fermentation

Review

Fermentation as a Strategy to Valorize Olive Pomace, a By-Product of the Olive Oil Industry

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Abstract: In the Mediterranean region, where olive oil is mostly produced, high amounts of olive oil by-products are generated, which creates an ecological concern, due to their phytotoxic phenolic components (e.g., oleuropein, hydroxytyrosol, tyrosol). However, these compounds also represent a relevant source of antioxidants for health and well-being. The food and beverage, cosmetic, and pharmaceutical industries can all greatly benefit from the treatment and proper exploitation of olive oil by-products for their health-promoting benefits in various fields. Additionally, recovery and treatment procedures can support effective waste management, which in turn can increase the sustainability of the olive oil sector and result in worthwhile economic advantages. Due to their high phenolic content, olive pomace could be viewed as a good matrix or primary supply of molecules with high added value. The purpose of this review was to give a thorough overview on how the primary solid olive oil by-products, particularly olive pomace, are currently valued through fermentation, emphasizing their applications in several industries—ethanol production, enzyme production, animal feeding, and human nutrition. It was possible to conclude that the olive pomace has a microbiota profile that allows spontaneous fermentation, a process that can increase its value. In addition, its phenolic content and antioxidant activity are relevant to human health; thus, further studies should be carried out in order to implement this process using olive pomace as the main substrate.

Keywords: olive pomace paste; by-product valorization; fermentation; food security

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1. Introduction

The world's population is growing. In 15 November 2022, it reached 8 billion and it is expected to reach almost 10 billion by 2050 [1,2]. Simultaneously, the Earth's resources are decreasing. Estimations predict that the global food demand will increase from 35% to 56% between 2010 to 2050, and the population risk of hunger is expected to change from −91% to +8% in the same period [3]. Since the global production of plant-based products is constantly increasing and produces significant waste, the upcycling, valorization and utilization of these by-products is a priority, aiming at responsible consumption and production [4]. Recently, the circular economy and residue valorization concepts have come to the forefront to reduce waste, conserve resources, improve production sector efficiency, and value by-products in order to create value-added products that will help the economy to become more sustainable and to solve environmental issues. One of the greatest challenges is to valorize residues from the food industry when they still have advantageous nutritional properties. The olive leaf and its extracts are known for their human health beneficial properties, due to its richness in phenolic compounds [5]. These compounds have relevant antioxidant, anticancer, antimicrobial, antifungal, and anti-inflammatory activities [6–11].

One of the biggest residue-making industries is the olive oil sector. It leads to the production of by-products (e.g., olive pomace paste and olive leaves) and wastes (e.g., wood and wastewater), representing an important environmental issue in the Mediterranean areas, where they are generated in huge quantities in short periods of time [12]. Olive mill waste (OMW) is a main environmental concern. It is related to possible negative physical, chemical, and biological effects on soil; potential phytotoxicity to crops; and potential risk to groundwater. The inhibition of soil microbial activity may in turn reduce soil fertility by inhibiting key processes in nutrient cycling responsible for the formation of labile forms of macro- and micro-elements; thus, the release of OMW into the environment is not recommended [13–15]. Therefore, the possibility of OMW composting has been highlighted [12]. According to the International Olive Council, about 3,000,000 ton of olive oil are produced every year, but olive oil represents only 20% of the fruit. Indeed, 80% of the olive remains as olive pomace (OP) [16,17], and more than 12,000,000 ton of this by-product are available each year as an alternative ingredient (after stone removal) that can be valorized [18].

2. Olive Pomace (OP) Importance

Olive oil production (Figure 1) begins with defoliation and washing, followed by milling, where the oil is extracted from the olive; malaxation, a step that allows the olive oil drops to assembly and facilitate the separation from the aqueous phase; horizontal centrifugation, a step where the OP and the oil are separated; and the vertical centrifugation, to remove all the remaining impurities (Figure 1) [19].

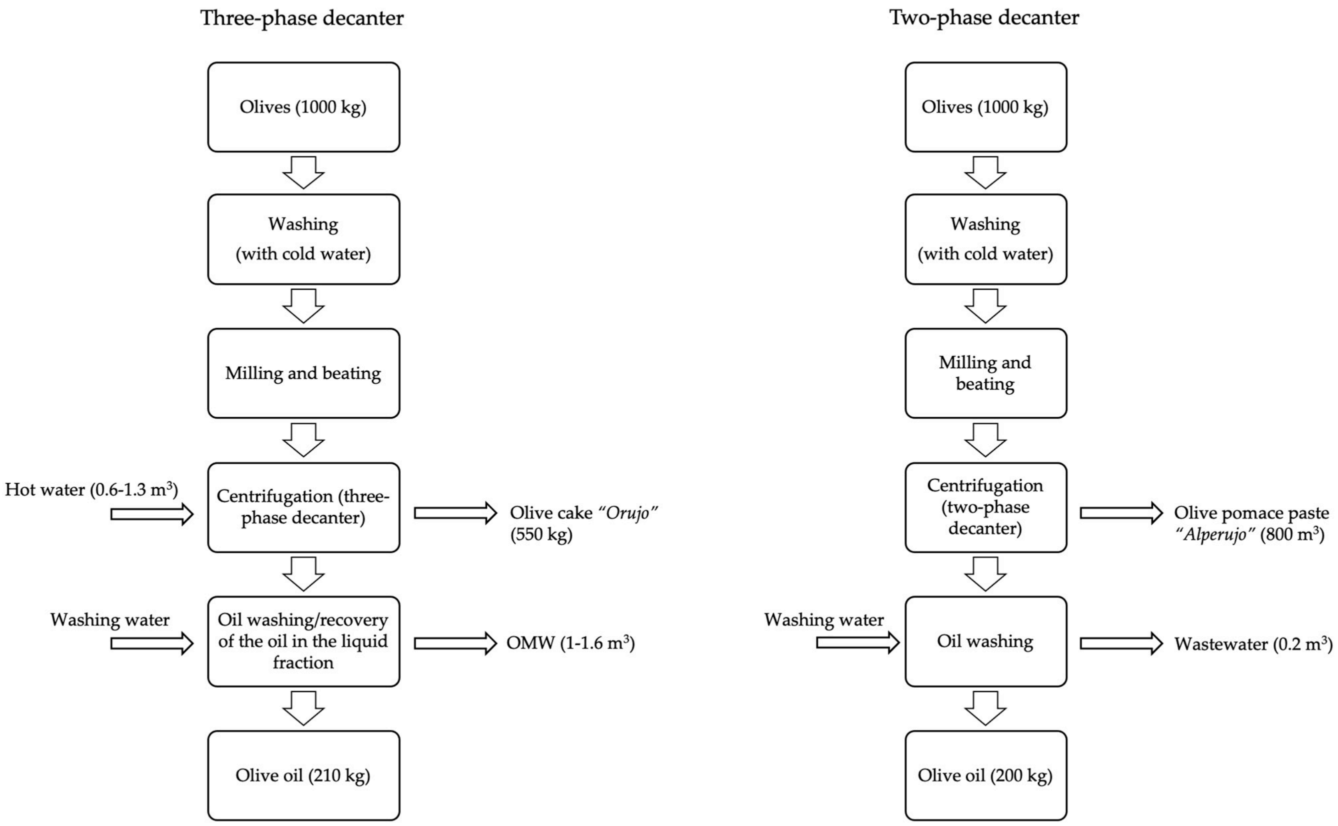

Figure 1. Representation of olive oil extraction, adapted from Albuquerque et al. [20].

The raw OP contains crushed hull, skin, pulp, water, and residual oil [21]. It is composed of small amounts of crude protein and a high percentage of fiber, mainly composed of lignin (27%), followed by cellulose (15%) and hemicellulose (10%) [12]. In OP, the cellulose amount varies between 14% and 26%, although this energy source is blocked in the lignocellulosic matrix being inaccessible to most microorganisms of interest—non-pathogenic microorganisms with probiotic potential [21,22].

Currently, OP is mainly used to recover the residual oil via solvent extraction [23]. It is possible to recover the stone fragments that can be used as fuel for heating the kilns or to produce activated carbon [24,25]. Nevertheless, the OP derived from the two-phase decanter and the pitted one are difficult to manage for the oil extraction, because more time and energy are necessary for pomace dehydrating [26]. Thus, researchers have been focusing their findings on sustainable uses of olive pomace involving the extraction of molecules of interest, such as hydroxytyrosol, tyrosol, oleuropein, caffeic acid, and squalene, intended for cosmetics purposes, considering their UV filter profile [27].

The direct use of OP has been mainly proposed for non-edible purposes, such as clay bricks, since wet OP forms pores allowing to produce construction materials with insulation properties [28]. Due to its adsorption characteristics, OP also has been used as pollutant remover from soil, being effective in removing pollutants such as heavy metals and triazinic herbicides, and due to its chemical properties, composted OP has been used as a conditioner and fertilizer [29]. Composting of solid wastes requires adjustments of conditions such as temperature, pH, moisture, oxygen level, and nutrients, to permit microbial development [30,31]. A carbon–nitrogen ratio between 20 and 40 of the composting material, moisture content of 50% to 65%, and an oxygen supply are optimal conditions for the composting process; however, they are not enough if the mass transfer during the process is limited. The main issue with this procedure using olive oil by-products is odor emission, as well the produced wastewater, which needs to be treated. Biofilters are used to treat the emitted gas from the composting process in an effort to minimize this issue, raising the technology's overall cost [31]. This method could be a low-cost alternative to combustion for recycling solid wastes with complete decontamination of raw materials [12].

Nunes et al. (2021) verified that an OP extract can be considered an all-in-one advantageous ingredient, since it presents a mixture of lipidic and hydrophilic bioactive compounds usually not present in other plant extracts (Table 1). In addition, the authors also observed an OP antibacterial activity against Gram-positive and Gram-negative bacteria [32]. OP is a natural source of phenolic compounds (Table 2), and several studies are focused on the development of new extraction methods to improve the extraction yield [33–35]. Studies also confirmed the OP antioxidant activity, for instance, by shielding the gut from H_2O_2-induced oxidative stress [36]. According to Quero et al. (2022), the bioactive components of OP have the potential to be used in food, nutraceutical, and medicinal applications in the future, reducing waste and advancing the circular economy [36].

Table 1. Chemical composition of olive pomace [32].

Compounds	Levels
Total fat (g/100 g)	4.6–10.5
Fatty acids (relative %)	
C16:0 (Palmitic)	11.6–14.3
C16:1 (Palmitoleic)	0.6–1.3
C17:0 (Heptadecanoic)	0.12–0.19
C18:0 (Stearic)	2.3–3.6
C18:1n9*cis* (Oleic)	71.1–72.9
C18:2n6*cis* (Linoleic)	8.4–10.5
C20:0 (Arachidic)	0.43–0.47
C18:3n3 (α-Linoleic)	0.72–0.9
C20:1n9 (*cis*-11-Eicosenoic)	0.22–0.3

Table 1. *Cont.*

Compounds	Levels
C22:0 (Behenic)	0.15–0.21
C24:0 (Lignoceic)	0.06–0.08
Total vitamin E (mg/100 g)	0.87–2.25
α-Tocopherol	0.77–1.96
α-Tocotrienol	0.04–0.21
β-Tocopherol	0.02–0.05
γ-Tocopherol	0.04–0.07
Total protein (g/100 g)	0.9–4.4
Ash (g/100 g)	9.9–16.7
pH	5.2–5.6

Table 2. Phenolic compounds present in olive.

Phenolic Compounds	References
Phenolic acids	
4-hydroxyphenyl acetic acid	[37]
Caffeic acid	[38–41]
Cinnamic acid	[39,41]
Ferulic acid	[39–41]
Gallic acid	[41]
Homovanillic acid	[40]
p-coumaric acid	[37,40,41]
Sinapic acid	[37,41]
Syringic acid	[37,40,41]
Vanillic acid	[40,41]
Seroidoids and derivatives	
D3,4-Dihydroxyphenylethanol-elenolic acid dialdehyde	[37,38]
Demethyloleuropein	[38]
Hydroxytyrosol	[42]
Ligstroside	[40]
Oleuropein	[38,42,43]
Tyrosol	[40,42]
Verbascoside	[38,40,43]
Flavonoids	
Apigenin	[42]
Apigenin 7-O-glucoside	[43]
Apigenin 7-O-rutinoside	[42]
Cyanidin 3-O-glucoside	[39,43]
Cyanidin 3-O-rutinoside	[39,43]
Hesperidin	[39,41]
Luteolin	[42]
Luteolin 4′-O-glucoside	[42,44]
Luteolin 7-O-glucoside	[42,43]
Luteolin 7-O-rutinoside	[42]
Quercetin	[41,44]
Quercetin 3-O-glucoside	[42]
Quercetin 3-O-rhamnoside	[40]
Quercetin 3-O-rutinoside	[43]
Rutin	[42–44]

3. Microbiological Traits of OP

The OP chemical composition is influenced by the growing conditions, extraction process, regional area of the olive cultivar, and weather, and it directly influences the OP microbiota. Previous research has suggested that the microbiome of OP is made up of bacteria and yeast and is quite comparable to other olive oil by-products such olive mill wastewater (OMWW). Proteobacteria were found to be the most prevalent microorganism, followed by *Actinobacteria* (*Streptomyces*), *Firmicutes* (*Staphylococcus*), and *Acidobacteria*, according to Vivas et al. [30]. Furthermore, members of *Pseudoxanthomonas*, *Hydrocarboniphaga*, and *Stenotrophomonas* (*Gammaproteobacteria*) were detected, with *Comamonas* (*Betaproteobacteria*) as the main microbial group. The cultivar seems to have a significant influence on the fungus population. The dominant yeasts were *Pichia caribbica* (syn. *Meyerozyma caribbica*), *Pichia holstii* (syn. *Nakazawaea holstii*), and *Zygosaccharomyces fermented* (syn. *Lachancea fermenta*), which were followed to a lesser extent, by *Zygosaccharomyces florentinus* (syn. *Zygotorulaspora florentina*), *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*), *Saccharomyces cerevisiae*, and *Saccharomyces rosinii* (syn. *Kazachstania rosinii*).

A study carried out by Lanza et al. (2020) shows that OP indigenous microflora activity, via spontaneous fermentation, enhances the byproduct organoleptic profile by debittering it [45].

4. OPP Valorization via Fermentation

4.1. OP for Energy Production

The direct combustion of biomass to generate electricity or heat, because it is a well-established industry, has not been the object of study of many research publications. The use of olive by-products as biofuel for heating is quite widespread in olive oil producing regions, especially in agro-industries, livestock farms, greenhouses, and domestic heating systems. The research on energy use has focused on the improvement of methane generation in anaerobic digestion processes [46].

There are alternatives regarding OP fermentation, as the anaerobic digestion of OP biomass leads to biogas (a mixture of CH_4 and CO_2) production as well as partially stabilized matter, recovering energy and increasing the environmental sustainability [47,48]. According to an analysis about the utilization of olive by-products in Andalusia (which produces 50% of the EU-28's olive oil), 80% of the olive by-products are used to generate energy from biomass (47% for electricity and 33% for thermal energy) [49]. Landfill accounts for 0.7%, whereas composting or direct field application accounts for 14.3%. The olive sector in Andalusia is the most developed one in EU; therefore, their parameters probably represent an optimistic version of the European olive sector. The remaining EU countries may use less olive waste for energy generation (electricity) and more for composting and waste destinations.

However, the high level of phenolic compounds and phytotoxicity presents a limiting factor [50]. The primary restriction is the presence of a high number of phenolic compounds and organic acids in the residue, which blocks methanogenic microorganisms. Therefore, a pre-treatment is required to get rid of unwanted chemicals [51]. The utilization of the "cascading use" approach, which forbids energy use until valuable substances have been extracted, presents an intriguing possibility. Given the inhibitory effect that phenolic compounds can have on sugar fermentation, the separation and purification of these value-added chemicals may pave the way for further research. Elimination of these substances from the aqueous extract may also make it easier to produce ethanol from the glucose found in the extractive fraction, which would increase the production of biogas or bioethanol [52].

Olive pomace has been considered as a potential substrate for bioethanol production; however, pre-treatments, such as saccharification, are necessary since olive has a lignocellulosic complex and the sugars need to be more accessible to the microorganisms responsible for the fermentation, to optimize the process [32,53]. A study carried out in the University of Minho showed the potential that OP has as a fermented product and methods that improve the carbohydrates availability to ferment, namely using fungi, such as *Aspergillus niger* in order to perform the saccharification [54]. Another option is physical pre-treatment, which enhances accessible surface area and pore size while lowering cellulose polymerization and crystallinity levels. To promote biodegradability or enzymatic hydrolysis of these residues, various physical treatments can be used to lignocellulosic waste materials, such as milling and irradiation [55].

4.2. OP in Enzyme Production

OP has been used as a main source of nutrients for enzyme production using solid-state fermentation by *Aspergillus* species [56–58]. The interest in the enzyme manufacture, mainly lipase, is due to their large applications options, such as additives in the food industry, fine chemicals, detergents, wastewater treatment, cosmetics, pharmaceuticals, leather processing, and biomedical assays [59]. The global market of enzymes, in 2022, was about $12.46 billion and it is projected to surpass around 20.5 billion by 2030 [60]. Thus, the utilization of OP to its production is economically appealing.

4.3. OP in Animal Feeding

Additionally, a common approach for the use of this by-product is its use for feed [61]. In fact, the nutritional value and low cost involved are the ideal parameters for a feed purpose. Ibrahim et al. (2021) verified the implementation of fermented olive pomace paste (FOPP) as poultry feed [62]. The increment of FOPP in the feed led to an increase on defense system response, reduce body weight gain, protein efficiency ratio, better nutrient digestibility, and lower serum cholesterol concentration comparing to the ones fed with a standard feed [62]. Furthermore, there were more phenolic compounds and flavonoids in the FOPP fed chickens' breast meat, followed by a decrease on meat oxidative stress, improving the meat quality, and prolonging meat storage time.

Finding alternative feeds, such as those obtained from the agro-industrial sector, that can be used efficiently for animal nutrition is necessary due to the ongoing rise in feed prices and the need to improve the sustainability of animal production. These by-products (BPs) may be a valuable resource for enhancing the nutritional value of animal-derived products as they are sources of bioactive substances, particularly polyphenols. They are also effective at controlling the biohydrogenation process in the rumen, which affects the composition of milk fatty acids (FAs). The findings showed that while switching out some of the ratio's ingredients, namely concentrates, often has no effect on milk output or its primary constituents, it can lower yields by up to 12% at the highest tested levels. However, employing nearly all BPs at various tested levels made the overall beneficial effect on milk FA profile obvious. These BPs, which ranged from 5% to 40% of the dry matter (DM) in the ration, did not reduce the production of milk, fat, or protein, suggesting benefits for both economic and environmental sustainability as well as a decrease in the competition between humans and animals for food. The general enhancement in the nutritional quality of milk fat associated with the inclusion of these BPs in dairy ruminant diets is a significant benefit for the commercial marketing of dairy products resulting from the recycling of agro-industrial byproducts [63].

According to studies, knowledge of the usage of olive cake (OC) is consistent with the evidence for FA. Increased levels of OC in the diet resulted in a noticeable alteration in the composition of goat milk. The contents of milk fat and milk total solids, as well as milk yields, increased under an OC diet, with a reduction in saturated fatty acids and an increase in monounsaturated fatty acids compared to the control [64,65]. These authors conclude that adding small amounts of olive oil by-products to dairy goat diets improves milk FA composition from the perspective of the consumer while having no detrimental effects on animal performance.

4.4. OP in Food Fortification

As this residue comes from a human food industry, so would be ideal to use it for this purpose. Nowadays, several studies verified that OP can be a food fortifier in bread, pasta, and granola, after a drying process. The results showed that the enrichment of bread and pasta with OP improved phenolic contents and antioxidant activity, before and after the cooking process [66–68]. Moreover, the addition of polyphenol-rich extracts to dairy products increased their stability and prevented rancidity [69]. Studies also confirmed OP as a potential functional ingredient with prebiotic activity using OP-added formulations subjected to simulated gastrointestinal digestion followed by in vitro fecal fermentation [21,32,70]. Since this by-product in its original form has a highly bitter taste, direct intake is not recommended; as a result, a pretreatment stage is needed to change this organoleptic profile. According to studies, the bulk of the phenolic compound that causes the bitterness could be eliminated after three weeks in a brine with a low sodium chloride content (6%) [71]. The surface area of the pomace compared to the entire olive is a crucial component to consider, because the process time is shortened when the natural protective barrier of the olive skin ruptures [72–74]. Furthermore, additional research suggests that the acidic circumstances operate as chemical hydrolysis factors for oleuropein, one of the principal compounds responsible for the bitterness [75–77]. Oleuropein is hydrolyzed in a pH range of 3.8 to 4.2 and fermentation would be indicated as treatment for this by-product since it is a natural debittering process. Moreover, the phenolic content has an industrial high value associated, so extraction processes aimed at the recovery of phenolics would also be a valuable strategy [71].

4.5. OP as a Fermented Food

A study was conducted using OP that had previously undergone sequential fermentation with yeast and lactic acid bacteria (LAB) (namely *Saccharomyces cerevisiae* and *Leuconostoc mesenteroides*) on taralli, a characteristic Apulian product. By including 20% fermented OP made from black olives, the taralli was improved [19]. During storage for 180 days, the profiles of both the bioactive substances and the fatty acids were observed. In comparison to the control, the experiment produced significantly increased quantities of bioactive substances (hydroxytyrosol, tyrosol, verbascoside, oleacin, oleocanthal, maslinic acid, and lutein). Moreover, the enriched taralli retained a high level of polyphenols and a low concentration of saturated fatty acids for up to 90 days of storage. Yet it appears that the scientific community is becoming increasingly interested in using OP for human consumption. OP has already been recommended by several authors as a new dietary or nutraceutical supplement, proving its positive effects on human health.

According to Cecchi et al., 1 g of dried OP has the equivalent of phenolic total content of 200 g of virgin olive oil [78]. Using the sequential fermentation of *S. cerevisiae* and *Leuc. mesenteroides* on a pilot scale, Tufariello et al. (2019) developed a novel product [79]. The sequential inoculum demonstrated that, as in the case of table olives, the initial half of fermentation was dominated by yeasts and the second part by LAB, and considerably increased fermentation performance. When compared to an unfermented sample, the overall phenol levels in fermented OP were somewhat lower; however, the hydroxytyrosol content was higher, and the content of triterpene acids, carotenoids, and tocochromanols did not change. Due to the formation of alcohols, esters, and acids during fermentation, a favorable shift in volatile chemicals was seen [79]. A study utilizing the SHIME®, a sophisticated gastrointestinal simulator, was suggested to investigate the relationship between OP and the human gut flora. The goal of the study was to comprehend how the phenolic fraction in OP affected bacterial development and how it might have a dose-dependent antibacterial effect. Verbascoside and luteolin were also present, but in smaller amounts. The fiber content was made up of both insoluble and soluble fractions (20.4% and 3.7%, respectively), while the monosaccharide and protein contents were present at 16.8% and 9%, respectively. The main substances discovered were oleuropein-derived molecules, free hydroxytyrosol, and insignificant amounts of verbascoside and luteolin. The same study confirmed that the OP had no antimicrobial effect on the intestinal microbial community because the production of SCFA was unaffected, while *Fusobacteriaceae*—a group of bacteria typically associated with inflammation—were decreased and *Lactobacillaceae* and *Bifidobacteriaceae* were clearly increased [80]. The presence of esterase, which are frequently active in gut microbiota and are known to be involved in the hydrolysis of various phenolic compounds, was validated in terms of phenolic content by a decrease in hydroxytyrosol along with a contextual increase in tyrosol, reported after 9 days [80].

5. Fermentation as a Byproduct Valorization Approach

Researchers concluded that fermentation is an effective approach when the objective is to improve the food digestibility and bioavailability, since macro and micro molecules have value in human diet and their digestibility is an important factor [81,82]. In addition, the functional properties of the food are intrinsically correlated with the content of the components, such as proteins, starch, fats and sugars, and they are important factors that define the food application [83]. A study carried out in order to determine the value *Citrus unshiu* byproducts, a major agricultural waste in Korea, verified that the fermented citrus byproducts exhibited greater polyphenol content, an inhibition effect on radical scavenging abilities of salt and superoxide anion compared to non-fermented citrus, and antibacterial activity against *Listeria monocytogenes* and *Escherichia coli*, proving that the fermentation process increases the byproduct bioactive compounds [84]. The valorization of fish byproducts, i.e., the reduction of their environmental impact and the addition of economic value through fermentation, was the objective of study by Martí-Quijal et al. (2020) [85]. The improvement of antioxidant activity and phenolic acid content was verified. In their work, the lactic acid bacteria isolated from sea bass had proteolytic capacity, giving it the ability to synthesize phenolic acids with antioxidant capacity. The metabolites from the fermentation process normally have antimicrobial activity, such as alcohols, organic acids, and phenols. They are important to the food industry due to their capacity to prevent foodborne diseases and extend shelf-life, since they inhibit the growth of undesired microorganisms. Ricci et al. (2021) verified, using tomato, melon, and carrot byproducts, that their fermentation had antimicrobial activity, in vitro and in foodstuff, even higher when compared to commercial preservatives [86]. These encouraging findings point to an area that needs more research in the development of novel natural preservatives that may be used to extend the shelf life and increase food safety across a variety of product categories.

The production of fragrance using biotechnological routes have increased in recent years, developing different methods to obtain natural flavor. Fermentation is a biochemical process alternative to produce a natural source of flavor and aroma metabolites, making the final product more attractive for consumption, with market acceptability. Lindsay et al. [87] verified that secondary metabolites from the fermentation of by-products had high potential for adding value. The study was carried out using filamentous fungi and diverse by-products, such as apple pomace, onion pulp, orange pomace, kiwifruit peels, carrot pomace, and olive pomace. Although numerous studies on lactic acid bacteria shows that their fermentation improves food sensory qualities, the application of LAB for the creation of aroma from waste and byproducts has been rarely studied [88–90]. The aroma production with agri-food waste/byproduct fermentation was proved successful using citrus pulp, coffee husks, apple pomace, soybean oil, cassava bagasse, sugarcane bagasse, apple peels, and citrus peels [91–97].

6. Conclusions

The utilization of by-products in the food industry continues to be an important task considering the production of virtuous upcycling, and the valorization of agri-food waste is a challenging opportunity for the sustainable and competitive growth of an innovative food system. Moreover, the exploitation of agri-food waste offers excellent substrates for microbial growth and enhances waste recovery and valorization, mitigating environmental consequences and increasing the economy. Therefore, the research in this field should be increased, firstly aiming to restore the byproduct purpose as human feeding and then, if it is not possible, the research should be focused on bioactive molecule production. Olive by-product has a great potential regarding valorization, mainly in fermented processes, due to its chemical composition and natural microbiota. Even though these solid by-products are a significant source of bioactive compounds that are relevant to human health, very few researches have specifically addressed the treatment and/or valorization of olive oil by-products as food products. We can conclude about the relevance of investing in OP valorization through fermentation as a promising approach, by improving the microbiota and transforming it into a functional food with high content of advantageous phenolic compounds and antioxidant activity.

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