husks are generated annually in Italy, considering an average annual production of about 110,000 tons of hazelnuts (FAOSTAT, 2023). The recent recognition of nuts as a health food (Alasalvar et al., 2020; Blomhoff et al., 2006; Fischer & Glei, 2013) gave a significant boost to the image of nuts, including hazelnuts, by initiating a series of studies certifying their beneficial effects (Alasalvar et al., 2009b). However, the research focused not only on the whole hazelnut but also on all the by-products that resulted from its processing, particularly the content of phenolic compounds and their antioxidant activity. The hazelnut skin was found to have the highest content of total phenolic acids, even more than the whole hazelnut both raw and roasted. The identified compounds consisted mainly of flavonoids, phenolic acids and related compounds, hydrolyzable tannins and related compounds, and other phenols (Pelvan et al., 2018). The antioxidant activity appeared to be higher than those of other hazelnut by-products (hard shell, green leaf cover, and tree leaf) (Shahidi et al., 2007), whole hazelnut (raw and roasted) (Pelvan et al, 2018) and if we consider the ferric reducing-antioxidant power skin had values 3 times the antioxidant activity of whole nuts, 7-8 times that of dark chocolate, 10 times that of espresso coffee, and 25 times that of blackberries (Del Rio et al., 2011). It should be noted that these properties vary according to the variety of hazelnut considered but in summary, the concentration of total phenolic compounds ranges between 51.9 and 203.1 mg of gallic acid equivalent/g of skin; the total flavonoid content is almost 60% of total phenolic compounds; and the total antioxidant capacity ranges between 309 and 1375 μmol of Trolox equivalent/g of hazelnut skin (Taş & Gökmen, 2015). Thanks to the properties found, hazelnut skin has been tested in various foods such as coffee to improve its physiologically positive phytochemical effects (Contini et al., 2012), as a source of dietary fiber in bread and yogurt (Anil, 2007; Bertolino et al., 2015), in cookies to improve their fatty acid composition and nutritional value (Costantini et al., 2023), and as an antioxidant to fortify fresh egg pasta (Zeppa et al., 2015). In meat, hazelnut skin has been tested to succeed in improving the oxidative stability and nutritional and technological characteristics of processed products such as in the case of chicken burgers or low-fat beef burgers (Longato et al., 2019; Turhan et al., 2005) and as an ingredient in new feed formulations. Specifically, its influence on the chemical

and sensory properties of cheese produced from the milk of sheep fed hazelnut skin (Caccamo et al., 2019), and the effects of its inclusion in feed on growth parameters, production performance, and final product quality of dairy cows (Renna et al., 2020), lactating ewes (Campione et al., 2020) and lambs (Menci et al., 2023) have been studied.

1.7.2. Tomato pomace

Tomato (*Lycopersicon esculentum* Mill.) is a widely grown horticultural crop, with a world production of more than 192 million tons in 2023 (FAOSTAT, 2023). In addition to being consumed as a fresh vegetable, tomatoes are consumed in various processed products, such as concentrate, juice, sauce, puree, and ketchup (Kaur et al., 2008). Worldwide, tomato production has increased exponentially over the past 30 years. In Italy, over the same period, production has been very erratic but in the last 5 years, it returned close to 6 million tons per year (FAOSTAT, 2023). As a seasonal fruit, only a relatively small proportion of tomatoes are consumed as fresh products while the vast majority of tomatoes are processed. From the processing of this product, mainly during the peeling, cutting, chopping and crushing, pulping, and extraction stages, various wastes are generated (Rajan et al., 2022), the main by-product being tomato pomace, which consists of skins, seeds, and a small portion of pulp and accounts for about 7-7.5% of the raw material (Nour et al., 2018). In recent decades, the consumption of tomatoes has been associated with the prevention of several diseases (Willcox et al., 2003) mainly due to the content of antioxidants, including carotenes (lycopene and β-carotene), ascorbic acid, tocopherol, and phenolic compounds (Martínez-Valverde et al., 2002, Jesús Periago et al., 2009). Tomato is the most abundant source of lycopene, which accounts for 80-90% of total carotenoids (Calvo et al., 2008). In recent years, this substance has attracted much attention for its potential health benefits (Kong et al., 2010), being the most efficient free radical scavenger, with a capacity found to be more than twice that of β-carotene (Capanoglu et al., 2010). For this reason, numerous studies investigated whether it is possible to find all these beneficial properties and this nutritional potential due to certain molecules also in individual by-products and not only in the whole fruit. The tomato pomace is sometimes composed mainly of peels but is often a heterogeneous product of peels and seeds that have very different nutritional characteristics. Seeds are mainly a source of protein (23-40%) and oil (17-25%) (Lu et al., 2019). In particular, tomato seed oil emerged due to its high nutritional quality as it is rich in linoleic (37-73%), oleic (15-30%), palmitic, stearic, and arachidic acids and has a total percentage of unsaturated fatty acids of about 80% (Ahmadi Kamazani et al., 2014; Botineştean et al., 2015). In contrast, the peel is rich in dietary fiber, lycopene, and phenols. Dietary fiber content ranges from 39 to 88% on average (Herrera et al., 2010; Savadkoohi & Farahnaky, 2012), and these values are slightly higher than those reported for grapefruit peel (44-62%), orange peel (64%) (Figuerola et al, 2005), banana peel (50%) (Wachirasiri et al., 2009) and mango peel (40-72%) (Ajila & Rao, 2013). Lycopene values, on the other hand, ranged from 51 mg/100g (Nour et al., 2018) to as much as 288 mg/100g (Zuorro & Lavecchia, 2013) while total phenols showed average contents of 12.3 mg GAE/g (Nour et al., 2018). There is a need to specify that the huge variety in the average contents of different biomolecules is because the chemical composition of a crop is a multifactorial result that depends on the variety, maturity, and growth

conditions. Furthermore, as by-products of food processing, the resulting chemical composition also depends on the processing parameters used (Lu et al., 2019). In the context of meat products, the use of this by-product has been tested in both feed and processed products. According to Persia et al. (2003), tomato seeds can be included up to 15% in chick diets without affecting their growth performance. In addition, King & Zeidler (2004) showed that tomato-pressing by-products are a valuable source of vitamin E in broiler rations, helping to reduce lipid oxidation during heating processes and long-term frozen storage, thus extending the shelf life of meat. In processed products this by-product has been used for several purposes, dietary fiber intake (Savadkoohi et al., 2014; Yadav et al., 2016) and improvement in textural properties (Calvo et al., 2008), color score (García et al., 2009; Kim & Chin, 2013) and oxidative stability (Candogan, 2002; Darwish et al., 2019; Kim et al., 2013; Sánchez-Escalante et al., 2003).

2. Research objectives

Considering the above, the objective of this doctoral work was to create a synergy between the quality of meat and meat products and the sustainability of the production chain in terms of using natural ingredients and reducing food waste. The strategies chosen involved both food and feed. The first step was to evaluate the possibility of modifying the lipid composition of the pig carcass through the animals' diet, also verifying its oxidative stability. Subsequently, we wanted to study the possibility of increasing oxidative resistance using agro-food by-products included directly in the pork-based products or the animals' diet. Specifically:

- To test the possibility of changing the fatty acid profile and key nutritional indices of pig tissues through the inclusion in the diet of natural substances as extruded flaxseed, characterized by a high content of n-3 polyunsaturated fatty acids, and verify their oxidative stability.

- To evaluate the effect of hazelnut skin and dry tomato peels added to the formulation of pork burgers against lipid oxidative phenomena and on some chemical-physical and sensory characteristics of the product during refrigerated storage.

- To characterize the antioxidant power of hazelnut skin and a green phenolic extract obtained from hazelnut skin and to explore and validate their effects on oxidative stability and chemical and sensory properties of pork burgers during refrigerated storage.

- To explore the formulation of new finishing diets for heavy pigs, including hazelnut skin and its green phenolic extract, and evaluate their effect on growth performance, carcass traits, and quality of *longissimus thoracis* muscle and backfat tissue.

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Effects of pig dietary n-6/n-3 polyunsaturated fatty acids ratio and gender on carcass traits, fatty acid profiles, nutritional indices of lipid depots and oxidative stability of meat in medium–heavy pigs

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Abstract

The effects of different dietary n-6/n-3 polyunsaturated fatty acids (PUFA) ratios and gender on key carcass traits, as well as the nutritional and technological quality of lipids in medium–heavy pig tissues have been poorly studied. To investigate the subject, 24 Large White, barrows and gilts, evenly divided into two groups of 12, were fed from 80 kg of live weight (LW) until slaughter at 150 kg LW, either a high (9.7:1) (HPR) or low (1.4:1) (LPR) dietary n-6/n-3 PUFA ratio. On individual samples of longissimus thoracis muscle (LTM), subcutaneous (SF), and perirenal (PF) adipose tissues (ATs), the fatty acid (FA) composition was determined by gas chromatography, and lipid nutritional indices (LNIs) were calculated. The oxidative stability of meat was evaluated by determining the malondialdehyde content on raw and cooked (24 h postmortem) and refrigerated (8 days postmortem) LTM samples. The carcass traits did not vary between genders and diets. The LPR group showed a higher n-3 PUFA level and a lower n-6/n-3 PUFA ratio in all the tissues examined and better LNI, especially in the ATs. Diet did not affect the oxidative stability of meat. Gender did not influence the n-6/n-3 PUFA ratio, while barrows showed improvements in some LNI in ATs. Reducing the n-6/n-3 ratio in the diet of growing–finishing medium–heavy pigs improved the FA profile in all tissues and most LNI in ATs without impairing the oxidative stability of meat.

Chapter 2. Effects of pig dietary n-6/n-3 polyunsaturated fatty acids ratio and gender on carcass traits, fatty acid profiles, nutritional indices of lipid depots, and oxidative stability of meat in medium–heavy pigs

1. Introduction

Nowadays, consumers are paying increasing attention to the nutritional quality and health benefits of what they eat. In particular, the lipid amount and characteristics in foods go under unprecedented scrutiny. It is well known that some dietary saturated fatty acids (SFAs) increase total serum LDL-cholesterol concentrations (EFSA, 2010; FAO/WHO, 2008; Mensink et al., 2003), while polyunsaturated fatty acids (PUFAs) have a hypocholesterolemic effect (Kris-Etherton & Yu, 1997). Additionally, PUFAs, most notably those of the n-6 and n-3 series, exert several beneficial effects on human health and are essential for many physiological functions. In particular, n–6 PUFAs show an antiatherogenic action, and n–3 PUFAs are known for their antithrombogenic effect (Garaffo et al., 2011) and their correlation with a low incidence of cardiovascular diseases and atherosclerosis (Crawford et al., 2000; Harris & Zotor, 2019; Mozaffarian & Wu, 2011; Simopoulos, 1999; Yagi et al., 2017; Yang et al., 2023). Intriguingly, the ratio between n-3 and n-6 PUFAs seems to play itself an important role, with several evidence indicating that diets with high n-3 PUFA content and low n-6/n-3 PUFA ratio are more beneficial to human health (Lee et al., 2018; O'Connell et al., 2017). However, the typical Western diet presents a n-6/n-3 PUFA ratio that ranges from 15:1 to 20:1 (Kobayashi et al., 2006; Simopoulos, 2006), while the recommended ratio varies between 1:1 and 4:1 (Simopoulos, 2002). In the light of the above, the content of individual fatty acids (FAs) and the ratio between FA classes are used to calculate common lipid nutritional indices (LNIs), used to evaluate nutritional quality and healthiness of the lipid profile of food. Monitoring, understanding, and tuning the FA profile in meat and related products is thus key for quality control and impact on human health. Meat and meat products are essential components of a balanced diet given their content of amino acids, vitamins, minerals, and FAs (Costa et al., 2011). Nonetheless, they are seen as potentially unhealthy foods, mainly because of their high content of SFAs, cholesterol, and high n-6/n-3 PUFA ratio (Rhee, 1992). Therefore, much attention is paid to their FA profile, and there is a strong interest in modifying their FA composition toward a more favorable n-6/n-3 PUFA ratio. In pigs, as in other monogastric animals, the FA composition of intramuscular fat (IMF) and adipose tissues (ATs) depends on many factors such as genetics (Piedrafita et al., 2001; Wood et al., 2008; Zappaterra et al., 2020), gender, age, live weight (LW) at slaughter (Kouba & Mourot, 2011; Lebret & Mourot, 1998; Lo Fiego et al., 2005b; Minelli et al., 2019) and feeding practices (Belmonte et al., 2021; Fanalli et al., 2022; Lo Fiego et al., 2005a). For example, at the same LW, lipids in entire males are more unsaturated than in females, while castrated males, which yield more adipose carcasses, show a higher degree of saturated fatty acids (Lebret & Mourot, 1998; Lo Fiego et al., 2010). As would be expected, total carcass fatness and SFA content increase with increasing LW (Lo Fiego et al., 2010; Lo Fiego et al., 2005b). In addition, pigs cannot convert dietary n-6 PUFAs into n-3 PUFAs due to the lack of the n-3 FA desaturase gene; the most common approach to improve n-6/n-3 PUFA ratio in pork meat is

thus providing a high content of n-3 PUFAs in the diet of pigs. The inclusion of flaxseed in pig diets has been successfully exploited to this end (Corino et al., 2014; de Tonnac et al., 2018; Guillevic et al., 2009; Tognocchi et al., 2023; Turner et al., 2014), and the results have shown that it is a suitable means to increase the n-3 PUFA content and to reduce the n-6/n-3 PUFA ratio in pork. However, most of the studies have involved lightweight pigs, and some parameters, such as gender and LNIs of Ats, are often overlooked. Very limited data are available on medium–heavy pigs (Dalla Bona et al., 2016). This class of pigs, slaughtered at a LW of 130–150 kg, is gaining more and more importance in the Italian pig industry because of better feed conversion compared to heavier pigs and because they are not subject to the limitations imposed by the Protected Denomination of Origin (PDO) rules. For this reason, further research on the effect of lowering the dietary n-6/n-3 PUFA ratio in the feeding of medium–heavy pigs is needed. However, a further concern is that the higher degree of unsaturation in meat and meat products has been shown to reduce their shelf life, due to increased lipid oxidation (Dominguez et al., 2019; Guillevic et al., 2009; Wood et al., 2004). This study aimed to investigate the effect of gender and n-6/n-3 PUFA ratio in the grower–finisher diet on key carcass traits, the oxidative stability of raw and cooked meat, the FA profile, and the lipid nutritional indices (LNIs) of intramuscular (IMF), subcutaneous (SF) and perirenal (PF) adipose tissues (ATs) in medium–heavy pigs.

2. Materials and methods

2.1. Livestock and diets

The study was performed on 24 Italian Large White pigs intended for Italian medium– heavy pig production. The subjects balanced for gender (12 barrows and 12 gilts) and live weight (average LW 79.9 ± 6.1 kg), were evenly housed in 8 concrete-floored pens of 9 m^2 each, 3 animals per pen (4 replicates), and assigned to two dietary treatments differing for the n-6/n-3 PUFA ratio (PR). The composition of feeds is shown in Table 1. The high PR (HPR) barley/soybean diet had a PR value of 9.97:1 in the growing (80–113 kg LW) and 9.42:1 in the finishing (113–150 kg LW) periods, respectively, whereas in the low PR (LPR) diet, where 5% of extruded flaxseed was included, the PR ratio was equal to 1.47:1 and 1.24:1 in the same periods. The two diets were isoenergetic and isoproteic, and they had the same lysine/digestible energy ratio. Feed was administered at a rate of 7.5% of LW 0.75 in the growing phase and 8.5% of LW 0.75 in the finishing phase. Animals had ad libitum access to water. Pigs were slaughtered at an average LW of 149.9 ± 10.6 kg after 104 days of trial. All the experimental procedures performed in this study complied with the Italian legislation and did not require special animal care authorizations, according to the decision of the Animal Welfare Committee of Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA; 14 September 2016), according to the Italian Legislative Decree 4 March 2014 n.26 art.2 point F.

Table 1. Ingredients (%), proximate composition (% on dry matter (DM) basis), and fatty acid composition (% of total fatty acids) of the diets.

Ingredients		HPR		LPR	
		Growing period	Finishing period	Growing period	Finishing period
Extruded linseed	%	0.00	0.00	5.00	5.00
Barley meal	%	85.50	91.00	80.50	86.60
Soybean meal	%	11.00	5.50	11.00	5.00
L-Lysine	%	0.31	0.29	0.30	0.29
DL-Methionine	%	0.06	0.04	0.06	0.03
L-Threonine	%	0.05	0.04	0.05	0.03
Calcium carbonate	%	1.18	1.13	1.19	1.15
Dicalcium phosphate	%	1.00	1.00	1.00	1.00
Salt (NaCl)	%	0.40	0.40	0.40	0.40
Vit/min pre-mix[1]	%	0.50	0.50	0.50	0.50
Proximate composition					
Dry Matter (DM)	%	88.30	89.50	88.60	89.80
On DM basis					
Digestible energy	MJ/kg	13.35	13.26	13.63	13.54
Crude protein	%	16.87	12.55	17.89	13.20
Crude fat	%	2.00	1.73	4.30	3.86
Crude fibre	%	4.76	4.50	4.91	5.08
Ashes	%	5.87	4.50	5.86	5.89
Fatty acid composition	**(% of total FAs)**				
C14:0 (myristic)	%	0.47	0.39	0.25	0.21
C16:0 (palmitic)	%	29.01	24.25	18.13	15.20
C16:1 (palmitoleic)	%	0.49	0.34	0.17	0.15
C18:0 (stearic)	%	2.03	1.51	4.00	3.18
C18:1n-9 (oleic)	%	14.92	13.50	20.60	18.12
C18:2n-6 (linoleic)	%	47.55	53.67	33.50	34.69
C18:3n-3 (α-linolenic)	%	4.77	5.70	22.83	28.02
C20:1 (eicosenoic)	%	0.74	0.64	0.53	0.41
n-6/n-3 PUFA ratio		9.97	9.42	1.47	1.24

HPR: high dietary n-6/n-3 PUFA ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1). Growing period, from 80 to 113 kg of live weight (LW); finishing period, from 113 kg LW to slaughter (150 kg LW). 1 Vitamin/mineral pre-mix: providing the following nutrients (per kg diet as-fed): vitamin A 15,000 IU; vitamin D3 2000 IU; vitamin E (α-tocopheryl acetate) 50 mg; vitamin K 2.5 mg; vitamin B1 2 mg; vitamin B2 5 mg; vitamin B5 15 mg; vitamin B6 4 mg; vitamin B12 0.036 mg; niacin 25 mg; folic acid 1 mg; biotin 0.15 mg; choline 346 mg; Cu 15 mg; Fe 150 mg; Mn 25 mg; Co 0.4 mg; I 1.5 mg; Zn 100 mg; Se 0.1 mg.

2.2. Slaughtering and sampling procedures

After overnight fasting, the pigs were individually weighed, transported to a commercial abattoir according to the Council Regulation (EC) 1/2005 on the protection of animals during transport and related operations, and slaughtered by exsanguination after electrical stunning, in agreement with the Council Regulation (EC) No 1099/2009 on the protection of animals at the time of the killing. After slaughtering, each carcass was graded in agreement with EUROP grid carcass grading, using the Fat-o-Meat'er (Frontmatec, Kolding, Denmark) device (MIPAAF. D.M. 24, 2018). Subsequently, each carcass was dissected into commercial cuts, and samples of *longissimus thoracis* muscle (LTM) and SF, both at the level of the last rib, and a sample of PF, were taken from each left half- carcass. All the samples were transported to the laboratory in a refrigerated box. Each sample of LTM was sliced into three subsamples: one for oxidative stability evaluation before and after the cooking procedure,

a second subsample; the SF and PF samples were vacuum packed (Elegen, Reggio Emilia, Italy) and stored at -20 °C until the lipid extraction for FAs analyses. A third subsample from each LTM was packed in MAP (70% O_2 and 30% CO_2) using a high-barrier tray lidded with a PET/EVOH/PE film (AERPACK System, supplied by Coopbox Group, Italy), and stored in a refrigerator (4 ± 1 °C) for 8 days for oxidative stability evaluation after storage. The whole-package oxygen transmission rate (OTR) was <0.1 cm^3 day^{-1} (air, 25 °C).

2.3. Oxidative stability of raw and cooked muscle

Oxidative stability was evaluated by the 2-thiobarbituric acid reactive substances (TBARS) measurements (Belmonte et al., 2021; Siu & Draper, 1978). For the cooking procedure, slices of LTM of about 2.5 cm thickness and 100 g weight were individually vacuum packed in plastic bags and put in a water bath at a temperature of 80 °C, until the core temperature, controlled during cooking with a temperature probe, reached 70 °C. Then, slices were cooled under running water. Absorbance at 532 nm was measured against a blank sample on two replicates of each sample on a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan) immediately after cooling. TBARS were expressed as mg of malondialdehyde (MDA) per kg of muscle using a tetraethoxypropane (TEP) (Sigma-Aldrich, Milan, Italy) as a standard.

2.4. Fatty acid profile

The total lipids of LTM, SF, and PF were extracted according to the method of Folch et al. (1957). In total, 50 mg of extract lipids were methylated before the gas-chromatographic analysis as detailed in previous papers (Ficarra et al., 2010; Zappaterra et al., 2020). The fatty acid methyl esters content (FAMEs) was expressed as g/100 g of total lipids.

2.5. Lipid nutritional indices (LNI) and iodine value (IV)

From the FA composition data, the total amounts of saturated FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), unsaturated FA (UFA = MUFA + PUFA), n-6 PUFA, n-3 PUFA, n-3 long-chain PUFA (n-3 LCPUFA = eicosatrienoic (C20:3n-3) + eicosapentaenoic (EPA, C20:5n-3) + docosapentaenoic (DPA, C22:5n-3) + docosahexaenoic (DHA; C22:6n-3), essential FA (EFA= linoleic (LA) + linolenic (ALA) + glinolenic (GLA) + arachidonic (C20:4n-6) and UFA/SFA, PUFA/SFA, and n-6/n-3 PUFA ratios were calculated. Moreover, some other nutritional indices as H/H (Mierlitã, 2018), HPI (Chen et al., 2004), AI (Ulbricht & Southgate, 1991), TI (Ulbricht & Southgate, 1991), UI (Logue et al., 2000) and PI (Erickson, 1992), were calculated according to the following equations:

Hypocholesterolemic/Hypercholesterolemic ratio (H/H) = (C18:1n-9+ΣPUFA) / (C12:0+C14:0+C16:0) (Mierlitã, 2018) (1)

Health-Promoting Index (HPI) = ΣUFA / [C12:0 + (4 * C14:0) + C16:0] (Chen et al., 2004) (2)

Atherogenic Index (AI) = [C12:0 + (4 * C14:0) + C16:0] / ΣUFA (Ulbricht & Southgate, 1991) (3)

Thrombogenic Index (TI) = (C14:0 + C16:0 + C18:0) / (0.5 * ΣMUFA) + (0.5 * Σn-6 PUFA) + (3 * Σn − 3 PUFA) + (Σn-3 PUFA / Σn-6 PUFA) (Ulbricht & Southgate, 1991) (4)

Unsaturation Index (UI) = (% monoenoics * 1) + (% dienoics * 2) + (% trienoics * 3) + (% tetraenoics * 4) + (% pentaenoics * 5) + (% hexaenoics * 6) (Logue et al., 2000) (5)

Peroxidizability Index (PI) = (% monoenoics * 0.025) + (% dienoics * 1) + (% trienoics * 2) +(% tetraenoics * 4) + (% pentaenoics * 6) + (% hexaenoics * 8) (Erickson, 1992) (6)

On SF and PF fat samples, starting from the FA profile and according to the following formula (Lo Fiego et al., 2016), iodine value (IV) was calculated:

IV = (85.703 + [C14:0] * 2.740 - [C16:0] * 1.085 - [C18:0] * 0.710 + [C18:2n-6] * 0.986) (7)

2.6. Statistical analysis

The data were subjected to analysis of variance using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included, within each anatomical location (LTM, SF and PF), dietary treatment (HPR and LPR), gender (gilts and barrows) and their interactions as fixed effects. The interactions were not found to be statistically significant ($p > 0.05$) and therefore were not presented in the tables. A Principal Component Analysis (PCA) was performed to describe the relationship between the dietary treatments and the principal classes of fatty acids and nutritional indices of lipid depots. The PCA was performed using RStudio software, version 2023.03.0 + 386.

3. Results

3.1. Carcass characteristics

Table 2 displays the effects of dietary treatment and gender on carcass traits, specifically comparing the hot carcass weight covariate for the slaughter live weight.

Table 2. Effect of dietary treatment and gender on carcass traits (the hot carcass weight was the covariate for the slaughter live weight).

	Dietary treatment			Gender			
	HPR (N=12)	LPR (N=12)	P-value	Gilts (N=12)	Barrows (N=12)	P-value	R-MSE
Slaughter live weight (kg)	146.9	152.9	0.185	150.5	149.2	0.760	10.599
Hot carcass weight (kg)	127.1	126.9	0.866	126.8	127.3	0.695	2.549
Backfat thickness (mm)	30.79	34.63	0.241	31.93	33.49	0.630	7.696
Lean meat content (%)	51.48	49.73	0.244	50.97	50.24	0.620	3.517
IMF LTM (%)	1.63	1.57	0.755	1.48	1.72	0.245	0.490

HPR: high dietary n-6/n-3 PUFA ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1); R-MSE: root means square error. IMF LTM: intramuscular fat of *longissimus thoracis* muscle.

The gender and the different dietary n-6/n-3 PUFA ratio had no significant effect on any of the parameters measured after slaughter ($p > 0.05$).

3.2. Fatty acid profile, lipid nutritional indices and oxidative stability of *longissimus thoracis* muscle (LTM)

Table 3 displays the effects of dietary treatments and gender on the intramuscular fatty acid profile of the *longissimus thoracis* muscle. The dietary treatment did not result in significant changes in the proportions of total SFA, MUFA or PUFA ($p > 0.05$). In a more detailed analysis, pigs fed with a low dietary n-6/n-3 PUFA ratio of 1.4:1 exhibited decreased contents of heptadecenoic (C17:1) ($p < 0.01$), arachidonic ($p < 0.05$) and docosatetraenoic (C22:4n-6) ($p < 0.01$) FAs. Conversely, they displayed increased contents ($p < 0.01$) of ALA, eicosatrienoic, EPA and DPA fatty acids, resulting in higher ($p < 0.01$) proportions of total n-3 PUFA and n-3 LCPUFA, except for DHA ($p > 0.05$). The contents of the remaining FAs in LTM were not significantly influenced by the dietary treatments ($p > 0.05$). The gender did not substantially modify the FA composition of LTM except for lauric, palmitoleic (C16:1), vaccenic (C18:1n-7) and eicosenoic (C20:1) FAs, which were higher ($p < 0.05$) in barrows, while DHA was lower ($p < 0.01$) than in gilts.

Table 3. Fatty acid composition (g/100 g of total lipids) of intramuscular lipids of the *longissimus thoracis* muscle from pigs receiving a diet with different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Fatty acids (FAs)	Dietary treatment			Gender			R-MSE
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	
C10:0 (capric)	0.08	0.08	0.82	0.07	0.08	0.07	0.019
C12:0 (lauric)	0.05	0.05	0.45	**0.04**	**0.05**	0.03	0.001
C14:0 (myristic)	0.80	0.84	0.60	0.75	0.88	0.05	0.150
C16:0 (palmitic)	15.69	15.73	0.97	14.90	16.52	0.08	2.103
C17:0 (heptadecanoic)	0.16	0.14	0.09	0.14	0.15	0.25	0.019
C18:0 (stearic)	8.54	8.52	0.97	8.16	8.89	0.17	1.225
C20:0 (eicosanoic)	0.10	0.10	0.97	0.09	0.10	0.13	0.014
C16:1 (palmitoleic)	2.17	2.07	0.54	**1.90**	**2.34**	0.01	0.384
C17:1 (heptadecenoic)	**0.20**	**0.16**	<0.01	0.18	0.18	0.63	0.021
C18:1n-7 (vaccenic)	2.81	2.73	0.58	**2.58**	**2.96**	0.02	0.377
C18:1n-9 (oleic)	26.32	25.13	0.46	24.32	27.12	0.09	3.786
C20:1 (eicosenoic)	0.42	0.44	0.58	**0.39**	**0.47**	0.03	0.078
C18:2n-6 (linoleic)	6.30	6.45	0.65	6.57	6.18	0.24	0.772
C18:3n-3 (α-linolenic)	**0.32**	**1.24**	<0.01	0.77	0.79	0.64	0.093
C18:3n-6 (γ-linolenic)	0.15	0.12	0.06	0.13	0.14	0.89	0.027
C20:2n-6 (eicosadienoic)	0.15	0.16	0.68	0.16	0.15	0.70	0.020
C20:3n-3 (eicosatrienoic)	**0.05**	**0.15**	<0.01	0.10	0.10	0.89	0.012
C20:4n-6 (arachidonic)	**2.36**	**1.89**	0.03	2.30	1.96	0.10	0.471
C20:5n-3 (eicosapentaenoic)	**0.10**	**0.38**	<0.01	0.25	0.23	0.16	0.042
C22:4n-6 (docosatetraenoic)	**0.35**	**0.20**	<0.01	0.30	0.26	0.07	0.057
C22:5n-3 (docosapentaenoic)	**0.30**	**0.53**	<0.01	0.45	0.38	0.07	0.085
C22:6n-3 (docosahexaenoic)	0.06	0.06	0.43	**0.07**	**0.05**	<0.01	0.013
∑ SFA	25.41	25.45	0.98	24.16	26.70	0.09	3.430
∑ MUFA	31.92	30.52	0.47	29.37	33.07	0.06	4.537
∑ PUFA	10.14	11.19	0.09	11.10	10.23	0.16	1.444
∑ UFA	42.05	41.71	0.83	40.47	43.30	0.08	3.721
∑ n-6 PUFA	9.32	8.83	0.37	9.46	8.69	0.16	1.288
∑ n-3 PUFA	**0.82**	**2.36**	<0.01	1.64	1.54	0.23	0.191
∑ n-3 LCPUFA	**0.50**	**1.12**	<0.01	0.87	0.75	0.06	0.137
∑ DFA	50.59	50.24	0.85	48.64	52.19	0.08	4.643
∑ EFA	9.12	9.71	0.28	9.77	9.06	0.19	1.266

HPR: high dietary n-6/n-3 polyunsaturated (PUFA) ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1); R-MSE: root means square error. SFA: saturated FAs; MUFA: monounsaturated FAs; PUFA: polyunsaturated FAs; UFA: unsaturated FAs (MUFA + PUFA); n-3 LCPUFA: n-3 long-chain PUFA (eicosatrienoic + eicosapentaenoic + docosapentaenoic + docosahexaenoic acids); DFA: desirable FAs (MUFA + PUFA + stearic acid); EFA: essential FAs (linoleic + α-linolenic + γ-linolenic + arachidonic acids).

Table 4 displays the effects of dietary treatment and gender on the lipid nutritional indices of intramuscular fat of LTM. The pigs that received the low n-6/n-3 PUFA dietary ratio (LPR group) showed a significantly ($p < 0.01$) lower n-6/n-3 PUFA ratio (3.73 vs. 11.40) as well as a lower thrombogenic index (0.92 vs. 1.08; $p < 0.01$). The other calculated indices did not differ significantly ($p > 0.05$) between the two groups. These indices did not vary between genders.

Table 4. Lipid nutritional indices (mean values and R-MSE) of intramuscular lipids of *longissimus thoracis* muscle from pigs receiving a diet with different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Item	Dietary treatment			Gender			
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	R-MSE
UFA/ SFA ratio	1.66	1.66	0.88	1.68	1.64	0.38	0.131
PUFA/ SFA ratio	0.41	0.46	0.29	0.47	0.39	0.09	0.107
n-6/n-3 PUFA ratio	**11.40**	**3.73**	<001	7.64	7.49	0.42	0.459
H/H ratio	2.22	2.21	0.92	2.27	2.16	0.16	0.178
HPI	2.23	2.21	0.72	2.27	2.18	0.23	0.178
AI	0.45	0.46	0.66	0.44	0.46	0.19	0.037
TI	**1.08**	**0.92**	<0.01	0.98	1.03	0.24	0.098
UI	59.56	61.60	0.18	60.15	61.01	0.56	3.514
PI	21.98	24.76	0.07	24.63	22.11	0.10	3.547

HPR: high n-6/n-3 PUFA ratio (9.7:1); LPR: low n-6/n-3 PUFA ratio (1.4:1). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids. H/H: hypocholesterolemic/hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/ (C12:0 + C14:0 + C16:0) (Mierlitã, 2018). HPI: health-promoting index = ΣUFA/ [C12:0 + (4 * C14:0) + C16:0] (Chen et al., 2004). AI: atherogenic index = [C12:0 + (4 * C14:0) + C16:0]/ΣUFA (Ulbricht & Southgate, 1991). TI: thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 * ΣMUFA) + (0.5 * Σn-6 PUFA) + (3 * Σn-3 PUFA) + (Σn-3 PUFA/Σn-6 PUFA)] (Ulbricht & Southgate, 1991). UI: unsaturation index = (% monoenoics * 1) + (% dienoics * 2) + (% trienoics * 3) + (% tetraenoics * 4) + (% pentaenoics * 5) + (% hexaenoics * 6) (Logue et al., 2000). PI: peroxidizability index = (% monoenoics * 0.025) + (% dienoics * 1) + (% trienoics * 2) + (% tetraenoics * 4) + (% pentaenoics * 6) + (% hexaenoics * 8) (Erickson, 1992).

Table 5 displays the effects of dietary treatment and gender on the lipid oxidation of LTM, measured as the malondialdehyde (MDA) content. Neither the diet nor gender affected ($p > 0.05$) the oxidative stability of fresh, refrigerated (8 days) or cooked LTM samples.

Table 5. Effect of dietary treatment and gender on MDA (malondialdehyde) content (mg/kg) of raw (at 24 h and 8 days) and cooked *longissimus thoracis* muscle.

	Dietary treatment			Gender			
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	R-MSE
MDA at 24 h post-mortem	0.105	0.113	0.715	0.097	0.122	0.263	0.053
MDA at 8 days of storage	0.144	0.198	0.112	0.157	0.185	0.404	0.078
MDA cooked	0.406	0.495	0.182	0.458	0.443	0.822	0.156

HPR: high dietary n-6/n-3 PUFA ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1); R-MSE: root means square error.

3.3. Fatty acid profile and lipid nutritional indices of subcutaneous adipose tissue and perirenal fat

Tables 6 and 7 display the effects of dietary treatment and gender on fatty acid profile of the SF and PF, respectively. The SFA content in SF did not vary ($p > 0.05$) with the treatments (Table 6), whereas it decreased ($p < 0.05$), mostly in stearic acid, in PF when the dietary n-6/n3 PUFA ratio (LPR group) is lowered (Table 7). In terms of MUFA content, while observing the same trend in both tissues,

lowering the dietary ratio of n-6/n-3 PUFA did not significantly affect ($p > 0.05$) the single components and the total MUFA contents in PF (Table 7); however, in SF (Table 6), this dietary modification resulted in a significant reduction ($p < 0.01$) in the total MUFA content, specifically affecting heptadecenoic, oleic ($p < 0.01$) and vaccenic ($p < 0.05$) fatty acids. The total content of PUFA, as well as most of the individual PUFA in both Ats, were higher ($p < 0.01$) in the LPR group. In detail, the linoleic acid content increased in both ATs but was significantly higher only in PF ($p < 0.01$), while the n-3 PUFA, such as ALA, eicosatrienoic, EPA, DPA, the essential fatty acids (EFA) and the n-3 LCPUFA, were higher ($p < 0.01$) in both ATs of the LPR group. Furthermore, in this group, DHA and DFA were significantly higher ($p < 0.01$ and $p < 0.05$, respectively) only in PF (Table 7). The LPR group showed higher UFA content in SF ($p < 0.05$) and PF ($p < 0.01$). The low dietary n-6/n-3 PUFA ratio led to an increase in the IV in both ATs, but it was statistically significant ($p < 0.01$) only in PF.

Table 6. Fatty acid composition (g/100 g of total lipids) of subcutaneous adipose tissue from pigs receiving a diet with different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Fatty acids (FAs)	Dietary treatment			Gender			R-MSE
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	
C10:0 (capric)	0.07	0.07	0.88	**0.07**	**0.08**	0.03	0.008
C12:0 (lauric)	0.01	0.01	0.18	0.01	0.01	0.61	0.001
C14:0 (myristic)	1.32	1.30	0.59	1.31	1.32	0.75	0.071
C16:0 (palmitic)	24.74	24.08	0.19	24.39	24.43	0.93	1.162
C17:0 (heptadecanoic)	**0.37**	**0.29**	<0.01	0.32	0.34	0.25	0.056
C18:0 (stearic)	15.68	14.70	0.09	15.35	15.03	0.57	1.352
C20:0 (eicosanoic)	0.20	0.19	0.25	0.20	0.20	0.96	0.023
C16:1 (palmitoleic)	1.80	1.73	0.36	1.72	1.80	0.36	0.190
C17:1 (heptadecenoic)	**0.34**	**0.28**	<0.01	0.30	0.32	0.20	0.045
C18:1n-7 (vaccenic)	**2.34**	**2.19**	0.02	2.20	2.33	0.06	0.156
C18:1n-9 (oleic)	**37.92**	**35.95**	<0.01	36.50	37.38	0.20	1.583
C20:1 (eicosenoic)	0.87	0.78	0.09	0.79	0.87	0.19	0.136
C18:2n-6 (linoleic)	8.67	9.52	0.14	8.77	9.40	0.28	1.358
C18:3n-3 (α-linolenic)	**0.74**	**3.80**	<0.01	2.14	2.40	0.14	0.417
C18:3n-6 (γ-linolenic)	**0.17**	**0.15**	0.02	0.15	0.16	0.19	0.021
C20:2n-6 (eicosadienoic)	0.42	0.44	0.38	0.40	0.45	0.07	0.057
C20:3n-3 (eicosatrienoic)	**0.13**	**0.55**	<0.01	0.32	0.36	0.30	0.047
C20:4n-6 (arachidonic)	**0.23**	**0.18**	0.01	0.20	0.21	0.57	0.037
C20:5n-3 (eicosapentaenoic)	**0.01**	**0.05**	<0.01	0.02	0.03	0.30	0.001
C22:4n-6 (docosatetraenoic)	**0.10**	**0.08**	<0.01	0.08	0.09	0.22	0.013
C22:5n-3 (docosapentaenoic)	**0.09**	**0.21**	<0.01	0.14	0.15	0.19	0.018
C22:6n-3 (docosahexaenoic)	**0.02**	**0.03**	0.10	0.03	0.02	0.39	0.005
∑ SFA	42.46	40.72	0.09	41.71	41.48	0.82	2.408
∑ MUFA	**43.28**	**40.93**	<0.01	41.51	42.69	0.13	1.811
∑ PUFA	**10.56**	**14.99**	<0.01	12.27	13.29	0.19	1.823
∑ UFA	**53.84**	**55.93**	0.03	**53.79**	**55.98**	0.02	2.153
∑ n-6 PUFA	9.58	10.37	0.20	9.62	10.32	0.25	1.448
∑ n-3 PUFA	**0.98**	**4.63**	<0.01	2.65	2.96	0.11	0.452

∑ n-3 LCPUFA	**0.24**	**0.82**	< 0.01	0.51	0.56	0.06	0.065
∑ DFA	69.52	70.63	0.15	**69.14**	**71.01**	0.02	1.777
∑ EFA	**9.80**	**13.65**	<0.01	11.27	11.18	0.22	1.746
IV	59.88	62.08	0.07	60.57	61.09	0.48	2.804

HPR: high dietary n-6/n-3 PUFA ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1); R-MSE: Root means square error. SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs; UFA: Unsaturated FAs (MUFA+PUFA); n-3 LCPUFA: n-3 long chain PUFA (eicosatrienoic + eicosapentaenoic + docosapentaenoic + docosahexaenoic acids); DFA: Desirable FAs (MUFA + PUFA + stearic acid); EFA: Essential FAs (linoleic + α-linolenic + γ-linolenic + arachidonic. IV: Iodine Value = (85.703 + [C14:0] * 2.740 - [C16:0] * 1.085 - [C18:0] * 0.710 + [C18:2n-6] * 0.986) (Lo Fiego et al., 2016).

Table 7. Fatty acid composition (g/100 g of total lipids) of perirenal fat from pigs receiving a diet with different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Fatty acids (FAs)	Dietary treatment			Gender			R-MSE
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	
C10:0 (capric)	0.09	0.09	0.62	0.09	0.9	0.72	0.013
C12:0 (lauric)	0.09	0.09	0.27	0.09	0.09	0.30	0.009
C14:0 (myristic)	1.42	1.46	0.17	1.47	1.42	0.05	0.068
C16:0 (palmitic)	28.02	27.49	0.22	27.99	27.52	0.28	1.019
C17:0 (heptadecanoic)	0.36	0.31	0.20	0.34	0.34	0.92	0.080
C18:0 (stearic)	**22.56**	**21.35**	0.03	22.21	21.67	0.33	1.333
C20:0 (eicosanoic)	0.21	0.20	0.41	0.21	0.20	0.39	0.024
C16:1 (palmitoleic)	1.35	1.26	0.23	1.31	1.31	0.98	0.175
C17:1 (heptadecenoic)	0.24	0.20	0.07	0.21	0.23	0.38	0.058
C18:1n-7 (vaccenic)	1.62	1.51	0.13	1.57	1.57	1.00	0.158
C18:1n-9 (oleic)	30.29	28.71	0.09	28.85	30.16	0.15	2.139
C20:1 (eicosenoic)	0.53	0.48	0.14	0.48	0.53	0.19	0.083
C18:2n-6 (linoleic)	**6.92**	**8.76**	<0.01	7.88	7.79	0.89	1.473
C18:3n-3 (α-linolenic)	**0.63**	**4.00**	<0.01	2.23	2.41	0.14	0.307
C18:3n-6 (γ-linolenic)	0.13	0.12	0.28	0.12	0.13	0.39	0.017
C20:2n-6 (eicosadienoic)	**0.24**	**0.29**	<0.01	0.26	0.27	0.54	0.038
C20:3n-3 (eicosatrienoic)	**0.07**	**0.40**	<0.01	0.22	0.25	0.14	0.036
C20:4n-6 (arachidonic)	0.20	0.17	0.06	0.19	0.18	0.40	0.040
C20:5n-3 (eicosapentaenoic)	**0.01**	**0.05**	<0.01	0.03	0.03	0.21	0.009
C22:4n-6 (docosatetraenoic)	**0.08**	**0.06**	<0.01	0.07	0.07	0.85	0.010
C22:5n-3 (docosapentaenoic)	**0.07**	**0.20**	<0.01	0.13	0.14	0.48	0.022
C22:6n-3 (docosahexaenoic)	**0.02**	**0.03**	0.02	0.03	0.02	0.13	0.005
∑ SFA	**52.76**	**50.97**	0.04	52.41	51.31	0.21	2.026
∑ MUFA	34.04	32.17	0.07	32.42	33.80	0.17	2.350
∑ PUFA	**8.37**	**14.08**	<0.01	11.16	11.30	0.86	1.834
∑ UFA	**42.42**	**46.25**	<0.01	43.57	45.09	0.13	2.307
∑ n-6 PUFA	**7.57**	**9.40**	<0.01	8.53	8.44	0.90	1.559
∑ n-3 PUFA	**0.81**	**4.68**	<0.01	2.63	2.85	0.14	0.358
∑ n-3 LCPUFA	**0.18**	**0.68**	<0.01	0.41	0.44	0.23	0.061
∑ DFA	**64.98**	**67.57**	<0.01	65.79	66.76	0.30	2.200
∑ EFA	**7.87**	**13.05**	<0.01	10.41	10.51	0.89	1.755
IV	**49.99**	**53.38**	<0.01	51.36	52.02	0.57	2.732

HPR: high dietary n-6/n-3 PUFA ratio (9.7:1); LPR: low dietary n-6/n-3 PUFA ratio (1.4:1); R-MSE: Root means square error. SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs; UFA: Unsaturated FAs (MUFA+PUFA); n-3 LCPUFA: n-3 long chain PUFA (eicosatrienoic + eicosapentaenoic + docosapentaenoic + docosahexaenoic acids); DFA: Desirable FAs (MUFA + PUFA + stearic acid); EFA: Essential FAs (linoleic + α-linolenic + γ-linolenic + arachidonic. IV: Iodine Value = (85.703 + [C14:0] * 2.740 - [C16:0] * 1.085 - [C18:0] * 0.710 + [C18:2n-6] * 0.986) (Lo Fiego et al., 2016).

Overall, gender did not affect the FA composition of both adipose tissues. However, barrows exhibited higher ($p < 0.05$) contents of both UFA and DFA in SF (Table 6).

Tables 8 and 9 display the effects of dietary treatment and gender on LNI in SF and PF, respectively. The data show that the LNI were significantly affected by dietary treatment in both ATs. In SF, the LPR group had higher UFA/SFA ($p < 0.05$), PUFA/SFA ($p < 0.01$) and H/H ratios, ($p < 0.01$), while the n-6/n-3 PUFA ratio was lower ($p < 0.01$) (Table 8). Additionally, HPI was significantly higher ($p < 0.05$) in the LPR group. This group showed lower values of TI ($p < 0.01$) and AI ($p < 0.05$). However, significantly higher ($p < 0.01$) values of UI and PI were observed in the LPR group. As already seen in LTM, gender did not influence these indices in subcutaneous adipose tissue ($p > 0.05$), except for UI, which was higher ($p < 0.05$) in barrows (Table 8). Regarding the lipid nutritional indices of PF (Table 9), the trend is similar to that observed for SF. In fact, the UFA/SFA, PUFA/SFA and H/H ratios were enhanced ($p < 0.01$) in the LPR group, while the n-6/n-3 PUFA ratio, AI and TI were lower ($p < 0.01$). Moreover, in this group, both UI and PI increased significantly ($p < 0.01$). In PF, there was no difference ($p > 0.05$) between barrows and gilts in UFA/SFA, PUFA/SFA and n-6/n-3 ratios (Table 9). Barrows had higher H/H ratio ($p < 0.05$) and HPI ($p < 0.01$) compared to gilts, while the latter showed higher ($p < 0.05$) values of AI and TI.

Table 8. Lipid nutritional indices (mean values and R-MSE) of subcutaneous adipose tissue from pigs receiving different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Item	Dietary treatment			Gender			R-MSE
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	
UFA/ SFA ratio	**1.27**	**1.38**	0.03	1.29	1.36	0.18	0.110
PUFA/ SFA ratio	**0.25**	**0.37**	<0.01	0.30	0.32	0.23	0.054
n-6/n-3 PUFA ratio	**9.55**	**2.24**	<0.01	5.90	5.89	0.45	0.192
H/H ratio	**1.86**	**2.01**	0.01	1.90	1.97	0.21	0.136
HPI	**1.80**	**1.91**	0.03	1.82	1.89	0.18	0.126
AI	**0.56**	**0.53**	0.04	0.55	0.53	0.22	0.037
TI	**1.42**	**1.01**	<0.01	1.25	1.18	0.14	0.108
UI	**66.48**	**76.80**	<0.01	**69.84**	**73.44**	0.04	4.027
PI	**14.31**	**22.74**	<0.01	17.80	19.26	0.16	2.434

HPR: high n-6/n-3 PUFA ratio (9.7:1); LPR: low n-6/n-3 PUFA ratio (1.4:1). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids. H/H: hypocholesterolemic/hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/ (C12:0 + C14:0 + C16:0) (Mierlitã, 2018). HPI: health-promoting index = ΣUFA/ [C12:0 + (4 * C14:0) + C16:0] (Chen et al., 2004). AI: atherogenic index = [C12:0 + (4 * C14:0) + C16:0]/ΣUFA (Ulbricht & Southgate, 1991). TI: thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 * ΣMUFA) + (0.5 * Σn-6 PUFA) + (3 * Σn-3 PUFA) + (Σn-3 PUFA/Σn-6 PUFA)] (Ulbricht & Southgate, 1991). UI: unsaturation index = (% monoenoics * 1) + (% dienoics * 2) + (% trienoics * 3) + (% tetraenoics * 4) + (% pentaenoics

* 5) + (% hexaenoics * 6) (Logue et al., 2000). PI: peroxidizability index = (% monoenoics * 0.025) + (% dienoics * 1) + (% trienoics * 2) + (% tetraenoics * 4) + (% pentaenoics * 6) + (% hexaenoics * 8) (Erickson, 1992).

Table 9. Lipid nutritional indices (mean values and R-MSE) of perirenal fat from pigs receiving a diet with different n-6/n-3 PUFA ratio: effect of dietary treatment and gender.

Item	Dietary treatment			Gender			
	HPR (N=12)	LPR (N=12)	P-*value*	Gilts (N=12)	Barrows (N=12)	P-*value*	R-MSE
UFA/ SFA ratio	**0.80**	**0.91**	<0.01	0.83	0.88	0.07	0.059
PUFA/ SFA ratio	**0.16**	**0.28**	<0.01	0.21	0.22	0.68	0.040
n-6/n-3 PUFA ratio	**9.14**	**2.01**	<0.01	5.64	5.52	0.27	0.256
H/H ratio	**1.31**	**1.47**	<0.01	**1.35**	**1.43**	0.03	0.076
HPI	**1.26**	**1.38**	<0.01	**1.28**	**1.36**	<0.01	0.071
AI	**0.80**	**0.73**	<0.01	**0.78**	**0.74**	0.02	0.040
TI	**2.23**	**1.43**	<0.01	**1.88**	**1.77**	0.04	0.123
UI	**52.52**	**66.18**	<0.01	58.41	60.29	0.25	3.807
PI	**11.47**	**21.54**	<0.01	16.31	16.70	0.70	2.364

HPR: high n-6/n-3 PUFA ratio (9.7:1); LPR: low n-6/n-3 PUFA ratio (1.4:1). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids. H/H: hypocholesterolemic/hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/ (C12:0 + C14:0 + C16:0) (Mierlitã, 2018). HPI: health-promoting index = ΣUFA/ [C12:0 + (4 * C14:0) + C16:0] (Chen et al., 2004). AI: atherogenic index = [C12:0 + (4 * C14:0) + C16:0]/ΣUFA (Ulbricht & Southgate, 1991). TI: thrombogenic index = (C14:0 + C16:0 + C18:0) / [(0.5 * ΣMUFA) + (0.5 * Σn-6 PUFA) + (3 * Σn-3 PUFA) + (Σn-3 PUFA/Σn-6 PUFA)] (Ulbricht & Southgate, 1991). UI: unsaturation index = (% monoenoics * 1) + (% dienoics * 2) + (% trienoics * 3) + (% tetraenoics * 4) + (% pentaenoics * 5) + (% hexaenoics * 6) (Logue et al., 2000). PI: peroxidizability index = (% monoenoics * 0.025) + (% dienoics * 1) + (% trienoics * 2) + (% tetraenoics * 4) + (% pentaenoics * 6) + (% hexaenoics * 8) (Erickson, 1992).

The biplot graphs simultaneously show the distribution of samples in the plane described by the principal components and the influence of the studied variables on this same distribution. The farther the arrow of a variable moved away from the center, the more significant the impact of that variable has been. For example, the variable n6/n3 PUFA ratio influenced the description of the distribution plane of the HPR samples with higher values. Similarly, we can observe that the LPR samples were influenced by the high values of the total PUFA, n-3 and n-6 PUFA in the case of both adipose tissues. It can be noted that, as anticipated, the increase in PUFA in the LPR samples led to an increase in UI, which, as seen, influenced the distribution of these same samples in the plane of the principal components. Similarly, in the two adipose tissues, it is visible how the clustering of HPR samples was influenced by high values of MUFA, SFA, AI, and TI. In Figure 1, through Principal Component Analysis, a clear separation between the two dietary groups is graphically visualized for all the tissues examined.

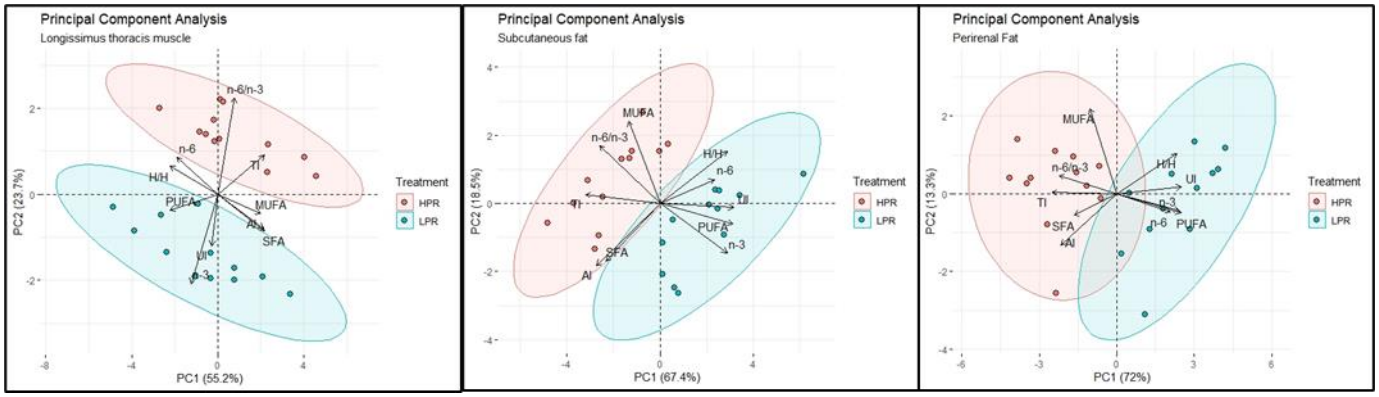

Figure 1. Principal Component Analysis. Biplot of the results of longissimus thoracis muscle (LTM), subcutaneous (SF) and perirenal (PF) adipose tissues (ATs). According to diet treatment, each plot discriminates the variables SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-3 PUFA, n-6 PUFA, n-6/n-3 PUFA ratio, H/H: hypocholesterolemic/hypercholesterolemic ratio; AI: atherogenic index; TI: thrombogenic index; UI: unsaturation index.

These distinctive patterns observed in the PCA plots suggest that the alterations in fatty acid composition and nutritional indices are not random occurrences. Rather, they are indicative of discernible trends closely linked to varying ratios of dietary n-6/n-3 polyunsaturated fatty acids (PUFAs). These trends underscore the significant impact of the experimental interventions on the biochemical composition of the investigated tissues. This observation reinforces the idea that the specific dietary manipulations exerted a notable influence on the metabolic profiles of the tissues, further emphasizing the importance of considering the role of dietary factors in shaping the biochemical landscape.

4. Discussion

4.1. Carcass characteristics

The pigs' carcass traits were not affected by different dietary n-6/n-3 PUFA ratios. This agrees with the findings of de Tonnac & Mourot (2018) who could not find any effect of varying the n-6/n-3 PUFA ratios on carcass characteristics, except for an increase in the weight of the liver. Liu & Kim (2018) found that the different dietary n-6/n-3 PUFA ratios did not influence backfat thickness and lean meat percentage. Feeding linseed or fish oil to raise the n-3 PUFA intake of growing–finishing pigs did not influence carcass parameters (Sobol et al., 2015). Furthermore, similar findings were reported in Heigai pigs fed diets supplemented with different n-6/n-3 PUFA ratios, although it was observed that the reduction from eight to five of the dietary n-6/n-3 PUFA ratio yielded lighter carcasses (Nong et al., 2020). Such discrepancy may be accounted for by differences in the duration of the dietary treatment, the genetic type involved in the study, and the slaughter weight. In the present study, the gender did not influence the carcass traits. Our findings also aligned with those of other researchers (Cisneros et al., 1996; Weatherup et al., 1998) who have observed gender-related impacts neither on carcass weight nor intramuscular lipid content. However, it is worth noting that some studies have reported that barrows tend to yield heavier carcasses compared to gilts (Latorre et al., 2004).

4.2. Lipid characteristics

Many studies have highlighted that the characteristics of dietary fats influence the composition of lipids in swine tissues (Corino et al., 2008; Duran-Montgé et al., 2008). Our results demonstrate that lowering the dietary n-6/n-3 PUFA ratio from 9.7:1 to 1.4:1 alters the FA profile in LTM, SF and PF of medium–heavy pigs, thus confirming previous reports (Li et al., 2015; Nong et al., 2020). In agreement with a previous study (Liu & Kim, 2018), the different n-6/n-3 PUFA dietary ratios did not change the total contents of SFA, MUFA and PUFA in LTM. However, in adipose tissues, the low dietary n-6/n-3 PUFA ratio significantly increased PUFA contents. This is in accordance with previous studies (Koch et al., 1968; Li et al., 2015), highlighting how the intramuscular fat of the LTM is comparatively less sensitive than adipose tissues to PUFA dietary incorporation. Moreover, it has been emphasized (Okrouhlá et al., 2013; Nuernberg et al., 2005) that, in pigs, n-3 long-chain PUFA are synthesized from dietary ALA. The long-term dietary administration of ALA can significantly increase the accumulation of this same fatty acid in body phospholipids and can improve the efficiency of its conversion to longer chain n3-PUFA, namely, EPA and DPA (Dugan et al., 2015). In the tissues we studied, the low dietary n-6/n-3 PUFA ratio, containing higher percentages of ALA, enhanced the content of ALA and total n-3 PUFA, except DHA, whose content did not vary. The lack of DHA production from linolenic acid has already been demonstrated by many authors (Kouba et al., 2003; Leikus et al., 2018; Minelli et al., 2020; Smink et al., 2012).

Consistent with another previous study (de Tonnac et al., 2018), our results also showed no effect of the dietary n-6/n-3 PUFA ratios on total n-6 PUFA in muscle, whereas the arachidonic and docosatetraenoic acids were reduced. The low dietary n-6/n-3 PUFA ratio enhanced the relative content of n-3 PUFA and n-3 LCPUFA in the intramuscular fat and adipose tissues at the expense of arachidonic and docosatetraenoic acids (Li et al., 2015). Additionally, the essential fatty acids (EFA) contents in muscle and adipose tissues were improved in pigs fed the low dietary n-6/n-3 PUFA ratio. In the perirenal fat, characterized by high concentrations of SFA, the low dietary n-6/n-3 PUFA ratio led to a reduction in the total SFA and, notably, stearic acid content.

In this study, the dietary n-6/n-3 PUFA ratio affected the MUFA content, particularly the oleic acid, in SF but not in LTM and PF. Oleic acid, the most abundant fatty acid in swine tissues (Kouba et al., 1997), is partly provided by diet and is also synthesized from C18:0 by D 9-desaturase (stearoyl-CoA-desaturase); the activity of this enzyme is higher in the subcutaneous adipose tissue than in perirenal or intramuscular fats (Jiang et al., 2018). The activity of the enzyme is inhibited by high linolenic acid dietary levels (Kouba et al., 2003). Eventually, this could partly explain the differences observed among tissues. As regards the LNI, we found that the n-6/n-3 PUFA ratio in LTM and ATs was positively correlated with its dietary ratio. In fact, the low dietary n-6/n-3 PUFA ratio reduced the n-6/n-3 PUFA ratio, which ranged from values above 9 to 3.73 in LTM, and 2.24 and 2.01 in SF and PF, respectively. Similar findings were obtained in previous studies (Okrouhlá et al., 2013; Wojtasik et al., 2012) that found that the increased n-3 PUFA content in the diet decreased the n-6/n-3 PUFA ratio in pork.

Consuming diets with n-6/n-3 PUFA ratio below 4.0 reduces the occurrence of cardiovascular diseases in humans (Simopoulos, 2010). Therefore, since the tissues examined from LPR diets showed values beneath the suggested maximum threshold, under this profile they could be regarded as beneficial for human food consumption. Meat and meat products with a more balanced n-6/n-3 PUFA ratio, as requested by the consumer, can be marketed as healthier foods, with premium prices in the market. Additionally, regulations and labelling standards that promote a balanced n-6/n-3 PUFA ratio could drive industry innovation and research to improve animal nutrition and rearing practices.
The UFA/SFA and PUFA/SFA ratios are used to value the healthiness of fats for human consumption. A balanced intake of dietary PUFA/SFA is thought to be important in regulating serum cholesterol (Kang et al., 2005). A ratio of PUFA/SFA greater than 0.45 is recommended in human diets to prevent some chronic diseases (Woloszyn et al., 2020). In our study, the low dietary n-6/n-3 PUFA ratio brought about an increase of this parameter in all tissues examined, though only in LTM the minimum suggested value was exceeded.
The H/H index expresses the relationship between hypocholesterolemic fatty acids (C18:1n-9 and PUFA) and hypercholesterolemic fatty acids (C12:0, C14:0 and C16:0). This index can be used to evaluate the cholesterolemic effect of dietary lipids (Murariu et al., 2023). In this study, the different dietary n-6/n-3 PUFA ratios did not affect the H/H index of LTM, while the low dietary n-6/n-3 PUFA ratio improved this parameter in both the ATs, mainly cause of an increase in PUFA deposition. The values of H/H observed in our study fall within the range indicated for meat and meat products in previous studies (Chen & Liu, 2020).
The atherogenic index (AI) is represented by the ratio between the sum of the main SFA, thought as proatherogenic (favoring the adhesion of lipids to the cells of the immunological and circulatory system), and the sum of the UFA, deemed antiatherogenic (inhibiting the aggregation of plaque and diminishing the level of cholesterol) (Woloszyn et al., 2020). The thrombogenic index (TI) is defined as the ratio between the prothrombogenic FAs (C14:0, C16:0 and C18:0) and anti-thrombogenic FAs (MUFA, n-6 PUFA, and n-3 PUFA). At increasing TI values, the tendency to form clots in blood vessels increases. Both AI and TI can be related to platelet aggregation (Ghaeni et al., 2013). Low values for AI and TI represent a protective potential for coronary artery health. In the present study, the low dietary n-6/n-3 PUFA ratio significantly reduced the AI value in SF and PF, as well as the TI values in all the tissues examined. The health promoting index (HPI) was the inverse of AI, therefore showing reciprocal values against the AI.
The unsaturation index (UI) and peroxidazibility index (PI) are potential tools to evaluate the susceptibility to oxidation of a tissue. These two indices are inversely related to the shelf-life of meat and meat products (Dal Bosco et al., 2022) and are positively related to the protective potential for coronary artery disease (Woloszyn et al., 2020). In the present study, the unsaturation index values were lower in the LTM than in ATs. Further, no changes in oxidative stability were observed in the muscle at varying diets neither on fresh nor cooked meat. In SF and PF, both UI and PI increased at lowering the dietary n-6/n-3 PUFA ratio. However, the calculated IV was below 70 for both diets, the maximum limit set by the regulations for Italian ham production (Lo Fiego et al., 2016).

Regarding gender, the intramuscular FA composition was very similar in barrows and gilts. These results conflict with experimental outcomes (Nuernberget al., 2005; Zhang et al., 2007) where barrows yielded more saturated intramuscular fat than gilts. In our study, carcass fatness and intramuscular fat were the same in both genders. In fact, many authors (Ramirez & Cava, 2007; Warnants et al., 1999; Zhang et al., 2007) have indicated that the difference in FA composition of intramuscular fat between genders was accounted for by the higher degree of intramuscular fat in barrows, given that the proportion of SFA increased with the fat content of the carcass. The similar FA composition of LTM could explain the close values of nutritional indices between genders. In subcutaneous adipose tissue, the barrows showed a higher UFA content and consequently a higher value of unsaturation index. Overall, gender did not influence lipid nutritional indices, though barrows showed higher values of H/H and HPI and lower values of AI and TI in perirenal fat.

5. Conclusions

Based on the results of this study, we inferred that a low dietary n-6/n-3 polyunsaturated fatty acid ratio led to higher n-3 PUFAs content and better n-6/n-3 PUFA ratio in all the tissues examined, as well as to improved lipid nutritional indices, particularly for subcutaneous and perirenal adipose tissues. These changes have potential beneficial effects on human health while preserving the technological properties of the lipids, in particular without exerting negative effects on the oxidative stability. Conversely, gender seemed to play only a marginal role. Furthermore, the carcass characteristics were adversely affected neither by the low dietary n-6/n-3 polyunsaturated fatty acid ratio nor by gender.

This study confirms dietary intervention as a powerful tool for the quality control and improvement of meat and meat products. Given that the meat from medium–heavy pigs is intended also for use in non-PDO cured products, it is essential to conduct additional research to confirm the oxidative stability of cured products and assess the residual content of n-3 polyunsaturated fatty acids at the end of the curing period.

Abbreviations

AI	Atherogenic index
ALA	Alpha-linolenic acid
ATs	Adipose tissues
CVD	Cardiovascular diseases
DFA	Desirable fatty acids
DHA	Docosahexaenoic acid
DM	Dry matter
DPA	Docosapentanoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
EVOH	Ethylene vinyl alcohol
FA	Fatty acid
FAMEs	Fatty acids methyl esters
GC	Gas chromatography
GLA	γ-linolenic acid
GLM	General linear model
H/H	Hypocholesterolemic/hypercholesterolemic ratio
HPI	Health-Promoting Index
HPR	High dietary n-6/n-3 polyunsaturated fatty acid ratio
IMF	Intramuscular fat
IV	Iodine value
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acids
LDL	Low density lipoproteins
LNI	Lipid nutritional indices
LPR	Low dietary n-6/n-3 polyunsaturated fatty acid ratio
LTM	Longissimus thoracis muscle
LW	Live weight
MAP	Modified atmosphere packaging
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
OTR	Oxygen transmission rate
PCA	Principal component analysis
PDO	Protected denomination of origin
PE	Polyethylene
PET	Polyethylene terephthalate
PF	Perirenal fat
PI	Peroxidizability index
PR	n-6/n-3 polyunsaturated fatty acids ratio
PUFA	Polyunsaturated fatty acids
R-MSE	Root means square error
SF	Subcutaneous fat
SFA	Saturated fatty acids
TBARS	2-Thiobarbituric acid reactive substances
TEP	Tetraethoxypropane
TI	Thrombogenic index
UFA	Unsaturated fatty acids
UI	Unsaturation index

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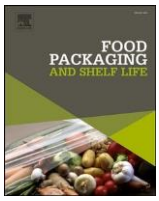

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Effect of hazelnut skin and dry tomato peel on the oxidative stability, chemical and sensory properties of pork burgers during refrigerated storage

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Abstract

The quality deterioration of meat products due to lipid oxidation could be controlled by utilizing agri-food by-products rich in antioxidants. This study evaluated the effect of adding hazelnut skin and dry tomato peel to pork burgers against oxidation phenomena. Three types of burgers were prepared: a control (C) with a basic formulation, and two formulations with 2.5% hazelnut skin (HS) or with 2.5% dry tomato peel (DTP). Microbiological, sensorial, and physio-chemical analyses were performed during 7 days of refrigerated storage (0-4°C). Results showed a high inhibition of oxidation in HS burgers at all sampling times, both raw and cooked burgers, while in DTP burgers this phenomenon occurred only when cooked. Both by-products provided a significant amount of fiber, increased the polyunsaturated fatty acids (PUFA) content, and improved the omega-6/omega-3 ratio.

Chapter 3. Effect of hazelnut skin and dry tomato peel on the oxidative stability, chemical and sensory properties of pork burgers during refrigerated storage

1. Introduction

Consumers nowadays are very concerned with the quality of the food they eat every day, but they are also becoming more aware of how food production affects the environmental impact. Regarding meat and meat products, healthiness plays a pivotal role in consumer choices. Consequently, the meat production chain is oriented to improve meat quality, and the main strategies adopted are the reduction of drugs in animal farming and the replacement of synthetic compounds with natural antioxidants in animal feed (Corino, Rossi, Cannata & Ratti, 2014) and in meat processing (Teixeira & Rodrigues, 2021; Saldaña et al., 2021), the reduction of fat content, improving fat composition by animal's nutritional strategies aimed to increase the omega 3 fatty acids (Lo Fiego, Belmonte, & Mezzetti, 2018). Lipid oxidation is the main non-microbial process responsible for the quality deterioration of meat and meat products (Min & Ahn, 2005; Domínguez et al., 2019). There are intrinsic factors that cause meat oxidation, such as the proportion of antioxidant molecules in animal tissues and the degree of lipid unsaturation (Ladikos & Lougovois, 1990). Among the different kinds of meat, pork is one of the most prone to lipid oxidation due to the high content of unsaturated fatty acids that are most susceptible to oxidative stress (Juntachote, Berghofer, Siebenhandl, & Bauer, 2006; Alvarez-Parrilla et al., 2014). Usually, the parameters in which a qualitative alteration is most obvious are color, texture, and flavor due to the appearance of rancid smells and flavours. In addition, this oxidative mechanism also gives rise to toxic compounds implicated in several pathologies such as atherosclerosis, cancer, inflammatory processes, and aging (Domínguez et al., 2019). Moreover, this phenomenon is promoted by mechanical actions such as grinding, cooking, and boning that cause the breakdown of muscle membranes (Ladikos & Lougovois, 1990). Due to the production process, items like burgers are highly delicate from an oxidative point of view. A possible strategy to control lipid oxidation processes could be the utilization of agri-food by-products rich in antioxidant compounds. In the Italian agri-food sector, there is a large availability of by-products from the tomato and hazelnut industries. Due to their characteristics and qualities, these co-products are already well-known and extensively studied in the research community (Navarro-González, García-Valverde, García-Alonso, & Periago et al., 2011; Elbadrawy & Sello, 2016; Del Valle, Cámara, & Torija, 2006; Özdemir, Yilmaz, Durmaz, & Gokmen, 2014; Locatelli et al., 2010; Taş & Gökmen, 2015; Müller et al., 2020; Pelvan, Olgun, Karadağ, & Alasalvar, 2018; Del Rio, Calani, Dall'Asta, & Brighenti, 2011). These attributes are mostly related to the intake of macronutrients such as fiber and unsaturated fatty acids as well as the presence of phenolic compounds, which have strong antioxidant properties. Tomato peels consist mainly of peel, residual pulp, and tomato seeds and correspond to about 7-7.5% of the raw material (Nour et al., 2018). This indicates that on average, just under 500 k tons are produced annually in Italy, with an average yearly production of tomatoes of about 6 million tons throughout the period of 2011 to 2021 (FAOSTAT, 2021). In addition to the high fiber content, the bioactive compounds that in tomato peels attract interest, mainly for their antioxidant and coloring properties,

are carotenoids, phenolic compounds, vitamins, and glycoalkaloids (Viuda-Martos et al., 2014; Andres, Petron, Delgado-Adamez, Lopez, & Timon, 2017). These constituents are extremely interesting for their anticarcinogenic, cardioprotective, antimicrobial, anti-inflammatory, and antioxidant potential, among others (Viuda-Martos et al., 2014). However, it's important to underline that the amount of active compounds present depends on the tomato variety, agricultural practices, environmental conditions, and industrial transformation processes (Valdez-Morales, Espinosa-Alonso, Espinoza-Torres, Delgado-Vargas, & Medina-Godoy, 2014). Among all, lycopene is the most representative (80-90% of total pigments) (Doménech-Asensi et al., 2013) and promising bioactive compounds present for the implications associated with nutrition and human health thanks to the ability to interact with ROS and consequently mitigate the harmful effect of oxidation (Luisa García, Calvo & Dolores Selgas, 2009). As regards hazelnut skin, it is removed from the core during the roasting process and represents about 2.5% of the weight of the whole hazelnut, in shell (Alasalvar et al., 2009). This indicates that about 3k tons of hazelnut skin are made in Italy each year from an average annual hazelnut production of about 110k tons (FAOSTAT, 2021). In addition to excellent fiber content, several phenolic compounds, including flavan-3-ols, phenolic acids (mostly gallic acid), and procyanidins, are present in this by-product, which is now the subject of extensive research (Renna et al., 2020; Rondanelli et al., 2023). Despite being closely related to the cultivar (Taş & Gökmen, 2015), this results in the skin having an extremely higher total phenol content than natural hazelnut or roasted without skin (Pelvan et al., 2018), as well as an antioxidant capacity that is three times higher than nuts, 25 times higher than blackberries (Del Rio et al., 2011). These factors make hazelnut skin interesting from a health perspective because dietary traits like it have been connected to improvements in colon metabolism, a drop in total cholesterol and LDL, and a decrease in heart disease, hypertension, diabetes, and gastrointestinal disorders (Lairon et al., 2005; Liu et al., 1999; Montonen, Knekt, Järvinen, Aromaa, & Reunanen, 2003; Petruzziello, Iacopini, Bulajic, Shah, & Costamagna, 2006; Whelton et al., 2005). Although the food industry is gradually recognizing and utilizing these by-products, particularly tomato peels, considerable progress remains to be made in establishing them as co-products and not just waste, which currently imposes economic burdens on companies and contributes to environmental pollution. This study aimed to evaluate the effect of hazelnut skin (HS) and dry tomato peel (DTP) on the oxidative stability, and chemical and sensory properties of pork burgers during refrigerated storage.

2. Materials and methods

2.1 Characterization of by-products

The dry tomato peel was obtained from Packtin S.r.l. (Reggio Emilia), which utilizes an innovative circular drying process at low temperatures (35-40°C) while hazelnut skin was sent by Azienda Agricola Cascina Loreto (Piagera di Gabiano, Alessandria) and was mechanically separated during the hazelnut toasting process. Both by-products were reduced to powder, using a home mixer Moulinex DPA 141 (Moulinex Italy), and then sieved with a mesh width of 500 μm.

2.1.1 Proximate composition

The chemical composition of hazelnut skin and dry tomato peel was determined according to the AOAC official methods (AOAC, 1995) and the results were expressed on wet basis.

2.1.2 Microbiological analysis

For the microbiological analysis, 10 grams (g) of each by-product were diluted with 90 g of sterile sodium hypochlorite solution (0.9% NaCl), homogenized for 90 seconds in a laboratory Stomacher 400 blender (Seward Limited, Worthing, UK) and serial dilutions were created. Pour plate analysis was done using Plate Count Agar (PCA, Tryptic Glucose Yeast Agar, Biolife, Milan, Italy) for the aerobic mesophilic count and Violet Red Bile Glucose Agar for the Enterobacteriaceae count (VRBGA, Biolife, Milan, Italy). The plates underwent 24-48-hour and 48-72-hour incubations, respectively, at 30 °C. The bacterial load was expressed in terms of logarithm of colony-forming units (CFU) per g of by-products.

2.1.3 Fatty acid composition

Lipids from by-products were extracted with chloroform-methanol according to Folch, Lees, & Sloane Stanley (1957) and the fatty acid profile was determined by capillary gas chromatography. As reported by Zappaterra et al. (2020), 50 mg of lipid extract were diluted with 2 mL of hexane and methylated with 200 µL of 2N-methanolic potassium hydroxide solution (KOH supplied by Carlo Erba, Milan, Italy, and methanol supplied by ITW Reagents, Barcelona, Spain). Subsequently, the fatty acid methyl esters (FAMEs) were analyzed using a TRACE^{TM}GC Ultra gas chromatograph (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with Flame Ionization Detector, a PVT injector, and TR-FAME Column (30 m long, 0.25 mm i.d., 0.2µm film thickness) supplied by Thermo Fisher Scientific (Rodano, Milano, Italy). At this point 1 µL of the methylated esters sample was injected into the GC with a split flow rate of 10 mL/min, operating at a constant flow of 1 mL/min of helium as a carrier gas. The working temperature for the injector and detector was 240°C. The temperature program was raised from 140°C to 250°C. After 2 minutes at 140°C temperature increased by 4°C/min till 250°C, and it was then maintained for 5 minutes. The Chrom-card software (version 2.3.3, Thermo Electron Corporation Rodano, Milano, Italy) was used to record, identify, and integrate the peaks area. A solution of known concentrations standard FA mix (Supelco 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellafonte, PA, USA, and single FAMEs standard, Larodan, Fine Chemicals AB, Malmö, Sweden) was used to identify the retention times of the FAMEs. The amount of each FAME was expressed as its relative percentage of the total amount of FAMEs using the normalized and correct area method.

2.1.4 Antioxidant activity and phytochemicals

The extraction of free phenolic compounds from hazelnut skin and dry tomato peel was carried out following the procedure reported by Martini, Conte, & Tagliazucchi (2017) with some modifications. Briefly, 1 g of each by-product was homogenized with 20 mL of methanol/water/acetic acid solution (70:29:1, *v*/v/v) with an Ultra-Turrax homogenizer (IKA, Germany) for 1 min. The suspension was

then centrifuged (6000 rpm, 15 min, 4 °C). The supernatant was collected, and the pellet was resuspended with 20 ml of new solution. This procedure was repeated four times until the complete extraction of the phenolic compounds contained in the initial sample (1g) was achieved. The obtained polyphenol-rich extracts were stored at 0-4° C and then used for the subsequent analyses.

2.1.4.1 ABTS assay

To assess the antioxidant activity of by-products, the ABTS method was used according to the protocol described by Re et al. (1999). The ABTS test involves the use of chromogen 2, 2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS, AppliChem GmbH). The antioxidant activity is evaluated as a reduction of the absorbance at 734 nm of the ABTS+• radical cation in the presence of antioxidants. The ABTS radical cation (ABTS+•) was generated by mixing a total of 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate allowing the mixture to react in the dark for 16 h. The ABTS+• solution was diluted in methanol to obtain an absorbance value (A_0) of 0.705 ± 0.005 at 734 nm. Then 100 µl of the diluted sample was mixed with 1400 µL of ABTS+• solution and stored at 20°C for 15 min in darkness. The final absorbance at 734 nm (A_f) was read and the percentage of scavenging (S%) was calculated using the following Eq.:

$$S\ \% = (A_0 - A_f)/A_0) \times 100$$

Where A_0 indicates the initial absorbance (control), while A_f indicates the final absorbance (sample). Trolox (6-hydroxy 2,5,6,7-tetramethyl chroman-2-carboxyl acid) was used as standard and the ABTS scavenging capacity was expressed as mmol of Trolox equivalent per g of by-product, by means of a calibration curve obtained with Trolox 50- 500 mmol/L, in the same assay conditions.

2.1.4.2 FRAP assay

The antioxidant activity was measured also as ferric reducing/antioxidant power by using FRAP assays (Benzie & Strain, 1999). The method is based on the reduction of the Fe^{3+} -2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form at low pH. Briefly, 3 ml of FRAP assay solution (consisting of 20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was prepared daily and mixed with 100 µL of the sample. The absorbance was measured at 593 nm at room temperature after 6 min of incubation. Results were expressed as µM of $FeSO_4$ per g of by-product.

2.1.4.3 Total Phenolic Compounds (TPC)

The total phenolic compounds were determined using the Folin-Ciocalteu test (Singleton, Orthofer, & Lamuela-Raventos, 1999) with some modifications: 1975 µl of distilled water was mixed with 25 µl of phenolic compounds extract and 125 µl of Folin-Ciocalteu reagent. The solution was stirred and stored in the dark for 1 min. 375 µl of 20% (w/w) Na_2CO_3 solution was added and incubated for 2 h in the dark at room temperature. Then the absorbance value at 765 nm was measured. Gallic acid was used as a phenolic standard to create a calibration curve (concentration range of 0–500 mg/L). The results were expressed as mg of gallic acid equivalent/g by-products (mg GAE/g).

2.2 Manufacture of pork burgers

The raw pork loin (*longissimus dorsi muscle*) and the subcutaneous adipose tissue were purchased refrigerated, and vacuum-packed from a commercial market at each starting cycle of analyses. Burgers were formed using a conventional burger maker (50±0.5g patty, 1 cm thickness, and 6 cm diameter), and three different types of pork burgers were formulated: a basic burger (control group; C), with 88.5% of *longissimus dorsi* muscle, 10% of subcutaneous adipose tissue, and 1.5% of sodium chloride, and two groups with addiction of 2.5% hazelnut skin (HS) or 2.5% dry tomato peel (DTP), respectively. The concentration of the two by-products was chosen based on results from the literature. While higher concentrations could have been used for tomato peels, excellent results were also obtained with the addition of DTP from 0.30% to 4.5% (w/w) (Alves, Bragagnolo, da Silva, Skibsted, & Orlien, 2012; Kim et al., 2013; Luisa García et al., 2009). For hazelnut skin, good results were obtained with the addition of 1% and 2% (Turhan, Sagir, & Sule Ustun, 2005), while higher concentrations, such as 3%, negatively affected the sensory evaluation of the product. Therefore, to ensure comparability of results, the same concentration was chosen for both by-products. A moderate value of 2.5% was selected, which allowed for optimal technological performance without affecting the acceptability of the product.

The research was divided into four cycles, in which 54 pork burgers were produced, 18 for each type for a total of 216 burgers. For each test, 12 burgers of each group were packed in resealable polypropylene containers, without modifications in atmospheric gas concentration, and stored at 4±1 °C, for subsequent analyses carried out at 4 and 7 days of storage; the remaining 6 burgers were analyzed at day 0. On each sampling day (SD0, SD4, SD7) the samples were subjected to weight and diameter measurements, microbiological analysis, pH, color detection, water content, and oxidative status (TBARs) evaluation. Moreover, at day 0 chemical composition and fatty acid profile were determined. Subsequently, all burgers were cooked by a home electric double cast grill plat (Bosch, Germany) at 180 °C for 3 minutes and subjected to weight, diameter, color, moisture, and TBARs content measurements. Three burgers for treatments were destined for the sensory test.

2.3 Pork burgers analyses

2.3.1 Proximate composition

The chemical composition (moisture, crude lipids, crude protein, and crude fiber) of raw burgers was determined according to the AOAC methods (AOAC, 1995). The results were expressed as percentage of wet matter.

The fatty acid composition of raw burgers was done as previously described in section 2.1.3. Lipids were extracted with chloroform-methanol according to Folch, Lees, & Sloane Stanley (1957) and the fatty acid profile was determined by capillary gas chromatography after methylation as reported by Zappaterra et al. (2020). The results were expressed as the relative percentage of the total amount of FAMEs using the normalized and correct area method.

2.3.2 Microbiological analysis

The microbial load of raw burgers was performed according to the technique and with the soils described in the by-product characterization. The analyses were performed in duplicate, and the results were expressed as the logarithm of colony-forming units (CFU) per g of burgers.

2.3.3 Physicochemical analyses

The pH value of each raw burger was determined in duplicate using a pH-meter CyberScan 310 (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a Xerolite electrode (Crison Instrument, Allela, Spain).

Color was determined from the surface of raw and cooked burgers using a Minolta CM-600d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) with a window diameter of 8 mm and D65 as the illuminant source. Before color measuring the instrument was calibrated against a white plate supplied by the manufacturer. Each sample was measured at three different points and the measurements were averaged. Color detection was performed according to the CIE Lab color convention (CIE, 1986), where three basic coordinates are: L*- "lightness", a*- "redness", and b*- "yellowness". Further, Chroma (C*), the expression of the saturation index and color intensity, was calculated as $(a^{*2}+b^{*2})^{0.5}$, and Hue angle (h*) was calculated as arctan (b*/a*).

2.3.4 Diameter variation and cooking loss

A Borletti caliper was used and two measurements with different angles were made to define the diameter of the burger better. The measurement was carried out, before (RD) and after (CD) cooking on a home electric double-cast grill plat (Bosch, Germany) at 180 °C for 3 minutes.

(RD – CD)/RD*100 represents the diameter variation due to the cooking process.

The cooking loss was expressed as the difference (%) between the raw weight (RW) and cooked weight (CW) of burgers, according to the equation:

(RW-CW)/RW*100

2.3.5 Lipid oxidation analysis of raw and cooked burgers

The oxidative stability of raw and cooked burgers was evaluated according to Siu & Draper (1978), slightly modified. Approximately 2.5 g of minced sample were homogenized in 12.5 mL of distilled water for 2 min at 9500 rpm using an Ultra-Turrax tissue homogenizer (IKA, Germany). After that time 12.5 mL of 10% trichloroacetic acid (TCA) solution (Sigma-Aldrich, Milan, Italy) were added and then the sample was centrifugated at 2000 rpm for 20 min at 4°C. The supernatant was filtered through a paper filter (Whatman No. 5), and 4 mL of the clear filtrate were transferred into 15 mL pyrex test tubes. Then 1 mL of 0.06M 2-thiobarbituric acid (TBA, Sigma-Aldrich, Milan, Italy) was added and the samples were kept in a water bath at 80°C for 90 min. At the same time, the blank was run (2 mL of distilled water+2 mL of TCA solution+1 mL of TBA). The samples were cooled before reading and absorbance at 532 nm was measured against blank sample, using a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan). Using 1,1,3,3 tetraethoxypropane (TEP, Sigma-Aldrich, Milan, Italy) as a standard, TBARS were expressed as mg of malondialdehyde

(MDA) per kg of burger. In addition, the antioxidant potential, expressed as percentage of antioxidant activity (AOA), was calculated by the equation (Wijewickreme & Kitts, 1998):

$$\%\,\text{AOA} = \frac{[TBARS\ value\ of\ the\ control - TBARS\ of\ the\ test\ sample]}{[TBARS\ value\ of\ the\ control]} x100$$

2.3.6 Sensory properties

The cooked burgers were arranged in randomized order and served to the panelist at the same temperature and the participants were provided with unsalted crackers and water. A panel of 9 judges was selected among the staff of the Department. The subjects were equally distributed by gender, with an age range of 20 to 40 years. All participants had previous familiarity with sensory analysis and had been previously trained for the specific type of test chosen. Additionally, the judges were all regular consumers of hamburgers and pork, and the analyses were conducted in a teaching laboratory with natural lighting and a minimum distance of one and a half meters between each judge.

An acceptability test with a hedonic scale (from 0 to 5) was used; it was required to express an opinion on 7 parameters: color (0=not acceptable; 5=acceptable), olfactory evaluation (0=unpleasant aroma; 5=pleasant aroma), tenderness (0=not tender; 5=very tender), bitterness (0=absent; 5=high), sapidity (0=not sapid; 5=very savory), astringency (0=not astringent; 5=very astringent), overall liking (0=not appreciated; 5=greatly appreciated).

2.4 Statistical analysis

Data regarding the characterization of by-products (proximate composition, crude fiber, TPC, ABTS, microbiological load, and fatty acid composition were expressed as mean ± standard deviation (SD) of three different samples analyzed in triplicate. Data from burger analyses were submitted to analysis of variance using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA), assuming a level of at least $P< 0.05$ for statistical significance. The statistical models included as fixed effect the treatments (C, HS, and DTP) for moisture, crude protein, crude fat, crude fiber, and fatty acid composition of raw burgers. Treatments, storage days (0, 4, 7), and relative interactions were included for sensory analysis, pH, cooking loss, diameter variation, and microbiological load. Moreover, for MDA content and color parameters, cooking treatment was included in the model. The differences between means were tested by t-test (SAS/GLM PDIFF option).

3. Results and discussion

3.1 By-product characterization

The results of by-products characterization are shown in Table 1.

Table 1. Proximate composition, fatty acid composition, total phenolic contents (TPC), microbiological count, and antioxidant activity (ABTS and FRAP) of hazelnut skin and dry tomato peel (Mean ± SD).

	Hazelnut skin (n=3)	**Dry tomato peel (n=3)**
Moisture %	5.30 ± 0.001	5.05 ± 0.002
Crude lipids %	24.44 ± 1.30	11.36 ± 0.02
Crude protein %	5.99 ± 0.24	17.10 ± 0.10
Crude fiber %	21.70 ± 1.82	43.40 ± 1.95
Saturated fatty acids (SFA) %	9.20 ± 0.60	21.90 ± 0.20
Monounsaturated fatty acids (MUFA) %	77.20 ± 0.05	22.30 ± 0.50
Polyunsaturated fatty acids (PUFA) %	13.60 ± 0.01	55.80 ± 0.20
Aerobic mesophilic count (log UFC/g)	2.11 ± 0.21	4.19 ± 0.04
Enterobacteriaceae count (log UFC/g)	n.d.	3.14 ± 0.08
TPC (mg GAE/g)	125.91 ± 5.10	0.94 ± 0.07
ABTS (μM Trolox eq/g)	1041.26 ± 54.33	1.81 ± 0.13
FRAP (μM $FeSo_4$/g)	296.39 ± 5.92	--

n.d.: not detectable.
TPC: Total phenolic compounds
ABTS: 2, 2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic)
FRAP: Ferric reducing antioxidant power

In the present study, the moisture content of dry tomato peels agrees with Navarro-González et al. (2011); in contrast, Darwish, El-Hakim, El-Rahman, & Megali (2019) found a higher value. This difference may be directly related to the technological process the product goes through and, as a result, to the amount of residual pulp that is still present. The total lipid was found to be substantially higher in percentage than the values in the literature (Del Valle et al., 2006; Navarro-González et al., 2011; Elbadrawy & Sello, 2016; Darwish et al., 2019) and this could be due to the presence of seeds traces in our by-product that raised the level of lipids. The same goes for protein values that in other studies ranged from 10.50 % to 24.67 %, so our results were within the range (Elbadrawy & Sello, 2016). The fiber content and fatty acid composition were also comparable to that observed by other Authors (Darwish et al., 2019; Elbadrawy & Sello, 2016).

The chemical properties of the hazelnut peel vary greatly depending on the cultivar examined. Our results agree with Longato et al. (2019). This could be because the Authors used hazelnuts from a similar geographical region to ours. The moisture content has been found to be below the literature average (Locatelli et al., 2010; Longato et al., 2019). Instead, the lipid content has been found to be higher than most published research (Anil, 2007; Bertolino et al., 2015). While protein and, particularly, fiber contents were deficient compared to the values observed by other Authors (Locatelli et al., 2010; Turhan et al., 2005). The fatty acid composition was consistent with the literature (Özdemir et al., 2014).

From a microbiological standpoint, hazelnut skin had low loads while a significant microbial load was found in the dry tomato peel (Table 1) but given that there are no regulations governing microbiological limits for these by-products, keeping in mind those of vegetable products and meat to be consumed after cooking, these two by-products fell within the established limits (ICMSF, 1986; EC No 2073/2005).

Dry tomato peels exhibited values for TPC and ABTS that were lower than those determined by Darwish et al. (2019); this finding may be explained by the genetic type of the cultivar as well as the fractions of peel, seeds, and pulp present (Chandra, Shanmugaraj, Srinivasan, & Ramalingam, 2012). Hazelnut skin had a lower level of total phenolic compounds and FRAP, but ABTS value according to other Authors (Del Rio et al., 2011; Özdemir et al., 2014; Bertolino et al., 2015).

3.2 Proximate composition of burgers

The effect of treatment on the chemical composition and fatty acid profile of pork burgers are reported in Table 2.

Table 2. Effect of treatment on chemical composition (%) and fatty acid content (% of total fatty acid detected) of pork burgers.

	C	HS	DTP	R-MSE($)
Moisture	64.53	64.60	66.41	4.65
Crude lipids	10.24	10.23	11.13	1.95
Crude protein	24.84	24.97	25.14	2.08
Crude fiber	Tr (§) c	0.58^{b}	1.03^{a}	0.12
SFA	39.75^{a}	37.52^{b}	39.17^{a}	1.04
MUFA	48.01^{b}	49.71^{a}	47.04^{c}	1.02
PUFA	12.24^{c}	12.77^{b}	13.78^{a}	0.59
ω3	0.65	0.80	0.78	0.08
ω6	11.59^{b}	11.98^{b}	13.00^{a}	0.55
ω6/ω3	18.28^{a}	15.09^{c}	16.78^{b}	1.73

C: control burger; HS: hazelnut skin burger; DTP: dry tomato peel burger.
(§): Traces.
($): Root Mean Square Error
a, b, c: different letters on the same line indicate differences for $P<0.05$.

Based on the data (Table 2), it can be observed that adding 2.5% of hazelnut skin did not have a significant effect on moisture, crude lipid, and crude protein contents of pork burgers. This is consistent with a study by Turhan et al. (2005) who did not observe an increase in protein content in beef burgers with the addition of hazelnut skin. As regards dry tomato peel the result showed that its addition tended to increase slightly moisture, protein, and fat content. This tendency is in contrast with a study by Candogan (2002), in which no increase in protein and fat content was observed in beef balls added 5% and 10% of tomato paste.

As shown in Table 2, the fiber content was 0.58% for burgers with hazelnut skin and 1.03% for burgers with dry tomato peels. This indicated that the addition of these by-products provided fiber intake ($P<0.05$) in pork burgers, which commonly lack this component. The levels reached can be

further improved to meet the nutritional requirements of increasingly elaborate diets. Therefore, it is necessary to precisely establish the levels of these by-products to be incorporated into meat-based products without altering their sensory characteristics that are acceptable to consumers.

Regarding the effect of by-products on the fatty acid composition, hazelnut skin led to a significant increase ($P<0.05$) in monounsaturated and polyunsaturated fatty acids, while dry tomato peel resulted in a significant increase ($P<0.05$) in polyunsaturated and omega-6 fatty acids. Both treatments contributed to a decrease significantly ($P<0.05$) in the omega-6/omega-3 ratio. Making this kind of change in the product is important because nutritional guidelines (e.g., FAO/WHO, 2008) suggest that in order to reduce the incidence of various non-infectious diseases, such as diabetes, some forms of cancer, and cardiovascular disease (CVD), the intake of total fat, saturated and polyunsaturated fatty acids (SFAs and PUFAs), and the ratio of ω6: ω3 PUFAs should be within well-defined limits. Despite the significant reduction, we are still far from the optimal level, which should range between 1:1 and 4:1 (Simopoulos, 2002; 2010). However, it should be noted that this value is recommended for the specific diet, and therefore, this reduction can still be considered a contribution to the nutritional improvement of the product.

The effect of treatment and storage time on pH value, cooking loss, diameter variation, and microbial load of pork burgers were reported in Table 3.

Table 3. Effect of treatment and storage time on pH, cooking loss, diameter variation, and microbial load of pork burgers

	TREATMENT									R-MSE[($)]
	C			HS			DTP			
	SD0	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	
pH	5.58	5.48	5.52	5.53	5.42	5.42	5.53[a]	5.36[b]	5.36[b]	0.14
Cooking loss (%)	13.91[e]	13.14[e]	13.94[e]	11.06[f]	9.55[f]	9.43[g]	10.57[f]	10.32[f]	12.41[e]	2.91
Diameter variation (%)	3.34	5.59	4.07	3.29	4.77	2.93	5.23	4.48	3.90	2.90
Aerobic mesophilic count (log UFC/g)	5.03[c]	6.47[b]	7.14[a]	4.53[c]	6.68[b]	7.37[a]	4.62[c]	6.60[b]	7.40[a]	0.56
Enterobacteriaceae count (log UFC/g)	3.97[b]	4.93[a]	5.18[a]	3.99[b]	5.16[a]	5.26[a]	3.95[b]	5.29[a]	5.21[a]	0.57

C: control burger; HS: hazelnut skin burger; DTP: dry tomato peel burger; SD: storage days.

[a, b, c]: different letters on the same line indicate differences for P<0.05 between storage days within each treatment.

[e, f, g]: different letters on the same line indicate differences for P<0.05 between treatments within the same storage day.

[($)]: Root Mean Square Error

The pH values of both treated groups showed a tendency to be lower than the control group, although the difference was not statistically significant (P>0.05). This finding is consistent with previous studies on hazelnut skin, where it was observed that this by-product did not cause a significant decrease in pH value in beef burgers (Turhan et al., 2005) and chicken burgers (Longato et al., 2019). Regarding DTP, García et al. (2009) reported a significant reduction in pH with an increase in the concentration of DTP in beef burgers. In our research, during the storage period, the pH value significantly decreased (P<0.05) only in the samples with DTP from day 0 to day 4. The diameter of the burgers was not influenced by the treatments or storage time. Both by-products reduced significantly (P<0.05) the cooking loss, instead storage time did not affect this parameter (Tab.3). Our results agree with Turhan et al. (2005), who observed that hazelnut skin added to beef burgers reduced cooking loss. The microbial load was not influenced by the by-products inclusion but increased significantly (P<0.05) during storage for both classes of microorganisms sought. Considering the limits reported by Regulation (EC) No. 2073/2005 the microbial load fell within these limits until the 4^{th} day of storage, on the 7^{th} day the limit was exceeded. This may be due to the mode used of storage that did not involve changes in atmospheric gas concentration in the container, regardless of the formulation of the hamburger.

3.4 Effect of treatment, storage time, and cooking on color parameters of pork burgers

The data presented in Tables 4 and 5, respectively for raw and cooker burgers, show that the color parameters of burgers were significantly affected (P<0.05) by treatment and storage time.

Table 4. Effect of treatment and storage time on color parameters of raw pork burgers

	TREATMENT									R-MSE($)
	C			HS			DTP			
	SD0	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	
L*	50.39$^{b\,e}$	52.68$^{ab\,e}$	54.51$^{a\,e}$	35.84^{f}	36.68^{g}	37.87^{g}	48.39^{e}	48.66^{f}	49.97^{f}	3.09
a*	6.39$^{a\,f}$	3.51$^{b\,g}$	2.21$^{c\,g}$	6.36$^{a\,f}$	5.92$^{ab\,f}$	4.82$^{b\,f}$	15.72$^{a\,e}$	12.51$^{b\,e}$	10.37$^{c\,e}$	1.69
b*	13.97$^{a\,f}$	12.46$^{ab\,f}$	12.12$^{b\,f}$	8.27^{g}	7.97^{g}	7.71^{g}	24.21^{e}	22.92^{e}	23.03^{e}	3.22
C*	15.42$^{a\,f}$	12.97$^{b\,f}$	12.34$^{b\,f}$	10.45^{g}	9.94^{g}	9.12^{g}	28.89$^{a\,e}$	26.13$^{b\,e}$	27.27$^{b\,e}$	4.26
H*	65.72$^{c\,e}$	74.39$^{b\,e}$	79.78$^{a\,e}$	52.46$^{b\,g}$	53.38$^{b\,g}$	57.75$^{a\,g}$	57.11$^{c\,f}$	61.42$^{b\,f}$	65.71$^{a\,f}$	5.67

C: control burger; HS: hazelnut skin burger; DTP: dry tomato peel burger; SD: storage days.

$^{a, b, c}$: different letters on the same line indicate differences for P<0.05 between storage days within each treatment.

$^{e, f, g}$: different letters on the same line indicate differences for P<0.05 between treatments within the same storage day.

($): Root Mean Square Error

Table 5. Effect of treatment and pre-cooking storage time on color parameters of cooked pork burgers

	TREATMENT									R-MSE($)
	C			HS			DTP			
	SD0	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	**SD0**	**SD4**	**SD7**	
L*	61.28$^{b\,e}$	64.46$^{a\,e}$	65.23$^{a\,e}$	40.86$^{b\,g}$	42.90$^{ab\,g}$	45.00$^{a\,g}$	*56.05*$^{b\,f}$	55.22$^{b\,f}$	59.18$^{a\,f}$	3.09
a*	5.50$^{a\,f}$	3.91$^{b\,g}$	3.26$^{b\,g}$	6.45^{f}	5.67^{f}	5.50^{f}	11.80$^{a\,e}$	11.96$^{a\,e}$	10.08$^{b\,e}$	1.69
b*	21.60$^{a\,f}$	18.72$^{b\,f}$	17.92$^{b\,f}$	11.95^{g}	10.89^{g}	10.64^{g}	31.07$^{a\,e}$	27.46$^{ab\,e}$	27.00$^{b\,e}$	3.22
C*	22.35$^{a\,f}$	19.15$^{b\,f}$	18.23$^{b\,f}$	13.60^{g}	12.30^{g}	11.99^{g}	28.89$^{a\,e}$	30.01$^{b\,e}$	28.84$^{a\,e}$	4.26
H*	76.34$^{b\,e}$	78.70$^{a\,e}$	79.97$^{a\,e}$	61.64^{g}	62.23^{g}	62.47^{g}	67.90$^{b\,f}$	66.96$^{b\,f}$	69.52$^{a\,f}$	5.67

C: control burger; HS: hazelnut skin burger; DTP: dry tomato peel burger; SD: storage days.

$^{a, b, c}$: different letters on the same line indicate differences for P<0.05 between storage days within each treatment.

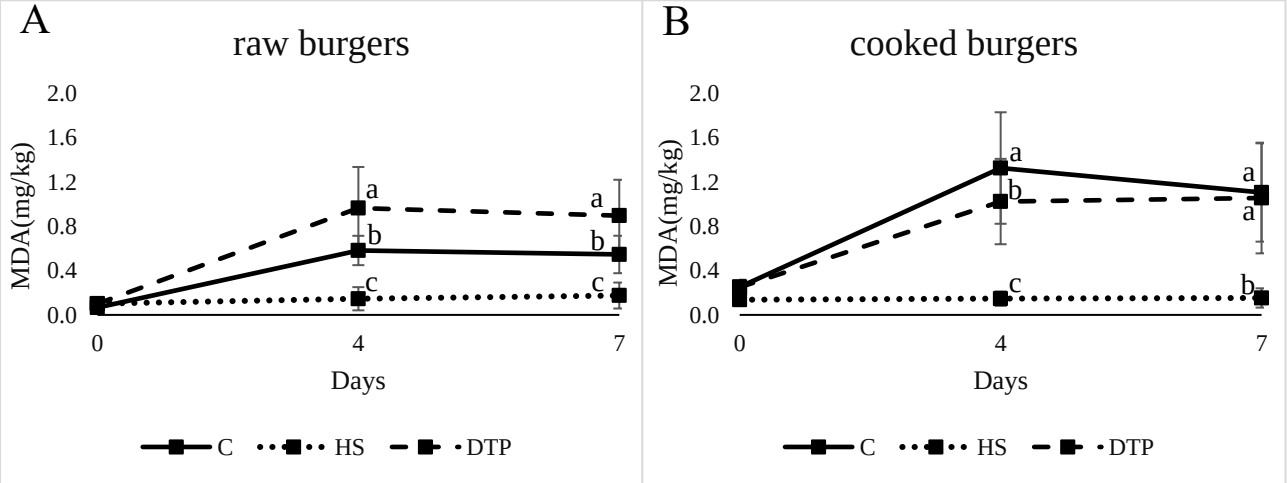
$^{e, f, g}$: different letters on the same line indicate differences for P<0.05 between treatments within the same storage day.

($): Root Mean Square Error

The addition of hazelnut skin and tomato peel has respectively given a brown and red color to the burgers. The brown color of the hazelnut film is evident from the values of L* and b*, which are always lower ($P<0.05$) than the other two groups both in row (Table 4) and cooked (Table 5) burgers. On the other hand, DTP samples show higher ($P<0.05$) values of a*, b*, and C* than C and HS raw and cooked burgers, indicating a reddish coloration and a higher intensity of color, as also reported in previous research (Candogan, 2002; Luisa García et al., 2009; Kim et al. 2013). The color changed significantly ($P<0.05$) over time (Tab. 4) mostly in C and DTP groups, as it appeared brighter on day 0 and decreased in intensity on days 4 and 7 of storage, as evidenced by the lower ($P<0.05$) values of the parameters a*, b*, and C*, while the HS group showed more stability. As regards cooked burgers (Table 5), their higher L* values than raw burgers could be explained by a surface coating of melted fat that formed during cooking. The same trend could be observed in b*, C*, and h* values while the a* value was lower in cooked than in raw burgers except for the HS group, and these variations may be due to the Maillard reaction effects on cooking color changes (Luisa García et al., 2009).

3.5 Lipid oxidation of raw and cooked burgers

Figure 1. Lipid oxidation of (A) raw and (B) cooked burgers (MDA mg/kg) at different days of refrigerated storage



C: control burgers; HS: hazelnut skin burgers; DTP: dry tomato peel burgers
Treatment: ^{a,b,c} $P<0.05$
Day: $P< 0.05$ only for C and DTP between 0 to 4 and 0 to 7 days in both raw and cooked burgers

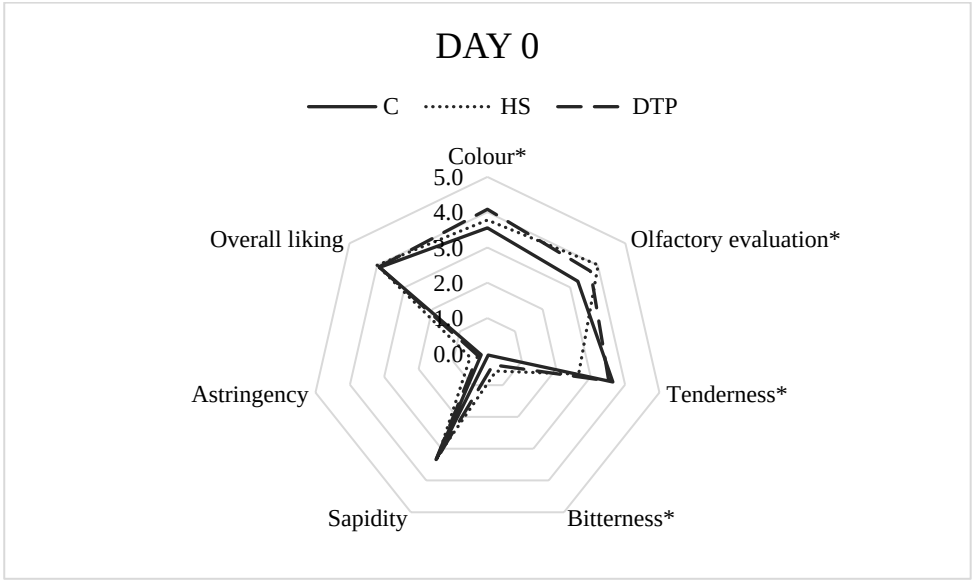
The results highlighted that HS burgers exhibited a high inhibition of oxidative phenomenon during all storage times of 7 days in both raw and cooked burgers (Figure 1). This group showed very high AOA% values, with peaks of 88% in raw samples and 94% in cooked samples (data not shown). There are few studies that have explored the use of hazelnut skin in meat preparations; Longato et al. (2019) reported that increasing the amount of hazelnut skin in chicken burgers decreased oxidative stability over a storage period of 4 days, attributing this to the high content of polyphenols, which can have a pro-oxidizing effect. This result contrasts with Olszowy (2019) that attributes to polyphenols an antioxidant effect as confirmed in our study.

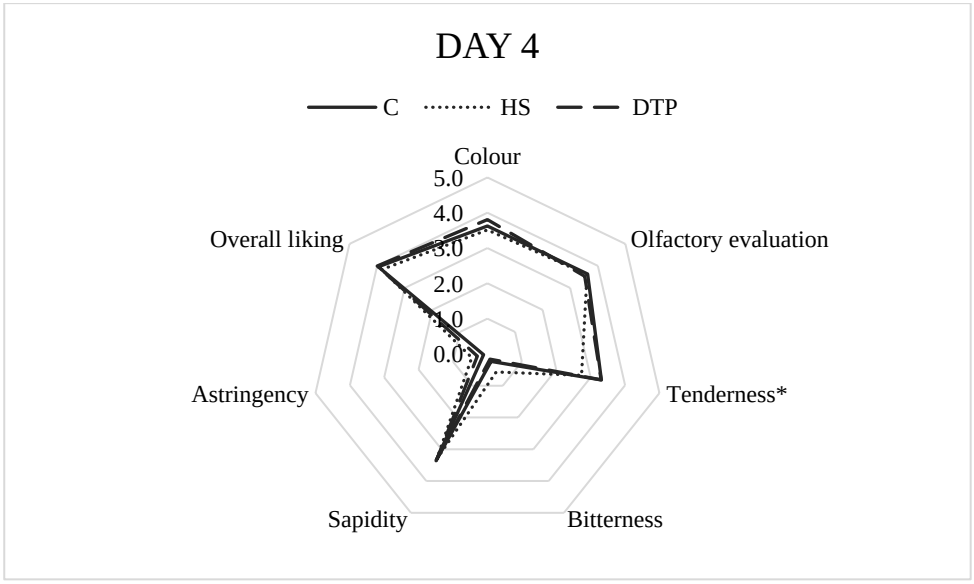
During storage time, dry tomato peels did not have any protective effect in raw samples, where MDA values were higher (P<0.05) than the other two groups both at the 4th and 7th days of refrigerated storage. A possible explanation of this result could be that in general the antioxidant effect of this by-product may be dose- and time-dependent, as verified by Candogan (2002), in which increasing the percentage of tomato by-products in beef burgers resulted in increased antioxidant capacity and by Kim & Chin (2013) who found that the tomato powder added to pork sausages did not show an antioxidant effect until after 21 days of storage. In cooked DTP samples, however, an effect against the oxidative phenomenon occurred, indicating that the by-product protected the lipids during cooking, but only on 4th day of storage. Overall (data not shown), regardless of storage period and treatments, the cooking process increased the MDA content of burgers by 42% (0.40 vs 0.69 mg/kg, P<0.05) but the increase was different between the three groups (+169% in C, P<0.05, +26.2% in DTP, P<0.05 and +6.3% in HS, P<0.05) showing a strong antioxidative activity of hazelnut skin.
Although there are no legal limits for these types of products regarding MDA content, it is believed, as reported by Trindade, Mancini-Filho, & Villavicencio (2010) for beef and by Longato et al. (2019) for chicken, that the level of MDA during storage should be kept below 2 mg/kg. In the present study, the MDA value was found to be significantly lower even after cooking in all formulations used, further highlighting the protective effect exerted by the hazelnut skin.

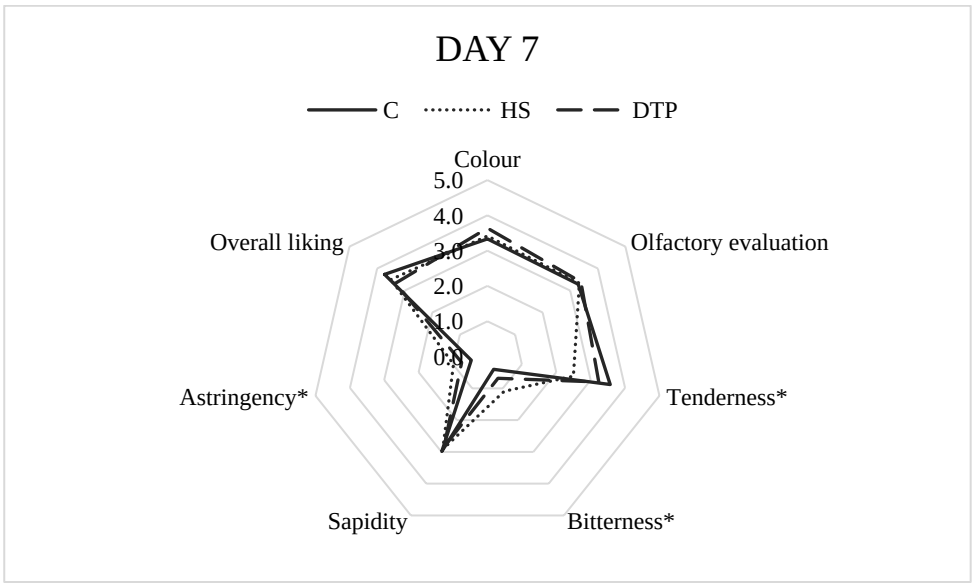
3.6 Sensory evaluation

Overall, the sensory properties of the burgers were not significantly impacted by the addition of by-products, except for bitterness, astringency, and tenderness, which were negatively influenced by the treatments (Figure 2).

Figure 2. Sensory evaluation of the pork burgers at different sampling days







C: control burger; HS: hazelnut skin burger; DTP: dry tomato peel burger.
* Treatment: P<0.05

Bitterness and astringency had higher values in HS and DTP samples, inversely tenderness tended to decrease in supplemented burgers compared to the control group. However, it is important to note that these negative effects did not reach critical values and did not significantly affect overall satisfaction, which was always equal in the treated groups compared to the control. The negative effects on tenderness may be attributed to the fibrous component present in the by-products, which can alter the chewability of the finished product. Additionally, tannins present in the hazelnut film may cause a common sensation of astringency on the palate. Similar results were found in studies conducted by Longato et al. (2019) on chicken burgers with hazelnut skin and by Eyiler & Oztan (2011) on sausages containing tomato powder, where tenderness was compromised by the added products. On the other hand, some sensory qualities such as color and smell were found to be improved by the presence of by-products, as reported in studies conducted by Kim, Jin, Mandal, & Kang (2011), Kim et al. (2013), and Longato et al. (2019). These improvements may be due to the flavor produced by the by-products during cooking and to the color properties that they apport that

can mask the characteristic smell and white color of cooked pork meat, which is not appreciated by everyone. It is worth noting that during refrigerated storage, even for a short period of only 7 days, all sensory parameters tended to worsen in all treatments, regardless of the presence of by-products. This could be due to the type of storage that only involved refrigeration without modification of the atmosphere inside the box.

4. Conclusions

Our work demonstrates that the presence of hazelnut skin in pork burgers reduced lipid oxidation making the product more stable both during storage and cooking. While dry tomato peel presented a protective potential only in cooking, suggesting that research on this by-product should be implemented perhaps by changing the dose and storage time. Both by-products provided a significant contribution to the amount of fiber, especially in the case of dry tomato peel, and affected the fatty acid composition of burgers by increasing PUFA, contributing to a decrease in the omega-6/omega-3 ratio. According to these results, incorporating hazelnut skin and dry tomato peel into pork burgers improves their nutritional profile maintaining microbial stability and without affecting sensory acceptability. This innovative approach offers a sustainable solution for utilizing food by-products and producing healthier meat products.

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Hazelnut skin polyphenolic green extract as a promising natural antioxidant in pork burgers: Assessment of quality parameters and consumer acceptance

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Abstract

Given the increasing consumer focus on healthier and environmentally friendly foods, the use of natural antioxidants in food production is becoming more common. The recovery of these antioxidants from agri-food waste is crucial for a circular economy, as it revalues matrices that would otherwise become waste. This study aimed to assess the antioxidant capacity of hazelnut skin and its green polyphenolic extract and to evaluate their effect on some qualitative parameters of pork burgers. Three types of burgers were formulated: a control group, and two experimental groups with the addition of 2.5% of hazelnut skin or 1% of hazelnut green extract. On days 0 and 7 of refrigerated storage (0-2 °C) parameters such as color, cooking losses, tenderness, lipid oxidation, and volatile profile were evaluated. Additionally, a group of panelists was asked to assess the acceptability of color and the potential for purchase. In both raw and cooked burgers, at all times examined, the two experimental groups showed a significant improvement in oxidative stability and lower production of volatile fat oxidation compounds compared to the control in which the main indicators of pork meat spoilage were detected. Although, even if on the 7th day of storage, the HS and HSE burgers exhibited better color stability, these groups showed a worsening in terms of color acceptability and tenderness. Overall, despite trade-offs, the hazelnut skin and their green extract showed high potential to emerge as food additives in meat products.

Chapter 4. Hazelnut skin polyphenolic green extract as a promising natural antioxidant in pork burgers: assessment of quality parameters and consumer acceptance

1. Introduction

Lipid oxidation represents one of the main chemical processes that affect the quality and shelf-life of meat and meat products. This phenomenon involves the degradation of unsaturated fatty acids present in lipids, leading to the formation of secondary compounds such as aldehydes and ketones, which are responsible for sensory and nutritional alterations (Wang et al., 2023). In addition to compromising the taste, aroma, and color of the meat, lipid oxidation is associated with a reduction in food safety, as it can promote the formation of potentially harmful compounds such as hexanal, pentanal, heptanal and octanal that are responsible for quality deterioration and represent health risks, including carcinogenesis (Arabshahi-Delouee et al., 2007). Generally, synthetic antioxidants are used to prevent this phenomenon but in recent years, the discussion around the use of synthetic antioxidants in animal production has intensified, driven by growing health concerns and the demand for more natural food products. The most commonly used synthetic antioxidants in food are phenolic antioxidants (Rodil et al., 2012), for example, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ). The toxicity of BHA, BHT, and TBHQ has been investigated extensively using a variety of experimental conditions and the results show that an excessive addition or incorrect use of synthetic phenolic antioxidants results in carcinogenicity, cytotoxicity, oxidative stress induction, endocrine disrupting effects, which warrant attention (Xu et al., 2021).

This problem and the shift in consumer preferences have catalyzed a surge in research focused on developing and applying natural antioxidants in food production systems (López-Pedrouso et al., 2022; Novais et al., 2022; Pateiro et al., 2021). Notably, several studies reported that various plant extracts, that support consumer health, might even have an antioxidant capacity stronger than synthetic antioxidants (Jayawardana et al., 2019; Zhang et al., 2018). In particular, polyphenol extracts, renowned for their antioxidant properties, are increasingly being studied for their application in meat products (Gutiérrez-del-Río et al., 2021; Manessis et al., 2020; Munekata et al., 2020). It is well known that agro-industrial wastes are a great source of phenolic compounds with antioxidant power.

Agro-industries generate numerous waste materials rich in bioactive compounds very interesting from a technological and nutritional point of view. The recovery of these bioactive compounds from agricultural and food industry waste presents a unique opportunity to enhance the sustainability of food production. Utilizing these compounds not only contributes to the creation of healthier animal products but also supports the principles of a circular economy by revaluing waste materials that would otherwise contribute to environmental degradation aligning seamlessly with the goals of the Green New Deal.

However, it is noteworthy that most natural extracts are currently obtained using solvent-based methods (Bubalo et al., 2018), which may pose sustainability and food safety challenges. Since most

bioactive compounds are not soluble in water, conventional extraction methods are very time-consuming, labor-intensive, and require large amounts of solvents like alcohols, hydrocarbons, and chloroalkanes. In the end, these methods may result in some target molecule degradation and partial volatile loss (Cravotto et al., 2008).

Above all, the yield is frequently extremely low despite the considerable energy consumption and the vast number of solvents (Chemat et al., 2012).

To address these challenges, it is necessary to develop and implement green extraction techniques such as supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction, that use less harmful solvents or none at all, reducing the environmental footprint and improving the safety profile of the extracts (Carpentieri et al., 2021).

An innovative example that combines a valuable by-product and a green extraction technique is the phenolic extract from hazelnut skin. Italy is the second-largest producer of hazelnuts, accounting for nearly 20% of global production and 15% of exports. It also has the highest per capita annual hazelnut consumption among producing countries, at 0.520 kg per person. It is estimated that 90% of the hazelnuts produced in Italy go to processors while the remaining 10% are destined for fresh consumption (Forte et al., 2022; Misachi, 2018), this means that just under 3 k tons of hazelnut skin are made in Italy yearly from an average annual hazelnut production of about 110 k tons (FAOSTAT, 2023). The well-documented properties of hazelnut skin, particularly its high polyphenol content, have spurred interest in its application within the food industry (Ollani et al., 2024). Various studies have already explored the potential uses of hazelnut skin (Bertolino et al., 2015; Costantini et al., 2023) and demonstrated its effectiveness as a natural preservative in meat (D'Ambra et al., 2023). Its potential is continuously being studied, particularly Capaldi et al. (2025), who investigated the development of a green phenolic extract through subcritical water extraction of bioactive compounds. The evaluation of polyphenolic profiles and antioxidant activities of this extract provided promising results compared to the benchmark of reflux maceration, both in the laboratory and on a semi-industrial scale.

This study aims to characterize the antioxidant power of hazelnut skin and a green phenolic extract obtained from hazelnut skin and to explore and validate their effects on the oxidative stability and chemical and sensory properties of pork burgers during refrigerated storage.

2. Material and methods

2.1. Hazelnut skin (HS) and hazelnut skin extract (HSE)

The selection of HS as by-product for this study is rooted in its dual potential as a functional ingredient and a sustainable raw material. Our previous study confirmed the potential of hazelnut skin as an effective antioxidant and nutritional additive (D'Ambra et al., 2023). Building on these findings, the current study utilizes it as a positive control and further investigates its functionality to validate and expand upon its beneficial properties. Different Italian companies supplied HS that had the following nutritional composition: moisture 5.3%, lipid 24.4%, protein 6%, fiber 21.7%, and saturated fatty acids 9.2%, monounsaturated fatty acids 77.2%, polyunsaturated fatty acids 13.6% as % of total fatty

acids. Instead, the HSE was produced by the University of Turin as described by Capaldi et al. (2025) from the same hazelnut skin.

2.2. Extraction of phenolic compounds and antioxidant activity

The HS required the free phenolic extraction process to undergo assays to characterize the total phenolic content and antioxidant activity while HSE was directly diluted in water and used for the assays. The extraction of free phenolic compounds from the HS was performed according to the method described by D'Ambra et al. (2023) with some modifications. Briefly, 2.5 g of this by-product was homogenized with 12.5 mL of a methanol/water/formic acid solution (in the ratio 70:28:2, v/v/v) using an Ultra-Turrax homogenizer (IKA, Germany) for 1 min. The resulting suspension was centrifuged at 6000 rpm for 15 min at 4 °C, using a Remi Elektrotechnik LTD (Model NEYA 16R, Mumbai, India). The supernatant was accumulated, and the pellet was resuspended with 12.5 mL of fresh solution. This process was repeated thrice to fully extract the phenolic compounds from the initial 2.5 g sample. The polyphenol-rich extracts obtained were stored at 0-4 °C for subsequent analyses.

2.2.1. Total phenolic content (TPC)

The total phenolic content of HS and HSE was determined using the Folin-Ciocalteu assay (Singleton et al., 1999) with some modifications: 1975 µL of distilled water was mixed with 25 µL of the sample and 125 µL of Folin reagent (1.8-2.2 mol/L). After exactly one minute, 375 µL of 20% Na_2CO_3 was added, and the samples were stored in the dark for 2 h. At the end of incubation, absorbance values were measured at 765 nm using a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan). The standard used to create the calibration curve was gallic acid, so the results are expressed as milligrams of gallic acid equivalent/grams by-products (mg GAE/g).

2.2.2. ABTS assay

The antioxidant activity of HS and HSE was evaluated as described by Re et al. (1999) with the ABTS method. This assay uses the chromogen 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, AppliChem GmbH). The antioxidant capacity is measured by the reduction in absorbance at 734 nm of the ABTS•+ radical cation in the presence of antioxidants. The ABTS radical cation (ABTS•+) is produced by combining a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to react in the dark overnight. The ABTS•+ solution is then diluted in methanol to achieve an absorbance (A_0) of 0.705 ± 0.005 at 734 nm. Next, 100 µL of the diluted sample was combined with 1400 µL of ABTS•+ solution and kept at 20 °C for 10 min in the dark. The final absorbance at 734 nm (A_f) was measured. The percentage of scavenging ($S\%$) was calculated using the ensuing formula:

$$S\% = ((A_0 - A_f) / A_0) \times 100$$

A_0 represents the initial absorbance (control), and A_f represents the final absorbance (sample). The standard for constructing the calibration curve is Trolox (6-hydroxy-2,5,6,7-tetramethylchroman-2-

carboxylic acid), therefore, the antioxidant activity was expressed as mmol of Trolox equivalent per gram of by-product.

2.2.3 FRAP assay

The antioxidant capacity of HS and HSE was also measured as ferric reducing/antioxidant power by adopting FRAP assay as described by Benzie and Strain (1999). The procedure is grounded on reducing the Fe^{3+}–2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form under acidic conditions. Concisely, 3 mL of the FRAP assay solution (composed of 20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was prepared daily and united with 100 µL of the sample. The absorbance was recorded at 593 nm at ambient temperature after 6 min of incubation. The results were reported as µmol of $FeSO_4$ per gram of by-product.

2.2.4 DPPH assay

The DPPH method for estimating the antioxidant activity of HS and HSE was performed according to the method described by Helal et al. (2012). A DPPH (2,2-diphenyl-1-picrylhydrazy) solution (0.1 mM) was prepared in methanol and kept in darkness for 30 min to complete the reaction. After that 200 µL of the samples were mixed with 2 mL of DPPH solution and incubated in the dark in a shaker for 30 min. Then the absorbance of the resulting solutions was measured at 517 nm using a UV–visible spectrophotometer against a blank without a sample. The activity was calculated after the sample blank subtraction and expressed as mg of vitamin C per gram of sample.

2.2.5. Identification and quantification of phenolic compounds in hazelnut skin and hazelnut skin green extract by high-resolution mass spectrometry (UHPLC/MS)

The phenolic compound profiles of HS and HSE were determined as reported in Cattivelli et al. (2023). Before the injection in the high-resolution mass spectrometer, phenolic compounds were extracted from the samples by following the protocol previously described in Section 2.2. of this paper. Phenolic compounds were firstly separated using a C18 column (Acquity UPLC HSS C18 Reversed phase, 2.1 × 100 mm, 1.8 µm particle size, Waters, Milan, Italy) in an UHPLC Ultimate 3000 module (Thermo Fisher Scientific, San Jose, CA, USA) before being analyzed with a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The chromatographic separation and mass spectrometry settings are fully described in Martini et al. (2020). Quantification was carried out by building external calibration curves with the available standard compounds, as reported in Table S1 of Supplementary Material.

2.3. Pork burgers formulation

Burgers were prepared by grinding pork loin, with an average value of pH of 5.71 ± 0.06, with backfat purchased from the commercial market using an electrical grinder with a sieve of 7 mm of diameter (Trita Express, R.G.V. s.r.l., Como, Italy). Three types of burgers were formulated: a control burger with 88.5% *longissimus dorsi* muscle, 10% subcutaneous adipose tissue, and 1.5% sodium chloride; and two other groups with the addition of 2.5% hazelnut skin (HS) and 1% phenolic extract (HSE), respectively. The burgers were modeled using a burger press (50 g ± 0.5 g minced meat, 6 cm in

diameter and 1 cm in thickness). The concentrations were chosen based on the study conducted by D'Ambra et al. (2023) and taking into account the total polyphenol content and the antioxidant activity shown *in vitro* by the products. The analyses were conducted three times in triplicate. For each cycle, 9 burgers (3 of each type) were analyzed at day 0 (T_0), and 9 burgers were stored in a refrigerator at a temperature of 4 °C in a resealable polypropylene package with a capacity of 750 cc, normally used for domestic food storage (Contital s.r.l), in an unmodified atmosphere, for the analysis at day 7 (T_7). The cooking was done using a double plate grill (Bosch, Germany) at 180 °C for 3 min (core temperature reached 88.7 ± 4.5 °C).

2.4. Pork burger analysis

On each sampling day, the samples were subjected to various analyses, including the determination and evaluation of color, cooking loss, oxidation status (TBARS), volatile profile (HS-SPME), tenderness, fatty acid profile, and visual sensory acceptability analysis.

2.4.1. Color

Color of burgers was assessed on raw samples at T_0 and T_7 using a Minolta CM-600d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) with a window diameter of 8 mm, D65 as the light source, and 10° observer value. The colorimeter was calibrated with a standard white plate before each measurement session. Three readings were taken at different side points of burgers per treatment and the measurement was made by pointing the colorimeter perpendicularly to the surface of the burgers. The values obtained from three measurements for each sample were collected, and the average was recorded. Color determination was executed according to the CIE L*a*b* color convention (CIE, 1986), in which there are three basic coordinates: L^* - "lightness"; a^* - "redness"; b^* - "yellowness". The results were expressed as

$$\Delta E = \sqrt{(L^*_0 - L^*_7)^2 + (a^*_0 - a^*_7)^2 + (b^*_0 - b^*_7)^2}$$

2.4.2. Cooking loss

For the determination of cooking loss, burgers were weighed before and after cooking on a home electric double-cast grill plate (Bosch, Germany) at 180 °C for 3 min. The parameter was determined in triplicate. Cooking loss percentages were then calculated as follows and as reported in a previous paper (D'Ambra et al., 2023):

$$Cooking\ loss\ (\%) = \frac{weight\ raw - weight\ cooked}{weight\ raw}\ x\ 100$$

2.4.3. Determination of oxidative stability

Lipid oxidation of raw and cooked burgers was determined by the thiobarbituric acid reactive substances (TBARS) assay using the method by Siu and Draper (1978). Initially, 2.5 g of minced burger were placed in an ice bath and homogenized with 12.5 mL of distilled water at 9500 rpm for 2 min utilizing an Ultra-Turrax homogenizer (IKA, Germany). Then, 12.5 mL of trichloroacetic acid

(TCA) 10% (Sigma-Aldrich, Milan, Italy) was added and the sample was centrifugated at 2000 rpm for 20 min at 4 °C. The supernatant was taken and filtered across Whatman No. 5 filter paper and 4 mL of this filtrate was relocated into Pyrex test tubes. A blank sample was prepared simultaneously (2 mL of distilled water + 2 mL of TCA solution + 1 mL of TBA). After that, 1 mL of 2-thiobarbituric acid (TBA, Sigma-Aldrich, Milan, Italy) 0.06 M was added in each tube and all samples were incubated in a water bath at 80 °C for 90 min. After cooling, the absorbance at 532 nm was measured using a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan). TBARS were expressed as mg of malondialdehyde (MDA) per kg of burger, using 1,1,3,3-tetraethoxypropane (TEP, Sigma-Aldrich, Milan, Italy) as a standard. Moreover, the antioxidant power was calculated as the following equation and expressed as a percentage of antioxidant activity (AOA) (Wijewickreme & Kitts, 1998):

$$\%\ AOA = \frac{[TBARS\ value\ of\ the\ control - TBARS\ of\ the\ test\ sample]}{[TBARS\ value\ of\ the\ control]}\ x\ 100$$

2.4.4. Determination of volatiles profile

Volatile profile was determined by headspace solid phase microextraction (HS-SPME) followed by gas chromatography/mass spectrometry (GC/MS) as described by Lopez-Moreno et al. (2023) with some modifications. The determination was performed on C, HS, and HSE samples, to evaluate the influence of both treatments on the formation of volatile lipid oxidation compounds during storage and cooking. The analysis was performed after 0 and 7 days of refrigerated storage. Four grams of raw burgers were weighted into 25-mL screw-cap glass vials provided with Mininert® valves (Merck KGaA, Darmstadt, Germany). Samples were conditioned at 80 °C for 30 minutes using a thermoblock (Falc Instruments, Treviglio, Italy). Subsequently, a triphasic SPME fibre DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was exposed in the headspace for 30 min at 80 °C to extract the volatile compounds. The analysis was carried out using an Agilent 6890 Gas Chromatograph coupled to a mass spectrometer Agilent 5973N Mass Selective Detector (MSD) (Agilent Technologies Inc., Santa Clara, CA, USA). Desorption of analytes from the fibre was performed in splitless mode at 240 °C for 5 min into the GC injector port. Helium was used as carrier gas for the chromatographic separation at a flow rate of 1 mL/min and the detector temperature was set at 240 °C. The column was maintained at 40 °C for 5 min, incrementally warmed to 150 °C at a rate of 5 °C/min, and ultimately elevated to 240 °C at 8 °C/min, where it was sustained for an additional 5 min. The mass spectrometer was recorded in positive mode m/z = 30-180 in full scan. Peak identification was carried out by comparison with system libraries (Wiley, NIST). Compounds were correctly identified if the library match factor was 50% or more. The circumstance that the same compound appeared in at least 50% of the samples was also considered. The abundance of aromatic compounds was determined by normalising the area of a compound to the total peak area of the chromatogram. The analyses were performed in duplicate.

2.4.5. Instrumental tenderness

To evaluate the tenderness of burgers by Warner-Bratzler shear force (WBSF) measurements reported by Belmonte et al. (2021) 3 cylindrical subsamples of approximately 10 mm in diameter were excised in triplicate from each type of cooked burger. The height of each sample was measured with a caliper. The analysis used a dynamometer (Z1.0, ZwickRoll, Ulm, Germany) with a 1kN load cell. The test involved uniaxial compression with a flat cylinder. The specific settings were: distance between tools of 50 mm; maximum deformation of 50%; test speed of 10 mm/min; and preload dynamometer of 1 N. The data were processed using the software TestXpert® II 161 (v3.31, ZwickRoll GmbH & Co. KG, Ulm, Germany). The results were expressed as the maximum force (N) required to reach 50% deformation.

2.4.6. Fatty acid profile

Following the method, lipids from raw pork burgers were extracted using a chloroform-methanol mixture (Folch et al., 1957), and the fatty acid composition was analyzed through capillary gas chromatography. According to Zappaterra et al. (2020), 50 mg of lipid extract was mixed with 2 mL of hexane and subjected to methylation using 200 µL of a 2 N potassium hydroxide methanolic solution (KOH provided by Carlo Erba, Milan, Italy, and methanol provided by ITW Reagents, Barcelona, Spain).

Consequently, the fatty acid methyl esters (FAMEs) were analyzed using a TRACE™GC Ultra gas chromatograph (Thermo Electron Corporation, Rodano, Milan, Italy) equipped with a Flame Ionization Detector, a Programmable Temperature Vaporizing (PVT) injector, and a TR-FAME Column (30 m long, 0.25 mm i.d., 0.2 µm film thickness) furnished by Thermo Fisher Scientific (Rodano, Milan, Italy). One µL of the methylated esters sample was injected into the gas chromatograph with a split flow rate of 10 mL/min, working at an uninterrupted helium flow of 1 mL/min as the carrier gas. The injector and detector temperatures were set at 240 °C. The temperature program began at 140 °C and increased by 4 °C/min to 250 °C, where it was retained for 5 min. The Chrom-card software (version 2.3.3, Thermo Electron Corporation, Rodano, Milan, Italy) was used to designate, identify, and integrate the peaks area. A solution of known concentrations of a standard fatty acid mix (Supelco 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellefonte, PA, USA, and single FAMEs standard, Larodan, Fine Chemicals AB, Malmö, Sweden) was used to distinguish the retention times of the FAMEs. The measure of each FAME was expressed as the relative percentage of the total amount of FAMEs using the normalized and correct area method.

2.4.7. Sensory analysis

The sensory analysis was conducted exclusively on raw burgers to evaluate acceptability of color and purchase intent (Lawless and Heymann, 2010). Panels of 15 judges from the Department of Life Science staff were selected for sensory analysis. The panel was regularly split by gender, with ages ranging from 20 to 40 years. All participants had prior experience with sensory analysis and had been specifically trained for this type of test. The panel was conducted in a teaching laboratory with natural

lighting, and each judge was seated at least one and a half meters apart. An affective test with a hedonic scale (from 0 to 5) was used; it was required to express an opinion on 2 parameters: color (0 = not acceptable; 5 = acceptable) and willingness to purchase (yes or not), and any notes or comments on the product. The results are expressed as the average of 45 sensory tests conducted at both sampling times across the three trials of the experiment.

2.5. Statistical analysis

Data regarding the characterization of by-products (TPC, ABTS, FRAP, DPPH, and phenolic compound profiles) were expressed as mean ± standard deviation (SD) of three samples analyzed in triplicate. Data from burger analyses were submitted to analysis of variance using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA), assuming a level of at least $P<0.05$ for statistical significance. The statistical models included as fixed effects the treatments (C, HS, and HSE), the storage days (0 and 7), and relative interactions. The differences between means were tested by t-test (SAS/GLM PDIFF option).

3. Results and discussion

3.1. Total phenolic content and antioxidant activity of by-products

HS had lower levels of total phenolic compounds and FRAP, but its ABTS and DPPH values were comparable to or higher than those reported by other Authors (Bertolino et al., 2015; Del Rio et al., 2011; Kruk et al., 2024; Özdemir et al., 2014). As for HSE, the TPC value was lower compared to that reported by the researchers who produced it, which was close to 800 mg/g GAE eq (Capaldi et al., 2025). Table 1 reports the phenolic content and antioxidant activity of the by-products.

Table 1. Total phenolic content (TPC) and antioxidant activity (ABTS, FRAP, and DPPH) of HS and HSE (Mean ± SD).

	Hazelnut skin	**Hazelnut skin green extract**
TPC (mg GAE/g)	174.61 ± 17.3	605.21 ± 11.3
ABTS (mmol Trolox eq/g)	1096.29 ± 19.3	2472.73 ± 154.3
FRAP (μmol $FeSO_4$/g)	784.34 ± 10.2	2951.37 ± 13.6
DPPH (mg Vitamin C/g)	272.68 ± 5.9	793.10 ± 32.2

TPC: Total phenolic compounds
ABTS: 2, 2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic)
FRAP: Ferric reducing antioxidant power
DPPH: 2,2-diphenyl-1-picrylhydrazy

3.2. Phenolic compound profiles of hazelnut skin and hazelnut skin green extract

As expected, the phenolic profiles of HS and HSE were dominated qualitatively and quantitatively by flavan-3-ols. The quantitative data are reported in Table 2.

Table 2. Amount of phenolic compounds identified in HS and HSE. Results are expressed in mg of phenolic compound/100g of sample.

Compound	Hazelnut skin	Hazelnut skin green extract
Protocatechuic acid	3.05 ± 0.05	7.49 ± 0.28
Gallic acid	2.48 ± 0.07	13.13 ± 0.39
Coumaric acid-*O*-pentoside	5.56 ± 0.06	2.36 ± 0.03
Ellagic acid	0.83 ± 0.11	71.77 ± 5.52
Galloyl shikimic acid (two isomers)	1.64 ± 0.03	6.35 ± 0.17
Galloyl-hexoside (three isomers)	0.22 ± 0.01	1.56 ± 0.02
Syringic acid-4-*O*-hexoside	3.20 ± 0.01	5.85 ± 0.22
Total phenolic acids	***16.98 ± 0.34***	***108.52 ± 0.58***
Epicatechin	81.82 ± 5.46	446.89 ± 11.96
Catechin	22.14 ± 1.06	242.28 ± 7.76
Epigallocatechin	1.39 ± 0.01	19.04 ± 0.41
Gallocatechin	0.40 ± 0.02	9.94 ± 0.35
Catechin-3-*O*-sulphate	n.d.	4.85 ± 0.33
Epicatechin-3-*O*-gallate	4.20 ± 0.13	27.16 ± 0.98
Catechin-3-*O*-hexoside	0.10 ± 0.00	0.24 ± 0.03
Epigallocatechin-3-*O*-gallate	0.15 ± 0.01	0.71 ± 0.02
Gallocatechin-3-*O*-gallate	n.d.	0.97 ± 0.02
Procyanidin dimer A-type (three isomers)	3.60 ± 0.24	3.41 ± 0.31
Procyanidin dimer B-type (three isomers)	133.78 ± 2.76	114.01 ± 2.29
(Epi)catechin-(epi)gallocatechin (2 isomers)	22.15 ± 1.68	15.29 ± 0.56
Procyanidin dimer B-type gallate (2 isomers)	4.54 ± 0.32	5.02 ± 0.35
Procyanidin trimer B-type	23.84 ± 0.76	29.36 ± 0.84
Prodelphinidin trimer B-type	5.11 ± 0.33	5.39 ± 0.17
Total flavan-3-ols	***303.22 ± 12.78***	***924.55 ± 4.67***
Quercetin	50.59 ± 5.08	39.08 ± 1.37
Myricetin	5.09 ± 0.05	7.34 ± 0.12
Kaempferol-3-*O*-rhamnoside	1.31 ± 0.09	1.11 ± 0.03
Phloretin-2'-*O*-glucoside	10.62 ± 0.79	17.78 ± 0.44
Quercetin-3-*O*-rhamnoside	30.63 ± 0.84	17.24 ± 0.43
Myricetin-3-*O*-rhamnoside	5.56 ± 0.22	4.55 ± 0.05
Quercetin-3-*O*-rutinoside	0.53 ± 0.05	0.48 ± 0.03
Isorhamnetin-3-*O*-rutinoside	0.59 ± 0.00	0.43 ± 0.02
Total flavonols	***104.92 ± 7.11***	***88.01 ± 0.72***
Total phenolic compounds	***425.11 ± 20.23***	***1121.08 ± 4.76***

n.d. means that the compound was not detected in the sample

The total amount of phenolic compounds was significantly higher in the HSE (1121.08 ± 4.76 mg/100g) with respect to the HS (425.11 ± 20.23 mg/100g). The most representative class of phenolic compounds in both samples was flavan-3-ols, which represented 71.3% and 82.5% of total phenolic compounds in HS and HSE, respectively. The amount of total flavan-3-ols was significantly higher in HSE with respect to HS. Considering the individual flavan-3-ols, the profile between the two samples was quite different. The compound present in the highest amount in HSE was epicatechin, which accounted for 48.3% of total flavan-3-ols, followed by catechin (accounting for 26.2% of total flavan-3-ols) and procyanidin dimer B-type (three isomers accounting for 12.3% of total flavan-3-

ols). Differently, in HS, the most concentrated compound was procyanidin dimer B-type (three isomers accounting for 44.1% of total flavan-3-ols), followed by epicatechin, which accounted for 27.0% of total flavan-3-ols.

The second most representative class of phenolic compounds in HSE was phenolic acids, which represented 9.7% of total phenolic compounds. In this sample, the most representative phenolic acids were ellagic acid (71.77 ± 5.52 mg/100g) and gallic acid (13.13 ± 0.39 mg/100g). In contrast, flavonols were found in minor amounts, with quercetin being the most representative (39.08 ± 1.37 mg/100g). Otherwise, in HS, the second most representative class of phenolic compounds was flavonols, which accounted for 24.7% of total phenolic compounds. The compound present in the highest amount was quercetin (50.59 ± 5.08 mg/100g), followed by quercetin-3-O-rhamnoside (30.63 ± 0.84 mg/100g). Only low amounts of phenolic acids were detected in HS. Previous studies showed that flavan-3-ols (and, in particular, monomeric and dimeric catechins) constitute the most abundant class of phenolic compounds in HS (Capaldi et al., 2025; Del Rio et al., 2011). Concerning the class of flavonols, the most representative compounds were quercetin and quercetin-3-*O*-rhamnoside as already reported by Del Rio et al. (2011). In addition, some phenolic acids, such as gallic acid and protocatechuic acid, have already been identified in HS (Pelvan et al., 2018). Anyway, some phenolic compounds present in high concentrations, such as ellagic acid, were identified in the HS for the first time in this study.

3.2. Oxidative state of pork burgers

3.2.1. Lipid oxidation

During storage and cooking the two experimental groups (HS and HSE) showed a significant ($P<0.01$) improvement in oxidative stability (Figure 1).

Figure 1 reports the effect of treatment on MDA content (mg/kg) of burgers during refrigerated storage and cooking.

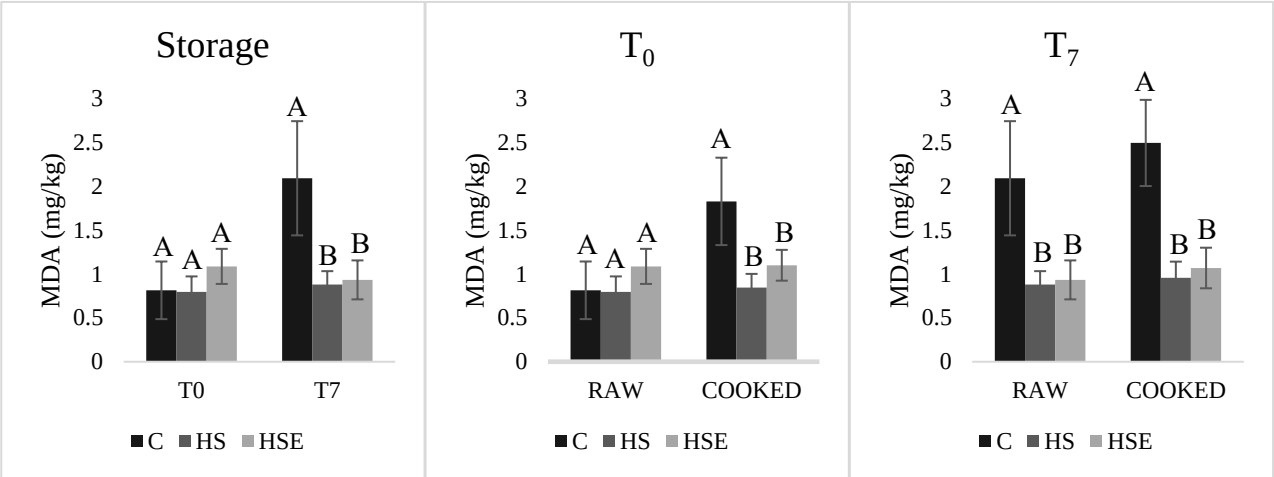

Figure 1. Effect of treatment on MDA content (mg/kg) of burgers during refrigerated storage and cooking.
C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
Storage: A, B: different letters indicate statistical differences between groups in each sampling time for $P<0.01$
T_0 and T_7: A, B: different letters indicate statistical differences between groups within raw and cooked samples for $P<0.01$

Both treatments provided an increasing antioxidant capacity (AOA%) consistently close to 50%. In fact, during storage, HS presents an AOA% of + 51% while HSE + 49%. During cooking, HS presents an AOA% of + 51% at day 0 and + 59% at day 7 while HSE + 37% at day 0 and + 54% at day 7 (data not shown).

Although there are no legal limits for these types of products regarding MDA content, it is believed, as reported by Trindade et al. (2010) in beef and by Longato et al. (2019) in chicken, that the level of MDA during storage should be kept below 2 mg/kg. In the present study, the MDA value was significantly lower during storage and even after cooking (Figure 1) in the experimental formulations used, further highlighting the protective effect exerted by the hazelnut skin and its phenolic extract. While the MDA content of the control group was always close to or above the target value of 2 mg/kg (Figure 1).

The results confirmed the antioxidant ability of HS in pork, as previously observed in our study (D'Ambra et al., 2023) and highlighted that HSE has the same antioxidant power when used by balancing their antioxidant compound content. This result is in line with results obtained with other natural extracts used as antioxidants in meat products. In particular, Jayawardana et al. (2019) reported that TBARS values of uncured pork sausages significantly reduced with the addition of different concentrations of black or green tea extract during 5 days of storage and that the inhibition increased as the concentration used increased. Šojić et al. (2020), on the other hand, achieved a significant reduction in lipid oxidation using supercritical extracts of wild thyme by-products as natural antioxidants in ground pork patties. Zamuz et al. (2018) indicated that the addition of chestnut by-product extracts to the beef patties had a positive effect on the decrease in the TBARS values, even at the lowest concentrations, especially with leaf and bur extracts.

The findings, however, differ from those reported by Longato et al. (2019), where the application of hazelnut skin as an antioxidant at concentrations of 2-3% in chicken burgers exhibited a pro-oxidant effect.

3.2.2. Volatiles profile

Table 3 reports volatile compounds detected in burgers.

Table 3. Volatile compounds detected in burgers by SPME analysis. Values are expressed as percentages of the total chromatographic area (%).

RT (min)	Volatile compound	C	HS	HSE
3.129	Pentanal	1.51^{b}	3.10^{a}	2.83^{b}
5.183	Hexanal	22.54^{A}	2.21^{B}	n.d.
8.649	Heptanal	1.69	n.d.	n.d.
9.333	Dodecane	n.d.	1.42	n.d.
9.838	Furan, 2-pentyl-	1.07	1.12	n.d.
10.637	1-Pentanol	1.08	n.d.	n.d.
11.826	Octanal	2.73^{a}	n.d.	0.56^{b}
12.403	Tridecane	n.d.	1.21	n.d.
12.965	2,3-Octanedione	3.09^{A}	0.51^{B}	n.d.
13.617	1-Hexanol	0.51	n.d.	n.d.
14.759	Nonanal	7.99	7.01	6.26

15.171	Tetradecane	n.d.	0.74	0.65
16.042	trans-2-octenal	0.94	n.d.	n.d.
16.136	1 Octen 3 ol	7.60^{Aa}	2.40^{Bb}	1.21^{Bc}
16.337	1-heptanol	0.85	1.04	n.d.
17.151	1-Hexanol, 2-ethyl-	n.d.	1.13	0.82
18.658	2-Nonenal, (E)-	0.90	n.d.	n.d.
18.794	Benzaldehyde	2.35	n.d.	n.d.
18.859	1-Octanol	2.98^{A}	3.28^{A}	0.67^{B}
20.214	2-Octen-1-ol, (E)-	1.50^{a}	0.77^{b}	n.d.
21.143	E-2-decenal	1.49	n.d.	n.d.
21.179	Benzeneacetaldehyde	n.d.	3.65	2.10
22.405	Dodecanal	0.37	0.51	0.65
22.801	2,4 nonadienal	0.37	n.d.	n.d.
23.493	3-dodecen-1-al	1.42	n.d.	n.d.
23.954	2,4-Decadienal	0.29	n.d.	n.d.
24.991	trans, trans-2,4-Decadienal	0.97	n.d.	n.d.
25.323	E-15-heptadecenal	n.d.	0.58	1.22
27.653	1-Dodecanol	n.d.	3.05	2.55

C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
RT: retention time
n.d. means that the compound was not detected in the sample
$^{a, b, c}$: different letters on the same line indicate differences for $P<0.05$
$^{A, B, C}$: different letters on the same line indicate differences for $P<0.01$

The most important source of aroma compounds is the lipid fraction of meat, which generates acids, aldehydes, ketones, and alcohols through the oxidation of phospholipids. By-products of fat oxidation are short-chain compounds responsible for the aroma and flavor of meat (Lopez-Moreno et al., 2023). In our study, we found 30 main compounds, as shown in Table 3. The difference between the three treatments is extremely noticeable, especially in the HSE samples, which had the lowest production of volatile compounds. When considering the total percentages of the classes of compounds detected, it is clear that the presence of alkanes (HS 3.37%, HSE 0.65%) and the low presence of alcohols (HS 11.67%, HSE 5.25%) and aldehydes (HS 17.57%, HSE 13.62%) in the treated burgers is a sign of an oxidation process blocked in the early stages of degradation, while the absence of alkanes, a moderate presence of alcohols (14.52%), and a high percentage of aldehydes (48.65%) in the control burgers indicate that oxidation is in an advanced state. In this group, all the compounds identified as the main indicators of pork meat spoilage, such as hexanal, heptanal, octenal, nonanal, (E)-2-octenal, (E)-2-nonanal, (E,E)-2,4-nonadienal, 1-pentanol, 1-octen-3-ol, and 3 octen-2-one (Liu et al., 2023) were detected. As for the treated samples, they showed either absence or minimal presence of such compounds, except for nonanal, a degradation compound from oleic acid, which is the main fatty acid provided by the hazelnut film. Aldehydes were the most abundant compounds detected in all samples, which is consistent with the literature, as aldehydes occupy the largest proportion (about 40%) in pork organic compounds, and aldehydes have a relatively low threshold and are volatile, which greatly influences the aroma (Duan et al., 2023). Hexanal, the major compound from the oxidation of n-6 fatty acids, mainly linoleic and arachidonic acid, has been commonly found as the major volatile in

the aldehyde group in other research published by authors who have explored the presence of volatile compounds in raw, cured, and cooked meats (Iglesias et al., 2009; Song et al., 2021; Xu et al., 2014). In our case as well, hexanal was the most abundant compound detected, but exclusively in the control samples, highlighting how, in these samples, the oxidative state of lipids led to the formation of characteristic degradation compounds, whereas this was not observed in the treated samples ($P<0.01$). Regarding alcohols, they are mainly produced by the degradation of linoleic acid and can be divided into saturated and unsaturated alcohols. Unsaturated alcohols have a lower threshold and exert an important impact on the overall flavor. For example, 1-octen-3-ol is an unsaturated alcohol with a low threshold and strong flavor, which may be the main contributor to pork's degraded aroma (Meynier et al., 1998). In this case too, this compound was significantly higher in the control group ($P<0.01$). Noteworthy is the presence of an aldehyde, E-15-heptadecenal, in the treated burgers, as this compound was also found in an extract of *Halimeda discoidea* as a bioactive compound capable of inhibiting the growth of *Klebsiella pneumoniae* ATCC, a Gram-negative bacterium associated with diseases such as pneumonia, meningitis, and liver abscess (Supardy et al., 2012).

Based on the results regarding the oxidative state and the oxidation-related volatile compounds in the treated burgers, it is evident that the added powders improved the product's healthiness by enhancing the stability of the lipid fraction during storage and consequently improving the volatile profile developed. The final healthiness of the product is further reinforced by the fact that hazelnut skin does not compromise the microbiological profile of the final product, as confirmed in a previous study (D'Ambra et al., 2023) and that the extract demonstrated antimicrobial activity (Capaldi et al., 2025).

3.3. Color and sensory parameters of burgers

The effect of treatment on color stability of the burger during refrigerated storage is shown in Figure 2.

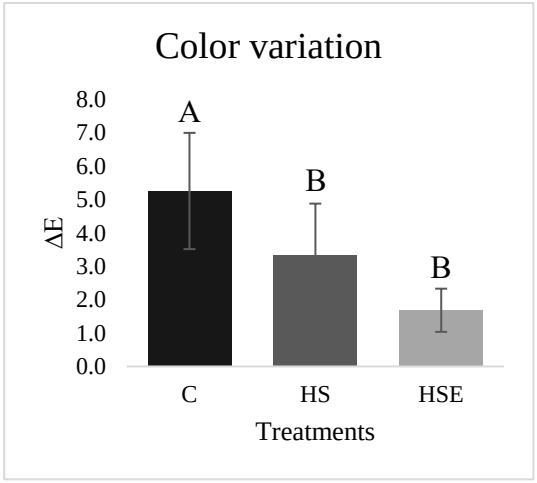

Figure 2. Impact of treatment on color stability of burgers on the 7th day of storage.
C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
A, B: different letters indicate statistical differences between groups for $P<0.01$

During the storage, the HS and HSE groups exhibited better color stability (P<0.01). The delta E values of 3.34 and 1.68 for the HS and HSE samples (Figure 2), respectively, indicate that the visual changes in the color of the burgers are minimal unlike the control group, which showed visible color changes as explained by Cserhalmi et al. (2006) according to which color changes could be estimated such as noticeable when ΔE values are between 1.5 and 3.0, visible with ΔE of 3.0–6.0, and great between 6.0 and 12.0. It is now known that plant extracts modifying the color of the product tend to cover the loss of gloss and greying of the raw material, making the product more technologically durable while preserving its color characteristics. This ability of natural extracts has also been observed by Šojić et al. (2020) and Zamuz et al. (2018). These studies reported, respectively, that the application of supercritical extracts of wild thyme by-products decreased discoloration in ground pork patties and the application of hull, bur, and leaf chestnut extracts had a positive effect on the color of beef patties during storage.

The effect of treatment on color acceptability and potential for purchase of burgers is shown in Figure 3.

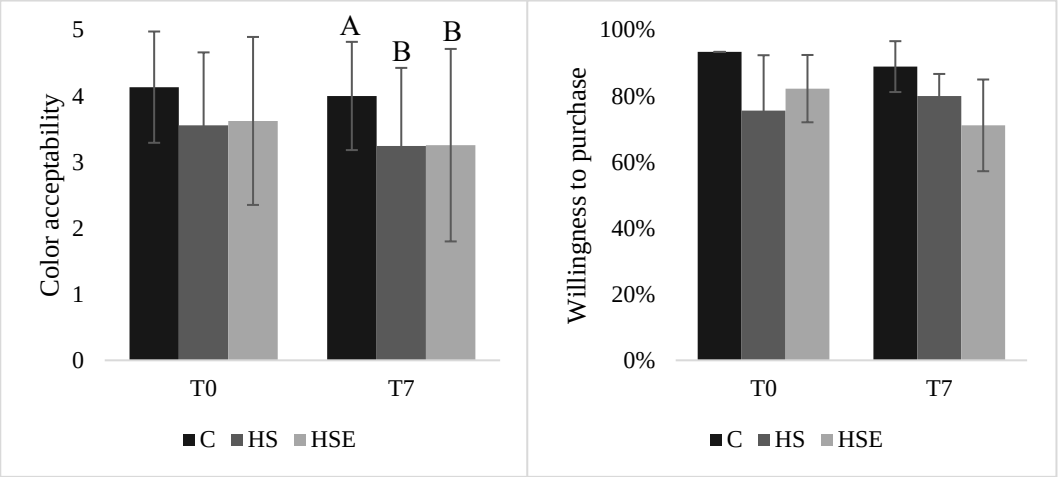

Figure 3. Influence of treatment on color perception and potential for purchase of burgers. Results are expressed as averages of ratings made by 15 panelists during the three replications of the experiment. C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
A, B: different letters indicate statistical differences between groups within each sampling time for P<0.01

Even if during storage treatments improved the color preservation, the two treated groups showed worsening (P<0.01) in terms of color acceptability (Figure 3). Despite this, the acceptability of the product was not compromised, as the willingness to purchase remained high (Figure 3). This is because most panelists who rated the color of the burgers as less acceptable but were still inclined toward a potential purchase explained their choice by stating that, if the label or packaging had specified that the coloration was due to the addition of natural plant-based antioxidants, it would not have impacted their decision to buy the product. The same trend about color perception was observed by Turhan et al. (2005) who used hazelnut skin in low-fat beef burgers. The control beef burgers received the highest appearance scores. Increasing the level of hazelnut skin resulted in beef burgers

with lower appearance scores and decreased overall acceptability ratings. The same was observed in the study of Al-Juhaimi et al. (2017) in which sensory evaluation of chicken burgers treated with pistachio hull water extracts revealed no significant difference in all sensory attributes between control and treated burgers except color. They observed that the overall mean value for color for the control burger was found to be higher than that of the chicken burger treated with 5% and 7% extracts. It is interesting to note that in previous tests on the inclusion of HS in pork burgers (D'Ambra et al., 2023), the evaluation of color by panelists, conducted on cooked burgers, showed results opposite to those of the evaluation on raw burgers carried out in the present study. In fact, when cooked, the burgers with HS tended to receive higher ratings compared to the control, indicating greater appeal.

3.4. Tenderness and cooking loss of burgers

The results regarding cooking loss and tenderness of the burgers are reported in Table 4.

Table 4. Effect of treatment on tenderness and cooking loss of burgers.

	Tenderness (N)		**Cooking loss (%)**	
	T_0	T_7	T_0	T_7
C	16.9^{B}	19.9^{B}	19.8^{A}	20.4^{A}
HS	24.6^{A}	25.9^{A}	13.4^{B}	13.2^{B}
HSE	23.3^{A}	26.2^{A}	17.5^{A}	17.8^{A}

C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
$^{A, B, C}$: different letters on the same column indicate differences for $P<0.01$

Cooking losses were significantly reduced by the addition of the HS at T_0 at T_7 ($P<0.01$). This confirms the result obtained in the previous study, where HS improved cooking losses (D'Ambra et al., 2023). Our results agree with Turhan et al. (2005), who observed that hazelnut skin added to beef burgers reduced cooking loss and the effect increased as the skin concentration increased. Certainly, this result will be due to the integration of dietary fiber that increases yield and prevents cooking loss in meat-based products by enhancing water-binding capacity, offering significant economic benefits for both consumers and processors (Biswas et al., 2011). Despite lower cooking losses treatments compromised the tenderness of the burgers ($P<0.01$). The dynamometer results showed that in the HS and HSE groups, the force imparted for maximum deformation was higher respect to C group (Table 4). It is possible that tiny particles or insoluble dietary fibre parts might be filled into the three-dimensional gel net structure of the meat protein available to enhance hardness (Hu et al. 2017). This outcome confirms the results of the sensorial analysis of our previous study, where the burgers with HS had received lower tenderness scores compared to the control group (D'Ambra et al., 2023). An increase in hardness was also observed by Zhao et al. (2021) in low-fat meatballs with kiwi fruit insoluble dietary fiber superfine powder at levels above 3%.

3.5. Fatty acid composition of burgers

Table 5 reports the effect of treatment on the fatty acid composition of pork burgers.

Table 5. Effect of treatment on fatty acids content (% of total fatty acids detected) of pork burgers.

	C	HS	HSE
C18:1n-9 (oleic)	37.99[b]	43.95[a]	37.37[b]
C18:3n-6 (γ-linolenic)	0.08[a]	0.03[b]	0.06[a]
C20:1 (eicosenoic)	1.09[a]	0.60[b]	1.06[a]
C20:2n-6 (eicosadienoic)	0.67[a]	0.37[b]	0.67[a]
Total monounsaturated	43.45[b]	49.03[a]	42.87[b]

C: control burgers; HS: hazelnut skin burgers; HSE: hazelnut skin extract burgers.
[a, b, c]: different letters on the same line indicate differences for $P<0.05$

Only fatty acids and fatty acid classes that showed significant differences between hamburger groups are reported. The addition of hazelnut skin significantly increased the content of monounsaturated fatty acids, mainly due to the increase in oleic acid. This result is consistent with our previous study (D'Ambra et al., 2023), although even though the oleic acid contribution was greater this time, no significant reduction in the omega-6/omega-3 fatty acids ratio was achieved (data not shown in table), unlike in the formulations studied previously.

4. Conclusions

The development and application of natural antioxidants, along with transforming the supply chain into a more sustainable one, are two major challenges faced by the meat production and processing industry. The application of hazelnut skin, an important by-product of our agro-industrial chain, and its green phenolic extract demonstrates significant potential as a natural antioxidant in meat products, particularly in improving the oxidative stability of pork burgers. This enhancement in oxidative stability contributes to better preservation of the volatile profile and color of the meat. While minor alterations in sensory attributes, such as color and tenderness, were noted, acceptability remained largely unaffected. In fact, consumer perceptions were positively influenced when they were informed about the natural origin and health benefits of the additives, highlighting the growing demand for clean-label, environmentally sustainable ingredients in food products. These findings emphasize the dual benefits of hazelnut skin as both a functional ingredient and a strategy for upcycling agro-industrial waste. Certainly, there are needs to be explored the development of technologies and processes to efficiently extract and utilize these materials on a commercial scale. Investigating potential barriers to market entry and establishing value chains for these by-products can significantly contribute to reducing waste and promoting sustainability within the agri-food industry.

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6. Supplementary material

Compound	*m/z*	*Fragments*	*Retention time (min)*	*Quantified as*	*Standard purity*	*Supplier*
Protocatechuic acid	153.0184	109.0283	5.74	3,4-Dihydroxybenzoic acid	≥97%	Sigma-Aldrich (Milan, Italy)
Gallic acid	169.0134	125.0233	3.55	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Epicatechin	289.0721	245.0820; 109.0283; 125.0235; 205.0499	7.86	Epicatechin	≥98%	Sigma-Aldrich (Milan, Italy)
Catechin	289.0721	245.0822; 109.0280; 125.0232	9.03	Catechin	≥98%	Sigma-Aldrich (Milan, Italy)
Coumaric acid-O-pentoside	295.0461	119.0490; 163.0395	9.71	4-Hydroxycinnamic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Ellagic acid	300.9992	257.0092; 229.0140	11.34	Ellagic acid	≥95%	Sigma-Aldrich (Milan, Italy)
Quercetin	301.0353	151.0028; 178.9976	15.05	Quercetin	≥95%	Sigma-Aldrich (Milan, Italy)
Epigallocatechin	305.0669	125.0234; 137.0234; 167.0341; 109.0284; 179.0342; 219.0661; 57.0333	5.68	Epigallocatechin	≥95%	Sigma-Aldrich (Milan, Italy)
Gallocatechin	305.0669	125.0234; 137.0234; 167.0341; 109.0284; 179.0342; 219.0661; 57.0333	7.18	Gallocatechin	≥95%	Sigma-Aldrich (Milan, Italy)
Myricetin	317.0305	151.0034; 181.0015	13.95	Myricetin	≥95%	Sigma-Aldrich (Milan, Italy)
Galloyl-shikimic acid isomer 1	325.0486	169.0136; 125.0235	7.76	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Galloyl-shikimic acid isomer 2	325.0486	169.0136; 125.0235	9.24	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Galloyl-glucose isomer 1	331.0674	169.0135; 125.0233	2.30	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Galloyl-glucose isomer 2	331.0674	169.0135; 125.0233	3.48	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Galloyl-glucose isomer 3	331.0674	169.0135; 125.0233	5.00	3,4,5-Trihydroxybenzoic acid	≥98%	Sigma-Aldrich (Milan, Italy)
Syringic acid-4-O-hexoside isomer 1	359.0988	123.0077; 138.0314; 197.0448; 182.0218; 153.0550	5.96	4-Hydroxy-3,5-dimethoxybenzoic acid	≥95%	Sigma-Aldrich (Milan, Italy)
Syringic acid-4-O-hexoside isomer 2	359.0988	197.0450; 182.0211; 123.0078; 153.0554	6.81	4-Hydroxy-3,5-dimethoxybenzoic acid	≥95%	Sigma-Aldrich (Milan, Italy)
Syringic acid-4-O-hexoside isomer 3	359.0988	197.0450; 182.0211; 123.0078; 153.0554	7.85	4-Hydroxy-3,5-dimethoxybenzoic acid	≥95%	Sigma-Aldrich (Milan, Italy)

Catechin-3-O-suplhate	369.0983	289.0718; 245.0822; 205.0507; 125.0235	10.19	Catechin	≥98%	Sigma-Aldrich (Milan, Italy)
Kaempferol-3-O rhamnoside	431.0988	285.0408	14.41	Quercetin-3-O-rhamnoside	≥95%	Sigma-Aldrich (Milan, Italy)
Phloretin 2'-O-glucoside	435.1302	273.0774	14.43	Quercetin-3-O-glucoside	≥95%	Sigma-Aldrich (Milan, Italy)
Epicatechin-3-O-gallate	441.0835	169.0137; 125.0235; 289.0718; 245.0819	11.97	Epicatechin-3-O-gallate	≥98%	Sigma-Aldrich (Milan, Italy)
Quercetin-3-O-rhamnoside	447.0932	300.0280; 255.0302; 301.0359; 271.0255; 227.0349; 151.0029; 107.0128; 163.0034; 65.0020	13.01	Quercetin-3-O-rhamnoside	≥95%	Sigma-Aldrich (Milan, Italy)
Catechin-O-hexoside	451.1252	289.0720; 245.0823; 109.0283; 125.0234	7.31	Epicatechin	≥98%	Sigma-Aldrich (Milan, Italy)
Epigallocatechin-3-O-gallate	457.0783	305.0668; 169.0137; 125.0235; 289.0718; 245.0819	9.48	Epigallocatechin-3-O-gallate	≥98%	Sigma-Aldrich (Milan, Italy)
Gallocatechin-3-O-gallate	457.0783	305.0668; 169.0137; 125.0235; 289.0718; 245.0819	9.96	Gallocatechin-3-O-gallate	≥98%	Sigma-Aldrich (Milan, Italy)
Myricetin-3-O-rhamnoside	463.0891	317.0320	11.55	Quercetin-3-O-rhamnoside	≥95%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer A type isomer 1	575.1201	449.0721; 407.0772; 289.0725; 287.0726	10.86	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer A type isomer 2	575.1201	449.0721; 407.0772; 289.0725; 287.0726	11.80	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer A type isomer 3	575.1201	449.0721; 407.0772; 289.0725; 287.0726	13.15	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer B type isomer 1	577.1359	125.0234; 289.0721; 407.0771; 245.0846	7.06	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer B type isomer 2	577.1359	125.0234; 289.0720; 407.0791; 245.0822	7.33	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer B type isomer 3	577.1358	125.0231; 289.0711; 407.0792	9.58	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Catechin-gallocatechin isomer 1	593.1308	177.0185; 125.0233; 407.0775; 289.0724; 109.0284; 57.0333	6.30	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Catechin-gallocatechin isomer 2	593.1308	177.0185; 125.0233; 407.0775; 289.0724; 109.0284; 57.0333	6.65	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Quercetin-3-O-rutinoside	609.1472	300.0279; 271.0255; 255.0295; 301.0350; 178.1124	11.46	Quercetin-3-O-rutinoside	≥94%	Sigma-Aldrich (Milan, Italy)
Isorhamnetin-3-O-rutinoside	623.1623	315.0513; 299.0199; 300.0277; 271.025	12.73	Quercetin-3-O-rutinoside	≥94%	Sigma-Aldrich (Milan, Italy)

Procyanidin dimer B type gallate isomer 1	729.1462	125.0233; 289.0720; 407.0760; 287.0569	9.96	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin dimer B type gallate isomer 2	729.1462	125.0233; 289.0720; 407.0760; 287.0569	12.36	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Procyanidin trimer B type	865.2001	125.0233; 289.0720; 407.0760; 287.0569	8.51	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)
Prodelphinidin trimer B type	881.1945	125.0233; 289.0720; 407.0760; 287.0569; 577.1264	7.73	Procyanidin B1	≥90%	Sigma-Aldrich (Milan, Italy)

Chapter 5. Influence of pig dietary supplementation with hazelnut skin (HS) or hazelnut skin green extract (HSE) on live performance, carcass characteristics, and meat quality of heavy pigs

Abstract

The environmental impact of intensive farming has sparked public debate, emphasizing the need for eco-sustainable food production. Using agro-industrial by-products in animal feed supports this ecological transition while also enhancing feed functionality. For example, by-products rich in bioactive substances, such as phenolic compounds with strong antioxidant properties, can improve meat stability, particularly in oxidation-prone pork. This work aimed to evaluate the effects of dietary addiction of a by-product, hazelnut skins (HS) in two different forms: raw hazelnut skins or their green polyphenolic extract (HSE) on growth performance, carcass traits and meat quality of heavy pigs. A total of 72 pigs were allotted to 3 dietary treatments (each n=24): control group (C) fed a conventional diet and two groups with a similar diet supplemented with 0.6% of HS or 0.1% of HSE, respectively. After the evaluation of live performance, 36 subjects, balanced for gender and treatment, were slaughtered for carcass and meat quality evaluation. Dietary treatments did not significantly impact growth performance or carcass yield, demonstrating nutritional adequacy. However, HSE showed an increase in thigh yield ($P<0.01$), suggesting a potential beneficial effect on lean cuts yield. Negligible effects on meat quality were observed; oxidative stability of the meat was slightly enhanced during cooking in the HS group after 7 days of storage ($P<0.08$). Moreover, HSE reduced cooking loss, enhancing water retention. HS also slightly improved omega-3 levels and reduced the omega-6/omega-3 ratio ($P<0.05$), showcasing its potential for improving meat nutritional value. The subtle improvements observed and the absence of interference with growth performance, carcass characteristics, and final meat quality indicate for HS and HSE the possibility of notable commercial advantages in sustainable livestock systems.

1. Introduction

The quality of food, especially those of animal origin, is closely scrutinized by consumers, who are increasingly aware of the strong correlation between diet, health, and the environment. This is particularly relevant in light of growing concerns about the sustainability of production processes. The environmental impact of intensive farming has been at the center of public debate for several years, highlighting the urgency of making this food supply chain more eco-sustainable. However, despite increasing concern, a survey conducted among Norwegian consumers by Austgulen et al. (2018) concluded that consumers may not yet be ready to make dietary choices based on what is best for the climate or environment. Therefore, greater sustainability must primarily come from more sustainable animal production systems. Feed production, in addition to being the main cost of livestock production in the EU, accounting for 55% in the case of pigs in 2022 (FEFAC, 2023), is one of the main environmental burdens of the livestock sector. For pig production, feeds are responsible for the most significant share (70%) of the environmental impact (Andretta et al., 2021; Pomar et al., 2021). Specifically, depending on the production system in question, animal feed accounts for 55–75% of climate change effects, 70–90% of energy use, and 85% of land occupation associated with production (Reckmann et al., 2012). This is due to the global network of crops used for feed production. Producing each ingredient requires resources and energy and involves large-scale transportation networks, further increasing the environmental impact of pig production. Consequently, feed production generates more globalized impacts, while livestock production and waste management create more localized impacts (McAuliffe et al., 2016). A circular food system is increasingly seen as a solution to producing food within the Earth's biophysical limits. This approach would largely disconnect livestock feed from arable land, thereby avoiding competition for land between food and feed. Currently, up to 40% of all global arable land is used to produce high-quality feed for livestock, nearly half of which is allocated to monogastric animals (Mottet et al., 2017). Among the various strategies proposed and adopted to address this issue are the formulation of feeds using local ingredients and by-products from the food and bioenergy industries. However, these alternative feed options are environmentally sustainable only if they do not compromise growth performance (Monteiro et al., 2017). In recent years, interest in using food waste for livestock feed has grown. For example, in 2015, the European Commission adopted an action plan for the circular economy to prevent waste along the food production chain and promote more sustainable production, including reusing such waste as animal feed (Boumans et al., 2022; EC, 2011). It is estimated that the total food waste not currently used in feed or bio-based products represents about one-fifth of the EU's total production, amounting to 88 million tons per year (Stenmarck et al., 2016). Of the approximately 100 million tons of waste produced annually in the EU, only 5% is converted into animal feed (Colović et al., 2019). The use of agro-industrial by-products for animal feed not only aids in the ecological transition of livestock production but also offers numerous functions within a feed due to the composition and substances contained in various by-products. One example is the high content of bioactive substances such as phenolic compounds, which have demonstrated significant antioxidant activity, making them valuable for food preservation and enhancement (Ollani

et al., 2024). This property is crucial as these substances can help preserve feeds by preventing the oxidation of polyunsaturated fatty acids and potentially improving meat quality. Pork, for example, is among the best in fatty acid composition due to its higher unsaturation level. However, this also makes it the most susceptible to lipid oxidation, necessitating the accumulation of bioactive compounds, such as metabolites from polyphenols with high antioxidant capacity, in its tissues. The simplest and most direct way to achieve this is through diet. This strategy explores whether the metabolism of these bioactive compounds within the animal affects the stability and quality of the resulting meat products. It is hypothesized that these bioactive compounds can be metabolized and transferred into the animal's tissues. This could enhance the oxidative stability of meat, reducing lipid peroxidation and improving overall quality (Minelli et al., 2023). The Italian agri-food sector has a wide availability of by-products, such as hazelnut skin (HS). Italy is the second-largest producer of hazelnuts, accounting for nearly 20% of global production and 15% of exports. Among producer countries, Italy also has the highest per capita annual consumption of hazelnuts, with 0.520 kg per person. It is estimated that 90% of hazelnuts produced in Italy are destined for processors, while the remaining 10% is for fresh consumption (Forte et al., 2022; Misachi, 2018). This means Italy produces just under 3,000 tons of hazelnut skins annually, out of an average annual hazelnut production of about 110,000 tons (FAOSTAT, 2023). Due to their characteristics and quality, this by-product is already well-known and widely studied by the scientific community (Del Rio et al., 2011; Locatelli et al., 2010; Müller et al., 2020; Özdemir et al., 2014; Pelvan et al., 2018; Taş & Gökmen, 2015). These characteristics are mostly linked to the contribution of macronutrients such as fibers and unsaturated fatty acids, as well as the presence of phenolic compounds with strong antioxidant properties. Some research on dairy caw (Renna et al., 2020) and dairy sheep diets (Caccamo et al., 2019) has highlighted the possibility of replacing a portion of concentrated feed with raw hazelnut skin without any negative impact on live performance. Moreover, in sheep was reported an improvement of the sensory profile of the cheese (Caccamo et al., 2019) and a reduced lipid oxidation of meat (Menci et al., 2023). Hazelnut skin could also have beneficial effects in monogastric animals like pigs. Polyphenols in the skin may reduce oxidative stress, potentially improving animal product quality (e.g., oxidative stability of meat).

However, no studies have yet investigated the inclusion of this by-product in pig diets. The aim of this study was to characterize hazelnut skins and incorporate them into a finishing feed for gilts and borrows in two different forms: raw HS or their green polyphenolic extract (HSE) to evaluate their effect on growth performance and meat quality.

2. Materials and methods

2.1. Hazelnut skin and hazelnut skin extract characterization

The hazelnut (*Corylus Avellana* L.) skin (HS) is an agro-food by-product obtained during the conventional industrial roasting process of hazelnut; it was supplied by Dalma Mangimi S.p.A. (Marene, Cuneo, Italy). Hazelnut skin extract (HSE) is a polyphenolic green extract from the same hazelnut skin, provided by Turin University and obtained by a subcritical fluid extraction technique

reported by Capaldi et al. (2025). For chemical analyses, HS was ground using a home mixer Moulinex DPA 141 (Moulinex Italy), then sieved with a mesh with 500 microns. All the analyses on HS and HSE were made in triplicate, and the results were reported as mean value ± SD.

2.1.1. Proximate composition

The chemical composition of HS and HSE was determined according to the AOAC official methods (AOAC, 1995) and the results were expressed on wet basis.

2.1.2. Fatty acid profile

The total lipids from HS, HSE, feed, and animal tissues were extracted following the Folch et al. (1957) method. According to Zappaterra et al. (2020), 50 mg of lipid extract was mixed with 2 mL of hexane and subjected to methylation using 200 µL of a 2 N potassium hydroxide methanolic solution (KOH provided by Carlo Erba, Milan, Italy, and methanol provided by ITW Reagents, Barcelona, Spain). The fatty acids analyses were made using a TRACE^{TM}GC Ultra (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with a Flame Ionization Detector, PVT injector and TR-FAME Column (30 m long, 0.25 mm i.d., 0.2 µm film thickness) supplied by Thermo Scientific (Rodano, Milano, Italy). One µL of the methylated esters sample was injected into the GC with a split flow rate of 10 mL/min, operating at a constant flow of 1 mL/min of helium as a carrier gas. The detector and injector had the same operating temperature, 240 °C. After 2 min, the program temperature was increased at a rate of 4 °C per min from 140 °C to 250 °C and then maintained for 5 min. The Chrom-card software (version 2.3.3, Thermo Electron Corporation Rodano, Milano, Italy) was used to record, identify, and integrate the peaks of the fatty acid methyl esters (FAMEs). To identify the retention times of the FAMEs, a solution of standard FAMEs mix with known concentrations was used (Supelco 37 Component FAME mix, PUFA standard n.2, Animal Source (Supelco, Bellafonte, PA, USA), and single FAMEs standard, (Larodan, Fine Chemicals AB, Malmö, Sweden). The quantity of each FAME was expressed as the relative percentage of the total amount of FAMEs using the normalized and correct area method.

2.1.3. Extraction of phenolic compounds and antioxidant activity

The HS required the process of free phenol extraction to undergo assays for the characterization of total phenolic content and antioxidant activity, while the HSE was directly diluted in water and used for the assays. The extraction of free phenolic compounds from HS was performed following the method described by D'Ambra et al. (2023) with some modifications. Briefly, 2.5 g of this by-product was homogenized with 12.5 mL of a methanol/water/formic acid solution (in a ratio of 70:28:2, v/v/v) using an Ultra-Turrax homogenizer (IKA, Germany) for 1 min. The resulting suspension was incubated for 30 min at 37 °C and then centrifuged at 6000 rpm for 15 min at 4 °C using a Remi Elektrotechnik LTD centrifuge (model NEYA 16R, Mumbai, India). The supernatant was collected, and the pellet was resuspended with 12.5 mL of fresh solution. This procedure was repeated three times to completely extract the phenolic compounds from the initial 2.5 g sample. The polyphenol-rich extracts obtained were stored at 0–4 °C for subsequent analyses.

2.1.4. Total phenolic content (TPC)

The total phenolic content of HS and HSE was assessed using the Folin-Ciocalteu assay (Singleton et al., 1999) with some modifications. Briefly, 1975 µL of distilled water was combined with 25 µL of the extracted sample and 125 µL of Folin reagent (concentration 1.8–2.2 mol/L). After 1 min 375 µL of a 20% Na_2CO_3 solution was added, and the mixture was incubated in the dark for 2 h. Following incubation, absorbance was measured at 765 nm using a Jasco spectrophotometer (model V550, UV/VIS, Tokyo, Japan). Gallic acid was used for the calibration curve, and the results were reported as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

2.1.5. ABTS assay

The antioxidant capacity of HS and HSE was evaluated using the ABTS assay, based on the protocol described by Re et al. (1999). This method employs the chromogen 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, AppliChem GmbH) to measure antioxidant activity. The assay was conducted by observing the decrease in absorbance at 734 nm of the ABTS•+ radical cation in the presence of antioxidants. To generate the ABTS•+ radical cation, a 7 mM aqueous solution of ABTS was mixed with 2.45 mM potassium persulfate and incubated in the dark overnight to allow the reaction to occur. The resulting ABTS•+ solution was diluted with methanol to achieve an initial absorbance value (A_0) of 0.705 ± 0.005 at 734 nm. For the assay, 100 µL of the diluted sample was mixed with 1400 µL of the ABTS•+ solution and incubated at 20 °C for 15 min in the dark. The final absorbance at 734 nm (A_f) was registered, and the scavenging percentage ($S\%$) was calculated using the consequent equation:

$$S\% = \frac{(A_0 - A_f)}{A_0} x\ 100$$

Where A_0 represents the initial absorbance (control), while A_f expresses the final absorbance (sample). Trolox (6-hydroxy 2,5,6,7-tetramethyl chroman-2-carboxyl acid) served as the standard, and the ABTS scavenging capacity was quantified as mmol of Trolox equivalent per gram of by-product, based on a calibration curve acquired with Trolox ranging from 50 to 500 mmol/L under the same assay conditions.

2.1.6. FRAP assay

The antioxidant capacity of HS and HSE was further evaluated using the ferric reducing/antioxidant power (FRAP) assay, as outlined by Benzie and Strain (1999). This method is based on the reduction of the Fe^{3+}–2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form (Fe^{2+}) under acidic conditions. In this assay, 3 mL of freshly prepared FRAP reagent (20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was mixed with 100 µL of the sample. The absorbance was recorded at 593 nm at ambient temperature after 6 min of incubation. The results were reported as µmol of $FeSO_4$ per gram of sample.

2.1.7. DPPH assay

The antioxidant activity of HS and HSE was also assessed using the DPPH assay, following the procedure described by Helal et al. (2012). A 0.1 mM solution of DPPH (2,2-diphenyl-1-

picrylhydrazyl) was prepared in methanol and allowed to react in the dark for 30 min to stabilize. For the assay, 200 μL of the sample was combined with 2 mL of the DPPH solution and incubated in a shaker in the dark for 30 min. The absorbance of the mixture was then measured at 517 nm using a UV-visible spectrophotometer, with a blank containing no sample used as a reference. The antioxidant activity was determined after subtracting the absorbance of the sample blank and expressed as mg Vitamin C equivalent /g of the sample.

2.1.8. Identification and quantification of phenolic compounds by high-resolution mass spectrometry (UHPLC/MS)

The phenolic compound profiles of HS and HSE and feeds were determined as reported by Cattivelli et al. (2023). Before the injection in the high-resolution mass spectrometer, phenolic compounds were extracted from the samples by following the protocol previously described in Section 2.1.3 of the cited paper. Phenolic compounds were firstly separated using a C18 column (Acquity UPLC HSS C18 Reversed phase, 2.1 × 100 mm, 1.8 μm particle size, Waters, Milan, Italy) in a UHPLC Ultimate 3000 module (Thermo Fisher Scientific, San Jose, CA, USA) before being analyzed with a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The chromatographic separation and mass spectrometry settings are fully described by Martini et al. (2020). Quantification was carried out by building external calibration curves with the available standard compounds.

2.2. Animal diets

Given the lack of research that considers the use of hazelnut skin in the pig diet and considering the high crude fiber content of HS and what reported by Tunçil (2020) that more than 96% of fiber of HS is water insoluble and contains about 55% of lignin and about 45% of fiber polysaccharides, the level of inclusion of HS and HSE in the feed was based on the content of total polyphenols, expressed in mg of gallic acid equivalent (GAE)/g of HS and HSE. Therefore, based on the data of the scientific literature relating to the supplementation of the pig diet with polyphenols provided by grape seed extract (GSE) or bearberry (O' Grady et al., 2008) or by apple (Xu et al., 2019), ranging from 400-850 mg GAE/kg feed, it was decided to add to the feed a quantity of by-product or its polyphenolic extract such as to provide an aliquot of total polyphenols added (mg GAE/kg feed) of approximately 800-850 mg. Three types of diets were formulated: a maize-barley-soya bean meals basal diet (control diet, C), and two experimental formulations, one where 0.6% of ground hazelnut skin, providing 843 mg GAE/kg feed (particle size 2.5 mm), was included as replacement of 0.6% of wheat bran (hazelnut skin diet, HS) and one where the basal diet was supplemented with 0.1% of polyphenolic green extract from hazelnut skin providing 837 mg GAE/kg feed (hazelnut skin extract diet, HSE). The three diets, isoenergetic and isolipidic, were formulated to meet the requirements for all nutrients (NRC, 2012) and feed was administered as flour ad libitum. The main characteristics of the diets are reported in Table 1. The dietary composition was determined in triplicate by spectroscope NIR FOSS 5000 (Hilleroed, Denmark) at a laboratory specialized in animal feed analysis, while the fatty acid

composition and the phenolic compound profiles were determined following the same methodology previously described for HS and HSE in 2.1.2 and 2.1.8 paragraphs respectively.

Table 1. Ingredients (%), proximate composition (%, as fed basis), fatty acid composition (% of total fatty acids), and phenolic compounds (mg/kg of feed) of the diets.

Ingredients		C	HS	HSE
Maize	%	50.00	50.00	50.00
Barley meal	%	20.00	20.00	20.00
Soybean meal	%	10.30	10.30	10.30
Wheat bran	%	10.00	9.40	10.00
Wheat middling	%	6.00	6.00	6.00
Calcium carbonate	%	1.30	1.30	1.30
Animal fat	%	1.00	1.00	1.00
Hazelnut skin	%	--	0.60	--
Hazelnut skin extract	%	--	--	0.10
Dicalcium phosphate dihydrate	%	0.50	0.50	0.50
Sodium chloride	%	0.50	0.50	0.50
Pre-mix1[1]	%	0.20	0.20	0.20
L-Lysine	%	0.20	0.20	0.20
Analysed composition (on fed basis)[2]				
Dry Matter	%	89.01	89.22	89.02
Crude protein	%	13.27	13.95	13.38
Crude fat	%	4.23	3.92	3.95
Crude fiber	%	4.13	3.53	4.03
Ashes	%	5.11	4.68	5.02
Calculated nutrients composition (on fed basis)[3]				
Digestible energy (DE)	MJ/kg	13.75	13.80	13.75
Calcium	%	0.71	0.71	0.71
Phosphorus	%	0.49	0.48	0.49
Digestible phosphorus	%	0.24	0.23	0.24
Lysine	%	0.76	0.76	0.76
Digestible lysine	%	0.66	0.66	0.66
Fatty acid composition (% of total FAs)				
C14:0		0.62	0.57	0.44
C16:0		17.90	16.95	17.59
C16:1		0.62	0.61	0.63
C17:0		0.11	0.19	0.16
C18:0		4.62	4.55	4.79
C18:1n-9		31.34	27.95	26.59
C18:1n-7		0.47	0.37	0.86
C18:2n-6		40.48	45.71	45.50
C18:3n-3		2.60	2.21	2.28
C20:0		0.43	0.23	0.35
C20:1		0.50	0.44	0.50
C20:2-6		0.12	0.12	0.15
C22:5-3		0.19	0.10	0.17
Phenolic compound (mg/kg feed)				
Total phenolic acids		0.37	38.41	38.96
Total flavonoids		0.96	88.64	104.63
Total phenolic compounds		1.33	127.05	143.59

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group; n.d.: not detected. [1]Providing the following nutrients (per kg diet as-fed): Vitamin A 5,200 IU; Vitamin D3 1,200 IU; Vitamin E (α-tocopheryl acetate) 16 mg; Vitamin K 1 mg; Vitamin B1 1.6 mg; Vitamin B5 8 mg; Vitamin B6 1.6 mg; Niacin 20 mg; Biotin 0.08 mg; Betaine 117 mg; Cu 14 mg; Fe 160 mg; Mn 48 mg; I 1.19 mg; Zn 60 mg; Se 0.24 mg; L-Lysine monohydrochloride 1,560 mg. [2]NIR (Near infrared spectroscopy). [3] Sauvant et al., 2004

2.3. Animals

All the experimental procedures performed in this study followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for scientific purposes,

complied with the Italian Legislative Decree 4th March 2014 n.26 art.2 point F, and have been authorized by Ethics Committee of Animal Experimentation (OPBA) of University of Modena and Reggio Emilia on May 19, 2022. The study was performed on 72 Italian Large White x (Italian Landrace x Italian Large White) pigs (Topigs Norsvin Italy) intended for Protect Denomination Origin (PDO) Italian heavy pig production. The subjects balanced for gender (36 barrows and 36 gilts) and live body weight (LBW) (average LBW 113.9±11.0 kg), were evenly allotted in 9 concrete-floored pens of 10 m^2 each, 8 animals per pen (3 replicates), and randomly assigned to one of the following three dietary treatments for 101 days before slaughter (average LBW 175.8±14.6 kg): i) control group (C); ii) hazelnut skin group (HS); iii) hazelnut skin extract group (HSE). Animals were given water ad libitum from a nipple drinker system during the whole experiment. During the trial, the residual feed of each pen was weighed weekly, and pigs were individually weighed three times (at starting, after 54 days, and at slaughtering).

2.4. Slaughter and sampling procedures

At the end of the trial, a subsample of 36 pigs (12 for each treatment), balanced for pen and gender, were randomly selected and, following the Council Regulation (EC) No 1/2005 to protect animals during transport, after overnight fasting, transported to a commercial abattoir where the animals were electrically stunned and blended in agreement with the Council Regulation (EC) No 1099/2009 on the protection of animals at the time of the killing. All slaughter procedures were controlled by the Veterinary Service from the Italian Ministry of Health. After slaughter, hot weight was detected on each carcass and, at the level of the last rib, on the split line of the carcass in two halves, the backfat thickness was taken by calliper. Subsequently, carcasses were dissected in primal cuts and each lean (thig, loin, neck, shoulder), adipose (backfat, belly, jowl, and perirenal fat) cut and the head were weighed to determine their incidence on hot carcass weight. At carcass cutting, from each left side, was excised the *longissimus thoracis* muscle (LT) between the 4/5th and the last thoracic vertebras for subsequent analyses. Moreover, at the last rib level, was taken a sample of backfat tissue (BF) for fatty acid (FA) analyses. All the whole LT and BF samples were transported, in a refrigerated box, to the laboratory of the Department and stored at 4±1 °C till to reached 24 h *postmortem* (*p.m.*). Subsequently, each LT was sliced into 5 subsamples (~2.5 cm thickness). Three of these, randomly selected, were destined to measurements of pH, color, cooking loss, and lipid oxidative stability (LOS) at 24 h *p.m.*, and after 3 and 7 days of refrigerated storage at 4±1 °C, packed in resealable polypropylene containers, without modifications in atmospheric gas concentration. LOS was measured before and after cooking. The fourth subsample of LT was subjected to drip loss measurements while the fifth and the BF samples were vacuum packed (Elegen, Reggio Emilia, Italy) and stored at -20 °C until the subsequent chemical analyses.

2.5. pH and color measurements

After 24 h, 3 and 7 days of refrigerated storage, pH was measured on each LT muscle subsample using a portable Crison pH meter equipped with a Xerolite electrode (Crison Instruments, Alella, Spain). At the same times, instrumental color measurements were carried out by a Minolta CM-600d

spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) with a window diameter of 8 mm, and D65 as the light source, and 10° observer value. After calibration with a standard white calibration plate, 3 different measurements were made on each subsample and the values were averaged. The results were expressed as the CIE L*a*b* fundamental coordinates: L^*- "lightness", a^*- "redness", b^*- "yellowness". Further, Chroma (C^*), and Hue angle (H^*) were calculated as $\sqrt[2]{a^{*2} + b^{*2}}$, and as arctan ($b^*/a^*$) respectively.

2.6. Drip loss

Drip loss was evaluated on LT (starting at 24 h *p.m.*) according to Honikel, (1998) with some modifications. Briefly, a slice of fresh LT muscle, approximately 2.5 cm thick and with an initial weight of about 100 g, was weighed, placed in an inflated bag, avoiding contact between the sample and the bag, and stored at 4 ± 1 °C for 48 h. The weight loss percentage was calculated as weight difference recorded during storage.

2.7. Cooking loss

Cooking loss at 24 h, 3 and 7 days of storage were determined on a 4x4 cm LT muscle sample. The measurement was carried out, before and after cooking on a home electric double-cast grill plat (Bosch, Germany) at 80°C for 3 min (core temperature reached 77.5±8.5 °C). The parameter was determined in triplicate. Cooking loss percentages were then calculated as follows and as reported in a previous paper (D'Ambra et al., 2023):

$$Cooking\ loss\ (\%) = \frac{weight\ raw - weight\ cooked}{weight\ raw}\ x\ 100$$

2.8. Oxidative stability

Lipid oxidation was evaluated on fresh and cooked LT samples following Siu and Draper (1978) by measurement of 2-thiobarbituric acid reactive substances (TBARS) as described in detail in a previous paper (D'Ambra et al., 2023). TBARS were expressed as mg of malondialdehyde (MDA) per kg of meat using 1,1,3,3 tetraethoxypropane (TEP, Sigma-Aldrich, Milan, Italy) as a standard.

2.9. Proximate composition and fatty acid profile

The chemical composition of LT samples was determined according to the AOAC official methods (AOAC, 1995) and the results were expressed on wet basis. While fatty acid profile of LT muscles and BF samples was determined as previously described in paragraph 2.1.2.

2.10. Statistical analysis

The data from HS and HSE characterization were expressed as mean ± SD of three samples analyzed in triplicate. The data from the animal trial (live performance, carcass traits, meat quality, and lipid composition) were subjected to analysis of variance using the GLM procedure of SAS, PDIFF option (SAS Institute Inc., Cary, NC, USA). The statistical model included dietary treatment (C, HS, and HSE), gender (gilts and barrows), and their interactions as fixed effects. The interactions were not found to be statistically significant ($p > 0.05$) and therefore were not presented in the tables. Hot

carcass weight, carcass yield, and backfat thickness were covariate for slaughter LBW. For average daily feed intake (ADFI) and feed conversion ratio (FCR) calculation, the average data of each pen containing 8 pigs was considered as starting data.

3. Results

3.1. Hazelnut skin and hazelnut skin extract characterization

Table 2 shows the data characterizing the hazelnut skin and its green extract.

Table 2. Proximate composition, fatty acid composition, total phenolic content (TPC), antioxidant activity (ABTS, FRAP, and DPPH assay), and phenolic compounds of hazelnut skin (HS) and hazelnut skin extract (HSE) (Mean ± SD of 3 samples analyzed in triplicates).

	Hazelnut skin (n=3)	Hazelnut skin extract (n=3)
Moisture %	7.04 ± 0.04	6.99 ± 0.15
Crude lipids %	25.00 ± 0.001	n.d
Crude protein %	9.36 ± 1.25	n.d
Crude fiber %	16.97 ± 5.56	n.d
Ashes %	1.95 ± 0.00	0.06 ± 0.00
Fatty acid (FA) composition (% of total FAs)		
- C16:0	5.71 ± 0.07	--
- C16:1	0.15 ± 0.01	--
- C18:0	2.14 ± 0.02	--
- C18:1n-9	75.86 ± 0.12	--
- C18:2n-6	15.03 ± 0.02	--
- C18:3n-3	0.17 ± 0.00	--
- C20:0	0.13 ± 0.00	--
- C20:1	0.17 ± 0.00	--
- C22:5-3	0.64 ± 0.04	--
- n-6 PUFA	15.03 ± 0.02	--
- n-3 PUFA	0.82 ± 0.05	--
- Saturated (SFA)	7.98 ± 0.07	--
- Monounsaturated (MUFA)	76.17 ± 0.04	--
- Polyunsaturated fatty acids (PUFA)	15.85 ± 0.03	--
TPC (mg GAE/g)	140.44 ± 16.36	837.00 ± 0.02
ABTS (mmol Trolox eq/g)	1068.78 ± 12.22	2472.73 ± 154.3
FRAP (μmol $FeSO_4$/g)	540.36 ± 8.06	2951.37 ± 13.60
DPPH (mg Vitamin C/g)	262.69 ± 5.96	793.10 ± 32.20
High-resolution mass spectrometry		
Total phenolic acids (mg/100g)	17.02 ± 0.03	108.52 ± 0.58
Total flavonoids (mg/100g)	487.39 ± 0.56	1012.56 ± 2.69
Total phenolic compounds (mg/100g)	504.41 ± 1.01	1121.08 ± 4.76

TPC: Total phenolic content, detected by Folin-Ciocalteu assay
ABTS: 2, 2 -azinobis-(3-ethylbenzothiazoline-6-sulfonic)
FRAP: ferric reducing/antioxidant power
DPPH: 2,2-diphenyl-1-picrylhydrazy
n.d: not detected

Both products exhibit a low moisture level (7%), making them suitable for long-term storage, an important requirement for feed and food additives. HS is characterized by a good fiber content (16.97%) and a satisfactory protein content (9.4%). The lipid fraction (25%) is mainly composed of oleic acid (75.86%), followed by linoleic acid (15.03%) and palmitic acid (5.71%). As expected, proteins, lipids and fiber were not found in HSE.

The content of phenolic compounds (TPC) is approximately five times higher in the extract, demonstrating that the extraction method has a high yield. However, the antioxidant activity, determined through ABTS, FRAP, and DPPH assays, although higher in the extract, does not reflect the same ratio identified in the total phenolic content (TPC).

Regarding the classes of phenolic compounds found, the two products are qualitatively similar, with flavonoids being the most prevalent class (96.6% in HS and 90.3% in HSE), followed by phenolic acids class (3.4% and 9.7% in HS and HSE respectively). Flavan-3-ols are the main components of flavonoids (81.8% in HS and 91.3% in HSE), followed by flavonols (18.2% and 8.7% in HS and HSE respectively) (Data not reported in table).

3.2. Growth performance and carcass traits

Table 3 shows the effect of dietary treatment and gender on live performance.

Table 3. Effect of dietary treatment and gender on live performance

	Dietary treatments			Gender		
	C	HS	HSE	Gilts	Barrows	R-MSE
N. of pigs	24	24	24	36	36	
Initial LBW (kg)	114.6	114.5	112.6	111.8	116.0	10.96
ADG (kg)	0.59	0.64	0.61	0.60	0.62	0.11
ADFI[(1)] (kg/day)	2.40	2.42	2.39	--	--	0.09
FCR[(1)] (kg*kg^{-1})	4.05	3.81	3.92	--	--	0.21
Slaughter LBW (kg)	174.5	178.7	174.3	173.3	178.4	13.46

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group

R-MSE: root mean square error; LBW: live body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio

(1) mean values of each pen were considered as raw starting data

Different letters in the same line indicate statistically different means ($P<0.05$)

The in vivo performance of the animals was not affected ($P>0.05$) by either diet or gender. Animals that started the trial with a quit balanced live weight across diet and gender groups ($P>0.05$) showed in average the same daily feed intake and the same daily gain without differences in feed conversion rate, and slaughter weight. In general, even in the absence of statistically significant differences, the HS group showed a tendentially higher average daily gain (+0.05 kg) and slaughter weight (+4.2 kg) and a light better conversion index (-0.24 g*g^{-1}), when compared to the C group.

Table 4 shows the effect of dietary treatment and gender on carcass traits.

Table 4. Effect of dietary treatment and gender on carcass traits

	Dietary treatments			Gender		
	C	HS	HSE	Gilts	Barrows	R-MSE
Hot carcass weight (kg)#	147.8	146.9	144.8	145.0	148.0	4.70
Hot carcass yield (%)#	81.60	81.06	80.04	80.05	81.74	2.60
Backfat thickness (mm)#	25.42	25.44	26.05	24.13	27.15	4.20
Lean cuts (%)[1]						
• Thigh	26.33^{B}	26.66^{B}	27.72^{A}	26.89	26.91	0.85
• Loin	18.49	19.00	18.85	19.24^{a}	18.32^{b}	1.02
• Neck	7.20	7.17	7.22	7.34^{a}	7.06^{b}	0.38
• Shoulder	14.84	14.21	14.37	14.58	14.36	0.68
• Total lean cuts	66.86^{B}	67.04^{B}	68.16^{A}	68.05^{a}	66.65^{b}	1.89
Adipose cuts (%)[1]						
• Backfat	4.47	4.36	3.73	3.92^{b}	4.46^{a}	0.77
• Belly	13.21	13.41	13.08	13.27	13.20	1.02
• Jowl	7.81	7.24	7.32	7.21^{b}	7.70^{a}	0.60
• Perirenal fat	1.70	1.74	1.63	1.45^{B}	1.92^{A}	0.37
• Total adipose cuts	27.20	26.76	25.76	25.85^{b}	27.29^{a}	1.96
Head (%)[1]	4.65	4.91	4.79	4.79	4.77	0.40

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group
covariate for slaughter LBW
R-MSE: root mean square error.
[1] = as % of hot carcass weight
Within each effect, different letters in the same line indicate statistically different means for $P<0.05$ $^{(a,b,c)}$ or $P<0.01$ $^{(A,B,C)}$

Hot carcass weight, carcass yield, and backfat thickness, covariate for slaughter live weight, were not influenced by either diet or gender. However, concerning the main commercial cuts HSE group had the highest lean cuts percentage due to the greatest thigh percentage and the differences were statistically significant ($P<0.01$) compared to both the C and HS groups. No difference attributable to dietary treatment was found for adipose cuts, although carcasses from treated groups showed, overall, a slightly lower amount of this component ($P>0.05$).

Gilts had a higher content of lean cuts ($P<0.05$) compared with barrows, particularly loin and neck percentages ($P<0.05$). The opposite was observed for adipose cuts, which were more prevalent in barrows ($P<0.05$) than in gilts, especially backfat, jowl ($P<0.05$), and perirenal fat ($P<0.01$) percentages.

3.3. Chemical and physical characteristics of longissimus thoracis muscle

Table 5 reports the chemical and physical characteristics of *longissimus thoracis* muscle at 24 h *postmortem* and during two different times of refrigerated storage.

Table 5. Effect of dietary treatment and gender on chemical and physical characteristics of *longissimus thoracis* muscle at 24h *p.m.* and during refrigerated storage at 4±1 °C for 3 and 7 days

	Dietary treatments			Gender		
	C	HS	HSE	Gilts	Barrows	R-MSE
24 h postmortem						
pH	5.54[b]	5.64[a]	5.59[ab]	5.59	5.59	0.10
L*	57.33	56.45	55.40	57.78[a]	55.00[b]	3.81
a*	1.52	1.69	1.71	1.65	1.62	0.81
b*	11.49[a]	11.11[a]	10.65[b]	11.21	10.90	1.10
Chroma (C*)	11.60[d]	11.26[de]	10.73[e]	11.36	11.04	1.14
Hue angle (H*)	82.51	81.40	80.96	81.67	81.58	3.83
Moisture (%)	73.26	73.30	72.88	73.25	73.05	0.71
Ether extract (%)	2.19	1.68	2.21	1.87	2.18	0.87
Protein (%)	23.15	23.22	23.21	23.24	23.14	0.52
Drip loss (%) [(1)]	4.33	3.87	4.05	4.40	3.76	12.68
Cooking loss (%)	28.15[d]	26.33[cd]	24.84[e]	26.36	26.53	4.41
MDA (mg/kg) raw	0.172	0.134	0.154	0.168	0.138	0.10
MDA (mg/kg) cooked	0.389	0.338	0.320	0.371	0.327	0.13
3 days of refrigerated storage						
pH	5.57[b]	5.70[a]	5.60[a]	5.61	5.64	0.11
L*	59.26	58.22	57.52	58.23	58.43	3.23
a*	4.51	4.63	4.27	4.23	4.71	1.49
b*	13.44	13.17	12.71	12.89	13.32	1.40
Chroma (C*)	14.22	13.98	13.45	13.61	14.16	1.75
Hue angle (H*)	71.73	71.07	71.95	72.30	70.87	4.44
ΔE 24h_3g	4.61	5.13	4.94	4.31	5.48	2.11
Cooking loss (%)	27.14	27.05	26.97	26.79	27.31	4.12
MDA (mg/kg) raw	0.183	0.164	0.170	0.178	0.166	0.08
MDA (mg/kg) cooked	0.326	0.321	0.289	0.314	0.310	0.12
7 days of refrigerated storage						
pH	5.53[b]	5.64[a]	5.59[ab]	5.59	5.59	0.10
L*	59.69	59.30	58.60	59.72	58.74	3.61
a*	4.03	4.24	3.95	3.82	4.33	1.25
b*	13.26	13.20	12.87	13.01	13.22	1.23
Chroma (C*)	13.89	13.89	13.51	13.58	13.94	1.47
Hue angle (H*)	73.24	72.43	73.47	73.92	72.17	4.11
ΔE 24h_7g	4.04	5.03	5.55	4.18[e]	5.57[d]	2.10
Cooking loss (%)	25.62	26.36	24.53	25.43	25.58	3.67
MDA (mg/kg) raw	0.223	0.209	0.217	0.209	0.224	0.06
MDA (mg/kg) cooked	0.390[d]	0.322[e]	0.338[de]	0.347	0.353	0.09

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group
R-MSE: root mean square error
MDA: malondialdehyde
[(1)] After 48 hours of refrigerated storage (4°C)
Different letters in the same line indicate statistically different means for P<0.05 [(a,b,c)] or P<0.08 [(d,e,f)] within each effect

Overall, the dietary treatment did not significantly affect most of the qualitative traits examined in LT except for pH which was higher for the treated groups compared with the C group, but while HS resulted always significant (P<0.05), HSE group was different from C (P<0.05) only at 3 days of refrigerated storage. In addition, at 24 hours post-mortem, the HSE group showed lower values of b* (P<0.05), C* (P<0.08), and cooking loss (P<0.08) than the C group. The HS group showed intermediate values with no statistically significant differences from the other two groups.

There were no differences (P>0.05) in LT characteristics between barrows and gilts, except for muscle color lightness (L*) which was lower (P<0.05) at 24 h *p.m.* and a tendentially higher ΔE (P<0.08) on day 7 of storage in barrows. The chemical composition of the LT (moisture, ether extract, and protein)

was not affected by either gender or diet. The MDA content of muscle samples during storage is shown in Table 5 for raw and cooked LT. No statistically significant differences (P>0.05) were found between the control and treated groups, except in cooked samples on day 7, where the HS group had a tendentially lower MDA level (P<0.08) compared to the C group, while the HSE group showed intermediate values. Additionally, Figures 1 and 2 show graphically the variations in MDA content during refrigerated storage of raw meat (Figure 1) and after cooking (Figure 2). Data indicates that in raw samples, all three groups followed the same increasing trend over time. In cooked samples, instead, the C and HSE groups followed a similar trend, while the HS samples remained almost unchanged throughout the three sampling days, confirming what was found with the inclusion of hazelnut skin directly in pork burghers. However, despite not finding significant differences, the supplemented groups always showed numerically lower values than C, both in raw and cooked meat. MDA content was not affected by gender.

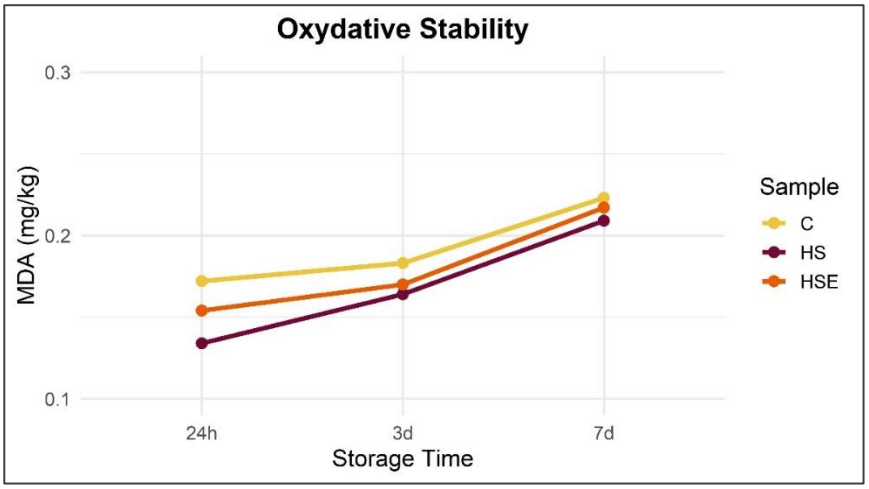

Figure 1. Variations of malondialdehyde (MDA mg/kg) content during refrigerated storage of raw *longissimus thoracis* muscle samples. C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group.

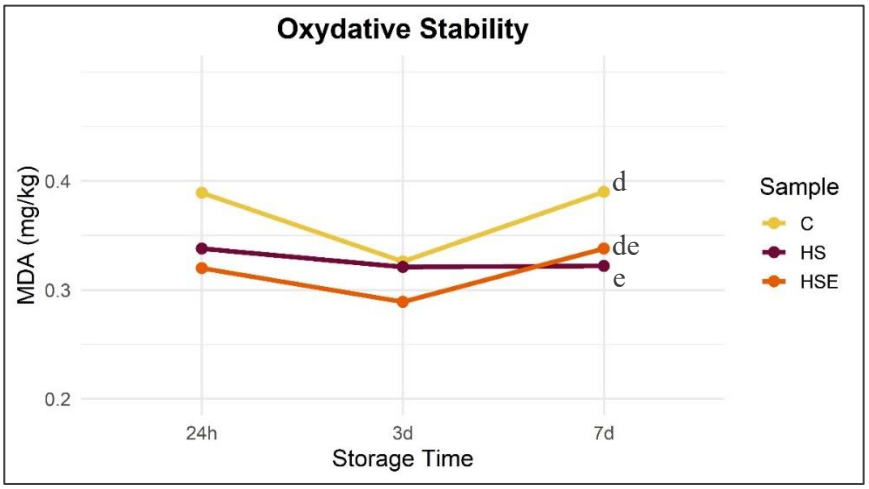

Figure 2. Variations of malondialdehyde (MDA mg/kg) content of cooked *longissimus thoracis* muscle samples after different storage times. C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group. Different letters indicate statistically different means for P<0.08 $^{(d,e)}$ within each effect.

3.4. Fatty acid profile of longissimus thoracis muscle and subcutaneous adipose tissue

The fatty acid profile of *longissimus thoracis* muscle is reported in Table 6.

Table 6. Effect of dietary treatments and gender on fatty acid profile (% of total fatty acid) *of longissimus thoracis* muscle

	Dietary treatments			Gender		
	C	HS	HSE	Gilts	Barrows	R-MSE
C10:0 (capric)	0.08^{b}	0.12^{a}	0.11^{ab}	0.10	0.11	0.04
C12:0 (lauric)	0.09	0.09	0.09	0.09	0.09	0.01
C14:0 (myristic)	1.37	1.35	1.37	1.33	1.40	0.13
C16:0 (palmitic)	24.15	23.98	24.10	23.76	24.38	1.08
C17:0 (heptadecanoic)	0.10^{b}	0.18^{a}	0.17^{a}	0.16	0.14	0.06
C18:0 (stearic)	12.38	12.40	11.83	12.40	12.01	0.82
C20:0 (eicosanoic)	0.13^{ab}	0.18^{a}	0.11^{b}	0.13	0.14	0.08
C16:1 (palmitoleic)	3.54	3.43	3.58	3.40	3.63	0.37
C17:1 (heptadecenoic)	0.18	0.18	0.22	0.21	0.18	0.07
C18:1n-7 (vaccenic)	4.05	3.98	4.12	4.23	3.86	0.73
C18:1n-9 (oleic)	39.61	38.36	39.66	37.99^{B}	40.43^{A}	2.67
C20:1 (eicosenoic)	0.67	0.65	0.65	0.62^{b}	0.69^{a}	0.08
C18:2n-6 (linoleic)	9.95	10.88	10.17	11.11^{a}	9.55^{b}	1.95
C18:3n-3 (α-linolenic)	0.30	0.31	0.30	0.31	0.30	0.04
C18:3n-6 (γ-linolenic)	0.06	0.05	0.05	0.06	0.05	0.11
C20:2n-6 (eicosadienoic)	0.32	0.29	0.33	0.31	0.31	0.07
C20:4n-6 (arachidonic)	2.54	2.84	2.58	3.07^{a}	2.23^{b}	1.24
C20:5n-3 (eicosapentaenoic)	0.06	0.06	0.06	0.06	0.05	0.02
C22:4n-6 (docosatetraenoic)	0.21^{b}	0.39^{a}	0.33^{ab}	0.37^{a}	0.25^{b}	0.16
C22:5n-3 (docosapentaenoic)	0.02	0.03	0.02	0.03	0.02	0.02
C22:6n-3 (docosahexaenoic)	0.17^{b}	0.26^{a}	0.15^{b}	0.23^{a}	0.15^{b}	0.10
Total saturated	38.30	38.30	37.78	37.98	38.27	1.74
Total monounsaturated	48.06	46.59	48.23	46.45^{B}	48.80^{A}	2.44
Total polyunsaturated	13.64	15.11	13.99	15.57^{a}	12.93^{b}	3.36
Total n-6	13.09	14.44	13.47	14.93^{a}	12.40^{b}	3.23
Total n-3	0.55^{b}	0.67^{a}	0.53^{b}	0.64^{a}	0.52^{b}	0.13
n-6/n-3 ratio	23.90^{ab}	21.57^{b}	25.70^{a}	23.55	23.90	3.29
Atherogenic index (AI)	0.48	0.48	0.48	0.47	0.49	0.04
Thrombogenic index (TI)	1.18	1.16	1.15	1.15	1.17	0.09

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group
R-MSE: root mean square error
AI=[C12:0+(4x C14:0)+C16:0]/[n-6PUFA+n-3PUFA+MUFA](Ulbricht & Southgate, 1991).
TI=[C14:0+C16:0+C18:0]/[(0.5xMUFA)+(0.5x n-6PUFA)+(n-3PUFA/n-6PUFA)] (Ulbricht & Southgate, 1991).
Within each effect, different letters in the same line indicate statistically different means for P<0.05 $^{(a,b,c)}$ or P<0.01 $^{(A,B,C)}$

In general, the diet did not result in consistent changes to the fatty acid profile; indeed, if we look at the totals of the fatty acid classes, we see only a slight increase in omega-3 (P<0.05) in HS and a lower omega-6/omega-3 ratio when compared with HSE, driven by a higher docosahexaenoic fatty acid content (P<0.05).

Regarding the effect of gender, barrows presented a higher content of monounsaturated fatty acids, specifically due to higher values of oleic fatty acid (P<0.01) and eicosenoic fatty acid (P<0.05). Gilts,

on the other hand, were characterized by a higher content of polyunsaturated fatty acids, both omega-3 and omega-6, due to higher percentages of linoleic acid, arachidonic acid, docosatetraenoic fatty acid, and docosahexaenoic fatty acid ($P<0.05$). Despite these differences, the omega-6/omega-3 ratio, atherogenic index, and thrombogenic index remained unchanged.

Table 7 reports the fatty acid profile of subcutaneous adipose tissue.

Table 7. Effect of dietary treatments and gender on fatty acid profile (% of total fatty acid) of subcutaneous adipose tissue

	Dietary treatments			Gender		
	C	HS	HSE	Gilts	Barrows	R-MSE
C10:0 (capric)	0.06	0.06	0.06	0.06	0.06	0.01
C12:0 (lauric)	0.10	0.10	0.09	0.10	0.10	0.01
C14:0 (myristic)	1.35^{a}	1.32^{ab}	1.28^{b}	1.30	1.34	0.09
C16:0 (palmitic)	24.68	24.46	24.33	24.35	24.64	0.92
C17:0 (heptadecanoic)	0.29	0.30	0.29	0.29	0.30	0.06
C18:0 (stearic)	13.85	14.31	14.01	14.30	13.81	0.82
C20:0 (eicosanoic)	0.30	0.29	0.27	0.29	0.28	0.06
C16:1 (palmitoleic)	1.79^{a}	1.59^{b}	1.62^{b}	1.62	1.70	0.16
C17:1 (heptadecenoic)	0.23	0.23	0.25	0.24	0.24	0.03
C18:1n-7 (vaccenic)	2.45	2.33	2.31	2.24	2.48	0.69
C18:1n-9 (oleic)	37.18^{a}	35.82^{b}	36.37^{ab}	36.22	36.69	1.41
C20:1 (eicosenoic)	0.85	0.85	0.84	0.82	0.87	0.10
C18:2n-6 (linoleic)	14.95^{b}	16.26^{a}	16.22^{a}	16.14	15.48	1.15
C18:3n-3 (α-linolenic)	0.74	0.75	0.77	0.76	0.75	0.05
C18:3n-6 (γ-linolenic)	0.07	0.06	0.06	0.06	0.06	0.03
C20:2n-6 (eicosadienoic)	0.70	0.74	0.74	0.72	0.73	0.06
C20:3n-3 (eicosatrienoic)	tr^{c}	0.13^{a}	0.07^{b}	0.06	0.07	0.04
C20:4n-6 (arachidonic)	0.22	0.22	0.24	0.23	0.22	0.03
C20:5n-3 (eicosapentaenoic)	tr	tr	tr	tr	tr	0.00
C22:4n-6 (docosatetraenoic)	0.11	0.09	0.11	0.11	0.10	0.03
C22:5n-3 (docosapentaenoic)	tr	tr	tr	tr	tr	0.00
C22:6n-3 (docosahexaenoic)	0.06	0.07	0.07	0.07	0.07	0.02
Total saturated	40.64	40.85	40.34	40.69	40.53	1.59
Total monounsaturated	42.51^{a}	40.82^{b}	41.38^{b}	41.15	41.99	1.16
Total polyunsaturated	16.85^{b}	18.33^{a}	18.28^{a}	18.16	17.48	1.29
Total n-6	16.04^{b}	17.38^{a}	17.37^{a}	17.27	16.60	1.22
Total n-3	0.81^{b}	0.95^{a}	0.91^{a}	0.89	0.89	0.09
n-6/n-3 ratio	19.88^{a}	18.28^{b}	19.34^{a}	19.50	18.84	1.27
Atherogenic index (AI)	0.51	0.50	0.50	0.51	0.50	0.03
Thrombogenic index (TI)	1.26	1.25	1.23	1.25	1.25	0.09
Iodine value (IV)	67.52	68.50	68.85	68.60	68.08	2.23

C: control group; HS: experimental Hazelnut Skin group; HSE: experimental Hazelnut Skin Extract group

R-MSE: root mean square error; tr: trace

AI=[C12:0+(4*C14:0)+C16:0]/[n-6PUFA+n-3PUFA+MUFA] (Ulbricht & Southgate, 1991)

TI=[C14:0+C16:0+C18:0]/[(0.5*MUFA)+(0.5*n-6PUFA)+(n-3PUFA/n-6PUFA)] (Ulbricht & Southgate, 1991)

IV= (85.703 + [C14:0] x 2.740 - [C16:0] x 1.085 - [C18:0] x 0.710 + [C18:2n-6] x 0.986) (Lo Fiego et al., 2016)

Within each effect, different letters in the same line indicate statistically different means for $P<0.05^{(a,b,c)}$

HS and HSE groups showed a lower monounsaturated fatty acids content compared to the C group ($P<0.05$), due to lower palmitoleic and oleic fatty acid content, and a higher polyunsaturated fatty acids content, attributable to a greater amount of linoleic and eicosatrienoic fatty acid ($P<0.05$). This also translates into a higher omega-6 and omega-3 fatty acids content in the HS and HSE groups compared to the C ($P<0.05$). Regarding the omega-6/omega-3 ratio, the HS group showed lower values than the other two groups ($P<0.05$).

No significant differences were observed between the groups in terms of the atherogenic index, thrombogenic index, or iodine value.

Gender did not influence the fatty acid profile of backfat.

4. Discussion

Sustainable livestock production is increasingly vital for reducing environmental impact and optimizing resources. A key strategy is incorporating agricultural by-products into animal diets, which minimizes waste and enhances growth, carcass quality, and meat traits. This approach lowers feed costs, reduces dependence on traditional ingredients, and supports circular economy practices in animal agriculture. In this study, we applied this approach by exploring the possibility of incorporating hazelnut skin and its green phenolic extract into the finishing pig diet.

Hazelnut skin is an agri-food by-product generated by roasting hazelnuts. The results obtained (Table 2) show that it is characterized by a low moisture content (7%) which facilitates its transportation, conservation, and processing for use as an ingredient in animal feed. Its high fiber content (about 17%) could however represent a limit for its inclusion in high quantities in diets for monogastric animals such as pigs; a limit that can be overcome by using its polyphenolic extract, even if, at an economic level, the extraction process is subject to higher costs. However, HS is rich in lipids (25%), mainly consisting of oleic fatty acid (76%) and linoleic fatty acid (15%). It also shows a satisfactory amount of protein (9.4%) and high amounts of antioxidant substances mainly represented by flavonoids (487 mg/100g, 1.013 in the green extract) and phenolic acids (17 mg/100g, 109 in the extract). This resulted in a higher amount of all phenolic compounds in the feeds of the supplemented groups compared to the control (Table 1). However, the in vivo performance results indicate that diet did not significantly affect the overall growth, feed intake, or feed efficiency of animals. Considering that these parameters tended to have slightly better values in the treated groups, especially in the HS group where during the 101-day trial its live weight increased by about 57% compared with 53% in the C group and 56% in the HSE group (data not shown in the table), we believe that the absence of differences is probably due to the low levels of supplementation adopted. This suggests that the dietary interventions used in this study were equally effective in supporting normal growth patterns in Italian heavy pigs. This is not a given, as dietary changes can often disrupt growth performance, especially including in the feed high levels of some by-products with high fiber content such as hazelnut skin. Indeed, in a previous study Zhu et al. (2019), stated that the inclusion of 15% mulberry leaf powder in finishing pig feed reduced growth performance in terms of average daily gain and

feed-to-gain ratio, while, Biondi et al. (2020), adding 15% of tomato processing by-products to the diets of castrated pigs did not find significant effects on growth performance.

Gender did not significantly influence slaughter weight and average daily gains (Table 3), however, sex-specific differences emerged in the commercial cuts percentage (Table 4), demonstrating that physiological differences between gilts and barrows influence carcass characteristics. The higher yield of lean cuts in gilts, particularly in the loin and neck, aligns with previous research indicating that gilts tend to deposit less fat and produce leaner carcasses compared to barrows (Latorre et al., 2009; Minelli et al., 2013; Pérez-Ciria et al., 2021). Conversely, the increased adipose cuts content observed in barrows, particularly in backfat, jowl, and perirenal fat, highlights the different fat deposition patterns between sexes, driven by castration, which primarily promotes more fattening of castrated pigs (Barton-Gade, 1987; Yao et al., 2011). These results suggest that sex remains anyway a crucial factor for carcass composition.

The dietary effects were more subtle, with the only significant difference being the greater thigh yield observed in the HSE group. This result, which translates into an overall increase in lean cut weight, could suggest a marginal advantage of the HSE diet in promoting lean tissue growth. Such differences, although limited, could provide a competitive advantage in a commercial context where the thighs are intended for PDO production. The results obtained likely stem from the low level of by-products included in the feeds, therefore dietary changes did not interfere with pig growth performance, ensuring no negative repercussions on the carcass and main commercial cuts.

Muscle quality characteristics (Table 5) showed minimal variations between diets and sexes, indicating that the treatments did not compromise meat quality. The consistent trend of higher pH in the HS group, although statistically significant, suggests only slight alterations in muscle acidification, potentially reflecting differences in post-mortem glycolysis or muscle energy reserves. A similar result was also obtained by Xu et al. (2022) in finishing pigs fed grape seed proanthocyanidin extract (50, 100, and 200 mg/kg).

Color measurements and cooking loss further delineated minimal differences between groups, with the HSE diet resulting in lower cooking losses compared to the Control and HS groups. This suggests that the HSE diet may improve water retention during cooking, potentially leading to juicier meat products. This trend aligns with literature findings, as the addition of plant-based phenolic supplements and mulberry leaves to finishing pig feed respectively increased juiciness and reduced cooking loss and drip loss (Muzolf-Panek et al., 2024; Zhu et al., 2019).

The greater color saturation in the control and HS samples may indicate a more vivid meat color. As reported in the literature, such dietary supplementation may lead to colorimetric differences, primarily affecting the a* parameter due to changes in myoglobin content, which increases meat redness (Muzolf-Panek et al., 2024; Xu et al., 2022). However, our results seem to be supported by several studies where colorimetric parameters were not influenced by dietary supplementation (Pieszka et al., 2017; Scerra et al., 2022).

The effects of sex on meat color were minimal, with barrows showing slightly darker meat (lower L*) and greater color variation (higher ΔE) during storage. Although these differences may not

substantially affect consumer perception, agree with previous research (Alonso et al., 2009; Belmonte et al., 2021; Daza et al., 2018) that describes barrow meat as a globally redder meat.

The oxidative stability of muscle samples, reflected by malondialdehyde (MDA) content, showed limited dietary effects. However, reduced MDA levels in the HS group on day 7 suggest that the HS diet may confer some degree of antioxidant protection during initial storage, potentially delaying lipid oxidation. Literature results regarding lipid oxidation in such studies are varied. In some cases, dietary treatment influenced meat oxidative stability by increasing antioxidant capacity and consequently decreasing oxidation products, as observed with mulberry leaves (15%), plant-based phenolic supplements (0.1%), and grape seed proanthocyanidin extract (50, 100, 200 mg/kg) in studies by Zhu et al. (2019), Muzolf-Panek et al. (2024), and Xu et al. (2022), respectively. In other cases, the inclusion of 15% vegetable by-products (tomato or bergamot processing waste) in the diet did not affect or alter meat oxidative stability (Biondi et al., 2020; Scerra et al., 2022).

The antioxidant effect of such plant matrices may depend heavily on the nature of the bioactive compounds present and the metabolites formed during digestion, as well as the ability of these metabolites to be absorbed and transferred to the animal's muscles. As demonstrated in the literature, even at high concentrations (15%), effects are not always observed, whereas lower concentrations (0.1%) of different matrices may yield results. In our specific case, the trend toward lower MDA values in treated groups may suggest that the by-product selected for the trial is qualitatively suitable for use as an antioxidant supplement, but the quantities used may have been too low to observe significant differences.

Regarding fatty acid composition, the results suggest that supplementation with hazelnut derivatives can selectively influence fatty acid proportion, with potential benefits linked to higher omega-3 levels and a lower omega-6/omega-3 ratio, especially in HS group (Tables 6 and 7). Overall, the changes in fatty acid composition attributable to dietary treatment were much more pronounced in the backfat (Table 7) than in the muscle (Table 6) and this agrees with previous research (Li et al., 2015; Koch et al., 1968), highlighting how the intramuscular fat of the LT is comparatively less sensitive than adipose tissues to PUFA dietary incorporation.

However, the heterogeneity of effects between the HS and HSE groups indicates that hazelnut skin, as expected, may exert a more pronounced effect on improving lipid balance than the extract, highlighting the potential importance of using whole ingredients rather than purified extracts to achieve nutritional benefits. The results obtained with hazelnut skin in the HS group align with those observed with silage bergamot pulp or apple and strawberry supplementation (Scerra et al., 2022; Pieszka et al., 2017), where omega-3 levels increased, albeit more markedly. Similarly, the increased omega-6/omega-3 ratio in the HSE group is consistent with literature findings (Biondi et al., 2020; Xu et al., 2022), indicating the common challenge of balancing polyunsaturated fatty acids in meat through dietary supplementation without the direct addition of essential fatty acids.

Regarding the gender effect, the only differences in fatty acid composition were found in intramuscular lipids of LT (Table 6) where females showed lipids richer in polyunsaturated fatty acids,

and poorer in monounsaturated fatty acids compared to barrows, confirming previous research (Belmonte et al., 2021; Lo Fiego et al., 2010).

5. Conclusion

The incorporation of hazelnut skin and its green phenolic extracts into finishing pig diets demonstrates promising potential. Although the dietary interventions did not have a significant overall impact, the subtle improvements observed and the absence of interference with growth performance, carcass characteristics, and final meat quality indicate the possibility of notable commercial advantages. This by-product could indeed play a fundamental role in sustainable livestock production by reducing waste and enriching animal diets. Future research should focus on refining supplementation levels to maximize benefits and fully exploit the bioactive properties of hazelnut by-products, paving the way for broader adoption in commercial pig farming.

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Chapter 6. General conclusions

The food industry is facing significant challenges driven by the growing focus on issues such as sustainability and food safety. The quality of meat and meat products no longer depends only on objective characteristics that define their suitability for consumption but is now influenced by the choices of consumers who are increasingly aware of the strong correlation between food, health and the environment. Therefore, it is essential to develop new production and processing strategies that can improve the health properties of meat and its derivatives while fully respecting the environment. The focus of this doctoral work was to enhance the nutritional characteristics, oxidative stability, and technological properties of pork meat, without overlooking the possibility of making pig farming more sustainable by intervening in the diet. The first strategy addressed in this study was to demonstrate that including natural substances with specific nutritional characteristics in pig diets allows the production of healthier meat in terms of fatty acid composition. Indeed, the inclusion of extruded flaxseed in the diet, characterized by a low n-6 to n-3 polyunsaturated fatty acid ratio, resulted in an increase in n-3 PUFA content and a better balance between n-6 and n-3 PUFA in all analyzed tissues (intramuscular, subcutaneous and perirenal adipose tissues). Furthermore, it improved the nutritional indices of lipids, with particularly positive effects on subcutaneous and perirenal adipose tissues. These changes have potential health benefits for humans while preserving the technological properties of lipids, particularly without negatively impacting oxidative stability or carcass characteristics. This study confirmed that dietary intervention is a powerful tool for controlling and improving the quality of meat and meat products. Nevertheless, it must be noted that pork is particularly susceptible to lipid oxidation, especially considering its tendency to undergo various transformations. Therefore, subsequent strategies were developed to stabilize pork and its derivatives while simultaneously improving their nutritional and technological characteristics and promoting greater eco-compatibility within the supply chain. For this reason, attention was focused on agro-industrial by-products, which are valuable sources of biomolecules but are currently considered food waste and, at best, destined for underutilized disposal methods. The green strategies implemented targeted both food and feed. On one hand, the direct use of agri-food by-products, either in their raw form or as processed extracts, was explored as a means of introducing new ingredients into meat products to leverage their natural antioxidant properties, as well as their potential to provide nutritional enrichment and functional benefits. On the other hand, the inclusion of these by-products in animal feed was investigated to determine whether the metabolism of the bioactive compounds within the animal could influence the stability and quality of the animal's tissues, and consequently, the derived meat products, while also making the animal’s diet healthier and livestock production more sustainable. The formulation of pork-based foods, such as pork burgers, with the addition of by-products proved to be highly promising. The first trial revealed that the addition of hazelnut skins, a by-product of hazelnut roasting, to pork burgers reduced lipid oxidation, thereby improving product stability during both storage and cooking. Conversely, dried tomato peel, a by-product of the tomato processing industry, exhibited a protective effect limited to the cooking phase, highlighting the need

for further research on this ingredient, potentially by modifying the dose used or the storage period. Both by-products significantly increased the fiber content, with dried tomato peel providing a particularly notable contribution, and influenced the fatty acid composition of the burgers by increasing PUFA levels and reducing the omega-6/omega-3 ratio. They also maintained microbial stability without affecting sensory acceptability. The observation of the antioxidant properties of hazelnut skins in pork led to a second trial that validated the antioxidant efficacy of hazelnut skins in pork burgers and explored the potential use of their phenolic green extract. Both products substantially improved oxidative stability across all sampling days and during sample handling. While slight changes in sensory attributes, such as color and tenderness, were noted, overall acceptability remained largely unaffected. Moreover, consumer perceptions were positively influenced when informed about the natural origin and health benefits of the additives, underscoring the growing demand for eco-friendly and clean-label ingredients in food products. These same products, hazelnut skins and their phenolic green extract, were used to formulate new finishing diets for heavy pigs to explore the potential for enhancing farm sustainability while simultaneously improving meat stability and quality. The integration of these by-products showed promising potential. Although dietary interventions did not produce significant overall effects, subtle improvements were observed, such as increased ham yield and reduced cooking losses in the group fed with the extract, along with slight improvements in oxidative stability after 7 days of storage and an increase in omega-3 levels, which resulted in a lower omega-6/omega-3 ratio, in the group fed with hazelnut skins. These improvements, combined with the absence of negative effects on growth performance, carcass characteristics, and meat quality, suggest interesting commercial opportunities for this by-product. However, further research is necessary to optimize integration levels, maximize benefits, and fully exploit the bioactive properties of hazelnut by-products, paving the way for wider adoption in commercial pig farming. Improving meat quality starting from the animal's muscle and stabilizing its qualitative and technological characteristics once transformed by using what are considered waste materials addresses both directly and indirectly the issue of food waste and supply chain sustainability. This strategy effectively revalorizes agro-industrial waste by treating it as a valuable raw material and reduces the creation of additional food waste by increasing the product's storage stability and enhancing its technological and nutritional properties. This reduces the likelihood of the product degrading before consumption or being unappreciated by consumers, who might otherwise discard it. The role of research in such topics is undoubtedly crucial, but several external factors must be considered, including consumer acceptance and marketability. Consumer perceptions, taste preferences, and product labelling play a vital role in determining the market success of foods based on by-products. Therefore, it is essential that products recovered from co-product streams are marketed and presented in a way that eliminates associations with waste. Furthermore, the materials and energy outputs must be competitively priced in the global market to be fully implemented. These are complex challenges that require extensive data support, but by adopting a circular economy approach, the use of by-products can thrive, helping to optimize resource utilization and minimize waste, contributing to sustainability and efficiency within the food chain.

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