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Spoilage yeasts in fermented vegetables: conventional and novel control strategies

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Abstract

Fermented vegetables are produced by the growth of different microorganisms present in raw products. Yeasts are essential in food fermentations, but they are also potential spoilage agents that can cause several alterations to the final product. This review provides an overview of the most relevant spoilage yeasts present in fermented vegetables, like table olives, fermented pickles, and sauerkraut, and the strategies that can be followed to prevent their presence in the final product by extending their shelf life. Conventional treatments have been applied for years to fermented products to reduce or control microbial contamination. In this group, although the application of thermal treatments and the use of chemical additives are remarkable, these technologies have some drawbacks. Nowadays, the food industry seeks techniques that are lethal to spoilage microorganisms, but have no adverse effects on the nutritional value, organoleptic characteristics, and beneficial product microbiota. Non-thermal technologies, such as high hydrostatic pressure, UV-C light, and electrolyzed oxidizing water treatments, applied alone or combined, are effective alternatives to conventional preservation treatments to achieve secure fermented vegetables with a high-quality.

Keywords: *vegetable fermentation; spoilage yeasts; conventional treatments; alternative treatments; novel technologies*

1. Introduction

Fermented foods are generally defined as those food or beverages made from controlled microbial growth and enzymatic conversions of major and minor food components [1, 2]. The original and primary purpose of food fermentation was to achieve a preservation effect. However, fermentation is currently more appreciated because it leads to sensory differentiation, enhances nutritional value and food composition, and improves health by enhancing immune system response [3-6].

Fermented vegetables are broadly consumed in Eastern and Western countries [7]. The major fermented vegetables in Western countries are table olives and sauerkraut. Table olives are made from both alkaline- or brine-treated raw olives and they have an important nutritional, culinary, and economic impact in the Mediterranean countries, whereas sauerkraut is produced in many European countries, as well as in United States. On the contrary, eastern countries produce a huge variety of fermented vegetables by using napa cabbage, radishes, cucumbers, beets, turnips, cauliflower, celery, and carrots [6, 7].

During the fermentation and storage stages of vegetables different aspects might be considered to avoid microbial contamination, especially by spoilage yeasts [8], being difficult to demarcate the boundary between beneficial fermenting activity and microorganisms' spoilage activity. *Candida* spp., *Saccharomyces* spp., *Pichia* spp., *Issatchenkia* spp., and *Zygosaccharomyces* spp. are the most relevant spoilage yeasts associated with vegetable deterioration during fermentation, mainly producing non-typical aromas, cloudy brines, and the degradation of structural polysaccharides, which lead to less firmness [8-11].

To control the presence of spoilage yeasts, different conventional treatments, alternative strategies, and novel technologies have been applied by food processors (Fig. 1). In the case of conventional treatments, chemical additives have been used for years to prevent the presence of spoilage yeast during fermentation [12-15]. Moreover, the biocontrol agents and the utilization of alternative preservatives, such as salts or natural antimicrobials, have been widely applied as alternative strategies to avoid microbial contamination during fermentation [14, 16]. Finally, different novel technologies have been developed and used to improve the security of fermented products, leading to the manufacture of foods with high quality and safe from a microbiological point of view. The most relevant novel technologies employed in fermented vegetables, such as table olives, fermented pickles, and sauerkraut, are non-thermal treatments, the combination of the non-thermal treatments with natural antimicrobials, and the application of antifungals [17, 18].

The purpose of this review is to highlight the most relevant spoilage yeasts present in the most consumed fermented vegetables in European countries, such as table olives, fermented pickles, and sauerkraut, and the strategies that can be employed to control their presence in final products to prolong their shelf life.

2. Spoilage yeasts in fermented vegetables

2.1 Table olives

The presence of yeasts in table olives is associated with different alterations. *Candida famata*, *Candida boidinii, Saccharomyces cerevisiae*, *Pichia membranifaciens, Pichia manshurica,* and *Issatchenkia occidentalis* have been found to cause table olive deterioration. Table 1 presents the most relevant alterations of fermented products associated with yeasts and the conditions that affect their growth.

During table olive production and preservation, the development of oxidative yeasts leads to fungal-type veils forming on brines, which can be smooth or rough. These veils constitute an instability factor for the preserved product by affecting the product’s quality, which indicates that brine is not sufficiently protected from spoiling microorganisms.

Table olive contamination has been associated with different environmental elements, such as transmission pollution vectors. On the one hand, internal airflow in industry is an important vector of spoilage yeast transmission [19], as is the water used in processing plants [20, 21]. The origin of some spoilage yeast species has also been associated with contaminated surfaces, containers and other devices that come into contact with the product while it is being prepared [22]. These alterations can be observed mainly in olive texture by causing drupe softening, gas formation, and brine muddying. Drupe softening is caused by pectinolytic enzymes of yeast origin, mainly from *S. cerevisiae, C. boidinii,* and *P. manshurica* [8, 12, 23-25]. In the event of gas production during table olive storage, *I. occidentalis* is considered to be the main gas-producer yeast. This yeast presents slight resistance to weak acids and uses ethanol as a source of carbon to produce CO_2 for its growth [26]. It is noteworthy that CO_2 can penetrate olive pulp and cause severe damage to fruit due to the formation of internal cavities that end up breaking the olive structure, which is called "alambrado" [27].

For salted black olives with dried salt, *C. famata* has been identified as one of the most important deterioration agents. Its presence in olives causes color changes that affect the product's typical brightness. It is worth mentioning that this yeast can resist high salt concentrations. Panagou et al. [28] observed yeast growth at 15% salt and tolerance to concentrations of 24% salt.

2.2 Fermented pickles

A considerable variety of fermented pickles is produced from different vegetables, of which the most widespread are cucumber, cabbage, radish, carrot, beet, turnip, eggplant, and capers. In addition to these fermented pickles, other products such as kimchi, gundruk, tursu, sunki or khorisa can be found. The microorganisms involved in traditionally fermented pickles are restricted to a few bacteria categories, mainly LAB, *Bacillus* spp. and Micococcaceae, yeasts and fungi [6].

Cucumber is the leading vegetable used in pickle production around the world [6]. During the pickling cucumber process, acid fermentation starts by the action of starter cultures, such as mainly homofermentative LABs (*Pediococcus* sp.) and facultatively heterofermentative LABs (*L. plantarum* and *L. pentosus*). The main problem of cucumber pickles is related to secondary fermentations that take place during bulk storage and can lead to the complete loss of the fermented product. Moreover, these secondary fermentations are characterized by atypical fermentation odors.

The main yeasts associated with cucumber pickle deterioration during fermentation are *I. occidentalis*, *P. manshurica, P. membranifaciens, S. cerevisiae, Zygosaccharomyces rouxii, and Zygosaccharomyces globiformis* [29-31] (Table 1).

Secondary fermentations are caused by the microbial use of lactic acid, and the subsequent formation of acetic, butyric, and propionic acids [9]. These abnormal fermentations cause non-typical fermentation odors and gas formation [23]. This fermentation type is characterized by raising pH levels in fermentation brines. However, this increased pH is associated with not only partial lactic acid degradation, but also with the chemical reduction of the matrix and increased dissolved oxygen, as a result of the activity of oxidative yeasts. Changes in fermentation brines favor the development of certain LAB species, such as *L. buchneri* or *Pediococcus ethanolidurans*, specific Enterobacteriaceae like *Enterobacter cloacae,* and some species of *Clostridium*, which are mainly responsible for the formation of propionic and butyric acids that confers the product bad, and even fetid, odors. The production of these gases due to yeast activity is related to the formation of swelling in the containers that store pickles [31]. Excessive ethanol production can cause uncontrolled deterioration. Although secondary contaminations during the fermentation processes of pickles are considered sporadic episodes, their appearance is generally very difficult to predict, so not many studies have focused on the characterization of the microorganisms responsible for such spoilage.

2.3 Sauerkraut

In sauerkraut, the most relevant spoilage yeast is *Candida* spp. (Table 1). The fermentation temperature and the amount of salt added during the fermentation procedure are two critical points in sauerkraut production. This product is made of 2-3% salt concentrations, which help to control the growth of pathogenic microorganisms and spoilage yeasts. However, some strains of *Candida* genera are resistant to high salt concentrations, which lead to product spoilage [11, 23].

3. Strategies to preserve fermented vegetable products from spoilage yeasts

Several strategies have been employed by food processors to inhibit or reduce spoilage yeast growth in fermented vegetable products. In this review, the strategies have been classified into three different groups: i) conventional treatments; ii) alternative treatments; iii) novel technologies.

3.1 Conventional treatments to preserve fermented vegetable products from spoilage yeasts

Conventional treatments have been applied for years to fermented products to reduce or control microbial contamination. One of the commonest preservation strategies is the use of chemical additives, such as sorbic and benzoic acids and their salts, as well as acetic, citric, hydrochloric, and lactic acids, which can also be combined with modified atmosphere packaging (MAP) [12-15, 41]. Here it is important to point out the preservation effect of sodium chloride during food preparation given its inhibitory action against numerous spoilage yeasts [32]. However, increasing salt content to preserve fermented vegetables leads to final products with large amounts of sodium. In 2012, the World Health Organization (WHO) [33] recommended a reduction in daily salt intake to 5 g. Several authors reported that reducing sodium content in table olives would benefit consumer health [10, 34]. Hence employing calcium chloride as sodium substitutes to store fermented products for short periods of time has been investigated [15, 35]. Nevertheless, the use of combinations of sodium, potassium, calcium, and magnesium chlorides should be studied in-depth as they could affect the fermentation and microbial composition of the final product [16, 36].

Different authors have elucidated the impact of using suitable temperatures during product fermentation to control the growth of spoilage yeasts, and the recommended temperatures during fermentation lie between 15 ºC and 25 ºC [37]. Spoilage yeasts can be completely inhibited when fermentation is performed at 7 ºC [38]. Furthermore, in the presence of oxygen, yeasts employ the lactic acid present in media to grow. Therefore, anaerobic conditions are recommended for inhibiting yeast growth, even though this aspect can favor the development of other microorganisms like *Clostridium* spp. [28]. Lastly, the pH of media plays an important role in fermented products as lower pH levels (pH 3.5-4.0) inhibit the growth of pectinolytic yeasts, which are responsible for the most important microbial changes and spoilage in fermented vegetable products [24, 38]. Hence carrying out exhaustive pH control of fermentation brines, and their readjustment if necessary, could be a viable strategy to avoid the uncontrolled growth of spoilage yeasts. Moreover, in table olive fermentation and storage stages, numerous molds and yeasts might be deposited on product surfaces, which lead to several contamination problems, such as gas production and leaking packs. For years, thermal pasteurization has been employed to prolong the shelf life of table olives, but this process results in the loss of the characteristic olive color and in the generation of a cooked aftertaste from condiments [39].

Table 2 presents the most relevant studies that have employed conventional treatments to preserve table olives from spoilage yeasts. MAP could be considered as one of the most important preservation treatments, regulating the ripening process, extending shelf life, and controlling or reducing microbial spoilage during storage and distribution [40]. In this sense, Doulgeraki et al. [41] evaluated the heterogeneity of LAB and yeasts throughout the storage of Conservolea black olives in different MAP confirming that the combination of modified atmospheres and cold storage temperatures could became a feasible method to preserve table olives. Rodríguez-Gómez et al. [42] evaluated the impact of the packaging material, packaging atmosphere, and storage temperature on the microbial survival of packed Manzanilla olives. Regarding the microbial population in olive biofilm, it is noteworthy that yeast counts were similar in the olives packed in glass jars with brine, plastic bags covered with brine, and plastic bags in a vacuum. Nevertheless, these counts rose when plastic bags were used in an N_2 atmosphere (*ca.* 6.6 log CFU/olive). In brine, yeast populations were bigger in the olives packed in plastic bags (*ca.* 5.0 log CFU/mL brine) than in glass jars (*ca.* 3.3 log CFU/mL of brine), and the smallest significant yeast population was observed at 22 ºC. However, it is important to highlight that the changes observed in the physicochemical properties, especially the increase in the pH values of the packages containing the brine, limited the shelf life of the products.

On the other hand, the formulation of low salt fermented vegetables is a challenge to be addressed by food processors and researchers, as indicated previously. Nevertheless, product reformulations could lead to spoilage or support the growth of foodborne pathogens [10]. In this scenario, Bautista-Gallego et al. [10] studied the impact of partial sodium chloride replacement with potassium and calcium chloride on the microbial stability of seasoned cracked green Aloreña olives. The results of this work suggested that table olives could be manufactured with smaller amounts of sodium chloride without amending the final product’s microbial quality (Table 2). García-Serrano et al. [15] studied the effect of using calcium chloride and calcium lactate on the preservation stage of olives from the Manzanilla, Hojiblanca, and Cacereña cultivars. The results indicated lower yeast growth in the control vessels (*ca.* 3 log

CFU/mL) compared to the olives treated with calcium (*ca.* 4.5 log CFU/mL) for the first 120 days. Indeed, non-significant differences among treatments for yeast counts were noticed after 360 storage days, and both chloride and lactate maintained the olive texture during the preservation and darkening stages, without amending the flavor of the final product. Hence both calcium salts could be employed as an economic matter for the preservation stage of black ripe olives in salt-free solutions from a technical point of view (Table 2).

Besides after packing, table olives could not be completely stable having a short shelf life because of the leakage of brines and swelling of the containers mainly caused by spoilage yeasts, leading to high economic losses [13]. To overcome these problems, Alves et al. [13] investigated the effect of different acidifying agents and chemical preservatives on the microbial quality of packed olives of the Maçanilha cultivar. The data revealed that the lowest yeasts counts were detected in the brines containing hydrochloric acid or benzoic acid, being a feasible alternative to small or local industries for increasing the packed product's shelf life and preventing economic losses.

Concerning the fermented pickles, its microbial spoilage during storage produces high economic losses for manufacturers [43]. As previously mentioned, several works have reported a cucumber secondary fermentation process due to lactic acid conversion in acetic, propionic, and butyric acids [9, 31, 43]. In this context, film-forming yeasts have been described to be capable of using lactic acid during the bulk storage of fermented vegetables [29]. To solve these problems, food manufacturers have searched for effective methods to preserve pickles from their secondary fermentation. Table 2 also presents the most relevant studies that have employed conventional treatments to preserve fermented cucumbers from spoilage yeasts. As in the case of table olives, several contributions on optimizing the processing parameters of fermented cucumbers and sauerkraut dealt with sodium chloride reduction and partial substitution with other salts. For instance, Franco and Pérez-Díaz [31] investigated the microbial interactions between bacteria and yeasts, as well as the association of their activity with secondary cucumber fermentations. To this end, some cucumbers treated with brine (calcium chloride and acetic acid) were subjected to a first fermentation by using three *L. plantarum* strains, inoculated with *P. manshurica* Y089 and *I. occidentalis* Y090. The results obtained in this study suggested that the presence of spoilage yeasts and oxygen in fermentation tanks are relevant factors for the initiation of the spoilage process, leading to high economic losses for the pickling industry. Additionally, Pérez-Díaz et al. [35] studied the feasibility of fermentation procedures in brines containing calcium chloride rather than sodium chloride. The results revealed that potassium sorbate was unable to totally inhibit yeast development at the beginning of storage. Indeed, the yeast community grew in the fermentation tanks containing calcium chloride, while the LAB population declined. The highest counts for yeasts were 3.5 ± 0.4 log CFU/mL, which were in 1 log lower to that noted for the fermentations performed with sodium chloride. Based on the results, it can be stated that calcium chloride can be used to manufacture fermented cucumber stored for short periods of time. Therefore, alternative treatments and/or innovative techniques are needed to extend the shelf life of these products.

With sauerkraut, it is important to mention that yeasts also contribute to the typical sauerkraut flavor [44]. Nevertheless, Wolkers-Rooijackers et al. [45] reported that the presence of oxidative yeasts in fermentation vessels could lead to subsequent sauerkraut spoilage. To prevent spoilage yeast growth in sauerkraut, Viander et al. [46] studied the impact of a low salt concentration and salt quality during sauerkraut fermentation (Table 2). The results showed that the total number of yeasts and molds was bigger in the juice manufactured with mineral salt (*ca.* 10^5 CFU/mL) than in the juice containing 1.2% of sodium chloride salt (*ca.* 10^4 CFU/mL). Therefore, it can be stated that sauerkraut can clearly be produced with low amounts of sodium chloride, as well as with low mineral salt percentages. Nonetheless, the high pH values observed when using low amounts of sodium chloride and mineral salts could represent an important microbial risk during fermentation. Besides, Wolkers-Rooijackers et al. [45] evaluated the impact of sodium reduction on the sauerkraut fermentation and quality. The authors compared the conventional treatment (15 g/kg of sodium chloride, taken as a control) with two alternative treatments: salt reduction and 40% partial sodium replacement with a mixture of potassium chloride, magnesium chloride and calcium chloride. At the beginning of fermentation, there were more yeasts and molds in the control treatment (sodium chloride). However, after 9 fermentation days, yeast and mold counts were below the detection limit (3.3 log CFU/g) in spite of the applied treatment. Indeed, the sensory evaluation indicated that sodium chloride replacement led to products with great aroma, taste, and texture, suggesting that the partial replacement of sodium chloride can maintain the quality of the fermented product. Similarly, Müller et al. [32] tested the influence of iodized and non-iodized salt (1%) on fermentation characteristics and bacterial diversity during sauerkraut fermentation. The authors observed that for the fermentation conducted in the presence of non-iodized salt and without starters, the numbers of yeasts increased more than during the fermentation performed with starters and non-iodized salt. On the other hand, fermentations done with iodized salt had the lowest yeast counts, which indicates that iodine may have an inhibitory effect on yeast and mold development. Further studies on the fermentation conditions and their effects on the growth of starters and the sensory properties of the final fermented vegetables should be conducted. Finally, Yang et al. [47] studied the effect of salt concentration on sauerkraut fermentation quality. *Leuconostoc mesenteroides* ORC 2 and *L. plantarum* HBUAS 51041 were utilized as starter strains. Four salt brine concentrations were used: 0.5%, 1.5%, 2.5% and 3.5%. Although yeast counts reached the highest value after 5 fermentation days, regardless of treatment, higher yeast counts were detected at the end of the fermentation process in the samples with 2.5% and 3.5% salt. Contrarily, when employing 2.5% and 3.5% salt, LAB growth was inhibited, and sauerkraut maturation was postponed (Table 2).

3.2 Alternative treatments to preserve fermented vegetable products from spoilage yeasts

Among the alternative treatments employed to preserve fermented products from spoilage yeasts, it is important to highlight the application of biocontrol agents and the use of alternative preservatives like formic, hexanoic and hexenoic acids, zinc salts, and allyl isothiocyanate (AITC) [14, 16]. These alternative preservatives present strong inhibitory effects against yeast growth over time, which prolong the shelf life of fermented vegetable products [9, 16, 48, 49]. Their utilization can also be interesting when developing foods with different flavors.

On biocontrol agents, some works have reported the antagonistic behavior of *Pichia kudriavzevii* against pectinolytic yeast as a feasible alternative to be utilized to control spoilage yeast development in fermented vegetables [8, 23]. Biocontrol agents have been used in table olives with the aim of extending the shelf life and improving the safety of foods [40]. Golomb et al. [8] studied the influence of microbiota of Sicilian-style green olives on the development of different pectinolytic yeasts. The fermentations carried out by *P. manshurica* 09-406 and *P. kudriavzevii* 0-427 presented no spoilage olives. Indeed, yeast growth was inhibited and LAB growth rose during those fermentations conducted by *P. kudriavzevii* 09- 427, which avoided the recovery of pectinolytic yeasts. The latter suggests that *P. kudriavzevii* 09-427 might inhibit yeast development, which may be associated with its capacity to attach to olives or internalize in them. Thus, the observed *P. kudriavzevii* 09-427 antagonistic activity to pectinolytic yeasts, can be utilized as an alternative treatment to control pectinolytic yeast during olive fermentation by starter culture applications, leading to the obtention of a more natural product with an improved shelf-life.

Several preservative agents have been used as alternative treatments to preserve table olives from spoilage yeasts to prolong the product's shelf life. The use of zinc chloride has been proposed to fortify fermented vegetables and as an alternative preservative to potassium sorbate. In this context, Bautista-Gallego et al. [16] investigated the use of zinc chloride as a preservative agent in Aloreña table olive packing and tested different brine solutions: i) brine with 0.12% potassium sorbate, 5.0% sodium chloride, 0.21% citric acid, 0.06% ascorbic acid and 0.15% lactic acid; ii) brine with 0.050% zinc chloride; iii) brine with 0.075% zinc chloride; iv) brine with 0.100% zinc chloride. The yeast counts at the end of the process were particularly low when employing 0.075% zinc chloride, which indicates that replacing potassium sorbate with zinc chloride could be a feasible alternative given its stronger inhibitory effect on yeast communities and improved sensory profile.

For cucumbers, the most commonly salts used in their fermentation can selectively promote LAB development without amending the survival of the salt tolerant yeasts responsible for product spoilage [49]. Therefore, alternative treatments are needed to preserve fermented cucumbers from spoilage yeast growth. In this sense, Pérez-Díaz and McFeeters [48] studied the combination of fumaric acid (20 mM) and AITC (2.0 mM) to control yeast growth (*Z. globiformis*) in acidified cucumbers. Samples were stored in a brine solution containing 150 mM acetic acid, pH 3.5, in combination with 5 mM or 20 mM fumaric acid to prevent LAB growth, and 2.0 mM AITC to prevent spoilage yeast growth. No lactic acid production and minimal ethanol generation occurred in the non-pasteurized acidified cucumbers when fumaric acid and AITC were applied (pH 3.5 of media). Therefore, the results suggested that AITC prevented spoilage yeast growth, being a potential alternative to the thermal processes. Similarly, Doan et al. [49] investigated the inhibition of yeasts in commercial pickle brines by using 2,4-hexadienoic (sorbic), hexanoic, and (E)-3-hexenoic acids. These authors evaluated the influence of salt content on yeast development. The results exhibited that hexanoic and (E)-3-hexenoic acids brought about a reduction of 4 and 2 log CFU/mL in the yeast communities, respectively. Indeed, hexanoic and (E)-3-hexenoic acids at 200 mg/kg displayed stronger inhibitory effects on yeast growth than 2,4-hexadienoic acid traditionally used in fermentation brine throughout the study. Hence hexanoic or hexenoic can be utilized instead of sorbic acid to prevent yeast development during cucumber fermentation brine storage.

Lastly, Franco et al. [9] studied the growth inhibition of the microorganisms responsible for secondary fermentations by using AITC as an alternative preservative in cucumber juice. The use of the AITC was effective to stabilize the product, without using a thermal treatment. Nevertheless, it is important to mention that increasing consumer demands for natural ("clean label") and healthy foods have forced food processors to look for the application of non-thermal processes that guarantee product stability and safety, minimizing at the same time the loss of nutrients.

3.3 Novel technologies to preserve fermented vegetable products from spoilage yeasts

In the last decade, different novel technologies have been applied to olives to extend their shelf life [18, 39] with few or no negative effects on the product's quality and nutritional value [17, 39]. Table 3 summarizes the most relevant studies that have applied novel methods to preserve table olives from spoilage yeasts. Hondrodimou et al. [50] investigated the impact of natamycin on controlling fungal development during natural black olives fermentation. The employed brine was supplemented with 0.01% (w/v) natamycin. The authors observed that natamycin was able to suppress yeast growth. Natamycin, a preservative obtained as a result of bacterial fermentation [51], is authorized for surface applications with certain cheeses and dry cured sausages [52]. This additive displayed good inhibitory activity against fungi, which prolongs the shelf life of food [51].

Amongst the non-thermal technologies, high hydrostatic pressure (HHP) is applied to liquid and solid foods, in the range of 50–1000 MPa [39, 40]. Moderate pressure (>300 MPa) decreases the microbial reproduction rate and growth, meanwhile higher pressure inactivates the microbial activity [40]. Nevertheless, only few studies evaluating the application of HHP in fermented vegetables are available on the literature. In this context, Pradas et al. [53] evaluated different HHP treatments to preserve table olives (*Olea europaea var. europaea etnovar.* Cornezuelo). In their study, samples were subjected to different HHP treatments (400-600 MPa for 5-10 min). Their results revealed that yeast and mold counts were below 10^6 CFU/g, regardless of the applied treatment (treated and untreated samples). Indeed, HHP treatment could be employed to control the formation of gas in the packed olives and to enhance the sensory properties of olives. Abriouel et al. [39] evaluated the effect of HHP treatments (from 200 to 700 MPa), singly or in combination with natural antimicrobials (nisin and essential oils), on Manzanilla Aloreña olive preservation. Their study also evaluated the effect of ascorbic acid or purge with nitrogen gas, and HHP treatments. Non-viable yeasts were noticed after applying treatments at 300 MPa or higher. Nisin and thyme oil had no effect on yeast populations. Contrarily, rosemary oil reduced viable counts to levels below those observed in the controls after 1 month, and non-viable yeasts were detected in the samples containing rosemary oil after 2-3 storage months. Lastly, non-viable yeasts were noticed when preserving samples in low salt concentration brines supplemented with ascorbic acid or purged with nitrogen gas, and subjected to 450 or 550 MPa. Argyri et al. [17] also determined the impact of HHP treatments on fermented green table olives. For this purpose, olives were subjected to different HHP treatments: 400, 450, and 500 MPa for 15 and 30 min. The results evidenced that yeasts were more resistant than LAB, which recovered in all cases, except when subjecting samples to 500 MPa for 30 min. The HHP application has also been investigated as a non-thermal treatment to improve sauerkraut microbial quality [54]. These authors observed that HHP drastically lowered aerobic mesophilic bacteria and LAB counts. According to these studies, the combination of HHP, moderate temperatures, and low pH in fermented vegetables could be a valuable alternative to heat treatments to minimize microbial contamination by improving their quality and shelf life. However, HHP treatments would also lead to the inactivation and inhibition of the product’s beneficial microbiota, and could modify the color of olives [17, 39, 53], which plays an important role in consumer acceptability. To overcome this problem, some authors suggested to use ascorbic acid or gaseous nitrogen for purging the products [12]. The use of HHP has also been applied to control microbial spoilage of fermented cabbaged, founding that yeast were the most sensitive microorganisms to HHP, compared to total aerobic bacteria and lactic acid bacteria [55].

Finally, UV-C irradiation is an approved disinfection technique for surface treatment of foods [18], such as minimally fresh processed or ready-to-eat fruits and vegetables, water, and food processing surface. As far as we know, only one study regarding the use of UV-C light and electrolyzed oxidizing water on table olives is available on the literature [18]. Olives were exposed to UV-C radiation at several intensity levels (2.65, 2.11 and 1.52 mW/cm^2) and different UV-C radiation doses (0–4770 mJ/cm^2). Furthermore, table olives were subjected to electrolyzed oxidizing water treatments (15, 30, 50, and 80 mg/L) and to sodium hypochlorite treatments (15, 30, 50 and 80 mg/L chlorine). The most marked reductions in yeast and mold counts were obtained in electrolyzed oxidizing water treatments and sodium hypochlorite treatments at the 80 mg/L concentration, when counts were lowered. Indeed, the highest inactivation rate was noticed at the highest radiation dose. It is worth mentioning that the emerging technologies have also been applied to control spoilage yeast in other fermented products such as wine [56-58], dairy fermented products [59], and other fermented beverages [60, 61].

4. Conclusions and future trends

Fermentation is used to prolong the shelf life of vegetables, to increase their nutritional value and to facilitate flavors, aromas and textures developing. Although yeasts play an important role in vegetable fermentation, the line between the beneficial and altering effect of yeasts is sometimes a difficult one to draw. The presence of spoilage yeasts causes loss of product quality, which renders it unfit for consumption. Profound knowledge of spoilage yeast characterization, origin, and growth conditions can be helpful to establish effective control strategies. Thermal treatment is a useful tool to control microbial spoilage, but high temperatures also inhibit the growth of beneficial microorganisms, such as probiotic bacteria. Moreover, the use of antimicrobial compounds and additives is not completely effective in controlling product spoilage and, additionally, they are not well considered by consumers. In this scenario, the application of novel techniques, which are lethal to spoilage yeasts, but have a mild effect on beneficial bacteria and on organoleptic attributes and nutritional composition, is necessary. Non-thermal technologies, such as high hydrostatic pressure, UV-C light, and electrolyzed oxidizing water treatments, applied either alone or combined with natural antimicrobial compounds, are effective alternatives to conventional preservation treatments to achieve secure fermented vegetables with a high-quality. However, further studies on the use of these new preservation technologies to control yeast spoilage and to extend the shelf life of table olives, fermented pickles, and sauerkraut, are needed, in order to ensure survival of beneficial microorganisms and the sensorial stability of the product. Plus, although non-thermal technologies have been effective to control spoilage yeasts, further research is required to allow an efficient technology transfer at industrial level.

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Figure captions

Fig. 1 Vegetables fermentation: nutritional, organoleptic, and health benefits *vs.* development of spoilage microorganisms and preservation techniques employed to prolong their shelf life.