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CONTROLO DA QUALIDADE

Formulation/development of a food product with an agri-food by-product

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**Formulation/development of a food product
with an agri-food by-product**

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DECLARAÇÃO DE REPRODUÇÃO

É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA DISSERTAÇÃO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.), APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

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RESUMO

A pasta de bagaço de azeitona termicamente tratada (HT-OPP) é um ingrediente alimentar interessante devido ao seu baixo teor de gordura, elevado teor em fibra e especialmente de hidroxitirosol. Este trabalho teve por objetivo valorizar a HT-OPP, como ingrediente alimentar, incorporando-o num produto alimentar: paté de azeitona.

Numa primeira fase, decidiu-se incorporar percentagens crescentes de HT-OPP num paté de azeitona até se atingir um nível de aceitabilidade adequado. Para tal, realizou-se uma análise sensorial preliminar de aceitabilidade, mostrando os resultados que os consumidores aceitam a incorporação de 20%, e possivelmente de 25% de HT-OPP nos patés de azeitona. Numa segunda fase, decidiu-se comparar as propriedades sensoriais e químicas de um paté de azeitona comercial (CP) com as de um paté de azeitona incorporando 0%, 20% e 25% de HT-OPP. Assim, realizou-se outro teste sensorial (CATA, aceitabilidade e intenção de compra) usando um paté de azeitona, sem incorporação de HT-OPP, mais dois com 20% e 25% de HT-OPP e um CP. Os patés produzidos no laboratório foram submetidos a *High Pressure Processing* (HPP) para garantir a sua segurança microbiológica. Todos os patés de azeitona foram quimicamente avaliados: composição nutricional, perfil e teor total de vitamina E (HPLC-DAD-FLD), perfil de ácidos gordos (GC-FID) e de hidroxitirosol (HPLC-DAD-FLD). Os compostos fenólicos totais e a atividade antioxidante (TPC, FRAP, DPPH*) foram determinados por métodos espectrofotométricos.

Os resultados obtidos mostraram que o uso de HPP não afetou nenhum dos parâmetros químicos avaliados ($p > 0,05$). Em relação aos patés, CP registou um teor de humidade, proteínas, hidratos de carbono restantes, sal, Σ PUFA, TPC e atividades antioxidantes significativamente inferiores aos das outras amostras ($p < 0,05$). Por outro lado, apresentou teores significativamente superiores de gordura, Σ MUFA e MUFA/PUFA ($p < 0,05$), não tendo sido detetado/quantificado hidroxitirosol. Além disso, os patés com incorporação de 20% e 25% de HT-OPP submetidos a HPP (P20* e P25*) apresentaram atributos químicos interessantes ao nível dos teores de fibra, vitamina E, ácidos gordos e teor de hidroxitirosol. No teste CATA, os consumidores detetaram diferenças sensoriais entre os patés de azeitona. Os testes de aceitabilidade e intenção de compra revelaram que os consumidores gostaram mais de P20* e P25* do que do CP, estando também mais dispostos a comprá-los.

Concluindo, P20* e P25* são patés de azeitona promissores devido aos seus atributos de qualidade e avaliação sensorial positiva. O P25* foi o produto escolhido para ser apresentado à indústria alimentar visando a sua comercialização.

Palavras chave: Pasta de bagaço de azeitona termicamente tratada; Pâté de azeitona; *High Pressure Processing* (HPP); Hidroxitirosol; CATA.

ABSTRACT

Heat-treated olive pomace paste (HT-OPP) is an interesting food ingredient owing to its' low-fat, high-fibre and especially hydroxytyrosol contents. This work aimed to valorise HT-OPP, as a food ingredient, incorporating it in a food product: an olive pâté.

In a first stage, it was decided to incorporate increasing percentages of HT-OPP in an olive pâté until an adequate sensorial acceptability level was reached. For that, a preliminary acceptability sensory test was performed, showing the results that consumers accept the incorporation of 20%, and possibly 25% of HT-OPP in the olive pâtés. In a second stage, it was decided to compare the sensory and chemical properties of a commercial olive pâté (CP) with those of an olive pâté incorporating 0%, 20% and 25% of HT-OPP. Thus, it was performed another sensorial test (CATA, acceptability, and purchase intention) using an olive pâté without HT-OPP incorporation, two more with 20% and 25% of HT-OPP, and a CP. The pâtés produced in the laboratory were submitted to High Pressure Processing (HPP) to ensure their microbiological safety. All olive pâtés were chemically analysed: nutritional composition, profile and total vitamin E (HPLC-DAD-FLD), fatty acids profile (GC-FID), and hydroxytyrosol content (HPLC-DAD-FLD). Total phenolic compounds and antioxidant activity (TPC, FRAP, and DPPH•) were determined by spectrophotometric methods.

The results obtained showed that the use of HPP did not affect any of the chemical parameters evaluated ($p>0.05$). Regarding the pâtés, the CP registered a content of moisture, protein, remaining carbohydrate, salt, Σ PUFA, TPC and antioxidant activities significantly lower than all the other samples ($p<0.05$). On the other hand, it had a significantly higher levels of fat, Σ MUFA and MUFA/PUFA ($p<0.05$), and hydroxytyrosol was not detected/quantified. Furthermore, the pâté incorporating 20% and 25% HT-OPP submitted to HPP (P20* and P25*) showed interesting chemical attributes in terms of the contents of fibre, vitamin E, fatty acids, and hydroxytyrosol. In the CATA test, consumers detected sensory differences between the olive pâtés. The acceptability and purchase intention tests revealed that consumers liked P20* and P25* more than the CP, being also more willing to buy them.

To conclude, P20* and P25* are promising olive pâtés due to their quality attributes and positive sensory evaluation. P25* was the product chosen to be presented to the food industry aiming its commercialization.

Keywords: Heat-treated olive pomace paste; Olive pâté; High Pressure Processing (HPP); Hydroxytyrosol; CATA.

PUBLICATIONS AND COMMUNICATIONS PRESENTED ALONG THE MASTER COURSE

During this masters' course, different works were presented at national and international scientific congresses and meetings:

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LIST OF ABBREVIATIONS AND SYMBOLS

– ABTS	2,2'-azino-bis (3-ethylenbenzothiazoline-6-sulfonic acid) radical scavenging ability
– AOAC	Association of Official Agricultural Chemists
– CA	Correspondence Analysis
– CATA	Check-all-that-apply
– COVID-19	Coronavirus disease
– DAD	Diode Array Detector
– DPPH•	2,2-diphenyl-1-picrylhydrazyl radical
– dw	Dry weight
– EFSA	European Food Safety Authority
– EU	European Union
– EVOO	Extra-virgin olive oil
– FAME	Fatty acid methyl esters
– FAO	Food and Agriculture Organization
– FID	Flame ionization detector
– FLD	Fluorescence detector
– FRAP	Ferric reducing antioxidant power
– FSE	Ferrous sulphate equivalents
– fw	Fresh weight
– GAE	Gallic acid equivalents
– GC	Gas chromatography
– HC	Hydroxytyrosol content
– HPLC	High-performance liquid chromatography
– HPP	High Pressure Processing
– HT-OPP	Heat-treated olive pomace paste (88 °C/15 sec)
– ISO	International Organization for Standardization
– LDL	Low-density lipoprotein
– MUFA	Monounsaturated fatty acids
– n.d	Not detected
– OMWW	Olive mill waste waters
– OP	Olive pomace
– OPP	Olive pomace paste
– PCA	Principal Component Analysis
– PUFA	Polyunsaturated fatty acids

- **SDGs** Sustainable Development Goals
- **SFA** Saturated fatty acids
- **TE** Trolox equivalents
- **TFC** Total flavonoid content
- **TPC** Total phenolic content
- **TPTZ** 2,4,6-Tris(2-pyridyl)-s-triazine
- **UV** Ultra-violet
- **VOO** Virgin olive oil

CHAPTER I: INTRODUCTION

1.1 Food supply chain current challenges and solutions

Currently, the food supply chain must deal with undesirable circumstances: population growth, climate change, loss of land, and even pandemics. Therefore, sustainable measures should be adopted to overcome these challenges.

1.1.1 Population growth and climate change

Within the next decades, it is expected an increase in the global demand for nutritious food considering population growth, rising incomes, and consumer preferences shifts.

Earth's population is estimated to be 8 and 10 billion in 2023 and 2050, respectively (1). To feed the world's population in 2050, world food production will need to increase by 70% (2). In fact, the needed natural resources to support the present lifestyle is equivalent to three planets (3). Therefore, population growth poses an enormous challenge to the food supply chain, especially considering that climate change affects food production.

Climate change can be defined as a shift in the climate resulting from human activity in a direct or indirect way, which modifies the global atmosphere composition, in addition to the natural variability of the climate noticed during comparable time periods (4).

Climate change poses urgent threats to our environment since an increase in the global average temperature, severe weather incidents, inundations, hurricanes, tsunamis, occurrence of heavy precipitation, prolonged periods of dryness, acidification of the water, and potential sea-level rise are predicted (5). Specifically, a 1.8 to 4.0 °C increase in the global mean temperature is expected at the end of this century (6), and CO₂ levels are predicted to reach 450 ppm in 2030 (7). In the Mediterranean basin/area, a rise of temperature and drought frequency and a decline in precipitation are predicted (8, 9). In Portugal, a country where water is a limited resource (10), climate changes will significantly impact the food supply chain.

A wide range of consequences are predicted for the fishery, agriculture, and livestock production owing to climate change (5). Agriculture explores 40% of the earth's surface (5), meaning that this will probably be a vulnerable sector. Additionally, in Europe, 970 million tonnes of soil/year are lost, and at least 11% of the territory has been affected by moderate to high soil erosion (11). This means that the food supply chain faces an enormous challenge in producing food, and that agriculture is probably the most vulnerable sector. In

fact, it is expected that there will be a drop in the production of various crops. For example, there was already a decrease in the world mean yields of maize (4.1%), wheat (1.8%), and soybeans (4.5%) owing to climate change (12). Hence, the climate change impacts on food production are well understood.

In countries already experiencing high starvation levels, the impacts of climate change on food security will be worsen with time (6). Therefore, urgent actions must be taken to guarantee food security.

1.1.2 The consequences of the COVID-19 pandemic

The consequences of the COVID-19 pandemic in all industrial sectors worldwide were massive, including in the food industry. Indeed, despite not spreading via livestock or products (13), the COVID-19 pandemic significantly impacted the pillars of food security (14).

In particular, the lack of local or migrant labourers, because of illness or restrictions on travelling, significantly affected the food chain. Indeed, it resulted in severe disturbances in livestock production, harvesting, horticulture, cultivating, and crop production (13). The lack of workers caused a delay in food delivery and jeopardized the continuous supply of food to markets (13). This contributed to a lack of some food products and the inability to obtain enough food products to meet nutritional needs. For example, flour went missing on the European food shelves due to the home-cooking activities (13).

Due to the COVID-19 pandemic, millions of people are predicted to be unemployed, under-employed, and working in poverty (15). In fact, approximately 49 million people were predicted to fall into poverty in 2020 (16). The loss of income, food shortages, and the boost of food prices could have led millions of people to food insecurity. Indeed, because of financial regression triggered by the pandemic, more 83 million people might have hungry in 2020 (17).

This pandemic also shifted food consumption patterns: some people started preparing healthy meals, while others consumed more unhealthy snacks, likely due to anxiety and tedium (14). To conclude, the vulnerabilities of the food systems were exacerbated by the COVID-19 pandemic (18), and emphasized the importance of having a resilient and robust food system (14).

1.1.3 Food by-products: a sustainable solution

In 2015, the members of the United Nations adopted an agenda: “The 2030 Agenda for Sustainable Development”. This agenda has a total of 17 Sustainable Development Goals (SDGs), being some of them “no poverty (SDG1)”, “zero hunger (SDG2)”, and “climate action (SDG13)” (19).

The food supply chain should adopt more sustainable measures to assure food security. Food security occurs when all humans have access (physical, economical, and socially) to sufficient and safe food, which suits their needs and preferences for a healthy and active lifestyle (20).

Considering that undernutrition accounts for approximately 45 percent of the fatalities among 5-year-old children (21), and that 265 million people were projected to face food insecurity in 2020 (15), it can be said that food security is far from being guaranteed. This means that the food supply chain should adopt more sustainable measures like reusing food by-products.

Food by-products are secondary products that result from the main agriculture/industrial production, which might have valuable compounds with great functionality and/or bioactivity (22). Their conventional rejection on landfills has negative outcomes to the environment, and social and economic sectors (23). On the other hand, they may contribute to create new food ingredients or food products, which supports their valorisation in the food chain and generates income streams, economic prospects, and employment opportunities (23). There is a rising interest in fruit/vegetable by-products, because of their dietary fibre, polyphenols, carotenes, and flavonoid contents (24), which makes them exciting food ingredients for the food industry.

Globally, nearly 1.3 billion tonnes of foodstuff are wasted, representing an economical burden of \$990 billion (25). Regarding the European Union (EU), 20% of the food is lost or thrown away, which leads to a loss of €143 billion loss annually (11). Considering these numbers and the uncertainty of future food production, the reuse of food by-products seems an innovative and interesting industrial approach to assure the circular economy of the global and European food system.

To summarize, using food by-products as food or food ingredients might offer economic benefits, contribute to food security, produce health advantages, and even minimize the environmental consequences of their mishandling.

1.2 Olive oil production chain and by-products

Olive tree has been planted for at least 7 thousand years (26). Regarding olive oil, its' importance in both cultural and daily life dates to the 7th century BC, when Greek philosophers explored its nutritive and medical properties. At that time, olive oil intake was suggested by Aristotle and Hippocrates for stomach and dermatological ulcers (27).

The Mediterranean culture and diet are extremely linked to olive oil. In fact, olive oil intake counts for one to two-thirds of total vegetable fat ingested in the Mediterranean countries (28).

The production, consumption, and even exportation of olive oil occurs mainly in the EU. Additionally, 99% of the EU production is attained in Spain, Italy, Greece, and Portugal (29). Therefore, it is noticeable that the olive oil production chain is extremely important to the Portuguese economy.

It should be pointed out that the production of olive oil tends to grow since this is a major source of antioxidants and important fatty acids (30).

1.2.1 Types of olive oil

There are eight distinct types of olive oils and olive pomace oils. However, only extra-virgin olive oil (EVOO), virgin olive oil (VOO), olive oil composed of refined and virgin olive oils, and olive pomace oil can be purchased (31).

EVOO results from the first pressing of olives within 24 h after harvesting, being extracted by a non-chemical method and temperatures inferior to 28 °C (27). It has an acidity level inferior to 0.8% (27, 31).

VOO also results from the first olives pressing. However, its' acidity level can be inferior to 2% (27, 31).

Olive oil refinement requires the use of chemicals (acids, alkalis), and heat so that the maximum possible amount of oil can be extracted from the olive pulp (27). Refined olive oil cannot be put up for sale at retail (31). It can be blended with EVOO and/or VOO, leading to the production of olive oil with a maximum acidity level of 1% (31).

Olive pomace oil is obtained via a hexane extraction of olive pomace (OP) (27). After refining, this oil can be mixed with VOO, leading a refined olive pomace oil with a maximum acidity level of 1% (31).

1.2.2 Olive oil extraction systems

Different steps are involved in olive oil extraction: olive harvesting, defoliation, washing, crushing, malaxation, and pressure or centrifugation (30). Olive oil is extracted directly from olives' fruit solely with mechanical procedures, to preserve its' inherent organoleptic characteristics (30). Specifically, three different methods are used: pressing, three-phase, and two-phase (26).

In the pressing method, the paste resulting from malaxation is placed between fibre diaphragms piled on top of each other. The olive paste is next compressed by hydraulic pressure, and the liquid phases (oil and vegetative water) percolate. Finally, a vertical centrifugation or decantation separates olive oil from the remaining wastewaters (30). In this method, 1000 kg of olives generates 200 kg of olive oil (32).

The traditional pressing method has been currently replaced by the continuous centrifugal extraction methods, where malaxation is followed by centrifugation with a decanter. Two centrifugal decanters can be employed: three-phase and two-phase (30).

Hot water is required in the three-phase production system to wash the oil (30). Indeed, approximately 0.5 L of water are required for each kg of olive paste (26). This method produces three phases: OP, olive oil mill waste waters, and an oil phase, which is then centrifugated to obtain the olive oil (30).

On the opposite, no additional water is added to the olive paste in the two-phase production method. It allows the obtention of olive oil and OP (semi-solid residue) (26, 30). This can be considered an eco-friendly process since it demands less water and produces fewer amounts of waste.

1.2.3 Olive oil by-products

Despite being a vital agricultural sector, high quantities of several by-products are produced as a consequence of olive oil production, namely olive leaves, olive mill waste waters (OMWW), and OP (26). These by-products are economically valuable because of their high contents of important compounds (33).

Olive leaves correspond to a mix of leaves and twigs, which are collected throughout olive oil production (33). This by-product has high contents of phenolic compounds, triterpenes, and flavonoids (34). Olive leaf extracts were already incorporated in different food products: in an olive-based pâté as a natural preservative (35); in milk to inhibit the

growth of *Bacillus cereus* and even the activity of the α -glucosidase enzyme (36); and in soybean oil to improve its' oxidative stability (37).

OMWW is composed of olive fruit soft tissues and the water used during olive oil production. This by-product registers high levels of phenolic compounds, owing to their high-water solubility (33), representing a great environmental concern (26). Nevertheless, several methods were already developed to recover the polyphenols from OMWW (32, 38, 39).

OP (**Figure 1**) is a heterogenous by-product, which is composed by olives' skin, pulp, and stone (26).



Figure 1. Olive pomace. (Source: Author).

OP is an acidic by-product (pH 4.8-5.2) (40), mainly composed by water, polysaccharides, protein, fatty acids, and polyphenols (26). OP water content varies according to the employed olive oil production system: traditional press mills (22-25%); two-phase system (50-70%), and three-phase system (40-54%) (33).

OP also has a residual lipid fraction that is of primary interest in the food industry. In fact, the fatty acid profile of this by-product showed that oleic acid (MUFA) is the main fatty acid present. Palmitic (SFA) and linoleic (PUFA) acids are the second and third major fatty acids, respectively (41). Additionally, OP main components are cellulose, hemicellulose, and lignin (40).

OP is an interesting source of several functional compounds (42):

- Phenolic compounds (e.g., hydroxytyrosol)
- Tocopherols (α -, β -, γ -tocopherol)
- Monounsaturated and polyunsaturated fatty acids
- Dietary fibre
- Minerals.

OP is difficult to handle, representing the highest waste-management challenge to the sector (43). One way to minimize the harmful environmental impact is to use OP stones to produce activated carbon or generate heat and electricity (40). OP is also frequently used as biomass (26). It is frequently used to obtain olive pomace oil (40, 42). This process involves a hexane extraction, distillation, and solvent recycling (40). Nevertheless, all the mentioned processes are considered waste reduction strategies.

Considering that every year, at least 2 million tons of OP are produced (26), and the high bioactive compounds content registered in this by-product, it is possible to say that OP is of great interest to different industries: cosmetic, animal feed, and food.

OP can be regarded as an interesting ingredient for the cosmetic industry since it contains squalene (reduces oxidative skin damage and has emollient and moisturizing properties) and phenolic compounds (protect the skin against oxidative damage) (42).

OP was also incorporated in animal diets, resulting in healthier and better-quality animal products (42).

Currently, OP has been used as a functional food ingredient (**Table 1**). However, the commercial production of food products incorporating OP has been limited until now (44).

OP can be used as a food ingredient, after stones removal. This process originates a product denominated olive pomace paste (OPP), a homogenous biomass composed by olives' skin and pulp (**Figure 2**).

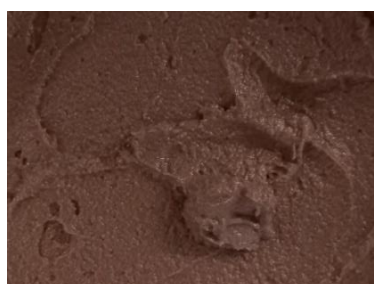


Figure 2. Olive pomace paste (OPP). (Source: Author).

Keeping in mind both the increasing demand for nutritive and healthy foods (45) and OPP nutritional quality attributes, it would be of great interest to create more innovative food products with this by-product. However, OPP incorporation in food products is a challenging process due to its characteristic bitter taste.

Table 1. Different food products fortified with olive pomace (OP).

Product	Olive pomace (OP) %	Results	Reference
Tagliatelle pasta	7% dried OP	- Fortified Tagliatelle pasta retained 6.6 mg/100 mg of hydroxytyrosol after cooking. - Fortified Tagliatelle pasta maintained a good resistance and texture after cooking.	(46)
	5% OP powder	Cooked pasta fortified with 5% OP had 6.3 times higher TPC, 1.9 times higher DPPH• scavenging activity, and 23 times higher ABTS than the control pasta (cooked).	
Pasta	10% OP powder	Cooked pasta fortified with 10% OP had 11.5 times higher TPC, 4.8 times higher DPPH• scavenging activity, and 77 times higher ABTS than the control pasta (cooked).	(47)
	5, 10% OP powder	Pasta fortification resulted in a decrease of the rapidly digestible starch content. On the other hand, there was an increase of the slowly digestible and resistant starch with the fortification.	
Spaghetti	10% dried OP	The fortification improved not only the phenolic content, but also the antioxidant activity of the Spaghetti.	(48)

Product	Olive pomace (OP) %	Results	Reference
		Fortification affected the sensory characteristics of uncooked (colour and overall quality) and cooked (colour, taste, elasticity, firmness, bulkiness, and overall quality) spaghetti.	
Bread	10% dried OP pre-treated with milk	- Breads' phenolic content improved with fortification. - Breads' antioxidant activity improved with fortification. - Breads' sensory properties (colour, odour, taste, crust and crumb firmness, and large bubbles) were impacted.	(48)
Granola bar	5% OP	- Granola bar fortified showed a high hydroxytyrosol and tyrosol contents of 3.64 and 0.65 mg/10 g, respectively. - To fulfil the European Food Safety Authority (EFSA) health claims, 12 g of granola bar fortified with OP is enough. - Colour and bitter taste were negatively affected by fortification.	(49)
Taralli	20% fermented OP	Fortified <i>Taralli</i> showed a significantly superior bioactive compounds content: hydroxytyrosol, tyrosol, and verbascoside ($p < 0.05$).	(45)
Fish burger	10% dry OP flour	Enriched cooked fish burgers showed a 12.8 times higher TPC, and 210.2 times higher TFC than the unfortified cooked burger.	(50)

Product	Olive pomace (OP) %	Results	Reference
		Enriched fish burger had a very bitter and spicy tastes. Indeed, this enrichment had a negative impact on the sensory attributes.	
Chinese biscuits	15% OP powder	- The incorporation of OP powder in biscuits improved the nutritional properties (dietary fibre increased 9 times). - Biscuits with 15% OP power received higher scores in the sensory evaluation (colour, appearance, taste, texture/donesse, and overall acceptability) than control biscuits (no OP).	(51)
		Cooked cod sticks breaded with OP showed a 4.4-times higher TPC, 7.7-times higher TFC, and a 2.9-times higher antioxidant activity compared to the cooked cod sticks breaded without OP.	
Cod sticks	0.2% OP powder	- Sensory acceptability of the cooked cod fish breaded with olive paste (0.2%) was not compromised. - Microbiological quality was maintained for more time in the cooked cod sticks breaded with OP.	(52)

OP- Olive pomace; TPC- Total phenolic content; DPPH•- 2,2-diphenyl-1-picrylhydrazyl radical; ABTS- 2,2'-azino-bis(3-ethylenbenzothiazoline-6-sulfonic acid) radical scavenging ability; EFSA- European Food Safety Authority; TFC- Total flavonoid content.

OP has also been subjected to different fermentation methods to improve its' organoleptic characteristics. It was already fermented by a solid-state fermentation process using *Kluyveromyces marxianus* NRRL Y-8281 yeast for 48 h at 45 °C (53).

In another study, a sequential inoculation with *Saccharomyces cerevisiae* and *Leuconostoc mesenteroides* was used to ferment an olive pâté cake (44).

The solid residue of OMWW was naturally fermented based on olive oil natural microbiota (54). The mentioned by-product was placed in a brine solution (6% NaCl) for 14 days at 20 °C, then it was washed, homogenized, and heat-treated.

1.3 The development of new food products

The creation of different products is vital to the food industry (55). Specifically, product development is creating, processing, and commercializing a new food product, and it involves four key stages: ideation, formulation, processing, and commercialization (56).

1.3.1 Food industry trends

The increasing demand for vegetables/fruits, organic food products, and concerns about animal welfare may improve the food industries' low research level and technological adaptation (55). Indeed, consumers' demand for organic, healthy, and low-calorie food products increased the difficulty of developing new food products (55).

Food research should keep up with the food trends to understand consumer preferences, expectations, and eating habits (57). In fact, the following points should be carefully evaluated while developing new food products: consumer demands and sensory attributes, seasonal ingredients, local products consumption, safety, traceability of both ingredients and final product, and environmental impact (55). To summarize, a variety of factors can impact the development of new food items.

A recent study has predicted how several food sectors will evolve in the future (58). The fruit/vegetable sector is growing rapidly worldwide, mainly because of consumers' rising interest in ready-to-eat food and the health benefits related to its' consumption. On the other hand, the meat/meat product sector tends to grow in the developing economies, and to steadily decrease in the developed economies. The predicted growth in the developing economies is related to the rising income, whereas the decrease predicted in the developed

countries is mainly justified by ethical, ecological, and health concerns. Regarding the seafood sector, there is an increasing demand projection.

Climate change, child labour, unfair trade, food waste, and animal welfare are just a few topics that are influencing consumer food purchases.

Climate change concerns are increasing among younger consumers, who adopt a minimalist and healthier way of living. Consumers are also keener toward plant-based, organic, natural, protein-rich, low-carb and low-sugar food choices (57).

In fact, today's food consumption is mainly decided by new vital factors: health concerns, sustainability, and convenience (59).

The increase in the number of illnesses related to unhealthy food habits, allergies, and intolerances to specific products or food components explain the health concerns. Sustainability concerns are due to the increasing awareness of the environmental consequences of traditional agricultural practices. Convenience relates to choices of foods that will save consumers time, like frozen food, fast food, and ready-to-eat meals (59). As a result, there is a growing requirement for health-enhancing food products (e.g., functional foods and clean label products).

"Functional food" is a food product that can deliver health benefits besides the ones provided by the traditional nutrients or food products, having a potential role in preventing diseases and promoting a better life quality (58).

Regarding the "Clean label" term, it should be noted that no objective definition has been made up till now. Indeed, its' definition is somewhat personal, depending on the consumers' familiarity with food ingredients and processing methods. It should be pointed out that "Clean label" cannot be used by producers as a claim (59). What happens is that some production techniques are recognized as being "less natural," and some ingredients as "unhealthy" or "unfamiliar", forcing the food industry to transmit the usage of a more "natural" production method (59).

Another trend among western countries is vegetarianism, veganism, and plant-based diets (60). The vegan or vegetarian diet movements can be explained by moral, ethical, religious, animal welfare, environmental concern, or health-related reasons (61). However, these diets have been criticized for being inconvenient because the food products are not easy to cook/prepare. In addition, their ingredients are not always readily available (60).

1.3.2 The choice of a food product: Olive pâté

“Olive pâté”, sometimes also referred as “olive fruit paste”, is a food product that can be used as a condiment for different food products (62). This is a Mediterranean food product (62), which is becoming increasingly popular due to its exceptional flavour and quality and its possible health benefits (63).

The term “Pâté” refers to a food product in which the ingredients are finely mashed (64). Commercial olive pâtés are mainly prepared with fermented table olives (54). They can contain both green and oxidized table olives (63), and a wide range of ingredients of both animal and vegetable origins (63). Some of the ingredients used to prepare olive pâtés are dried tomatoes, herbs, nuts, truffles, carp, olive oil, and garlic (62, 65).

Numerous studies have developed innovative olive pâtés using different ingredients (63-66). For example, OMWW was already used as an ingredient in the development of different olive pâtés (54, 62).

In one case, OMWW solid residue was fermented and incorporated into an olive pâté (54). In the other case, an OMWW concentrated was incorporated into an olive pâté (62). However, until now, no pâté has been developed incorporating heat-treated olive pomace paste (HT-OPP).

Portuguese food market offers several “olive pâté” brands. In order to identify the ingredients used in their formulation, a market research was performed, being the results presented in **Table 2**.

Keeping in mind that this product is highly present in the Portuguese market, developing an olive pâté with the incorporation of HT-OPP would be an interesting approach that simultaneously promotes the sustainability and the circular economy of the Portuguese olive oil production chain and valorise this ingredient. Furthermore, it would be an interesting way of providing a food product that attends to the consumers’ new trends: convenience, health, veganism, and sustainability. Finally, it could reduce the costs related to the production of olive pâtés.

Table 2. Olive pâtés from the Portuguese market.

Trade Name	List of ingredients
"Pasta de azeitonas pretas"	Black olives (83%), OO, honey, and aromatic herbs.
"Pasta de azeitona verde"	Green olives (83%), OO, honey, and aromatic herbs.
"Pasta de azeitona Preta BIO 90 g"	Black olives (83%), OO, honey, aromatic herbs, and citric acid (E330).
"Pasta de azeitona "negrinha do douro""	Douro black olives (81%), VOO, honey, spices, acidity regulator (citric acid), and salt.
"Pasta de azeitona verde"	Green olive pulp (95%), EVOO, oregano, garlic, citrus juices, and aromatic plants.
"Pasta de azeitona preta biológica caxineiro"	Olive pulp, lemon, salt, EVOO, oregano, and thyme.
"Pasta de azeitona preta"	Galega olive pulp (97%), EVOO, garlic, citrine juice, and aromatic herbs
"Pasta de azeitona com azeite virgem extra"	Olives (98%) (Aragão Olives, black olives), EVOO (1%), vinegar, salt, garlic, and thyme.
"Pasta de azeitona preta 150g Vale do Mestre"	Black olives, OO, garlic, and oregano.
"Pasta de azeitona preta biológica de Trás dos Montes - Casa S. Miguel"	Olive pulp, VOO, lemon, salt, oregano, and thyme.
"Pasta de azeitona preta"	Black olives in brine pitted and ground (90%), EVOO, salt, and aromatic herbs.
"Pasta de azeitona preta para barrar bio 350 g"	Olives (94%), salt, EVOO, balsamic vinegar, oregano, and organic bean sprouts.

OO- Olive oil; VOO- Virgin olive oil; EVOO- Extra-virgin olive oil.

1.3.2.1 *Table olives*

In raw fruit, phenolic compounds, specifically the bitter glucoside oleuropein, are responsible for their bitterness. Hence, a pre-treatment is necessary to make them edible fruits. Alkaline treatment breaks oleuropein in hydroxytyrosol and elenoid acid glucoside (non-bitter). In natural treatment with brine, some microorganisms hydrolyse the oleuropein molecule using the β -glucosidase and esterase activities (67).

Every year, table olive production reaches nearly 2.7 million tonnes. Furthermore, the EU is one of the leading manufacturers of this product (68). Table olive production involves a debittering process, and nearly 80% is produced by three methods: Spanish green olives, known as treated green olives; Californian style, known as olives darkened by oxidation; Greek style, known as natural olives (69).

Californian style olives are produced all over the world, including in Portugal. Compared to other kinds of table olives, this type of olives have the lowest sense of bitterness (70).

The Californian style (olives darkened by oxidation) production involves preservation in acidic solution with or without salt, lye treatment with a 3% NaOH solution, washing with water and air bubbling, treatment with ferrous gluconate or lactate solution, pitting/slicing/washing, canning, and sterilization (71).

The cracked green table olives from the Maçanilla cultivar are one of the most eaten and produced natural fermentation olives in Portugal. Their production requires: harvesting, transport, separation of the damaged olives, washing, breaking, and brining in a 6% NaCl solution. During brining, spontaneous fermentation happens at room temperature and is dependent on the development of natural bacteria (yeast) (72).

In Trás-Os-Montes, traditional stoned green olives (*“alcaparras”*) are commonly produced. These olives are debittered only using water, and their production requires: harvesting, separating the damaged olives, breaking with a wood hammer, slicing the pulp into two equal parts, immersing in water (changed every 1 or 2 days, for 1 or 2 weeks), and adding of herbs or condiments (73).

1.3.2.2 “Herbs, spices, seasonings, and condiments”

During European colonization between the 15-17th centuries, the spread of spices and condiments resulted in the diversification of their use around the globe (74). Apart from the flavouring ability, they can also have an essential role in the diet since they can prevent oxidative stress, inflammatory damage, cardiovascular diseases, and even cancer (75).

The *Codex Alimentarius* defines “Herbs, spices, seasonings, and condiments” as “*food flavouring substances, usually from botanical sources, dehydrated, ground or whole, added to food to improve aroma and taste*” (75).

Specifically, “Herbs” are regarded as plants with aromatic leaves, flowers, and stems, (75). Some examples are basil, oregano, and thyme (74).

Spices are extremely important in nutrition and food technology, being primarily utilized to contribute to sensory food qualities in gastronomy, home cooking, and industrial food. Currently, great importance is given to these products owing to research for new bioactive compounds with functional characteristics (76). Indeed, current research indicates that spices might have synergistic effects when combined, enhancing their health potential (75).

The term “Seasoning” refers to herbs, spices, and herb/spices mixtures used during food preparation (77). It can be understood as a mixture of species, salt, monosodium glutamate, and more ingredients (76).

“Condiments” are a combination of ingredients (e.g., mustard, chili sauce, garlic, onion, salt (75)), often added to food (e.g., bread, vegetables, potatoes, fish, and meat (78)). Due to several reasons, these products have a ubiquitous presence on the dining tables. First, their bright and unique colours add some colour and textural interest to the food products. Secondly, they allow consumers to personalize and modify their food flavour. Finally, they enhance the dishes taste and flavour by often including flavour-enhancing ingredients. All of this makes condiments the more-loved food category in the market (77).

“Sauces” are liquid or semi-liquid products that may or not include spices and can enhance food aroma, flavour, and appearance (75). It should be mentioned that the food category “sauces” (e.g., ketchup, mustard, mayonnaise) is not included in the “condiment” category in *Codex Alimentarius* (74).

1.3.3 Sensory analysis as a tool in new food development

Sensory analysis can contribute to numerous business activities like product improvement, development, reformulation, and even quality control (79).

Sensory analysis is a scientific technique utilised to evoke, measure, investigate and understand the responses given by senses (sight, smell, touch, taste, and hearing) (80). There are three main types of sensory tests: discrimination, descriptive, and affective (80).

Discrimination tests are merely used to perceive and determine whether or not there is a noticeable difference between two items. A total of 25 to 40 participants, formerly selected according to their sensory acuity, are needed for these sensory tests (80).

The descriptive tests are the most comprehensive and informative sensory tool (80). It should never be used with consumers since it requires a trained panel typically composed of 10-12 well-trained people, previously orientated through the definitions (80). These tests can be used to monitor competitors' offers, differentiate between our product and the competitor one and assess the suitability of a prototype (81). These techniques are helpful when redressing consumers complaints. However, they are too expensive for daily quality control situations (81).

Affective tests are used to assess how well the product is liked or which product is preferred (80). These tests have a principal aim to find an opinion of a product and can be classified into two main categories: preference tests and acceptability tests (82).

As the name suggests, the preference test allows choosing one product against another, forcing a choice (82). In contrast, an acceptability test is used to verify how well the product is liked by the consumers (82).

Usually, the acceptability test requires a 9-point hedonic scale, a high number of regular consumers, and is performed along with several steps: first to obtain the samples; to decide the test conditions (size, volume and temperature of the samples); to establish instructions to the panellists; to recruit potential consumers; set up counterbalanced orders; to label samples with three random digits; to conduct the test; to analyse and communicate the results (83).

The Check-all-that-apply (CATA) test can be used to assess the consumers' perception of food products (84). This is a highly effective method for analysing and collecting customer perceptions currently available (85). In reality, the CATA test provides an easy way of gathering the consumers' perceptions of food products, allowing the obtention of information comparable to that gained through descriptive analysis with experienced assessors (84).

The CATA test requires less time and has more flexibility than the traditional approaches, allowing for a more accurate assessment of consumers' impressions (86). Additionally, it is possible to analyse between 1 and 12 samples (84). It should also be pointed out that the CATA test and hedonic scales can be used simultaneously (84).

Specifically, the CATA test contains numerous sensory attributes (words/phrases) from where the panellists can choose the attributes believed to be suitable for describing each sample (87). To implement a CATA test, it is necessary to select the sensory terms to be used and order them (84). The selected terms can be organized alphabetically so that consumers can rapidly identify all the attributes to check (87). The terms can also be presented in the same order to all the consumers since it takes less time to answer the test (87). Regarding the number of participants, 50-100 consumers are needed to conduct this test (84).

The results from the CATA sensory test are usually summarized in a contingency table (88), containing the number of consumers who have chosen each term, for each sample (84). Then, to see whether there are any significant differences between products for each term, a Cochran's Q test should also be performed (84). In addition, a sensory map can be obtained. This map shows the resemblances and differences between the products, and their key sensory attributes (84).

CHAPTER II: OBJECTIVES

2.1 Main objective

This work aimed to valorise heat-treated olive pomace paste (HT-OPP), obtained after heat-treatment of olive pomace paste, as a food ingredient by developing a sustainable food product.

2.2 Specific objectives

The specific objectives were:

- A chemical characterization of HT-OPP: nutritional composition, vitamin E and fatty acid profiles, phytochemicals amounts, and antioxidant activities;
- To valorise HT-OPP as an innovative and sustainable food ingredient;
- To develop a sustainable, and convenient food product incorporating HT-OPP;
- To chemically characterize the developed food product (using the previously cited parameters);
- To evaluate the sensorial acceptability of the developed food product;
- To contribute to the sustainability and circular economy of the olive oil production system.

CHAPTER III: MATERIAL AND METHODS

3.1 Chemicals and reagents

Reagents and chemicals were of analytical grade:

- Total protein content: Kjeldahl tablets, sulfuric acid (H_2SO_4), sodium hydroxide (NaOH) from Merck (Darmstadt, Germany), and boric acid 4% (H_3BO_3) Panreac (Barcelona, Spain).
- Total fat content: Anhydrous sodium sulphate (Na_2SO_4) was acquired in Merck (Darmstadt, Germany), sand in VWR Chemicals (Alfragide, Portugal), and petroleum ether in Carlo Erba Reagents (Val de Reuil, France).
- Total dietary fibre content: fibre enzymes kit and celite were purchased in Sigma-Aldrich (St. Louis, USA), acetone in Fischer Chemical (U.K), ethanol 96% in AGA (Prior Velho, Portugal), chloridric acid in Carlo Erba (Val de Reuil, France), and sodium dihydrogen phosphate and di-sodium hydrogen phosphate anhydrous in Merck (Darmstadt, Germany).
- Salt content: silver nitrate (AgNO_3) and potassium dichromate ($\text{Cr}_2\text{K}_2\text{O}_7$) were purchased in Merck (Darmstadt, Germany).
- Vitamin E profile: BHT (butylated hydroxytoluene) and dioxane were purchased in Sigma-Aldrich (St. Louis, USA), absolute ethanol, *n*-hexane (HPLC grade), NaCl (1%) and anhydrous sodium sulphate (Na_2SO_4) in Merck (Darmstadt, Germany), tocopherols/tocotrienols standards and tocol in Calbiochem (La Jolla, CA, USA) and Matreya Inc. (Pennsylvania, USA).
- Fatty acids profile: dichloromethane (CH_2Cl_2) was acquired in Honeywell I Riedel-de HaënTM (Germany), potassium hydroxide (KOH) in Panreac (Barcelona, Spain), *n*-hexane and anhydrous sodium sulphate (Na_2SO_4) in Merck (Darmstadt, Germany), and boron trifluoride-methanol solution and fatty acids standards in Sigma-Aldrich (St. Louis, USA).
- TPC: sodium carbonate (Na_2CO_3) was purchased in Merck (Darmstadt, Germany), Folin-Ciocalteu reagent, and gallic acid in Sigma-Aldrich (St. Louis, USA).
- FRAP assay: TPTZ (2,4,6-tripyridyltriazine), ferric chloride (FeCl_3), heptahydrate ferrous sulphate, and sodium acetate were purchased in Sigma-Aldrich (St. Louis, USA).
- DPPH[•] scavenging activity assay: DPPH[•] (2,2-diphenyl-1-picrylhydrazyl radical) and trolox were purchased in Sigma-Aldrich (St. Louis, USA), and absolute ethanol in Merck (Darmstadt, Germany).

- Hydroxytyrosol content: Hydroxytyrosol standard was purchased in Sigma-Aldrich (St. Louis, USA).

A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to obtain ultra-pure water.

3.2 Formulation, development, and sensory analysis of the olive pâté incorporating Heat-Treated Olive Pomace Paste (HT-OPP)

3.2.1 HT-OPP obtention

OP was collected in a two-phases olive mill in Trás-Os-Montes in 2020. OP stones were manually removed with a stainless-steel sieve in the laboratory, leading to OPP production (**Figure 3**).



Figure 3. Olive pomace paste (OPP). (Source: Author).

Then, to assure the microbiological safety, OPP was submitted to pasteurization in a Vorwerk Thermomix TM 31[®] (**Figure 4**): 88 °C/15 sec.



Figure 4. Vorwerk Thermomix TM 31[®]. (Source: Author).

3.2.2 Formulation and development of the olive pâtés prototypes

A total of 4 olive pâtés were initially produced (**Table 3**). Depicted oxidized black olives, EVOO, vinegar, garlic powder, aromatic herbs, and salt were the used ingredients and were acquired in a supermarket.

The percentage (% m/m) of depicted black olives incorporated in the control pâté was selected according to Nanis *et al.* (2020). Then, increasing percentages (% m/m) of HT-

OPP were incorporated to use as much as possible of this by-product. EVOO and vinegar percentages (% m/m) were tested and selected in the laboratory to obtain a spreadable paste. Finally, garlic powder, aromatic herbs (oregano, chives, and basil), and salt were used to mask HT-OPP astringent flavour. Their percentages (% m/m) were also selected in the laboratory (**Table 3**).

Table 3. Olive pâté prototypes formulated in the laboratory.

Olive pâté	Ingredients
Olive pâté (P0)	75% depicted black oxidized olives, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.
Olive pâté with 10% HT-OPP (P10)	65% depicted black oxidized olives, 10% HT-OPP, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.
Olive pâté with 20% HT-OPP (P20)	55% depicted black oxidized olives, 20% HT-OPP, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.
Olive pâté with 30% HT-OPP (P30)	45% depicted black oxidized olives, 30% HT-OPP, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.

EVOO- Extra-virgin olive oil; HT-OPP- Heat-treated olive pomace paste (88 °C, 15 sec).

The olive pâtés were produced according to the flowchart below (**Figure 5**) and using a Vorwerk Thermomix TM 31® (**Figure 4**).

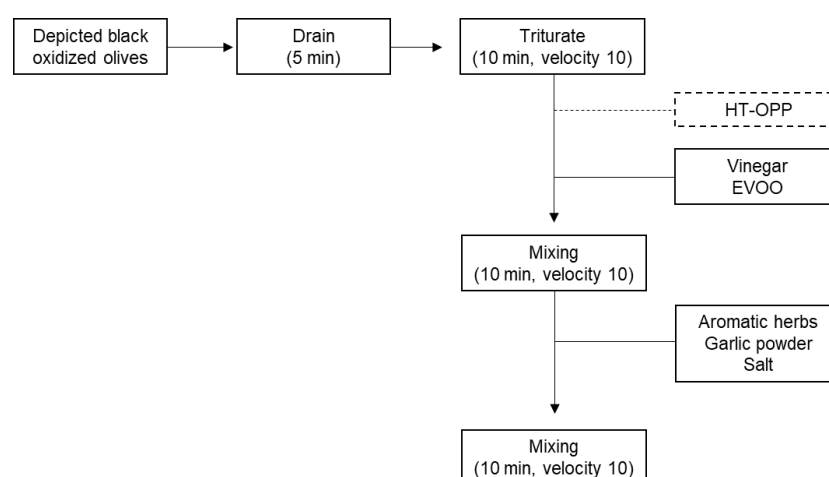


Figure 5. Flowchart of the production of the olive pâtés.

3.2.2.1 Acceptability test of the olive pâtés prototypes

In order to know the impact of HT-OPP incorporation on the acceptability of the olive pâtés, an acceptability sensory test, according to Lawless (2010), was performed (83). This aimed to select the highest acceptable percentage of HT-OPP that could be incorporated in the olive pâtés.

A total of 56 consumers were recruited in the Faculty of Pharmacy of the University of Porto (Porto, Portugal), according to their interest in participating in the sensory test. Consumers who had allergies or intolerances were excluded.

Consumers were informed that the olive pâtés were prepared on the testing day. They were also informed that the sensory analysis in which they were going to participate was carried out within the scope of this Masters' dissertation and that its' aim was to choose the highest acceptable percentage of HT-OPP that could be incorporated in an olive pâté.

The assessors had received disinfectant gel, a pen, wipes, a questionnaire (**Annex I**), and water to clean the palate between the olive pâtés tasting. All were told to taste the pâtés in the mentioned order in the questionnaire and then score their acceptability using a 9-point hedonic scale from 1 ("Dislike extremely") to 9 ("Like extremely") (83).

Olive pâtés were presented in plain flour mini toast as carrier, at room temperature, coded with three-random numbers (365- Olive pâté, 853- Olive pâté with 10% HT-OPP, 457- Olive pâté with 20% HT-OPP, 742- Olive pâté with 30% HT-OPP). An example of the table for testing is presented in **Figure 6**.



Figure 6. Acceptability test with the olive pâté prototypes. (Source: Author).

3.2.3 High Pressure Processing (HPP) of the olive pâtés

Keeping in mind the results obtained from the first sensorial acceptability test, it was decided to produce olive pâtés incorporating 20% and 25% of HT-OPP. Additionally, it was decided to explore another sensorial test using a total of four olive pâtés: olive pâté without HT-OPP, olive pâté incorporating 20% HT-OPP, olive pâté incorporating 25% HT-OPP, and a commercial olive pâté (CP) acquired in a supermarket.

Considering the consumers' microbiological safety, the olive pâtés produced in the laboratory (P0, P20, and P25) were processed by HPP. First, the olive pâtés were manufactured according to **Table 4** and the flowchart (**Figure 5**), using a Vorwerk Thermomix TM 31® (**Figure 4**).

Table 4. Olive pâtés produced in the laboratory to process by High Pressure Processing (HPP).

Pâté	Ingredients
Olive pâté (P0)	75% depicted black oxidized olives, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.
Olive pâté with 20% HT-OPP (P20)	55% depicted black oxidized olives, 20% HT-OPP, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.
Olive pâté with 25% HT-OPP (P25)	50% depicted black oxidized olives, 25% HT-OPP, 10% vinegar, 10% EVOO, 3% aromatic herbs, 1% garlic powder, and 1% salt.

EVOO- Extra-virgin olive oil; HT-OPP- Heat-treated olive pomace paste (88 °C, 15 sec).

Then, the olive pâtés were packed in vacuum in PA/PE bags (**Figure 7**) so that they could be processed by HPP.



Figure 7. Olive pâtés produced in the laboratory (P0, P20, and P25) packed in PA/PE bags. (Source: Author).

Finally, the olive pâtés (P0, P20, and P25) were processed by HPP at 550 MPa/3 min, at 10 °C. Processing was accomplished using a pilot-scale high hydrostatic pressure equipment with a 55 L vessel (Hiperbaric 55, Burgos, Spain) (**Figure 8**).



Figure 8. Pilot-scale high hydrostatic pressure equipment (Hiperbaric 55, Burgos, Spain).
(Source: Author).

3.2.4 Sensory characterization, acceptability, and purchase intention of the HPP-processed olive pâtés

As mentioned before, it was decided to proceed to another sensorial test with a larger number of people. This sensory analysis was carried out to obtain consumers' opinions about the olive pâtés with HT-OPP and assess their commercial potential.

For the four samples sensory characterization (P0*, olive pâté processed by HPP; P20*, olive pâté with 20% HT-OPP processed by HPP; and P25*, olive pâté with 25% HT-OPP processed by HPP; and a commercial brand), a CATA test was implemented.

Initially, a small focus group of assessors selected a total of 34 attributes to be used in the CATA analysis. The terms were selected while tasting several commercial olive pâtés, and then grouped in a sensory modality in an alphabetical order (87) (**Annex II**). Then, due to the COVID-19 pandemic situation, the taste session was adapted so that consumers could take samples home and evaluate them, safely. Thus, a bag containing soda crackers, a questionnaire (**Annex II**), and the olive pâtés (P0*, P20*, P25*, and CP) was prepared. The olive pâtés were coded with three-random numbers: 613- P0*, 278- P20*, 157- P25*, and 831- CP.

Consumers were instructed to analyse the samples, one time, and clean the palate with water and crackers between samples. They have also been instructed to pay attention to the questionnaire and to read it carefully before starting.

Assessors had to look at the pâté and select all the attributes related to “Appearance”, “Smell”, and “Texture (spreading in the cookie)”. Then, they had to taste the sample in the crackers and select all the attributes associated to “Taste” and “Texture (in the mouth)”.

Afterwards, consumers had to score the acceptability of the tasted pâtés on a 9-point hedonic scale from 1 (“Dislike extremely”) – 9 (“Like extremely”) (83). Assessors were then asked to evaluate the purchase intention of the tasted olive pâtés using a hedonic scale of 5-point hedonic scale from 1 (“Definitely will not buy”) to 5 (“Definitely will buy”) (82).

Finally, they were requested to select all the attributes they thought to be necessary for an ideal olive pâté (84).

3.3 Chemical analysis

A total of eight samples were chemically analysed (**Table 5**).

Table 5. Samples chemically analysed.

Samples	
HT-OPP	Heat-treated olive pomace paste (88 °C,15 sec)
P0	Olive pâté
P0*	Olive pâté processed by HPP
P20	Olive pâté with 20% HT-OPP
P20*	Olive pâté with 20% HT-OPP processed by HPP
P25	Olive pâté with 25% HT-OPP
P25*	Olive pâté with 25% HT-OPP processed by HPP
CP	Commercial olive pâté

3.3.1 Nutritional composition

Ash, fat, protein, and dietary fibre contents were accomplished according to AOAC methods (89). An infrared balance was used to determine the moisture content (KERN DBS, Balingen, German). The remaining carbohydrate and the salt content were also determined.

The results are presented in both fresh weight (fw) and dry weight (dw).

3.3.1.1 *Total ash*

Total ash was assessed according to official methods (AOAC 920.153 method).

Specifically, crucibles were tared, and numbered. Then, ≈ 5 g of samples were weighted, in triplicate, to the crucibles and then placed in a muffle furnace (Thermolyne 48000, F48010-26, Electrothermal Engineering Ltd, Essex, UK). The muffle furnace was gradually heated to 500 °C, and then maintained for 48 h to obtain white ashes (**Figure 9**). Finally, the ash content was obtained by subtracting the crucibles' weight after and before incineration, being the results presented in g/100 g in both fw and dw.



Figure 9. White ashes. (Source: Author).

3.3.1.2 *Total fat*

Total fat was determined according to official methods (AOAC 991.36 method).

Specifically, sample were weighted (≈ 5 g), in triplicate, to a porcelain capsule, and then mixed with anhydrous sodium sulphate (Na_2SO_4) and treated sand. Then, this mixture was transferred to Soxhlet extraction cartridges, and placed in Soxhlet extraction ampoules. The ampoules were then adapted to flat bottom balloons, previously dried, cooled, tared, and numbered.

The extraction was carried out with petroleum ether in a Huber minichiller and a heating mantle P Selecta (**Figure 10**), for 8 h. Afterwards, the petroleum ether was recuperated. Then, the flat bottom balloons were placed in an oven at 105 °C for 1 h. Subsequently, these

balloons were put in the oven for 30 min periods at 105 °C, until constant weight. The results are expressed in g/100 g in both fw and dw.



Figure 10. Fat extraction. (Source: Author).

3.3.1.3 Total protein

Total protein was assessed according to an official method (AOAC 928.8 method) and using the nitrogen conversion factor of 6.25 (90). The results are presented in g/100 g in both fw and dw.

In particular, the sample (≈ 1 g) was weighted in triplicate to free-nitrogen vegetal paper, which were then put in Kjeldahl tubes, together with 2 Kjeldahl tablets and 20 mL of sulfuric acid (96%).

Protein digestion took place for approximately 1 h, in an automatic digester (Buchi Digestion Unit K-424), and the produced vapours were neutralized in a Scrubber B-414. Then, a Kjeldahl Distillation Unit was used. Finally, samples were titrated with sulphuric acid (0.2 M), using methyl red as indicator.



Figure 11. Protein digestion (left) and distillation (right). (Source: Author).

3.3.1.4 Total dietary fibre

The total dietary fibre was accomplished by enzymatic digestion (AOAC 985.29 method) (**Figure 12**). Considering the high fat content of the olive pâtés, all were previously defatted. On the other hand, HT-OPP did not have to be previously defatted. Total dietary fibre results are presented in g/100 g in both fw and dw.

Samples were weighted (≈ 1 g), in quadruplicate, to beakers. Then, 50 mL of sulphate tampon (pH=6) and α -amylase were added to samples to proceed with the first digestion step (95 °C, 20 min).

After colling to room temperature, 10 mL of NaOH (0.275 M) were added. The pH was then adjusted to 7.5 ± 0.2 so that the protease (50 mg/mL) enzymatic activity could take place (60 °C, 35 min).

Then, after colling to room temperature, 10 mL of HCl (0.325 M) were added. The pH was adjusted to 4-4.6) so that the amyloglucosidase enzymatic activity could take place (60 °C, 35 min).

Finally, after cooling to room temperature, 200 mL of ethanol (95%) were added to the beakers, and the solution was left during the night to form a precipitate.

The precipitates were filtrated in pre-weighted VELP glass crucibles and using celite as a filter. Filtration took place using 60 mL of ethanol (78%), 20 mL of ethanol (96%), and 20 mL of ketone.

Finally, the VELP glass crucibles containing the residues were put in an oven at 105 °C for 1 night, cooled, and weighted. Two residues were used for protein quantification. The other two residues were incinerated (525 °C for 5 h), cooled, and weighted.



Figure 12. Enzymatic digestion (left) and filtration (right). (Source: Author).

3.3.1.5 Moisture

The moisture content was determined in an Infrared balance (KERN DBS, Baligen, German) (**Figure 13**). Specifically, ≈5 g of sample were put in aluminium dishes and dried at 105 °C until constant weight. Moisture results are presented in g/100 g in both fw and dw.



Figure 13. Moisture balance. (Source: Author).

3.3.1.6 Remaining carbohydrates

The remaining carbohydrates were determined by difference. The results presented in g/100 g in both dw and fw.

3.3.1.7 Salt content

The salt content of the olive pâtés was determined following the equation bellow (91), being the results presented in g/100 g in both fw and dw.

$$\text{Salt} = \text{Sodium} \times 2.5$$

To obtain the sodium content, the chloride ion content (Cl⁻) was determined according to bibliographic reference (92), being the steps briefly presented in **Figure 14**.

First, samples were incinerated according to the total ash method earlier described. Then, to dissolve the white ashes, 3 portions of 30 mL of hot deionized water were added to the crucibles. This solution was then transferred to a volumetric flask. After colling, the volumetric flask was filled to the calibration mark.

Then, the volumetric flask solution was filtrated (Whatman No. 4 filter paper) to an Erlenmeyer flask. A 10 mL aliquot was transferred into a new Erlenmeyer flask. Then, 2

drops of potassium chromate (10%) were added. Finally, the solution was titrated with silver nitrate (0.1 M) until a brick-red precipitate appeared.



Figure 14. Illustration of the steps for determining the chloride ion content. (Source: Author).

3.3.2 Total content and profile of Vitamin E

The Vitamin E profile and its total content were evaluated in the fat obtained by cold extraction (93). For that, first, the amount of sample necessary to obtain 20 mg of fat was weighed into a first Falcon® tube. Then it were added 75 µL of BHT (0.1% m/V), followed by 50 µL of tocol (0.1 mg/mL), and 1 mL of absolute ethanol.

Then, tubes were put under constant agitation for 30 min, and afterwards, 2 mL of *n*-hexane (HPLC grade) were added. The solution was then shaken for 5 min, constantly stirred for 30 min, being then added 1 mL of 1% NaCl solution (m/V). Afterwards, the tubes were vortexed for 2 min, and centrifugated at 5000 rpm for 5 min. Finally, the resulting supernatant was transferred to a second Falcon® tube, which was stored in the dark.

Subsequently, a re-extraction was performed. For that, 2 mL of *n*-hexane (HPLC grade) were added to the residue of the first Falcon® tube. After that, the tubes were constantly agitated for 30 min, centrifuged at 5000 rpm for 5 min, and finally, the resulting supernatant was transferred to the second Falcon® tube, mixing both organic phases.

To eliminate any water present in the second Falcon® tube, anhydrous sodium sulphate (Na_2SO_4) was added. Afterwards, the tubes were centrifuged at 5000 rpm for 5 min. Then, the *n*-hexane layer was transferred to a third Falcon® tube, concentrated in a nitrogen stream (Volume $\approx$ 500 µL), and later transferred to amber Eppendorfs®. The tube walls of the third Falcon® tube were washed with 500 µL of *n*-hexane (HPLC grade), and later

transferred to amber Eppendorfs®. Finally, the amber Eppendorfs® were centrifuged at 13000 rpm for 5 min, and 500 µL of the supernatant were transferred to an amber injection vial, containing an injection lid for HPLC.

A HPLC system (Jasco, Tokyo, Japan) was used to perform the chromatographic analysis (**Figure 15**). This system is equipped with a multiwavelength diode array detector (MD-2015) coupled to an FP-2020 fluorescence detector (Jasco, Tokyo, Japan), programmed at $\lambda_{\text{excitation}} = 290 \text{ nm}$, and $\lambda_{\text{emission}} = 330 \text{ nm}$.



Figure 15. HPLC system. (Source: Author).

A normal phase Supelcosil™ LC-Si HPLC Column (3 µm particle size, L x I.D. 15 cm x 4.6 mm) was used to separate the compounds. In addition, a standard stock solution containing individual compounds was prepared to obtain calibration curves for each vitamer (α -tocopherol: $y = 0.1757 x - 0.0470$; α -tocotrienol: $y = 0.2106 x - 0.0546$; β -tocopherol: $y = 0.2340 x - 0.0406$; γ -tocopherol: $y = 0.1622 x - 0.0437$; β -tocotrienol: $y = 0.1802 x - 0.0411$; γ -tocotrienol: $y = 0.1593 - 0.0505$; δ -tocopherol: $y = 0.1760 x - 0.0324$; δ -tocotrienol: $y = 0.1684 x - 0.0005$). The injection volume was 20 µL, eluted with 6% dioxane in *n*-hexane (HPLC grade), at a flow rate of 0.7 mL/min.

The different vitamin E vitamers (α , β , γ , δ -tocopherol and corresponding tocotrienols) were identified based on the UV spectra and on the comparison of their retention times with those of standards. Moreover, their fluorescence signals were used to quantification.

Results are presented in mg/100 g in both fw and dw.

3.3.3 Fatty acids profile

ISO 12966-2:2017 (2017) method was used to derivatize the fatty acids to fatty acid methyl esters (FAME) (94). First, 500 μL of the lipid fraction were solubilized in 1 mL of dichloromethane (CH_2Cl_2), being then added 2 mL of potassium hydroxide (KOH). Then, the tubes were closed, vigorously shaken, and placed in a heating block at 100 $^{\circ}\text{C}$ for 10 min. Afterwards, tubes were cooled for 1 min at room temperature, followed by cooling on ice for 5 min. Then, 2 mL of a boron trifluoride methanol solution ($\text{BF}_3/\text{CH}_3\text{OH}$) was added to tubes, which were then closed and placed in an eating block at 100 $^{\circ}\text{C}$ for 30 min. Next, tubes were cooled for 1 min at room temperature, followed by cooling on the ice for 5 min. Then, it was added 2 mL of deionized water and 3 mL of *n*-hexane. The tubes were closed, vortexed, centrifuged at 3000 rpm for 5 min, and left to rest until two completely distinct phases were obtained. The supernatant was transferred to vials, which contained anhydrous sodium sulphate (Na_2SO_4) to remove any water present. The vials were centrifuged at 3000 rpm for 5 min, and after that, 1.5 mL of supernatant were transferred into Eppendorfs[®]. Finally, the Eppendorfs[®] were centrifuged at 13000 rpm for 5 min, and at the end, 1 mL of the supernatant was transferred into injection vials.

A gas chromatograph (**Figure 16**) coupled with a flame ionization detector (GC-FID, Shimadzu GC-2010 Plus, Tokyo, Japan) and a split/splitless AOC-20i auto-injector (Shimadzu, Tokyo, Japan) was used to obtain the fatty acids profile.



Figure 16. GC system. (Source: Author).

A CP-Sil 88 silica capillary column (50 m x 0.25 mm i.d.; 0.20 μm film thickness, Varian, Middelburg, Netherlands) was used for FAME separation, being helium used as a carrier gas. The temperature program was: 120 $^{\circ}\text{C}$ /5 min; increased to 160 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$; hold for 15 min; increased to 220 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$. The injector and detector temperatures were at 250 $^{\circ}\text{C}$ and 270 $^{\circ}\text{C}$, respectively. The injection volume and split ratio were, respectively, 1.0 μL and 1:50. FAME identification was carried out by comparison of their retention times with standards (Supelco 37 Component FAME Mix), being the data examined based on relative peak areas. The results are expressed in the relative % of each fatty acid.

3.3.4 Phytochemicals and antioxidant activity

Phytochemicals extraction was performed in triplicate. Briefly, samples (100 mg) were mixed with 40 mL of an absolute ethanol and deionized water (70/30, V/V), under constant stirring, for 60 min. Later, extracts were centrifugated at 5000 rpm for 5 min. Then, aliquots (900 μ L) were stored in 1 mL Eppendorfs® at -80 °C for further analysis.

3.3.4.1 *Total phenolics content (TPC)*

TPC was determined according to the Folin-Ciocalteu assay as described by Singleton *et al.* (1965) (95) with minor adjustments by Alves *et al.* (2010) (96).

In a microplate, extracts (30 μ L) were first mixed with 150 μ L of Folin-Ciocalteu reagent (1:10). Then, 120 μ L of a sodium carbonate (Na_2CO_3) (7.5%, m/V)) were added. A gallic acid calibration curve was also prepared ($y = 0.009x - 0.004$; $R^2 = 0.999$). The microplate was then incubated at 45 °C for 15 min, being afterwards, placed in the dark at room temperature for 30 min.

Finally, a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) (**Figure 17**) was used to determine the samples' absorbance (765 nm). Results are presented in g of gallic acid equivalents/100 g (g GAE/100 g) in both fw and dw.



Figure 17. Microplate (left) and Microplate reader (right). (Source: Author).

3.3.4.2 *Hydroxytyrosol content (HC)*

Hydroxytyrosol analysis was carried out by injecting 1 mL of extract in an HPLC-DAD-FLD system (Jasco, Tokyo, Japan). This system is composed by a LC-NetII/ADC hardware interface, a pump (Jasco PU-2089), and a multiwavelength diode array detector (Jasco MD-2018 Plus) coupled to a fluorescence detector (Jasco FP-2020 Plus). It also contains an automatic sampler (Jasco AS-2057 Plus) and a column thermostat (Jasco CO-2060 Plus).

A Zorbax-SB-C18 chromatographic column (250 $\times$ 4.6 mm, 5 μ m; Agilent Technologies, Netherlands) was used at 20 °C. A gradient elution program was employed, using acetic

acid (A, 1%) and methanol (B, 100%): 0 min, 5% B; 30 min, 25% B; 50 min, 75% B; 55 min, 100% B; 60 min, 100% B; 63 min, 5% B. The flow rate was 1 mL/min. The injection volume was 20 μ L.

Hydroxytyrosol was evaluated by fluorescence and monitored at λ excitation= 280 nm, and λ emission= 330 nm. Results are expressed in g/100 g in both fw and dw.



Figure 18. HLPC system (Jasco, Tokyo, Japan). (Source: Author).

3.3.4.3 Antioxidant activity: Ferric reducing antioxidant power (FRAP) and DPPH[•] scavenging activity assays.

The FRAP assay was determined based on Benzie *et al.* (1996) (97), after slight modifications of Costa *et al.* (2014) (98).

In a microplate, the extracts (35 μ L) were mixed with 265 μ L of the FRAP reagent: acetate buffer (0.3 M), TPTZ solution (10 mM), and ferric chloride (20 mM). A ferrous sulphate (FeSO_4) calibration curve was also prepared ($y = 0.002x - 0.016$; $R^2 = 0.999$). The microplate was then incubated at 37 $^{\circ}\text{C}$ for 30 min.

A microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to read the samples' absorbance (595 nm). Results are presented in g of ferrous sulphate equivalents/100 g (g FSE/100 g) in fw and dw.



Figure 19. Microplate (left) and Microplate reader (right). (Source: Author).

Samples' DPPH• scavenging activity assay was determined according to Costa *et al.* (2014) (98). Specifically, in a microplate, the extracts (30 µL) were mixed with 270 µL of the DPPH• solution in ethanol (6×10^{-5} M). A trolox calibration curve was prepared ($y = -0.006x + 0.5278$; $R^2 = 0.999$). A microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to determine the samples' absorbance (525 nm), every 2 min for 40 min. The results are presented in g trolox equivalents/100 g (g TE/100 g) in fw and dw.



Figure 20. Microplate (left) and Microplate reader (right). (Source: Author).

3.4 Statistical analysis

Results from the chemical analysis are reported in mean $\pm$ standard deviation. Significant differences between samples were assessed using a one-way ANOVA test, followed by a Tukey HSD post-hoc test, with a significance level of 0.05. The IBM SPSS v. 26 (IBM Corp., USA) was used for data analysis.

In the first acceptability test, the statistical differences between the different olive pâtés were assessed by Kruskal-Wallis H-test (95% confidence level), followed by multiple comparison Wilcoxon/Mann-Whitney post-hoc tests (99), as data showed a non parametric behaviour. STATISTICA v.14 (Stat Soft Inc., Tulsa, USA) was used for this analysis and to obtain the boxplots.

In the CATA test, significant differences between olive pâtés for each attribute were assessed by a Cochran's Q test followed by multiple pairwise comparisons using the Critical difference (Sheskin) procedure, with a level of significance of 0.05, and using XLSTAT 2022 (Addinsoft, Paris, France) (100). The software STATISTICA v.14 (Stat Soft Inc., Tulsa, USA) was used to perform the principal component analysis (PCA) and the correspondence analysis (CA) to investigate the acceptability and purchase intention differences between the olive pâtés. Data were assessed by Kruskal-Wallis H-test (95% confidence level), followed by multiple comparison Wilcoxon/Mann-Whitney post-hoc tests (99), as data shown to have a non parametric behaviour. Boxplots were also obtained using this software.

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Chemical characterization of Heat-treated olive pomace paste (HT-OPP)

OP, from a two-phase system, was composed by olive skin, pulp, and stones. This is a promising valuable biomass owing to its chemical composition (26). Although to use it as a food ingredient, the stones must be removed resulting in OPP.

Different heat-treatments were applied to OPP (in a previous work) to eliminate its' natural microbiological load, aiming its' use as a microbiologically safe food ingredient. From all the applied treatments, 88 °C for 15 sec was the one selected as the best with potential to be applied at industrial level. A functional and microbiologically safe food ingredient was obtained: HT-OPP.

In this work, it was decided to develop a food product (olive pâté) incorporating the referred HT-OPP. This approach would contribute to the valorisation of HT-OPP as a functional food ingredient and promote both the circular economy and the sustainability of the olive oil production sector.

Nutritional analysis showed that HT-OPP had a protein content of 2.3% and a low-fat content (2.5%, fw). It also registered high moisture (65.3%), dietary fibre (12.2%), and remaining carbohydrates (15.1%) contents (**Table 6**). The high-fibre and low-fat contents are interesting features of this food ingredient.

Table 6. Nutritional composition of Heat-treated olive pomace paste (HT-OPP).

Nutritional analysis	g/100 g (dw)	g/100 g (fw)
Moisture	-	65.27 ± 0.14
Protein	6.74 ± 0.17	2.34 ± 0.06
Fat	7.19 ± 0.23	2.50 ± 0.08
Ash	7.66 ± 0.28	2.66 ± 0.10
Total fiber	35.04 ± 0.55	12.17 ± 0.19
Remaining carbohydrates	43.36 ± 0.43	15.06 ± 0.19

Results are presented in g/100 g of fresh and dry weight as mean ± standard deviation. dw- dry weight; fw- fresh weight.

A total of 3 different Vitamin E vitamers were found in HT-OPP: α -, β - and γ -tocopherol. Additionally, the main vitamer present was α -tocopherol. Keeping in mind that α -tocopherol is considered the most important lipophilic antioxidant (101), the vitamin E profile of HT-OPP is of great interest to the food industry.

Table 7. Vitamin E profile and total content of Heat-treated olive pomace paste (HT-OPP).

Vitamin E	mg/100 g (dw)	mg/100 g (fw)
α -tocopherol	3.39 ± 0.04	1.18 ± 0.01
β -tocopherol	0.14 ± 0.00	0.05 ± 0.00
γ -tocopherol	0.20 ± 0.00	0.07 ± 0.00
Total vitamin E	3.73 ± 0.04	1.30 ± 0.01

Results are in mg/100 g of fresh and dry weight as mean $\pm$ standard deviation. dw- dry weight; fw- fresh weight.

The fatty acids composition of HT-OPP is nutritionally interesting (**Table 8**). In fact, the residual lipid fraction of this ingredient is mostly composed of oleic acid (C18:1n9c). Additionally, linoleic (C18:2n6c) and palmitic (C16:0) acids were also found in high percentage.

Table 8. Fatty acid profile of Heat-treated olive pomace paste (HT-OPP).

Fatty acids	Relative (%)
Palmitic (C16:0)	10.29 ± 0.04
Palmitoleic (C16:1)	0.44 ± 0.01
Heptadecanoic (C17:0)	0.11 ± 0.01
Stearic (C18:0)	2.59 ± 0.13
Oleic (C18:1n9c)	77.30 ± 0.21
Linoleic (C18:2n6c)	7.86 ± 0.17
Arachidic (C20:0)	0.29 ± 0.02
α -linolenic (C18:3n3)	0.64 ± 0.03
<i>cis</i> -11-Eicosenoic (C20:1n9)	0.19 ± 0.01
Behenic (C22:0)	0.19 ± 0.01
Lignoceric (C24:0)	0.10 ± 0.01
Σ SFA	13.57 ± 0.14
Σ MUFA	77.93 ± 0.22
Σ PUFA	8.50 ± 0.17
MUFA/PUFA	9.17 ± 0.21

Results are presented in relative percentage (%) as mean $\pm$ standard deviation. SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids.

HT-OPP registered a high TPC (1.50 g GAE/100 g). It also registered a considerable HC (0.17 g/100 g). Additionally, it registered interesting antioxidant activities considering the FRAP (1.56 g FSE/100 g) and DPPH• scavenging activity (0.58 g TE/100 g) results.

The high hydroxytyrosol content of HT-OPP makes it an interesting food ingredient owing to several health benefits associated with this phenolic compound. For example, it is reported to have neuroprotective effects on several neurological conditions (102). Therefore, the high hydroxytyrosol content of this food ingredient could be perceived as a quality feature.

Table 9. Phytochemicals and antioxidant activity of Heat-treated olive pomace paste (HT-OPP).

Phytochemicals and antioxidant activity	dw	fw
TPC (g GAE/100 g)	4.31 ± 0.23	1.50 ± 0.08
HC (g/100 g)	0.49 ± 0.03	0.17 ± 0.01
FRAP (g FSE/100 g)	4.50 ± 0.26	1.56 ± 0.09
DPPH• scavenging activity (g TE/100 g)	1.68 ± 0.29	0.58 ± 0.10

Results are presented as mean ± standard deviation. TPC- Total phenolic content; GAE- gallic acid equivalents; HC- Hydroxytyrosol content; FRAP- Ferric reducing antioxidant power; FSE- Ferrous sulphate equivalents; DPPH•- 2,2-diphenyl-1-picrylhydrazyl radical; TE- Trolox equivalents; dw- dry weight; fw- fresh weight.

Nevertheless, it should be emphasized that consumption of foodstuffs rich in phenolic compounds might be challenging owing to their bitter, and astringency tastes (103). This makes the incorporation of HT-OPP a challenge since it has a high-phenolic content. Truly, the distinguishing organoleptic bitterness of HT-OPP could be a barrier to consume an olive pâté incorporating this functional food ingredient.

Hence, it was decided in the first approach to incorporate increasing percentages of HT-OPP (10%, 20%, and 30%) in an olive pâté, until reaching an adequate sensorial acceptability level. For that, a preliminary acceptability sensory test with the mentioned olive pâtés was performed, being the results presented in the next section.

4.2 Acceptability test of the olive pâté prototypes

The sensory quality of the developed olive pâtés (P0, P10, P20, P30) was initially evaluated by 56 tasters (38 females, 18 males), with ages between 18 and 57. The acceptability of the samples was accomplished using a 9-point hedonic scale, as mentioned before. **Table 10** reports the score.

Table 10. Frequency of mention (%) of each liking score by the consumers in the acceptability test of the four olive pâté prototypes: P0, P10, P20, and P30.

	P0	P10	P20	P30
"Like extremely"	19.64	19.64	7.14	0.00
"Like very much"	30.36	37.50	16.07	12.50
"Like moderately"	42.86	33.93	35.71	16.07
"Like slightly"	5.36	5.36	30.36	19.64
"Neither like nor dislike"	0.00	1.79	3.57	8.93
"Dislike slightly"	0.00	1.79	5.36	17.86
"Dislike moderately"	1.79	0.00	1.79	23.21
"Dislike very much"	0.00	0.00	0.00	1.79
"Dislike extremely"	0.00	0.00	0.00	0.00

Results are presented in frequency of mention (%). P0- Olive pâté; P10- Olive pâté with 10% HT-OPP; P20- Olive pâté with 20% HT-OPP; P30- Olive pâté with 30% HT-OPP.

Boxplots (**Figure 21**) show the consumers' acceptability of the four olive pâtés (P0, P10, P20, P30). P10 was the most appreciated by tasters (median=8), followed by P0 (median=7.5), and P20 (median=7). On the other hand, P30 was the less appreciated pâté (median=5). It should also be noted that the variability of the acceptability scores for P30 was substantial, ranging between 2 and 8. This high variability in the liking scores means that while some tasters liked this pâté, others did not (104). This suggests that HT-OPP bitterness is more evident in P30.

A Kruskal-Wallis test was performed to see if the acceptability was significantly different between the olive pâtés. Results showed that the acceptability ratings differed significantly between the olive pâtés ($H(3) = 71.687, p < 0.05$). Additionally, according to the applied pairwise comparisons post-hoc test, no significant differences were found between P0 and P10 ($p > 0.05$). On the opposite, significant differences were found between P0 and all the other olive pâtés ($p < 0.05$). Also, significant differences were found between P10, P20, and P30 ($p < 0.05$).

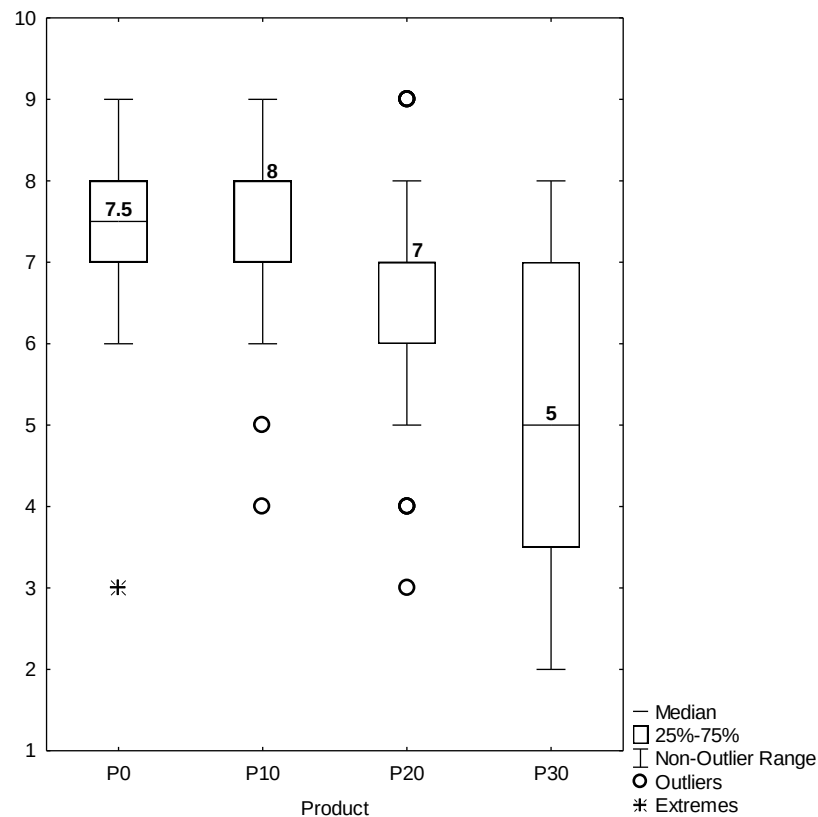


Figure 21. Boxplots for the acceptability: Evaluation on a 9-point hedonic scale from 1 (“Dislike extremely”) to 9 (“Like extremely”).

P0- Olive pâté; P10- Olive pâté with 10% HT-OPP; P20- Olive pâté with 20% HT-OPP; P30- Olive pâté with 30% HT-OPP.

On 9-point hedonic scale, an acceptability score of 6 (first score in the liking category) is considered the commercial or quality limit to a product (105). Hence, the median acceptability score for P10 and P20 achieved an adequate level on the 9-point hedonic scale (**Figure 21**). On the other hand, the median acceptability score for P30 was inferior to the reported adequate level.

These results suggest that 20% is the highest acceptable percentage of HT-OPP to incorporate in the olive pâtés. Additionally, a 10% increase from P20 to P30 resulted in a huge decrease in the median score acceptability. So, considering these results, it was decided to develop olive pâtés incorporating 20% and 25% of HT-OPP since consumers could accept these percentages.

4.3 Nutritional composition of the olive pâtés

Table 11 reports the nutritional composition of all the olive pâtés: CP, P0, P0*, P20, P20*, P25, P25*.

The commercial pâté (CP) had significantly lower moisture, protein, remaining carbohydrates, and salt contents than all the lab produced olive pâtés ($p < 0.05$). On the other hand, it registered a significantly higher fat content ($p < 0.05$). Regarding the ash amount, it was similar to those registered in the olive pâtés produced in the laboratory ($p > 0.05$).

All the olive pâtés produced (P0, P0*, P20, P20*, P25, P25*) registered similar moisture, ash, and remaining carbohydrates contents ($p > 0.05$).

Results showed that there was only a significant reduction in the fat content with the incorporation of 25% HT-OPP in the olive pâté ($p < 0.05$). On the other hand, no significant reduction in this parameter was observed with the incorporation of 20% HT-OPP ($p > 0.05$). Nevertheless, no significant differences were found between P20 and P25 and between P20* and P25* regarding the total fat contents ($p > 0.05$).

It should be mentioned that despite the high fat content registered in both P20* and P25*, their lipid fraction is of great interest considering the high MUFA content and MUFA/PUFA ratio, as it will be possible to see further in **Table 13**.

Regarding the total protein content, results showed that the incorporation of HT-OPP allowed a significant increase in this parameter ($p < 0.05$). Indeed, significantly lower contents were found in P0 than in P20 and P25, and in P0* than in P20* and P25* ($p < 0.05$). Also, no significant differences were observed between P20 and P25, and between P20* and P25* regarding the protein contents ($p > 0.05$).

Even though table olives have a low protein content, their proteins are rich in essential amino acids (106). For example, López-López *et al.* (2007) assessed the amino acid profile of different commercial table olives, namely Spanish, directly brined, and ripe olives. In this study, the authors showed that all table olives had a good balance of total essential amino acids (107). Therefore, it would be interesting to evaluate the amino acid profile of P20* and P25* in the future since both could have interesting amino acid profiles.

Results from **Table 11** also showed that the incorporation of HT-OPP resulted in a significant increase in the total dietary fibre content ($p < 0.05$). In fact, significantly lower contents were determined in P0 than in P20 and P25, and in P0* than in P20* and P25* ($p < 0.05$). Additionally, both P20* and P25* could be claimed as “high in fibre” considering

the registered contents. In fact, according to the European Regulation, a food product is classified as “high in fibre” when it has at least 3 g fibre/100 kcal or 6 g fibre/100 g (108).

Dietary fibre consists of the components, particularly carbohydrates, which are undigested by digestive enzymes of the small intestine (109). Dietary fibre is crucial for a healthy-balanced diet due to its health-related effects in cardiovascular, overweight, and digestive diseases (110). Indeed, a daily dietary fibre consumption of 25-35 g for adults is advisable (111).

Regarding the salt content of the olive pâtés, the incorporation of HT-OPP allowed a significant reduction of this parameter ($p < 0.05$). In fact, significantly higher contents were determined in P0 than in P20 and P25, and in P0* than in P20* and P25* ($p < 0.05$). Moreover, no significant differences between P20 and P25, and between P20* and P25* regarding the salt content were determined ($p > 0.05$).

The World Health Organization strongly suggests lowering the daily sodium consumption to less than 2 g (≈ 5 g salt), in adults (112). In Portugal, the daily average sodium intake is 2.962 g (≈ 7.4 g of salt/day), and men consume, on average, more sodium than women (3.431 vs. 2.547 g/day) (113). Therefore, the daily Portuguese salt intake is by far higher than the recommended values.

Nevertheless, the high salt content of both P20* and P25* can be a disadvantage to the overall nutritional quality of these olive pâtés, although these products are to be consumed occasionally and in small portions (20 g). Additionally, in the future, it would be interesting to reformulate these products to lower their salt contents.

Different practical strategies can be used to reduce the salt content: small steps over time, use of taste enhancers or modifiers (e.g., monosodium glutamate), and use of mineral salts (e.g., KCl, K_2SO_4 , $CaCl_2$, $MgCl_2$, among many others) (114). Additionally, according to a recent review work, the food industry’s willingness to take part in salt reduction food reformulation has been enthusiastic (115).

Recent works have reduced salt level in some foodstuffs, while preserving customer acceptability. For example, a 50% reduction of the sodium chloride content, using KCl was possible in bread without negatively affecting its’ sensory properties (116). In another work, 33% salt reduction using micronized salt in beef burgers did not affect their salty taste (117). Therefore, it would be interesting to reformulate P20* and P25* using some of the previously mentioned strategies.

Regarding the impact of HPP on the nutritional composition of the olive pâtés, no significant differences were found between unprocessed and HPP processed olive pâtés ($p>0.05$). This means that HPP processing did not significantly impact the nutritional composition/quality of the produced olive pâtés.

Studies by other authors in other food matrices also showed that this type of processing had an insignificant impact on the nutritional composition. Sardão *et al.* (2021) showed that HPP processing of an acorn beverage, at 450 MPa/5, 12.5, 20 min and at 600 MPa/5, 12.5, 20 min, did not lead to significant variations of the proteins and lipids total contents (118). In another study, no significant differences were found in the fat, crude fibre, ash, and available carbohydrates contents between unprocessed and HPP processed (500 MPa/10 min) apples (119).

Protein denaturation can take place due to HPP processing, which can positively or negatively influence texture and even the overall quality of food (120). Nevertheless, HPP has no impact on the primary structure of proteins, meaning that the amino acid content and thus the proteins' nutritional value is unaffected by this type of processing (121).

In a current study, Lin *et al.* (2020) revealed that processing of dry beans (≤ 450 MPa) resulted in a considerable increase in resistant starch content. On the other hand, this processing did not have any impact neither on the total dietary fibre content, nor on the insoluble/soluble dietary fibre ratio (122).

To conclude, the nutritional analysis results revealed that both P20* and P25* have the potential to be commercial olive pâtés. Their significant high-fibre content is a quality attribute, owing to their health-related properties mentioned previously. Additionally, similar contents of the other analyzed parameters (moisture, protein, fat, ash, remaining carbohydrates, salt) were registered. Therefore, both P20* and P25* are undeniably interesting olive pâtés to answer the rising interest of consumers in food that promotes well-being and is eco-friendly.

Table 11. Nutritional composition of all the olive pâtés: CP, P0, P0*, P20, P20*, P25, P25*.

		CP	P0	P0*	P20	P20*	P25	P25*
Fresh weight	Moisture	53.90 ± 0.54 ^b	59.02 ± 0.78 ^a	59.04 ± 0.79 ^a	58.93 ± 0.91 ^a	59.56 ± 0.55 ^a	59.17 ± 0.47 ^a	59.07 ± 0.38 ^a
	Protein	1.11 ± 0.03 ^c	1.65 ± 0.04 ^b	1.67 ± 0.08 ^b	2.04 ± 0.07 ^a	2.01 ± 0.01 ^a	2.03 ± 0.17 ^a	2.08 ± 0.12 ^a
	Fat	30.15 ± 1.33 ^a	18.38 ± 0.38 ^b	17.93 ± 0.20 ^{bc}	17.02 ± 0.18 ^{bcd}	16.34 ± 0.25 ^{cd}	16.31 ± 0.44 ^d	15.81 ± 0.26 ^d
	Ash	2.91 ± 0.25 ^a	2.79 ± 0.13 ^a	2.91 ± 0.12 ^a	2.77 ± 0.14 ^a	2.81 ± 0.03 ^a	2.58 ± 0.12 ^a	2.63 ± 0.29 ^a
	Total fiber	6.90 ± 0.22 ^c	7.09 ± 0.17 ^c	6.78 ± 0.04 ^c	8.53 ± 0.31 ^{ba}	8.18 ± 0.22 ^b	8.81 ± 0.19 ^a	8.92 ± 0.15 ^a
	Remaining carbohydrates	5.03 ± 0.70 ^b	11.07 ± 1.06 ^a	11.66 ± 0.63 ^a	10.71 ± 1.32 ^a	11.11 ± 0.54 ^a	11.11 ± 0.51 ^a	11.49 ± 0.09 ^a
	Salt	1.22 ± 0.03 ^c	2.24 ± 0.07 ^a	2.27 ± 0.03 ^a	1.75 ± 0.08 ^b	1.83 ± 0.05 ^b	1.75 ± 0.09 ^b	1.72 ± 0.09 ^b
Dry weight	Protein	2.42 ± 0.08 ^c	4.02 ± 0.10 ^b	4.08 ± 0.19 ^b	4.96 ± 0.16 ^a	4.97 ± 0.02 ^a	4.97 ± 0.43 ^a	5.08 ± 0.29 ^a
	Fat	65.40 ± 2.88 ^a	44.86 ± 0.92 ^b	43.78 ± 0.49 ^{bc}	41.44 ± 0.44 ^{bcd}	40.40 ± 0.61 ^{cd}	39.94 ± 1.09 ^d	38.64 ± 0.63 ^d
	Ash	6.31 ± 0.55 ^a	6.80 ± 0.31 ^a	7.11 ± 0.30 ^a	6.76 ± 0.33 ^a	6.94 ± 0.07 ^a	6.32 ± 0.30 ^a	6.42 ± 0.70 ^a
	Total fiber	14.96 ± 0.48 ^d	17.30 ± 0.41 ^c	16.54 ± 0.10 ^c	20.76 ± 0.74 ^{ba}	20.22 ± 0.55 ^b	21.57 ± 0.47 ^a	21.79 ± 0.37 ^a
	Remaining carbohydrates	10.91 ± 2.67 ^b	27.02 ± 1.00 ^a	28.49 ± 0.42 ^a	26.08 ± 1.35 ^a	27.47 ± 0.07 ^a	27.21 ± 1.55 ^a	28.07 ± 1.15 ^a
	Salt	2.65 ± 0.06 ^c	5.48 ± 0.17 ^a	5.55 ± 0.08 ^a	4.27 ± 0.20 ^b	4.54 ± 0.14 ^b	4.28 ± 0.23 ^b	4.20 ± 0.22 ^b

Results are presented in g/100 g as mean ± standard deviation, in both fresh and dry weight. Different superscript letters within each line represent significant differences ($p < 0.05$) between samples. CP- Commercial olive pâté; P0- Olive pâté; P0*- Olive pâté processed by HPP; P20- Olive pâté with 20% HT-OPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25- Olive pâté with 25% HT-OPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

4.4 Vitamin E total content and profile of the olive pâtés

The term “Vitamin E” refers to a group of chemical compounds: α - tocopherol and tocotrienol; β - tocopherol and tocotrienol; γ - tocopherol and tocotrienol; δ -tocopherol and tocotrienol (123).

Vitamin E usually prevents oxidative processes and stops the free radicals production (124, 125). In fact, it protects the polyunsaturated fatty acids (PUFA) from oxidation, being found in the plasma lipoproteins and cell membranes (126). Specifically, it gives a hydrogen to the peroxy radicals, inhibiting the production of another lipid radical, thus, preventing the propagation of the lipid peroxidation chain reaction (127).

Table 12 presents the results from the vitamin E profile and content of all the olive pâtés. According to the results, three tocopherols were detected: α -, β - and γ -tocopherol. Considering that the olive pâtés are composed by table olives and EVOO, ingredients with more than 90% of the total tocopherols are α -tocopherol (128, 129), it is expected that α -tocopherol was the major vitamin present in all the pâtés.

Additionally, higher quantities of γ -tocopherol than β -tocopherol were also expected, as the same was also determined in table olive flours (130). Regarding the tocotrienols, only in the naturally ripe olives, α -tocotrienol was found, disappearing, however, after processing (131). Furthermore, in table olives, no peaks corresponding to the other tocotrienols were detected (131).

According to the results presented in **Table 12**, the CP, and the control olive pâtés (P0 and P0*) had similar contents of total vitamin E, in dw ($p>0.05$). On the opposite, the olive pâtés incorporating HT-OPP (P20, P20*, P25 and P25*) had significantly lower contents of total vitamin E than the other samples ($p<0.05$). This significant reduction was already expected and may be related to the incorporation of HT-OPP, since this by-product registered inferior contents of vitamin E than the ones reported to table olives and EVOO.

The major vitamin present in table olives is Vitamin E (132). Additionally, the high α -tocopherol contents in table olives emphasize this products' nutritious worth (133). According to a review work, table olives can contain 1.3 to 9 mg of α -tocopherol/100 g edible portion (132). For example, a content of 6.44 mg α -tocopherol/100 g edible portion in fresh weight were registered in *Intosso d'Abruzzo* cv table olives (134). Regarding black oxidized olives, the vitamin E content is remarkably high, indicating that the strong processing conditions do not affect α -tocopherol amounts (135).

Tocopherols are also abundant in olive oils (from 1 to 35 mg/100 g) (133). For example, the total tocopherol concentration of Italian EVOOs ranged between 17.7 to 40.1 mg/100 g (136). Average contents of 13.4 and 33.5 mg α -tocopherol/100 g were described in different Italian EVOOs (137). In another study, both expensive and low-priced Italian EVOOs contained high contents of this vitamin (22.9 and 22.4 mg/100 g, respectively) (138).

Vitamin E must be consumed in the diet since it can only be synthesized by photosynthetic organisms (139). The deficiency of this vitamin can cause ataxia and enhance the risk of developing additional conditions related to fat malabsorption (126). Therefore, it is an essential vitamin that should be daily consumed for a healthy life. Indeed, the acceptable daily consumption levels of α -tocopherol are 13 mg and 11 mg for adult males and females, respectively (140).

Although the olive pâtés incorporating HT-OPP had lower contents than the other olive pâtés, they are still good sources of vitamin E. The α -tocopherol content on P20* and P25* are 6.88 and 6.50 mg/100 g (fw), respectively. This means that a serving (20 g) of the P20* would provide 12.5% and 10.6% of the acceptable daily intake for men and women, respectively. In the same way, a serving (20 g) of the P25* would provide 11.8% and 10% of the acceptable daily intake for men and women, respectively. Therefore, P20* and P25* seem interesting food choices for reaching the daily α -tocopherol recommended intakes.

Despite being a vital component, Vitamin E is reported to be a weakly stable molecule (125). HPP is highly accepted for not decomposing vitamins and other micronutrients (121). Different studies showed that the vitamin E content/profile was not significantly impacted by this type of processing. For example, HPP processing of açai juice did not lead to a significant impact neither in the α -tocopherol, nor in the γ -tocopherol contents (141). In another study, the δ -, γ -, and α -tocopherol contents in milk were not affected during HPP processing for 5 min, at different pressures: 400, 500, and 600 MPa (142).

Therefore, HPP was expected to have not a negative impact in the total vitamin E content of the olive pâtés. In fact, no significant differences were observed between HPP-processed and unprocessed olive pâtés' total vitamin E profile and content ($p>0.05$). Therefore, the vitamin E profile was not significantly affected by HPP processing in this study.

To conclude, both P20* and P25* can be considered extremely valuable olive pâtés considering their total Vitamin E and α -tocopherol contents.

Table 12. Vitamin E profile and total content of all olive pâtés: CP, P0, P0*, P20, P20*, P25, P25*.

		CP	P0	P0*	P20	P20*	P25	P25*
Fresh weight	α-tocopherol	10.34 ± 0.24 ^a	8.83 ± 0.10 ^b	9.57 ± 0.16 ^{ba}	6.62 ± 0.59 ^c	6.88 ± 0.15 ^c	6.50 ± 0.49 ^c	6.14 ± 0.22 ^c
	β-tocopherol	0.37 ± 0.00 ^a	0.37 ± 0.02 ^a	0.36 ± 0.04 ^a	0.30 ± 0.02 ^b	0.26 ± 0.01 ^b	0.30 ± 0.01 ^b	0.29 ± 0.01 ^b
	γ-tocopherol	0.64 ± 0.01 ^a	0.60 ± 0.01 ^{ba}	0.56 ± 0.02 ^b	0.40 ± 0.03 ^c	0.43 ± 0.00 ^c	0.41 ± 0.03 ^c	0.40 ± 0.02 ^c
	Total vitamin E	11.36 ± 0.25 ^a	9.80 ± 0.09 ^b	10.50 ± 0.15 ^{ba}	7.32 ± 0.63 ^c	7.57 ± 0.16 ^c	7.21 ± 0.50 ^c	6.82 ± 0.22 ^c
Dry weight	α-tocopherol	22.44 ± 0.51 ^a	21.84 ± 0.25 ^a	23.37 ± 0.39 ^a	16.15 ± 1.44 ^b	16.80 ± 0.37 ^b	15.93 ± 1.20 ^b	14.95 ± 0.52 ^b
	β-tocopherol	0.80 ± 0.01 ^{ba}	0.90 ± 0.05 ^a	0.89 ± 0.09 ^a	0.74 ± 0.05 ^{cb}	0.63 ± 0.02 ^c	0.73 ± 0.02 ^{cb}	0.70 ± 0.01 ^{cb}
	γ-tocopherol	1.40 ± 0.02 ^a	1.48 ± 0.03 ^a	1.38 ± 0.04 ^a	0.98 ± 0.05 ^b	1.05 ± 0.00 ^b	1.00 ± 0.07 ^b	0.97 ± 0.06 ^b
	Total vitamin E	24.64 ± 0.55 ^a	24.23 ± 0.22 ^a	25.64 ± 0.26 ^a	17.86 ± 1.55 ^b	18.49 ± 0.39 ^b	17.65 ± 1.23 ^b	16.61 ± 0.53 ^b

Results are presented in mg/100 g as mean ± standard deviation, in both fresh and dry weight. Different superscript letters within each line represent significant differences ($p < 0.05$) between samples. CP- Commercial olive pâté; P0- Olive pâté; P0*- Olive pâté processed by HPP; P20- Olive pâté with 20% HT-OPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25- Olive pâté with 25% HT-OPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

4.5 Fatty acids profile of the olive pâtés

Fatty acids are needed to human nutrition and health (143). Chemically, they can be categorized into distinct groups, according to the number of double bounds they contain. In fact, they can be classified in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) (144). Specifically, SFA do not have any double bond. On the other hand, MUFA have one double bond, whereas PUFA have at least two double bonds (144).

Table 13 reports the fatty acids profile of the olive pâtés. The main fatty acid present was oleic acid (C18:1n9c). Additionally, the second and third main fatty acids were palmitic (C16:0) and linoleic (C18:2n6c) acids, respectively. This means that all the olive pâtés evidenced a nutritionally rich fatty acid profile, which was expected owing to the ingredients used in the production: table olives, EVOO, and HT-OPP.

Table olives are mainly composed by oleic, palmitic, linoleic, and stearic acids (135). Nevertheless, it should be known that their fatty acid profile can be influenced by processing techniques, as well as the maturity stage, precipitation, and geographic origin (145). Californian-style black olives are mainly composed by oleic, palmitic, linolenic, and stearic fatty acids. Some minor fatty acids can also be found in this type of table olives: palmitoleic, linolenic, arachidic and eicosenoic (145).

The fatty acid composition of EVOO must comply with the percentages regulated by EU (146). For example, oleic acid can range between 55.00-83.00%, linoleic between 2.50-21.00%, and palmitic between 7.50-20.00% (146). In addition, it should be mentioned that olive oil fatty acid composition can be affected by many aspects, like the genetic factors and the environmental conditions experienced during the olives' growth and maturation (136). For example, only six fatty acids were detected in EVOO from the Italian market: oleic, palmitic, linoleic, stearic, palmitoleic, and linolenic acids (138). Another study showed that Italian EVOO had as minor fatty acids: myristic ($\leq 0.01\%$), heptadecanoic ($\leq 0.10\%$), and lignoceric ($\leq 0.10\%$) acids (136).

In general, P0, P20, and P25 had similar fatty acid profiles, according to **Table 13**. On the opposite, significant differences were observed between the fatty acid profile of the CP and of all the other olive pâtés ($p < 0.05$). Specifically, it registered a significant higher MUFA/PUFA ratio and $\sum$ MUFA and significant lower $\sum$ PUFA ($p < 0.05$).

Nevertheless, the olive pâtés incorporating HT-OPP still registered a high MUFA/PUFA ratio. They also have a high relative percentage of nutritionally essential fatty acids, namely linoleic (C18:2n6c) acid.

The MUFA/PUFA ratio is related to the oils' oxidative stability. In fact, olive oil rich in MUFA has a longer shelf lifetime, whereas olive oil rich in PUFA has a shorter one (147). Hence, olive oils with high MUFA/PUFA ratios have greater oxidative stability and longer shelf lives. Therefore P20* and P25* are predicted to have good oxidative stability having in mind their high MUFA/PUFA ratios.

MUFA participate in a variety of biological processes (e.g., energy metabolism, lipid biosynthesis, and membrane integrity maintenance). From all, palmitoleic and oleic acids play the most important roles in the human body (148). Oleic acid (C18:1n9c) is a non-essential fatty acid as it is produced by the human body. However, it is related to various health benefits, such as preventing SFA-induced inflammation (149). The intake of oleic acid has also been linked to a decrease in LDL cholesterol (133) and reported to prevent palmitic-SFA-induced mitochondrial dysfunction and insulin resistance (148).

PUFA protects from numerous illnesses like osteoarthritis, cancer, and even autoimmune disorders (150). However, because of a lack of appropriate enzymes, the human body is unable to produce PUFA that contains a first double bond on C3 and on C6, counting from the methyl-end (151). Therefore, linoleic acid is considered an essential fatty acid (149). Linoleic acids' main function is to be a biochemical precursor of long-chain PUFA with physiological importance (e.g., arachidonic acid) (148).

Regarding the impact of HPP on the fatty acids profile, no significant differences were determined between HPP processed and unprocessed olive pâtés ($p>0.05$). Also coinciding with our results, the human milk fatty acid proportions were not significantly impacted by any of the applied HPP treatments for 5 min: 400, 500, and 600 MPa (142).

To conclude, the lipid fraction of both P20* and P25* is of great interest owing to the contents of healthful fatty acids, namely oleic and linoleic acids. Additionally, the fatty acid profiles of P20* and P25* did not differ significantly ($p>0.05$), meaning that both are of extremely interest to the food industry.

Table 13. Fatty acid composition of all the olive pâtés: CP, P0, P0*, P20, P20*, P25, P25*.

Fatty acids	CP	P0	P0*	P20	P20*	P25	P25*
Palmitic (C16:0)	12.60 ± 0.02 ^b	13.29 ± 0.05 ^a	13.14 ± 0.03 ^a	13.07 ± 0.06 ^a	12.98 ± 0.23 ^a	13.26 ± 0.23 ^a	13.07 ± 0.10 ^a
Palmitoleic (C16:1)	1.00 ± 0.01 ^a	0.92 ± 0.03 ^{ba}	0.91 ± 0.02 ^{ba}	0.90 ± 0.03 ^{ba}	0.91 ± 0.06 ^{ba}	0.93 ± 0.07 ^{ba}	0.85 ± 0.06 ^b
Stearic (C18:0)	2.88 ± 0.03 ^a	2.46 ± 0.03 ^c	2.47 ± 0.04 ^c	2.59 ± 0.04 ^{cb}	2.49 ± 0.08 ^c	2.76 ± 0.07 ^{ba}	2.61 ± 0.10 ^{cb}
Oleic (C18:1n9c)	76.26 ± 0.06 ^a	74.62 ± 0.30 ^b	74.60 ± 0.11 ^b	74.35 ± 0.12 ^b	74.63 ± 0.15 ^b	74.15 ± 0.17 ^b	74.62 ± 0.33 ^b
Linoleic (C18:2n6c)	5.82 ± 0.08 ^b	7.26 ± 0.35 ^a	7.42 ± 0.06 ^a	7.60 ± 0.05 ^a	7.62 ± 0.02 ^a	7.38 ± 0.16 ^a	7.45 ± 0.13 ^a
α-linolenic (C18:3n3)	0.72 ± 0.01 ^a	0.81 ± 0.08 ^a	0.81 ± 0.05 ^a	0.83 ± 0.05 ^a	0.69 ± 0.06 ^a	0.73 ± 0.06 ^a	0.78 ± 0.06 ^a
Σ SFA	15.99 ± 0.07 ^b	16.24 ± 0.05 ^{ba}	16.09 ± 0.03 ^{ba}	16.13 ± 0.10 ^{ba}	15.91 ± 0.27 ^b	16.59 ± 0.34 ^a	16.14 ± 0.19 ^{ba}
Σ MUFA	77.47 ± 0.05 ^a	75.69 ± 0.28 ^b	75.68 ± 0.11 ^b	75.44 ± 0.14 ^b	75.77 ± 0.20 ^b	75.30 ± 0.14 ^b	75.63 ± 0.27 ^b
Σ PUFA	6.54 ± 0.08 ^b	8.07 ± 0.32 ^a	8.23 ± 0.08 ^a	8.43 ± 0.10 ^a	8.31 ± 0.07 ^a	8.11 ± 0.22 ^a	8.23 ± 0.13 ^a
MUFA/PUFA	11.85 ± 0.14 ^a	9.39 ± 0.42 ^b	9.19 ± 0.11 ^b	8.95 ± 0.12 ^b	9.12 ± 0.05 ^b	9.29 ± 0.24 ^b	9.19 ± 0.17 ^b

Results are presented in relative percentage (%) as mean ± standard deviation. Different superscript letters within each line represent significant differences ($p < 0.05$) between samples. CP- Commercial olive pâté; P0- Olive pâté; P0*- Olive pâté processed by HPP; P20- Olive pâté with 20% HT-OPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25- Olive pâté with 25% HT-OPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids. Heptadecanoic (C17:0), arachidic (C20:0), *cis*-11-Eicosenoic (C20:1n9), behenic (C22:0) and lignoceric (C24:0) fatty acids were found in relative % inferior to 0.5%.

4.6 Phytochemicals content and antioxidant activity of the olive pâtés

4.6.1 Total phenolics content (TPC) of the olive pâtés

Phenolic compounds are nowadays a highly investigated class of bioactive compounds (152). They include various families of compounds that are distributed widely in nature and mainly found in vegetable-derived foods (153). In addition, phenolic compounds interact with the salivary glycoproteins, being thus responsible for the fruit bitterness (154).

Chemically, phenolic compounds contain at least one aromatic ring that are linked to one or more hydroxyl groups (152). Furthermore, according to their basic chemical structure, they may be divided into ten distinct classes (155). These compounds are considered powerful antioxidants, and some even have antimicrobial or anticarcinogenic properties (153). Their capability of donating an electron and/or a hydrogen atom to free radicals is the reason they are considered antioxidants (152). Additionally, the presence of a high number of hydroxyl groups makes them toxic to microorganisms (154).

A popular technique to evaluate the amount of the phenolic compounds is the Folin-Ciocalteu assay (156). This methodology is based on the phenolics' capacity to reduce the Folin-Ciocalteu reagent, in alkaline conditions (156, 157). A calibration curve is also prepared, using a pure standard, mainly gallic acid (158).

Numerous works report the TPC of EVOO and table olives using different extraction methods and solvents than the ones used in this work, which compromises the comparison of results among studies. Nevertheless, some studies presented below demonstrate the interesting TPC of these ingredients.

The presence of bioactive components like phenolic compounds in EVOO contributes to its health-promoting qualities (159). In fact, the phenolic compounds of EVOO have numerous health-benefits (e.g., antitumoral activities) and antimicrobial properties (159). Therefore, despite being minor compounds in olive oil (159), the phenolic compounds are important to EVOO.

Commercial olive oils acquired in the local Portuguese market showed a TPC ranging between 9.74 and 21.97 mg GAE/100 g of oil (160). In another study, the TPC of high-priced Italian EVOO and low-priced Italian EVOO was of 17.66 mg and 12.68 mg GAE/100 g, respectively (138). In another study, the TPC of Spanish cv. Arbequina EVOO ranged between 7.81 and 11.8 mg GAE/100 g (161).

Ripe olives must be submitted to fermentation/curing to reduce the contents of bitter compounds (oleuropein and ligstroside), making them suitable for human consumption (70). In the Californian style, oleuropein, ligstroside, and verbascoside are hydrolysed into non-bitter phenolics during the lye treatment (162).

The Californian-style olives contain significantly lower phenolic contents than the other processed table olives (70, 132, 162). For example, in dw, commercial table olives darkened by oxidation showed a TPC ranging between 1.19 and 10.84 mg GAE/g pulp (163). It should be known that the table olives' phenolic content depends on the cultivar, agronomic factors, and even the olive fruits' ripening level (145).

According to **Table 14**, the CP had a significantly lower TPC than all the produced olive pâtés ($p < 0.05$). Results also show that the incorporation of HT-OPP in the olive pâté resulted in a significant increase of the TPC ($p < 0.05$). Indeed, a significantly lower TPC was registered in P0 than in P20 and P25, and in P0* than in P20* and P25* ($p < 0.05$). This increase may be associated with the incorporation of HT-OPP, a by-product with high TPC, as mentioned before.

According to the results (**Table 14**), HPP did not impact significantly the TPC. In fact, the TPC of treated and untreated olive pâtés was similar ($p > 0.05$).

According to the literature, HPP processing either has no impact or leads to an increase in the TPC. The enhancement of the extractability of antioxidant compounds after high-pressure processing, might explain an increase in the TPC (164). For example, HPP treatment (200-600 MPa) of cranberry bush purée caused non-significant increases in the TPC between 4-11% (165). On the other hand, some studies report small but not significant decreases in the TPC of pressurised food products. For example, HPP did not have a significant impact on the TPC of carrot/orange juice mixtures, probably owing to the use of mild pressuring conditions: 400 MPa/3 min; 400 MPa/1 min; 300 MPa/2 min (166). In another work, processing using combined high pressure/mild temperature conditions did not significantly affect the total polyphenol of strawberry halves (167). Indeed, the authors reported that no significant decreases in TPC were detected at pressures as high as 600 MPa.

To conclude, P25* has a significantly higher TPC than P20* ($p < 0.05$). Nevertheless, both can be regarded as interesting commercial options since they have higher contents than P0* ($p < 0.05$).

4.6.2 Hydroxytyrosol content (HC) of the olive pâtés

The most potent antioxidant presently known is hydroxytyrosol (3,4-dihydroxyphenylethanol) (168). The presence of the *o*-dihydroxyphenyl moiety is responsible for this great antioxidant efficiency (169). By giving a hydrogen atom to peroxy-radicals, it primarily acts as a chain-breaking antioxidant (169).

Hydroxytyrosol, a metabolite of oleuropein, can be found in olive products: olive leaves and fruits, EVOO, and OMWW (168). Indeed, numerous works report the HC of EVOO and table olives using different extraction methods and solvents, and chromatographic systems, which compromises the comparison of results among studies. Some studies are presented below to demonstrate that these ingredients can be an interesting source of HC.

For example, Fiorini *et al.* (2018) determined the HC (HPLC-DAD-ESI/MS) of high and low-priced Italian EVOO. High-priced EVOO registered a significantly higher HC than low-priced EVOO (12.26 and 7.26 mg/kg, respectively) (138).

Regarding the HC of Portuguese EVOO, Pereira and colleagues (2020) assessed the HC of different Portuguese monovarietal EVOO using a HPLC-DAD system. The HC ranged between 3.255 and 26.295 mg/100 g (170).

In table olives, the main phenolic compound is hydroxytyrosol (132). However, the Californian-style processing technique leads to the production of table olives with the lowest contents of phenolic compounds, particularly hydroxytyrosol (162). Rocha *et al.* (2020) reported that when compared to other table olives, the black ripe olives have minimal or null hydroxytyrosol contents (132). The hydroxytyrosol concentration in olives starts to decrease significantly during the rising steps (162). Additionally, this compound is oxidized during the ferrous gluconate treatment (162).

Johnson *et al.* (2018) determined the hydroxytyrosol content, using a UHPLC-(ESI) MS/MS system, of distinct types of table olives: Greek, Spanish, and California styles. The Greek and Spanish styles had comparable HC, but a significantly lower HC was determined in Californian-style olives (134.329, 133.685, and 19.981 mg/kg wet weight, respectively) (171).

In another study, table olives from the Portuguese “Douro” variety processed by the Californian-style method showed a hydroxytyrosol content of 0.54 mg/100 g (172).

Hydroxytyrosol consumption has numerous health-benefit effects: cardioprotective, anticancer, neuroprotective, antidiabetic, antiobesity, antimicrobial, antiviral, and

osteoprotective (169). In fact, hydroxytyrosol enrichment of food items has shown to have positive effects on consumers health.

For instance, Mateos *et al.* (2016) assessed the hydroxytyrosol bioavailability and pharmacokinetics in humans, using biscuits enriched with 5.25 mg of hydroxytyrosol/30 g. Results showed that the consumption of these cookies allowed a significant reduction of oxidised-LDL plasma levels (173)

An allegation on olive oil polyphenols was authorized by EFSA, having in mind the beneficial health effects on human cardiovascular diseases (174). The panel recommended daily consumption of 5 mg of hydroxytyrosol and its' derivatives in olive oil to maintain a balanced diet (174).

According to the results from **Table 14**, hydroxytyrosol was only detected and quantified in the olive pâtés incorporating HT-OPP: P20, P20*, P25, P25*. In fact, it was not detected neither in CP nor in the control olive pâtés (P0 and P0*).

Additionally, P25* had a significantly higher HC than the P20*, in both fw and dw ($p < 0.05$). Nevertheless, both can be considered olive pâtés rich in hydroxytyrosol. In fact, ≈ 5.5 mg of hydroxytyrosol is present in a serving portion (20 g) of P20*. A serving portion (20 g) of P25* contains ≈ 7.5 mg of hydroxytyrosol. This suggests that consuming both pâtés may bring some of the health benefits previously described.

Regarding the impact of HPP on phenolic compounds, Szczepańska *et al.* (2022) stated that decreases in simple polyphenols concentrations can be linked to HPP-induced degradation (175).

In this study, HPP did not have a significant impact on the HC. Indeed, small but not significant differences were noticed between HPP processed and unprocessed pâtés ($p > 0.05$).

To conclude, both P20* and P25* seem interesting commercial olive pâtés owing to their high HC. Indeed, they can be interesting options to provide a food product/snack that is both healthy and tasty.

4.6.3 Antioxidant activity of the olive pâtés: Ferric reducing antioxidant power (FRAP) and DPPH• scavenging activity assays

The antioxidant activity of all the olive pâtés was assessed by two methods: FRAP and DPPH• scavenging activity.

FRAP assesses the reduction of a ferric ion-ligand complex by an antioxidant to a ferrous complex (blue coloured), being considered an electron transfer reaction assay (176). Specifically, it allows the detection of compounds that have a redox potential inferior to 0.7 V (177).

DPPH• scavenging activity, used to estimate the antiradical activity of several chemicals, is based on the DPPH• neutralization by the donation of an electron or hydrogen (177).

Numerous works reported the antioxidant capacity of EVOO and table olives. However, the comparison of results is compromised owing to the usage of different solvents and extraction methods than those used in this work. Nevertheless, some results are presented below to demonstrate that these samples have interesting antioxidant activities.

EVOO has a range of commonly known healthy associated properties, like antioxidant properties that promote a healthy circulatory system and have a positive role on cancer, and degenerative illnesses (178). For example, high-priced Italian EVOO registered an antioxidant activity of 14.13 $\mu\text{M TE}$, whereas low-priced Italian EVOO registered an antioxidant activity of 9.90 $\mu\text{M TE}$ (138).

Antioxidant properties of table olives depend on the cultivars, maturation, agricultural practices, and processing method (145). In addition, table olive processing leads to a loss of phenolic compounds, which leads to a reduction of the antioxidant activity (163). Nonetheless, table olives are regarded as being rich sources of antioxidants (69).

Regarding the antioxidant activity of Californian olives, seven commercial black table olives from distinct cultivars, harvested green and darkened by oxidation, showed a DPPH• scavenging activity ranging between 1356 and 2533 $\mu\text{mol TE}/100\text{ g}$ (163).

Results (**Table 14**) show that the CP sample registered significantly lower antioxidant activities than all the other pâtés ($p<0.05$). Additionally, the incorporation of HT-OPP in the olive pâtés allowed a significant improvement of the antioxidant activities ($p<0.05$). Indeed, a significantly lower antioxidant activity was registered in P0 than in P20 and P25, and in P0* than in P20* and P25* ($p<0.05$). This may be related to the incorporation of HT-OPP, a by-product with high antioxidant activities, as mentioned before.

Additionally, P25* had a significantly higher antioxidant activity than P20*, in both FRAP and DPPH• scavenging activity assays ($p < 0.05$). Nevertheless, both olive pâtés registered interesting antioxidant activities, meaning that both have the potential to be commercial olive pâtés.

Here, HPP had little and no significant impact on the antioxidant activity, in both FRAP and DPPH• scavenging activity, according to **Table 14**. Numerous studies reported that the antioxidant activity of food products can be or not significantly impacted by HPP. Nonetheless, the influence of HPP on vegetable products is determined by the food form and processing conditions (179).

Zou and colleagues (2016) showed that the antioxidant activity of Mulberry juices (DPPH• scavenging activity assay) was not impacted by HPP. In fact, the antioxidant activities of unprocessed and HPP processed (500 MPa/5 min) juices, immediately after processing, did not differ significantly ($p < 0.05$) (180).

Also, no notable effects due to HPP treatment were observed regarding the antioxidant activity (ABTS⁺ radical cation assay) of carrot and tomato purées. Indeed, the antioxidant capacity of untreated purees was comparable with the values after two HPP treatments at 25 °C: 500 MPa and 800 MPa/5 min (181).

In another study, the antioxidant activity of melon purée exposed to various HPP treatments was evaluated after storage (4 °C) during 1, 6, and 10 days. Results revealed that the antioxidant capacity (ORAC assay) was not affected by the HPP since the results obtained for pressurized melon puree were comparable to the control purée (182).

On the opposite, HPP treatment has also been reported to result in a loss of antioxidant activities. For example, García-Cayuela *et al.* (2018) assessed the impact of HPP treatments, at 25 °C, on diced and peeled ripe persimmon fruits: 200 MPa/3 min and 200 MPa/6 min. Results showed that, right after processing (day of storage=0), there were decreases of 25% with HPP 200 MPa/3 min, and 30% with HPP treatment 200 MPa/6 min (183).

To conclude, the commercialization of both P20* and P25* seems of potential owing to the interesting antioxidant activities and the rising interest in foodstuffs enriched in natural antioxidants.

Table 14. Phytochemicals and antioxidant activity of all the olive pâtés: CP, P0, P0*, P20, P20*, P25, P25*.

		CP	P0	P0*	P20	P20*	P25	P25*
Fresh weight	TPC (g GAE/100 g)	0.20 ± 0.01 ^d	0.43 ± 0.01 ^c	0.45 ± 0.01 ^c	0.56 ± 0.01 ^b	0.56 ± 0.02 ^b	0.64 ± 0.02 ^a	0.64 ± 0.02 ^a
	HC (g/100 g)	n.d	n.d	n.d	0.03 ± 0.00 ^{ba}	0.03 ± 0.01 ^b	0.04 ± 0.00 ^a	0.04 ± 0.01 ^a
	FRAP (g FSE/100 g)	0.19 ± 0.01 ^d	0.48 ± 0.02 ^c	0.49 ± 0.02 ^c	0.62 ± 0.02 ^b	0.64 ± 0.02 ^b	0.82 ± 0.03 ^a	0.83 ± 0.03 ^a
	DPPH* (g TE/100 g)	0.17 ± 0.01 ^d	0.37 ± 0.02 ^c	0.34 ± 0.04 ^c	0.47 ± 0.03 ^b	0.45 ± 0.03 ^b	0.53 ± 0.03 ^a	0.54 ± 0.02 ^{ba}
Dry weight	TPC (g GAE/100 g)	0.43 ± 0.02 ^d	1.05 ± 0.01 ^c	1.10 ± 0.01 ^c	1.37 ± 0.02 ^b	1.38 ± 0.05 ^b	1.56 ± 0.04 ^a	1.56 ± 0.04 ^a
	HC (g/100 g)	n.d	n.d	n.d	0.08 ± 0.01 ^{ba}	0.07 ± 0.01 ^b	0.10 ± 0.01 ^a	0.09 ± 0.01 ^a
	FRAP (g FSE/100 g)	0.40 ± 0.02 ^d	1.16 ± 0.05 ^c	1.19 ± 0.05 ^c	1.51 ± 0.05 ^b	1.59 ± 0.05 ^b	2.00 ± 0.07 ^a	2.04 ± 0.07 ^a
	DPPH* (g TE/100 g)	0.38 ± 0.03 ^d	0.91 ± 0.06 ^c	0.85 ± 0.09 ^c	1.15 ± 0.06 ^b	1.12 ± 0.07 ^b	1.29 ± 0.08 ^a	1.33 ± 0.04 ^a

Results are presented as mean ± standard deviation, in both fresh and dry weight. Different superscript letters within each line represent significant differences ($p < 0.05$) between samples. CP- Commercial olive pâté; P0- Olive pâté; P0*- Olive pâté processed by HPP; P20- Olive pâté with 20% HT-OPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25- Olive pâté with 25% HT-OPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; TPC- Total phenolic content; GAE- Gallic acid equivalents; HC- Hydroxytyrosol content; n.d- not detected; FRAP- Ferric reducing antioxidant power; FSE- ferrous sulphate equivalents; DPPH*- 2,2-diphenyl-1-picrylhydrazyl radical; TE- Trolox equivalents.

4.7 Sensory characterization, acceptability, and purchase intention of the HPP-processed olive pâtés

The sensory evaluation of the olive pâtés was performed with a total of 100 consumers. The demographic characterization, such as age, gender, and occupation is presented in **Table 15**.

Consumers were from all age groups, with a predominance of individuals aged between 18 to 57 years old, distributed in three groups 18-25, 26-33, and 50-57. There was, however, a low proportion of assessors from the age groups of 66-73 and ≥ 74 .

Regarding gender, most of the tasters were women (55%).

Additionally, considering the occupation, most of the individuals were workers (54%) and students (33%). There was, however, a low proportion of tasters that were domestic (1%) and student-workers (1%).

Table 15. Characterization of the participants in the sensory analysis.

Information		Number of consumers	Frequency (%)
Age	<18	9	9.00
	18-25	27	27.00
	26-33	15	15.00
	34-41	13	13.00
	42-49	13	13.00
	50-57	14	14.00
	58-65	5	5.00
	66-73	2	2.00
	≥ 74	2	2.00
Gender	Female	55	55.00
	Male	45	45.00
Occupation	Worker	54	54.00
	Student	33	33.00
	Unemployed	5	5.00
	Retired	6	6.00
	Student-worker	1	1.00
	Domestic	1	1.00

Results are presented in total number and frequency (%).

4.7.1 CATA test

The CATA check list had a total of 34 terms grouped in sensory categories: appearance, texture, odour, mouthfeel, flavour, and taste. The frequency of selection of each term by the consumers (**Table 16**) was accomplished by dividing the total of assessors who selected that term to characterise each olive pâté by the total number of assessors.

The least and most selected attributes in each sensory category were, respectively: “not shiny” and “shiny” (aspect); “spicy” and “olive” (odour); “liquid” and “grainy” (texture while spreading in the cookie); “salty” and “olive” (taste); “liquid” and “creamy” (texture in the mouth).

To assess if consumers had perceived significant differences among the olive pâtés, it was performed a Cochran's Q test. This test was then followed by a post-hoc test: multiple pairwise comparisons using the critical difference (Sheskin) procedure. This type of evaluation was already used in different studies (100, 184, 185). According to this test, the term “liquid” was the only one that was not used to discriminate the olive pâtés ($p > 0.05$). The remaining thirty-three attributes were used to discriminate the olive pâtés ($p < 0.05$). The fact a high number of attributes used by consumers indicates that they noticed differences in the sensory features of the olive pâtés (84). Nevertheless, not all were considered important to discriminate samples.

According to **Table 16**, consumers checked with significantly higher frequency the attributes spicy and salty (taste) in the ideal olive pâté, comparing to the other olive pâtés ($p < 0.05$). Therefore, consumers believe that a potential ideal olive pâté should be spicier and saltier than the olive pâtés produced in this study.

Table 16. Frequency of terms of the CATA test selected by consumers to describe the olive pâtés (CP, P0*, P20*, P25*) and their ideal olive pâtés (IP).

Attributes		Olive pâtés				
		CP	P0*	P20*	P25*	IP
Aspect	“Not shiny”	9 ^{ab}	7 ^{ab}	8 ^{ab}	13 ^b	0 ^a
	“Shiny”	50 ^a	65 ^{ab}	75 ^b	61 ^{ab}	70 ^b
	“Light”	40 ^b	9 ^a	4 ^a	3 ^a	12 ^a
	“Dark”	29 ^a	60 ^b	60 ^b	70 ^b	57 ^b
	“Grainy”	14 ^a	44 ^c	31 ^{bc}	39 ^{bc}	26 ^{ab}
	“Oily”	49 ^b	22 ^a	26 ^a	23 ^a	12 ^a

Attributes		Olive pâtés				
		CP	P0*	P20*	P25*	IP
Odour	"Aromatic"	8 ^a	31 ^{bc}	26 ^b	23 ^b	43 ^c
	"Vinegary"	11 ^a	36 ^{bc}	49 ^c	37 ^{bc}	21 ^{ab}
	"Olive oil"	18 ^a	47 ^b	26 ^a	34 ^{ab}	45 ^b
	"Olive"	33 ^a	63 ^{bc}	62 ^{bc}	58 ^b	77 ^c
	"Spicy"	0 ^a	4 ^{ab}	3 ^{ab}	6 ^{ab}	9 ^b
	"Rancid"	69 ^b	1 ^a	1 ^a	0 ^a	0 ^a
Texture (spreading in the cookie)	"Spreadable"	48 ^b	30 ^a	35 ^{ab}	31 ^a	47 ^b
	"Creamy"	45 ^{ab}	31 ^a	31 ^a	31 ^a	61 ^b
	"Grainy"	32 ^a	63 ^b	53 ^b	59 ^b	27 ^a
	"Liquid"	4 ^a	2 ^a	0 ^a	0 ^a	1 ^a
	"Soft"	12 ^b	4 ^{ab}	8 ^{ab}	0 ^a	13 ^b
	"Sticky"	16 ^b	6 ^a	9 ^{ab}	10 ^{ab}	2 ^a
Taste	"Acidic"	2 ^a	10 ^{ab}	20 ^b	22 ^b	14 ^{ab}
	"Astringent"	10 ^{ab}	1 ^a	12 ^{ab}	18 ^b	3 ^a
	"Bitter"	8 ^a	11 ^a	48 ^b	46 ^b	8 ^a
	"Vinegary"	5 ^a	46 ^c	34 ^{bc}	22 ^{ab}	33 ^{bc}
	"Olive oil"	24 ^a	54 ^{bc}	42 ^b	39 ^{ab}	61 ^c
	"Olive"	35 ^a	79 ^{bc}	79 ^{bc}	63 ^b	88 ^c
	"Spices"	11 ^a	65 ^c	50 ^{bc}	36 ^b	67 ^c
	"Very bitter"	5 ^a	3 ^a	4 ^a	15 ^b	0 ^a
	"Spicy"	0 ^a	4 ^a	4 ^a	2 ^a	18 ^b
	"Rancid"	72 ^b	0 ^a	0 ^a	1 ^a	0 ^a
	"Salty"	4 ^a	1 ^a	0 ^a	0 ^a	17 ^b
Texture (mouth)	"Consistent"	9 ^a	14 ^{ab}	18 ^{ab}	19 ^{ab}	22 ^b
	"Creamy"	58 ^{bc}	40 ^a	45 ^{ab}	41 ^{ab}	75 ^c
	"Grainy"	29 ^{ab}	53 ^c	44 ^{bc}	55 ^c	21 ^a
	"Liquid"	8 ^c	7 ^{bc}	1 ^{ab}	0 ^a	0 ^a
	"Sticky"	16 ^b	4 ^a	4 ^a	3 ^a	2 ^a

Results are presented in frequency of mention (%). Within rows, different lowercase superscripts letters represent significant differences between the frequency of mention of attributes for each pâté ($p < 0.05$). CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; IP- Ideal olive pâté.

PCA is a useful and helpful multivariate analysis method (186). Specifically, this method is used to reduce dataset size, rejecting not so relevant information while keeping as much as information as possible (187). Results from the applied CATA method in sensory analysis can also be analyzed via PCA. For example, in de Souza *et al.* (2020), PCA was used to analyse the results obtained in the CATA sensory test for untreated, HPP treated, UV-C treated, and thermal treated juices (188).

In this study, the data obtained with CATA was firstly organised into a matrix with the absolute frequency of each checked attribute per each olive pâté. Then, a PCA was performed (**Figure 22**). **Figure 22B** shows the projection of the attributes on the factor-plane.

The PCA plot explained 92.47% of the information using the first two principal components (PC1 and PC2). In particular, the principal component 1 (PC1) contains more than 74% of the total information, and the principal component 2 (PC2) contains more than 18% of the total information.

Studying PC1 (horizontal axis), it is possible to observe that CP was projected in an opposite side, separated from P0*, P20*, and P25*. Additionally, PC2 (vertical axis) explains the differences between P0* and P20* and P25*. In fact, P20* and P25* are located on the positive side on both PC1 and PC2, while P0* is projected on the bottom-right side of the plot. CP is located on the negative side of both PC1 and PC2.

Considering the position of the CP and the relevant attributes in the PCA, it is noticeable that this olive pâté is perceived by the consumers as having more intensity of light and oily aspect, rancid odour, spreadable and creamy textures while spreading in the cookie, rancid and salty tastes, and creamy and sticky textures in the mouth.

Regarding the olive pâtés produced in the laboratory, P0* is associated with the attributes: shiny and grainy (aspect); aromatic, olive oil and olive (odour); grainy (texture in the cookie); vinegary, olive oil, olive, spices and spicy (taste). On the other hand, P25* is associated with the attributes: not shiny (aspect); astringent, bitter, and very bitter (taste). P20* is more associated with the attributes: dark (aspect), vinegary (odour), bitter (taste), consistent, and grainy (texture in the mouth).

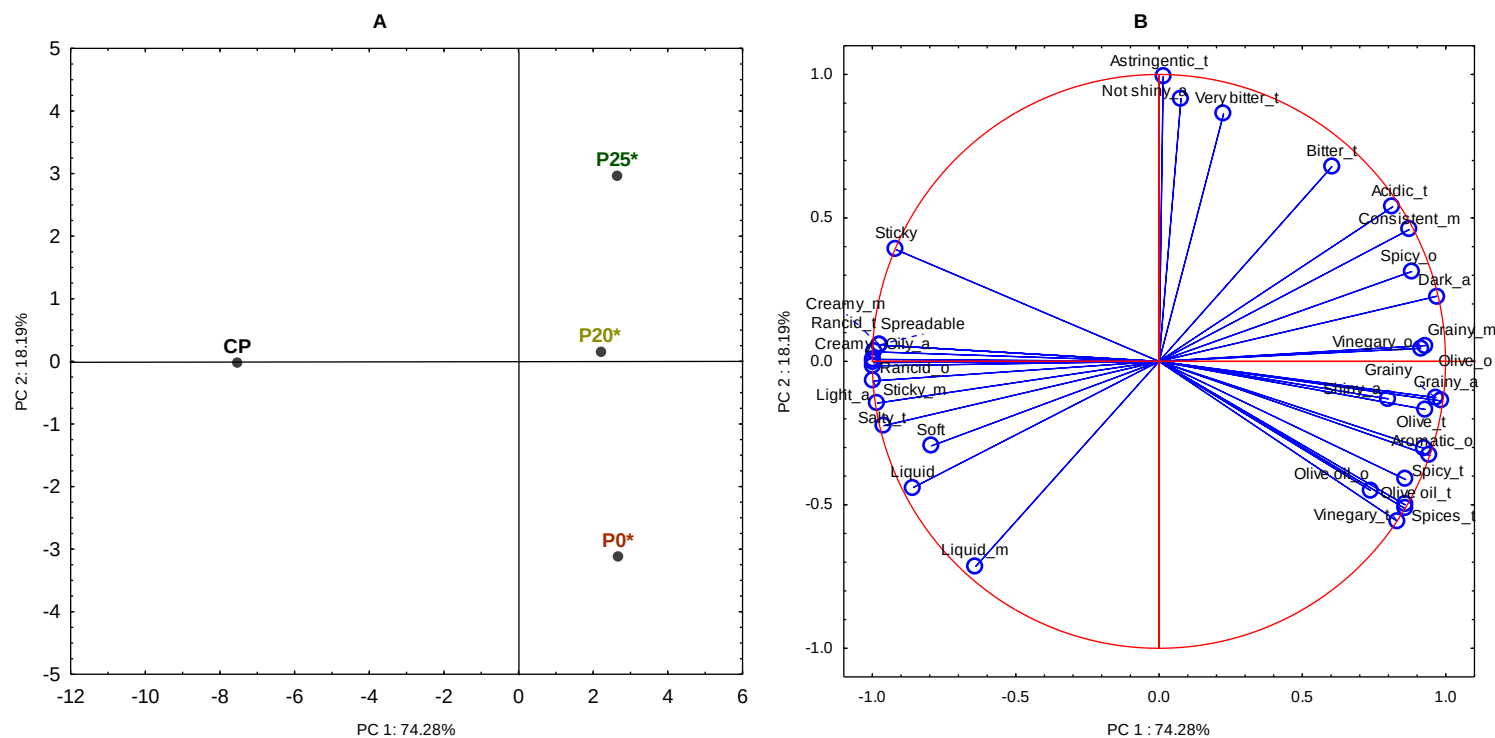


Figure 22. Principal component analysis performed on the matrix of the absolute frequency of mention of each attribute per each tasted olive pâté. (A) Projection of the olive pâtés on the factor-plane; (B) Projection of the variables on the factor-plane.

CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; a- aspect; o-odour; t- taste; m- texture (mouth).

4.7.2 Acceptability

The production of new foodstuffs at industrial level is strongly linked to sensory analysis (189). Specifically, the food industry uses the affective ratings of a tasted food as an orientation through the development, enhancement, and quality assurance of products (190).

The acceptance of a product will be determined by how well it answers to the consumers' needs and how much satisfaction it can provide (191). Many factors related to the individual, the product, or the environment where the food is consumed can affect its acceptability (192). For example, consumers' genetics, age, gender, family, cultural habits, religion, and education, among many other characteristics, can influence the acceptance/rejection of a food product (191). The food acceptance measurement attained through attitudinal judgments can be a weak indicator of consumption. Nevertheless, it is still used to forecast the behaviour of consumers towards innovative products at both industrial/academic levels, mainly because it is easy, quick, and simple to implement (193). Consumer acceptability tests assess the degree of liking based on its sensory appeal and are usually performed using a 9-point hedonic scale (83).

Here, 100 consumers evaluated the sensory quality of the following olive pâtés: CP, P0*, P20*, and P25*. The acceptability of the olive pâtés was performed using a 9-point hedonic scale. **Table 17** reports the frequency of mentioning of each liking score.

Table 17. Frequency of mention (%) of each liking score by the consumers in the acceptability test of the four tasted olive pâtés: CP, P0*, P20*, and P25*.

	CP	P0*	P20*	P25*
"Like extremely"	0.00	8.00	1.00	1.00
"Like very much"	2.00	26.00	28.00	18.00
"Like moderately"	11.00	35.00	33.00	27.00
"Like slightly"	5.00	17.00	12.00	16.00
"Neither like nor dislike"	6.00	6.00	5.00	2.00
"Dislike slightly"	4.00	3.00	10.00	13.00
"Dislike moderately"	14.00	2.00	5.00	13.00
"Dislike very much"	24.00	3.00	2.00	5.00
"Dislike extremely"	34.00	0.00	4.00	5.00

Results are presented in frequency of mention (%) of each liking score. CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

According to the results, only 18% of the consumers liked CP. On the other hand, the majority liked P0* and P20* (86% and 74%, respectively). Regarding P25*, 62% of the consumers liked this pâté.

Boxplots (**Figure 23**) were used to show the consumers' acceptability of the olive pâtés. As it can be seen, P0* and P20* were the most appreciated by consumers (median=7), followed by P25* (median=6). On the other hand, CP was less appreciated (median=2). Additionally, it should be pointed out that the variability of the acceptability scores for the olive pâtés incorporating HT-OPP was substantial (ranges between 1 and 9), meaning that some assessors liked this pâté while others did not (104).

The Kruskal-Wallis test showed that the acceptability ratings differed significantly among the olive pâtés ($H(3) = 128.966, p < 0.05$). Having in mind the applied pairwise comparisons post-hoc test, no significant differences were observed between P0* and P20* ($p > 0.05$). In the same way, no significant differences were observed between P20* and P25* ($p > 0.05$). In contrast, significant differences were found between P25* and P0*, and between the CP and all the other olive pâtés ($p < 0.05$).

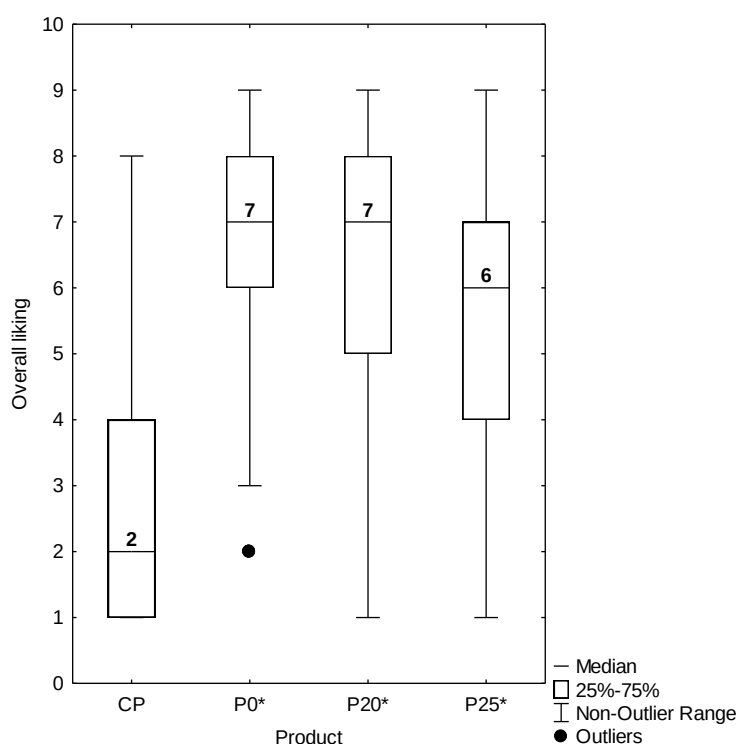


Figure 23. Boxplots for the acceptability of the tasted pâtés: Evaluation on a 9-point hedonic scale from 1 (“Dislike extremely”) to 9 (“Like extremely”).

CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

Correspondence analysis (CA) was performed to examine the relationship between acceptability and the characteristics of the assessors (sex, age group, and occupation) who tasted the olive pâtés (**Figure 24**).

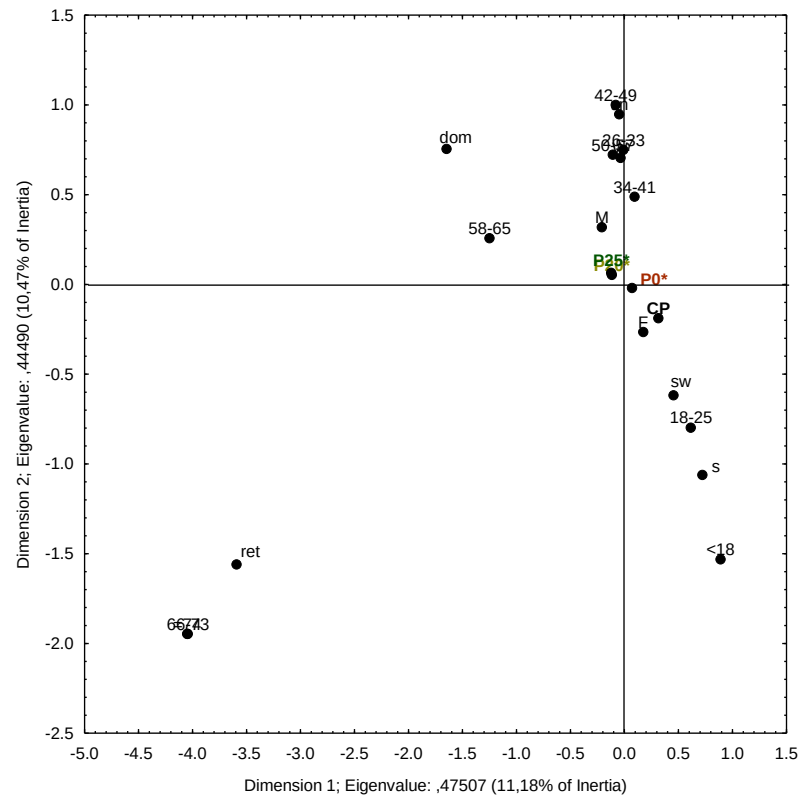


Figure 24. Correspondence analysis of the tasters' scores on the acceptability of the tasted olive pâtés, categorized by sex, age group, and occupation.

CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; F- Female; M- Male; dom- Domestic; w- Worker; s- Student; sw- Student worker; ret- Retired.

In **Figure 24**, the first and second dimensions separate females, projected on the bottom-right of the plot, from the male consumers, who are projected on the top-left side. Regarding the occupation, student and student-workers are projected on the bottom-right, whereas workers, unemployed and domestic consumers are projected on the top-left, and retired people are on the bottom-left. Concerning the age groups, consumers until 25 years old are projected on the bottom-right, and consumers with ages between 26-33 and 42-65 are located on the top-left, whereas consumers with ages between 34-41 are located on the top-right and consumers with more than 66 years old are located on bottom-left. Regarding the olive pâtés, the CP and P0* are projected on the bottom-right, while P20* and P25* are on the top-left.

On the left side of the Dim1 axis, it can be seen that the CP and P0* are closer to the female sex, so it appears that, in general, women scored better these olive pâtés. Regarding the age group, both are closer to consumers between 18-25, meaning that, in general, consumers from this age group scored better on these olive pâtés. Additionally, in the second dimension of this analysis, it is observed that these olive pâtés are closer to student-workers, meaning that, in general, consumers with this occupation scored them better.

On the other hand, on the right side of the Dim1 axis, it can be seen that the P20* and P25* are closer to the male sex, meaning that, in general, men scored better these olive pâtés. Regarding the age group, both are closer to consumers between 50-57 and 58-65, meaning that, in general, consumers from this age group scored better these olive pâtés. Additionally, they are closer to workers, meaning that, in general, consumers with this occupation scored better these olive pâtés.

Considering that no significant discrepancies were discovered between P20* and P25* in terms of acceptability, it would be interesting to assess whether consumers of a given gender, age, or occupation scored better one olive pâté or the other. According to the CA (**Figure 24**), P20* and P25* are projected close to each other, meaning that no acceptability differences should be considered between them, which is also in accordance with the acceptability scores given by the consumers. This means that both P20* and P25* could be considered of interesting commercial potential.

Phenolic compounds can affect the organoleptic characteristics of a food product since they contribute, for example, to their bitterness and astringency (194). It should be noticed that many consumers consider the bitter taste an unpleasant characteristic of a foodstuff, and that this attribute can even pose a challenge to the product consumption. For example, the bitterness or other organoleptic properties of vegetables are considered unpleasant, which poses a challenge for dietary guidelines to increase the intake of vegetables (195). Nevertheless, the phenolic compounds have been added to foodstuffs to expand their shelf-life time and even enhance their nutritive value, meaning that they can enhance their overall quality (194). Therefore, it was expected that the acceptability of the olive pâtés incorporating HT-OPP (P20* and P25*) would be affected by the considerably high TPC and HC, compared to those registered in P0*.

On 9-point hedonic scale, an acceptability score of 6 (first score in the liking category) is considered the commercial or quality limit to a product (105). Hence, the acceptability median scores of P0*, P20*, and P25* achieved an adequate level on the 9-point hedonic scale (**Figure 23**). On the other hand, the median acceptability score for CP was drastically

inferior to the reported adequate level, as can be seen in **Figure 23**. These results also suggest that both P20* and P25* could be considered interesting and potential commercial olive pâtés.

4.7.3 Purchase intention

Many aspects take part in purchasing intention of a food product, aside from the simple sensory interest. In fact, the purchase and, more importantly, repurchase depend on the price, idea, positioning, campaigns, package information, advertising, knowledge, and nutritional qualities (83). Therefore, as expected, studying the purchase intention with a blind-labelled sensory test is not straightforward considering that the products' price, position in relation to the competition, label claims, and advertising campaigns are not generally presented (196).

Nevertheless, many academic works study the purchasing intention of developed food products (49, 85, 197-199).

A high number of participants, typically more than 100, are required to assess the purchase intention of a food product (200). Additionally, a 5-point hedonic scale, varying between 1="definitely would not buy" and 5="definitely would buy", can be used to evaluate the purchase intention of a food product (82). In fact, some of the studies mentioned before used this hedonic scale (85, 198). The purchase intention of the tasted olive pâtés (CP, P0*, P20*, and P25*) was evaluated by 100 tasters. Tasters used a 5-point hedonic scale to evaluate the purchase intention of the developed olive pâtés, being the frequency of mentioning of each score presented in **Table 18**.

Table 18. Frequency of mention (%) of each purchase intention score by the consumers in the purchase intention test of the four tasted olive pâtés: CP, P0*, P20*, and P25*.

	CP	P0*	P20*	P25*
"Definitely would buy"	2.00	31.00	23.00	23.00
"Probably would buy"	6.00	35.00	38.00	24.00
"Maybe/Maybe not would buy"	14.00	22.00	17.00	19.00
"Probably would not buy"	19.00	8.00	12.00	15.00
"Definitely would not buy"	59.00	4.00	10.00	19.00

Results are presented in frequency of mention (%) of each purchase intention score. CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

According to **Table 18**, only 8% of the consumers would buy the CP. On the other hand, the majority would buy P0*, and P20* (66% and 61%, respectively). Regarding P25*, 47% of the consumers would purchase it.

Boxplots (**Figure 25**) were used to show the consumers' purchase intention for the tasted olive pâtés. As it can be seen, consumers showed more interest in purchasing the olive pâtés P0* and P20* (median=4), and then P25* (median=3). On the other hand, they showed little interest in purchasing the CP (median=1).

Results from the Kruskal-Wallis test showed that the purchase ratings differed significantly between the olive pâtés ($H(3) = 116.720, p < 0.05$). Considering the applied pairwise comparisons post-hoc test, no significant differences were found between P20* and P0* ($p > 0.05$), and between P25 and the P20 ($p > 0.05$). By contrast, significant differences were found between P25* and the P0* and between the CP and all the other olive pâtés ($p < 0.05$).

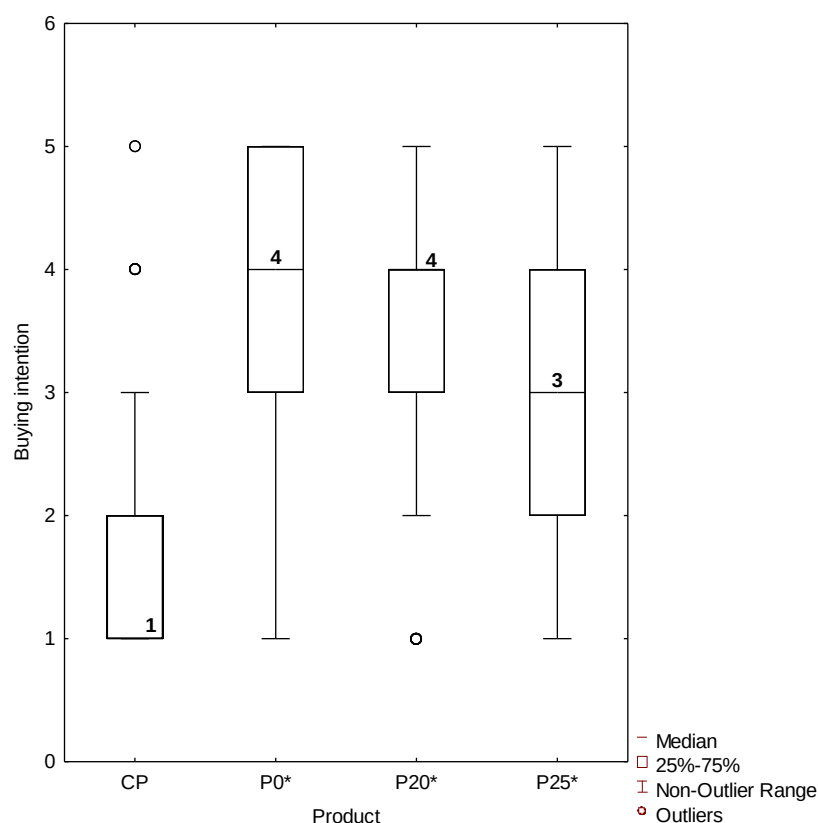


Figure 25. Boxplots for the purchase intention of the tasted olive pâtés: Evaluation on a 5-point hedonic scale from 1 (“Definitely would not buy”) – 5 (“Definitely would buy”).

CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP.

CA was performed to explore the relationship between the purchase intention and the characteristics of the individuals (sex, age group, and occupation) who tasted the olive pâtés (**Figure 26**).

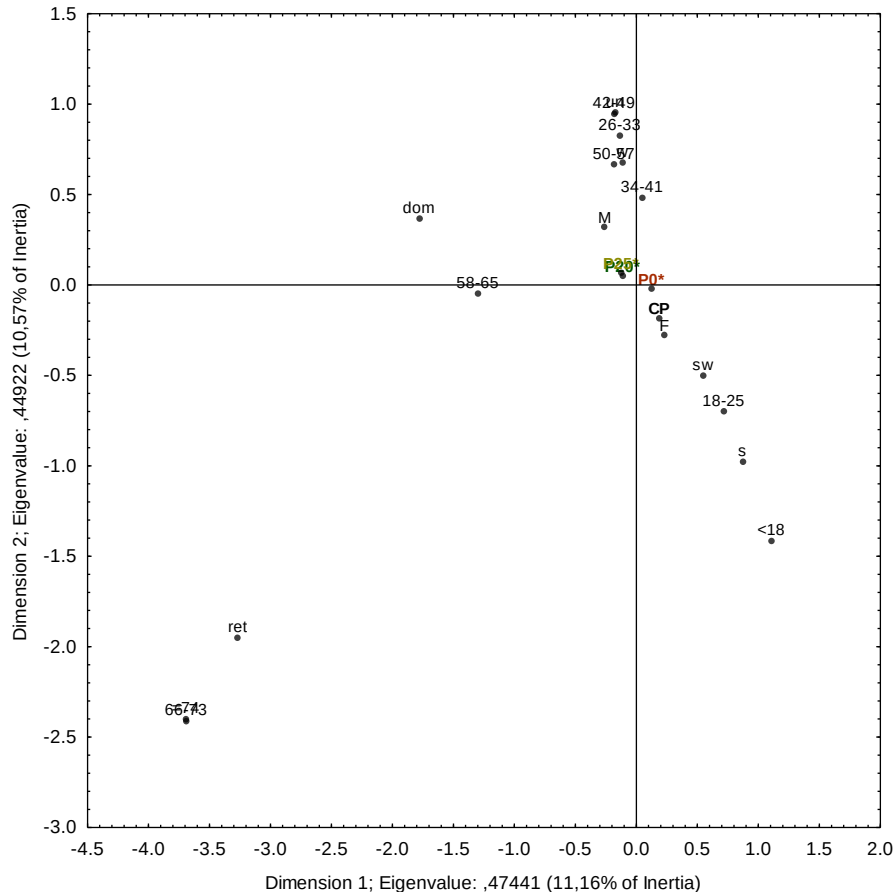


Figure 26. Correspondence analysis of the tasters' scores on the purchase intention of the tasted olive pâtés, categorized by sex, age group, and occupation.

CP- Commercial olive pâté; P0*- Olive pâté processed by HPP; P20*- Olive pâté with 20% HT-OPP processed by HPP; P25*- Olive pâté with 25% HT-OPP processed by HPP; F- Female; M- Male; dom- Domestic; w- Worker; s- Student; sw- Student worker; ret- Retired.

In **Figure 26**, the first and second dimensions separate females, projected on the bottom-right, from the male consumers, who are projected on the top-left. Regarding the occupation, student and student-workers are projected on the bottom-right, whereas workers, unemployed and domestic consumers are projected on the top-left, and retired people are on the bottom-left. Concerning age, consumers under 25 years old are projected on the bottom-right, and consumers with ages between 26-33 and 42-65 are located on the top-left, whereas consumers with ages between 34-41 are located on the top-right and consumers with more than 66 years old are located on bottom-left. Regarding the pâtés,

the CP and P0* are projected on the bottom-right, while P20* and P25* are projected on the top-left.

On the left side of the Dim1 axis, CP and P0* are closer to the female sex, appearing that, in general, women scored better these olive pâtés regarding the purchase intention. Considering the age group, both are closer to consumers with ages between 18-25, meaning that, in general, consumers from this age group scored better these olive pâtés regarding purchase intention. Additionally, in the second dimension of this analysis, it is observed that these olive pâtés are closer to student-workers, meaning that, in general, consumers with this occupation scored better these olive pâtés regarding the purchase intention.

On the right side of Dim1 of this plot, it can be seen that the P20* and P25* are closer to the male sex because men generally scored better these olive pâtés. Regarding the age, both are closer to consumers with ages between 50-57 and 58-65, meaning that, in general, consumers from this age group scored better these olive pâtés. Additionally, they are closer to workers, meaning that, in general, consumers with this occupation scored better these olive pâtés.

Considering that no significant differences were detected between P20* and P25* regarding the purchase intention, it would be interesting to assess whether consumers of a given gender, age, or occupation scored better one pâté or the other. As it was observed in the CA applied to the acceptability data, in this case, the CA presented in **Figure 26** showed that P20* and P25* are projected really close to each other, meaning that no differences can be pointed out between them regarding the scores given by consumers, in terms of their age, sex, or occupation. Therefore, both P20* and P25* could be considered interesting commercial olive pâtés.

CHAPTER V: CONCLUSION

Heat-treated olive pomace paste (HT-OPP) is an interesting food ingredient owing to its' low-fat, high-fibre content and especially hydroxytyrosol content. Nevertheless, it has a characteristic bitter and astringent taste that challenges its incorporation in food products.

In a first stage, it was decided to incorporate increasing percentages of HT-OPP in an olive pâté until an adequate sensorial acceptability level was reached. For that, a preliminary acceptability sensory test was performed with an olive pâté (P0), an olive pâté with 10% HT-OPP (P10), an olive pâté with 20% HT-OPP (P20) and an olive pâté with 30% HT-OPP (P30). The results showed that P20 could be a potential olive pâté and that a 10% increase from P20 to P30 resulted in a huge decrease in the median score acceptability. Therefore, it was decided to produce olive pâtés incorporating 20% and 25% of HT-OPP (P20 and P25).

In a second stage, it was decided to compare the sensory and chemical properties of a commercial olive pâté (CP) to an olive pâté and other two pâtés with 20% and 25% of HT-OPP incorporated. So, another sensorial test (CATA test, acceptability, and purchase intention) was performed using an olive pâté, olive pâtés with 20% and 25% HT-OPP and a commercial olive pâté. However, in this case, the olive pâtés produced in the laboratory had to be processed by High Pressure Processing (HPP) to assure microbiological safety.

Olive pâtés' chemical composition was assessed before and after HPP in order to evaluate if this treatment impacted their chemical qualities. Results demonstrated that HPP did not significantly impact the chemical composition of the olive pâtés: nutritional composition, vitamin E profile and total content, fatty acids profile, phytochemicals, and antioxidant activity ($p>0.05$). Therefore, HPP allowed the preservation of the quality attributes of the developed olive pâtés.

Regarding the chemical composition of the olive pâtés, the CP had significantly lower moisture, protein, remaining carbohydrates, salt contents, Σ PUFA, TPC and antioxidant activities than all the other olive pâtés ($p<0.05$). On the opposite, it registered a significantly higher fat content, Σ MUFA and MUFA/PUFA ratio ($p<0.05$). Additionally, hydroxytyrosol was not quantified in this pâté.

The olive pâté with 20% HT-OPP submitted to HPP (P20*) and the olive pâté with 25% HT-OPP submitted to HPP (P25*) showed interesting chemical attributes. Indeed, the nutritional composition analysis showed that both had significant high-fibre contents. Additionally, they are good sources of vitamin E and fatty acids related to healthy benefits. More important, these pâtés are excellent sources of hydroxytyrosol, having in mind that a

serving portion (20 g) is enough to meet the ESFA health claim. Therefore, the consumption of both pâtés could be associated with numerous health-benefit effects.

The sensorial analysis (CATA test, acceptability, and purchase intention) showed that both P20* and P25* could be considered potential commercial olive pâtés.

The CATA test showed that the CP was more associated with the following attributes: light and oily (aspect); rancid (odour); spreadable and creamy (texture in the cookie), rancid and salty (taste); creamy and sticky (texture in the mouth). P0* was associated with the attributes: shiny and grainy (aspect); aromatic, olive oil and olive (odour); grainy (texture in the cookie); vinegary, olive oil, olive, spices and spicy (taste). On the other hand, P20* was more associated with the attributes dark (aspect), vinegary (odour), bitter (taste), consistent, and grainy (texture in the mouth). P25* was associated with the attributes not shiny (aspect), astringent, bitter, and very bitter (taste).

The acceptability test showed that consumers liked both P20* and P25*. Actually, both attained the commercial acceptability score on a 9-point hedonic scale. More important, they registered considerably higher acceptability scores than the CP ($p < 0.05$). Additionally, no differences can be pointed out between P20* and P25* regarding the acceptability scores given by consumers, in terms of their age, sex, or occupation.

The purchase intention test showed that consumers would more likely buy the pâtés incorporating HT-OPP than the CP. Additionally, no differences can be pointed out between P20* and P25* regarding the purchase intention scores given by consumers, in terms of their age, sex, or occupation.

Truly, P20* and P25* are promising commercial olive pâtés due to their quality attributes (total fibre and vitamin E contents, fatty acid profile and hydroxytyrosol content) and positive sensory evaluation. Nevertheless, foreseeing the sustainable development and considerable amounts of OP produced every year, P25* was selected as the best product for further introduction to the food industry.

To conclude, P25* is an interesting olive pâté since it can meet current food trends: health concerns, sustainability, and convenience. In fact, a portion (≈ 20 g) of this new food product has interesting total fibre, vitamin E and hydroxytyrosol contents. Additionally, it is a sustainable food product since it incorporates HT-OPP, an ingredient that results from OPP pasteurization. Moreover, its' production can promote the circular economy of the Portuguese olive oil production sector. Finally, it is a convenient product that can be used

as a snack itself or as a condiment for other food products like pasta, boiled eggs, and salads.

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ANNEXES

ANNEX I



FICHA DE ANÁLISE SENSORIAL

 Idade: ☐ 18-25 ☐ 26-33 ☐ 34-41 ☐ 42-49 ☐ 50-57 ☐ 58-65 ☐ ≥ 66

 Sexo: ☐ Feminino ☐ Masculino

Caro provador,

Limpe o palato com água antes de começar. Por favor, prove as 4 amostras codificadas pela ordem apresentada, avaliando globalmente a sua aceitabilidade e assinale a sua escolha com uma cruz (x) na respetiva escala. Poderá limpar o palato com água entre cada amostra.

Código amostra: **365**

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Código amostra: **853**

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Código amostra: **457**

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Código amostra: **742**

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Obrigada pela sua participação!

ANNEX II

ESTUDO DO CONSUMIDOR: CHECK-ALL-THAT-APPLY

Caro provador, leia com atenção todas as instruções antes da prova.

Por favor, preencha o seguinte cabeçalho:

Idade: ☐ < 18 ☐ 18-25 ☐ 26-33 ☐ 34-41 ☐ 42-49 ☐ 50-57 ☐ 58-65 ☐ 66-73 ☐ ≥ 74

Sexo: ☐ Feminino ☐ Masculino

Situação profissional: ☐ Trabalhador ☐ Estudante ☐ Desempregado ☐ Reformado ☐ Outro:

Amostra 278

Por favor, coloque uma pequena porção da **amostra 278** em meia bolacha e assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Aparência geral	Odor	Textura (barrar na bolacha)
Baço	Aromático	Barrável
Brilhante	Avinagrado	Cremoso
Clara	Azeite	Granuloso
Escura	Azeitona	Líquido
Granuloso	Picante	Macio
Oleoso	Ranço	Pegajoso

Por favor, limpe o palato com água e prove a amostra em conjunto com a bolacha. Assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Sabor	Textura (boca)
Ácido	Consistente
Adstringente	Cremoso
Amargo	Granuloso
Avinagrado	Líquido
Azeite	Pegajoso
Azeitona	
Especiarias	
Muito amargo	
Picante	
Ranço	
Salgado	

Avalie globalmente a aceitabilidade da amostra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Avalie a sua intenção de compra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Definitivamente comprava
- ☐ Provavelmente comprava
- ☐ Talvez comprasse ou não
- ☐ Provavelmente não comprava
- ☐ Definitivamente não comprava

ESTUDO DO CONSUMIDOR: CHECK-ALL-THAT-APPLY

Amostra 157

Por favor, coloque uma pequena porção da **amostra** em meia bolacha e assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Aparência geral	Odor	Textura (barrar na bolacha)
☐ Baço	☐ Aromático	☐ Barrável
☐ Brilhante	☐ Avinagrado	☐ Cremoso
☐ Clara	☐ Azeite	☐ Granuloso
☐ Escura	☐ Azeitona	☐ Líquido
☐ Granuloso	☐ Picante	☐ Macio
☐ Oleoso	☐ Ranço	☐ Pegajoso

Por favor, limpe o palato com água e prove a amostra em conjunto com a bolacha. Assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Sabor	Textura (boca)
☐ Ácido	☐ Consistente
☐ Adstringente	☐ Cremoso
☐ Amargo	☐ Granuloso
☐ Avinagrado	☐ Líquido
☐ Azeite	☐ Pegajoso
☐ Azeitona	
☐ Especiarias	
☐ Muito amargo	
☐ Picante	
☐ Ranço	
☐ Salgado	

Avalie globalmente a aceitabilidade da amostra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Avalie a sua intenção de compra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Definitivamente comprava
- ☐ Provavelmente comprava
- ☐ Talvez comprasse ou não
- ☐ Provavelmente não comprava
- ☐ Definitivamente não comprava

ESTUDO DO CONSUMIDOR: CHECK-ALL-THAT-APPLY

Amostra 613

Por favor, coloque uma pequena porção da **amostra 613** em meia bolacha e assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Aparência geral	Odor	Textura (barrar na bolacha)
Baço	Aromático	Barrável
Brilhante	Avinagrado	Cremoso
Clara	Azeite	Granuloso
Escuro	Azeitona	Líquido
Granuloso	Picante	Macio
Oleoso	Ranço	Pegajoso

Por favor, limpe o palato com água e prove a amostra em conjunto com a bolacha. Assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Sabor	Textura (boca)
Ácido	Consistente
Adstringente	Cremoso
Amargo	Granuloso
Avinagrado	Líquido
Azeite	Pegajoso
Azeitona	
Especiarias	
Muito amargo	
Picante	
Ranço	
Salgado	

Avalie globalmente a aceitabilidade da amostra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Avalie a sua intenção de compra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Definitivamente comprava
- ☐ Provavelmente comprava
- ☐ Talvez comprasse ou não
- ☐ Provavelmente não comprava
- ☐ Definitivamente não comprava

ESTUDO DO CONSUMIDOR: CHECK-ALL-THAT-APPLY

Amostra 831

Por favor, coloque uma pequena porção da **amostra 831** em meia bolacha e assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Aparência geral	Odor	Textura (barrar na bolacha)
Baço	Aromático	Barrável
Brilhante	Avinagrado	Cremoso
Clara	Azeite	Granuloso
Escuro	Azeitona	Líquido
Granuloso	Picante	Macio
Oleoso	Ranço	Pegajoso

Por favor, limpe o palato com água e prove a amostra em conjunto com a bolacha. Assinale com uma cruz (x) os atributos que considera apropriados para caracterizar a amostra.

Sabor	Textura (boca)
Ácido	Consistente
Adstringente	Cremoso
Amargo	Granuloso
Avinagrado	Líquido
Azeite	Pegajoso
Azeitona	
Especiarias	
Muito amargo	
Picante	
Ranço	
Salgado	

Avalie globalmente a aceitabilidade da amostra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Gosto extremamente
- ☐ Gosto muito
- ☐ Gosto moderadamente
- ☐ Gosto ligeiramente
- ☐ Nem gosto/nem desgosto
- ☐ Não gosto ligeiramente
- ☐ Não gosto moderadamente
- ☐ Não gosto muito
- ☐ Não gosto extremamente

Avalie a sua intenção de compra e assinale a sua escolha com uma cruz (x) na respetiva escala.

- ☐ Definitivamente comprava
- ☐ Provavelmente comprava
- ☐ Talvez comprasse ou não
- ☐ Provavelmente não comprava
- ☐ Definitivamente não comprava

ESTUDO DO CONSUMIDOR: CHECK-ALL-THAT-APPLY

Pâté de azeitonas ideal

Por favor, assinale com uma cruz (x) os atributos que considera apropriados para caracterizar um pâté de azeitonas ideal.

Aparência geral	Odor	Textura (barrar na bolacha)
☐ Baço	☐ Aromático	☐ Barrável
☐ Brilhante	☐ Avinagrado	☐ Cremoso
☐ Clara	☐ Azeite	☐ Granuloso
☐ Escura	☐ Azeitona	☐ Líquido
☐ Granuloso	☐ Picante	☐ Macio
☐ Oleoso	☐ Ranço	☐ Pegajoso

Sabor	Textura (boca)
☐ Ácido	☐ Consistente
☐ Adstringente	☐ Cremoso
☐ Amargo	☐ Granuloso
☐ Avinagrado	☐ Líquido
☐ Azeite	☐ Pegajoso
☐ Azeitona	
☐ Especiarias	
☐ Muito amargo	
☐ Picante	
☐ Ranço	
☐ Salgado	

Muito obrigada pela participação na prova!