Process Conditions of Table Olive Fermentation

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Abstract

Table olive is one of the important fermented products in the food industry. There are mainly two types of table olive production methods in which fermentation is used. These are natural fermentation methods and Spanish style methods. Fermentation process in these methods take time and during this time the process progresses under the influence of different factors and so it must be managed well in order to achieve the desired quality, standard and safety production and minimum economic losses. In table olive fermentation, phenolic compounds contents, reducing sugar content, microbial profile, salt concentration, acidity and temperature are the main parameters that should be considered for providing a proper fermentation conditions. Understanding the basic parameters that determine the progress of the fermentation process in table olive production and the effects of these parameters on the process is of key importance for process control, acceleration and development of new production methods in table olive production. In this study, the factors determining the process conditions in table olive production were investigated.

Keywords: Fermentation, Olive, Process, Table olive, Parameter, Condition

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INTRODUCTION

Fermented food production has been carried out since ancient civilizations (Erten et al., 2015; Mannaa et al., 2021). Fermentation is the process of breaking down organic molecules by enzymatic activity of microorganisms (Sharma, 2020). Microorganisms used for fermentation are bacteria, yeasts and molds. In food technology, fermentation provides increased product shelf life and good organoleptic properties (Smid and Hugenholtz, 2010). Table olive is one of the important fermented products in the food industry (Kara ve Özbaş, 2013). Table olive production process aims to remove the bitterness caused by oleuropein in the fruit, improve the sensory quality and to increase the shelf life of the product (Gomez et al., 2006). There are mainly two types of table olive production methods in which fermentation is used. These are natural fermentation method (untreated) and Spanish style method (treated). In natural fermentation, olives are brought to eating maturity by fermentation (6-9 month) by removing their bitterness directly in brine (containing salt, 5-10 %). Since there is no alkali application in this method, the diffusion of phenolic compounds and fermentable components out of the olive and into the brine is limited, the removal of bitterness is delayed and the fermentation time is prolonged. (Gomez et al., 2006; Lanza, 2012).

However, this is a situation that increases the nutritional value and antioxidant activity of olives (Conte et al., 2020; Rocha et al., 2020). Spanish style production method is applied to green olives and the bitterness of the olive is removed with alkali. Removal of bitterness occurs in the form of hydrolysis of oleuropein glycoside, which causes bitterness in olives, to non-bitter components with alkali effect. With the alkali application, the permeability of the olive skin increases and a suitable medium for fermentation is provided. Then, the alkali is removed from the olive by washing processes. Afterwards, the olives are fermented (2-3 months) in brine (containing salt 5-10 %) so that brought to eating maturity (Minquez-Mosquera et al., 2008).

Although Spanish style treated olive fermentation takes a shorter time than natural fermentation, both of these two processes take time and during this time the fermentation process progresses under the influence of different factors. This process must be managed well in order to achieve the desired quality, standard and safety production and minimum economic losses.

PROCESS CONDITIONS OF TABLE OLIVE FERMENTATION

In table olive fermentation, phenolic contents, reducing sugar content, microbial profile, salt concentration, acidity and temperature are the main parameters that should be considered for providing a proper fermentation conditions (Corsetti et al., 2012; Fendri et al., 2012).

Microorganisms

Table olive fermentation is a complex biochemical process involving different microorganisms (Lanza, 2013). This process is generally a spontaneous process. The microbial composition is affected by several factors such as the composition of the brine, ambient acidity, salt concentration, olive variety, olive phenolic content, temperature, oxygen availability, alkaline concentration during fermentation (Botta and Cocolin, 2012; Santos et al., 2017). Mainly microorganisms that can be found in the fermentation medium are *Enterobacteriaceae*, lactic acid bacteria (LAB) and yeasts (Romeo, 2012). However, yeasts and LAB have the main role in the fermentation process and thanks to the synergy between them, high quality products are obtained (Botta and Cocolin, 2012; Ertan et al., 2015; Kiai et al., 2020). While in treated olive fermentation, LAB have a major role, in untreated natural olive fermentation yeasts or LAB can play a major role depending on factors like salt content and acidity of the brine (Özdemir, 1997; Santos et al., 2017). In a study (Tassou et al., 2002) conducted, it was reported that LAB dominate the medium in brine with 4% salt concentration, while yeasts dominate the medium in brine with 8% salt concentration. LAB are highly sensitive to salt concentration and phenolic compounds, whereas yeasts are more resistant to these compounds. The main LAB species involved in olive fermentation are *Lb. plantarum* and *Lb. pentosus* and yeasts species are *Saccharomyces cerevisiae*, *Wicherhamyces anomalus*, *Pichia klayveri*, *Candida boidinii* (Pereira et al., 2015; Perpetuini et al., 2020).

The main actions that yeasts perform in fermentation are: The formation of flavour and aroma compounds such as ethanol, glycerol, acetaldehyde, esters and higher alcohols, the formation of organic acids (acetic acid, succinic acid, formic acid), the formation of nutrients in the medium such as vitamins, amino acids, purines and reduced carbohydrates necessary for LAB development, the reduction of phenolic compounds and the prevention of the development of spoilage microorganisms (Botto and Cocolin, 2012; Bleve et al., 2014).

Esterase positive species take an important role in fermentation by forming esters from free fatty acids. Also, since many yeasts have lipase enzymes, they many cause an increase in free fatty acids in the medium (Botto and Cocolin, 2012). This contributes positively to the development of taste and aroma of the product. In addition, the use of sugar remaining in the product at the end of fermentation by yeasts prevents microbiological development in the product after packaging (Perpetuini et al., 2020). However, in some cases, it has been reported that some yeast species may cause softening in the product by causing degradation of pectic substances in the olive cell wall and in the middle lamella during fermentation, and gas packets may form on the product surface due to the carbon dioxide production (Fendri et al., 2012; Fadda et al., 2014). The all effects of yeasts on fermentation are not yet clear (Anagnostopoulos et al., 2017). The reasons for yeasts to form the majority in the fermentation medium are the increase in the acidity as a result of microorganisms activity, high salt concentration, lactic acid bacteria being more sensitive to phenolic compounds, higher presence of phenolic compounds in untreated olives obtained without alkali treatment, and higher acidity at the beginning of fermentation in olives preparing without alkaline application (Kara ve Özbaş, 2013). Oleuropein degradation is one of the main purposes for table olive processing. Many yeasts are known to exhibit β-glucosidase activity, which degrades oleuropein (Perpetuini et al., 2020; Zhang et al., 2021). So, some yeasts probably have a role in oleuropein degradation (Barilacqua et al., 2012). As for the source of yeasts in fermentation, Pereira et al. (2015) reported that yeasts were found in similar amounts in olive brine and olive pulp, and the source of these yeasts in fermentation was probably the fruit itself and the vats in which fermentation was carried out.

LAB increase the acidity of the medium by producing lactic acid from fermentable sugars, thus prevent the development of spoilage and pathogenic microorganisms and increases the shelf life of the product. In addition, they contribute to the development of taste and aroma of final product. LAB also perform the debittering process by hydrolyzing oleuropein (Botto and Cocolin, 2012; Bleve et al., 2014; Bonatsou et al.,2017; Romeo et al., 2018). It is known that LAB also form antimicrobial components such as organic acids, bacteriosins, diacetyl and reuterin that inhibit spoilage microorganisms (Corsetti et al., 2012). *Lb. plantarum* and *Lb. pentosus* are the main bacteria involved in olive fermentation. They stand out by their resistance to acidity and salt and their ability to degrade oleuropein (Romeo et al., 2018). It was reported that, in fermentation, yeasts were predominantly found on the olive surface and stomatal opening, while bacteria predominate in the intracellular cavities of the sub stomal cells (Nychas et al., 2002).

The microbial composition of the medium is quite complex during fermentation, depending on the changes in the biochemical conditions of the brine (Botto and Cocolin, 2012). Ambient acidity is low at the start of fermentation. Gram (-) bacteria are dominant in this medium. This period should be as short as possible and the acidity should increase so that the product does not deteriorate. Otherwise, these microorganisms will cause negative taste in the product and the formation of gas pockets on the olive surface (Botto and Cocolin, 2012; Lanza, 2013). Acetic acid can be added to inhibit Gram (-) bacteria (Medina et al., 2010). Bleve et al. (2014) studied the Cellina di Nardo and Leccino variety table olives obtained by natural fermentation. They found that microorganisms Gram (-) belonging to the *Enterobacteriaceae* family were present in the beginning and were not found in the fermentation medium a few days later. Low acidity allows undesirable microorganisms such as *Clostridium spp.* to growth in the medium. In the same study, it was reported that *Clostridium* and *Pseudomonas* microorganisms were not found at the end of fermentation and this was due to high acidity (pH <4.3).

In another study with similar results, it was determined that while *Enterobacteriaeceae* and Pseudomonas decreased in brine, LAB and yeast populations increased during fermentation in black olives produced by natural fermentation method (Nychas et al., 2002). As the fermentation progresses, the acidity rises and the LAB dominates medium, especially in treated olives production. The optimum growth acidity for the LAB is between pH 5.5-5.8 (Lanza, 2013; Botto ve Cocolin, 2012; Perpetuini et al., 2020). Since alkali treatment is applied in treated olives, the acidity at the beginning of fermentation (pH 8.0-9.0) is lower compared to olives obtained by natural fermentation (pH 5.0-6.5) (Lanza, 2013). Slow development of acidity is usually associated with slow growth of the LAB population (Martorana et al., 2017). In the untreated olive fermentation, if the salt content is not high and depending on the presence of phenolic secoiridoid derivatives in the medium, LAB and yeasts can develop together. Or one of them dominate the medium (Hurtado et al., 2012; Fendri et al., 2012; Fadda et al., 2014). It is stated that when the final pH of the product approaches 3.8-4.0 in untreated olives, yeasts become dominant in the fermentation medium (Kara ve Özbaş, 2013). Bleve et al. (2014) reported that the concentrations of yeasts in *Cellina di Mondo* and *Leccino* variety table olives obtained by natural fermentation increased from 3.0 log cfu/mL to 4.0-5.0 log cfu/mL throughout fermentation and they have an important role in fermentation. Aeration of the vat where fermentation takes place and also its size and types influence the fermentation development too. The size of the vat affects the ambient temperature. Fermentation takes place more irregularly in small volume vats (Hurtado et al., 2012).

It is possible to use starter culture to accelerate, control and standardize the fermentation process, to improve taste and aroma properties, and to increase the microbial safety of the product (Lanza, 2013; Bonatsou et al., 2017). By using starter culture, acidity formation takes place in a shorter time and thus the fermentation process is accelerated (Fendri et al., 2012). *Lb. plantarum* and *Lb. pentosus* are used as starter cultures, however it is possible to adding yeasts to the starter culture (Santos et al., 2017; Cosmai et al., 2018). The success of the starter culture in fermentation is directly related to the olive variety and the process method used (Hurtado et al., 2012). Perpetuini et al. (2018) in their study conducted with table olives inoculated with *Lb pentosus* (C8 ve C11) strains and spontaneously fermented, they determined that oleuropein was completely degraded in the samples produced by inoculation, LAB developed faster and a better acidification rate was provided. Pino et al. (2018) study conducted in which *Lb. paracasei* and *Lb. plantarum* strains were used as starters and they reported that starter culture accelerated fermentation, increased acidity from the 7th day, and inhibited spoilage bacteria also in this study, it was seen that yeast growth was correlated with main alcohols and phenols that cause undesirable taste and aroma and the use of starter suggested to prevent the growth of these autochthonous yeasts and spoilage bacteria. In another study, *Lb. plantarum* and *Lb. pentosus* strains were used as starters. In this study, it was determined that the fermentation time was shortened in the samples using starter, the antioxidant capacity was higher than the spontaneous samples, and the spoiling microbiata was lower (Comunian et al., 2017).

Features expected from starter culture are rapid development, high tolerance to salt, acidity and phenolic compounds, capacity to reduce phenolic compounds, showing enzyme activities that can contribute to the sensory properties of the final product, and being able to develop at low temperatures to improve the fermentation activities that continue especially in winter (Corsetti et al., 2012; Değirmencioğlu, 2016; Perpetuini et al., 2020).

Temperature

Ambient temperature is an important factor in the course of fermentation. The optimum temperature for fermentation is around 20 ºC. Therefore, fermentation slows down in the winter months when the temperature is low (Kayguluoğlu, 2018). By controlling the process temperature, fermentation can be accelerated and positive effects on product quality can be achieved. An increase in the amount of nutrients diffused from the fruit to the brine, enhancement of LAB growth and increase in acidity can be achieved with the application of high temperature (>18 ˚C). Also, less bitter product is obtained with the increased degradation of oleuropein due to the better growth of LAB (Campus et al., 2017; Vertedor et al., 2021). Although it accelerates the fermentation and has positive effects on the product, temperature control is not generally applied in the industry due to the energy cost (Aponte et al., 2010; Medina et al., 2010; Vertedor et al., 2021). There are limited number of studies in the literature about effects of temperature on the end product quality of table olives. Tassou et al. (2002) studied in their study, effects of different temperatures (25 ºC, 18 ºC and ambient temperature) and salt concentrations (4%, 6%, 8%) applications on the untreated olive fermentation. They reported that the best conditions for fermentation was providing with the sample of (25 ºC, %6) after five months of brining. The best acidity development was achieved with this sample. Also in this sample no off-flavors were detected indicating unfavorable fermentation by the panelists . In another study conducted, the effects of high temperature (20-24 ºC) applications on the brines of Spanish style treated table olives, during the fermentation was studied. It was reported that at the end of three months, the growth of LAB and yeasts and the development of acidity are higher and better color and optimum firmness are achieved in heated samples compared to the control samples without heat treatment (Vertedor et al., 2021).

Phenolic Compounds

The phenolics are minor compounds in olive fruit however they provide to gain functional properties to the fruit as they are the major antioxidant compounds in fruit. The phenolic compounds found in olives are mainly from classes the phenolic acids (gallic acid, caffeic acid), phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin-7-glucoside, luteolin) and secoiridoids (oleuropein, verbascoside) (Sahan et al., 2013; İzli, 2017). The phenolic fractions and content in product depends on different factors such as cultivar, climate, location, process conditions and stage of maturity (Perpetuini et al., 2018). Although it varies, the main phenols in olive fruits are oleuropein, tyrosol, hydroxtyrosol and verbascoside (Sahan et al., 2013). The most important of these compounds is oleuropein. Because, one of the main purposes of the table olive production process is to degrade oleuropein to eliminate the bitter taste caused by it. Oleuropein is degraded by microorganisms firstly to oleuropein-aglycone and glucose by the enzyme β-glucosidase and then to hydroxytyrosol and elenolic acid by the esterase enzyme. Also, oleuropein is degraded directly into hydroxytyrosol and elenolic acid glucoside with the application of alkali (NaOH) in the treated olive process. Then, during fermentation, elenolic acid glucoside is broken down into glucose and elenolic acid with the effect of acid (Ozdemir et al., 2014). During fermentation, the phenolic compounds in olives diffuse into the brine, and thus the amount of phenolic compounds in olives decreases. In a study conducted, in which using Gemlik and Edincik varieties of olive, it was determined that the amount of phenolic compounds of olive flesh in untreated olive fermentation decreased continuously during the fermentation of the fruit. Phenolic content decreased to 1.25% of the olive, about half of the amount in raw olives after 250 days of fermentation.

Also, the amount of phenolic compounds in the brine reached 1% towards the end of fermentation for both varieties (Borcakli et al., 1993). In another study conducted with four different olive cultivars, it was determined that flavonoid loss in olive flesh was 60% and total phenol loss was 79% during fermentation in olives produced by natural fermentation method. The main phenolic compounds detected in the brine after 71 days of fermentation were hydroxytyrosol, tyrosol, catechine and quercetin (Kiai and Hafidi, 2014). In a study using green and purple olives, it was determined again that there was a significant decrease in flavonoid and total phenol content in olive fleshes during fermentation, and this decrease was accompanied by a decrease in antioxidant activity. At the end of fermentation, it was determined that while the amounts of hydroxytyrosol and caffeic acid increased in both olive fleshes, the amounts of protocatechuic acid, ferulic acid, *p*-coumaric acid and quercetine decreased. Also, the main phenolic compound found in both brines was hydroxytyrosol (Kiai et al., 2020). Phenolics have important effects on colour, flavour and nutritional properties of products. They also show protective effects and extend shelf life of product because they have antimicrobial and antioxidative properties. They have antimicrobial effects on many microorganisms including LAB which have roles in the olive fermentation. They also can increase the shelf life of the product, thanks to their antioxidative properties (Borcakli et al., 1993; Özdemir, 1997; Pereira et al., 2006; Charoenprasert and Mitchell, 2012). Phenolic compounds show direct effects on table olive production processes and the final product with these properties.

Acidity

Acidity development in fermentation occurs mainly by the conversion of sugars to organic acids as a result of LAB metabolism, as well as the diffusion of organic acids from olives to the brine and hydrolysis of oleuropein (Pereira et al., 2015; Campus et al., 2017). Acidity is of prime importance for the development of the fermentation process because of it provides preservation of the product and LAB growth by preventing the development of undesirable bacteria (Borcakli et al., 1993; Pino et al., 2018). In treated olive fermentation, acidity is lower than in natural fermentation at the beginning due to the transition of the alkali remaining in the olive to the brine (Medina et al., 2010; Lanza, 2013). However, at the end of fermentation, acidity is lower in olives produced by natural fermentation method. The decrease in sugar content as the olive ripens and the lower permeability of the olive skin when alkali application is not used are some possible reasons for this situation (Gomez et al., 2006; Lanza 2012). It was reported that, the pH at the end of fermentation is 3.4-4.4, and as lactic acid total acidity value is 0.8-1.2 %, in treated olive fermentation and the final pH value is 4.3-4.5 and as lactic acid total acidity value is 0.5-1.0 %, in natural fermentation (Erten et al., 2015). The use of starter culture ensures that this acidity progress is faster and higher. In the study in which two different LAB strains are used as starters, it was determined that the pH value of the brine started at approximately 5 at the beginning of the 30-day fermentation, decreased to approximately 4 on the 7th day of fermentation, and was 3.7 at the end of the fermentation. In addition, it was observed that the acidity increased more slowly in the control samples without starter culture and the pH values determined as 4.8 at the end of fermentation were higher than the values of the inoculated samples (Perpetuini et al., 2018). Organic acids such as acetic acid, citric acid, sorbic acid, benzoic acid or their salts can be added with the purposes of accelerating the fermentation process and as preservative for product (Gomez et al., 2006; Fendri et al., 2012; Hurtado et al., 2012; Lanza, 2013; Erten et al., 2015).

Salt

One of the main parameters affecting the development of the process in table olive fermentation is the salt concentration of brine (Aponte et al., 2010). The brine salt concentration determines the diffusion rates of the soluble components. Because of osmotic pressure difference between brine and olive flesh, water soluble components diffuse from the olive flesh to the brine and vice versa during fermentation. In this context, as the sodium content increases in olives, some of nutrients of microorganisms such as reducing sugars, phenolic compounds, pectic substances, vitamins, alcohols and organic acids moves to the brine (Borcakli et al., 1993; Bautista-Gallego et al., 2013). This transition between brine and olive flesh continues until the amounts of ingredients on both sides reach equilibrium (Kanovouras et al., 2005).

In a study conducted with naturally fermented green olives brines with two different salt concentrations (4% and 7%), it was determined the diffusion of phenolic compounds and reducing sugars from olive flesh to brine was higher in samples containing 4% salt, brines containing 7% salt had higher polyphenol content and antioxidant activity. Also as expected, the salt content of olive fleshes in brine containing 7% salt was reported to be significantly higher (Fadda et al., 2014).

Salt is also used to prevent the growth of undesired microorganisms and improve the sensorial properties and texture of the product (Marsilio et al., 2002; Medina et al., 2010; Campus et al., 2015; Pino et al., 2018). Recently, the use of salt in olive production in industry tends to be reduced. The main reasons for this can be stated as masking of the fruit aroma by the excess salt, the prevention of lab growth by high salt concentrations, environmental causes related to chlorides, recommendation of low sodium intake in the diet for health reasons and shriveling of olives at high salt concentration (Borcakli et al., 1993; Medina et al., 2010; Değirmencioğlu, 2016).

Reducing Sugars

The water soluble compounds, must initially transition from the olive flesh to the brine, for the fermentation of table olives to take place. The most important of these soluble components for fermentation are reducing sugars. Mainly reducing sugars in olive are glucose, fructose and sucrose (Kiai et al., 2020). The transition of reducing sugars from olive flesh to the brine occurs depending on parameters such as olive skin permeability, salt concentration, temperature and olive/brine ratio (Borcakli et al., 1993; Kiai and Hafidi, 2014). Due to the transition of sugars to the brine, the amount of sugar in olive flesh is significantly reduced during fermentation (Özdemir, 1997).

Sugars in brine are the main energy source for microorganisms involved in fermentation. Microorganisms convert these sugars into organic acids (mainly lactic acid) and thus increase the acidity of the medium (Ünal and Nergiz; 2003; Kiai and Hafidi, 2014; Alak, 2016). In a study using green and purple olives, it was determined that the reduction in sugar content during fermentation was 73% in green olives and 60% in purple olives (Kiai et al., 2020).

Another study was done with Gemlik and Edincik varieties of olive. In the process using the Edincik variety, which has a higher reducing sugar content, it was determined that the transition of reducing sugars to the brine was faster, the pH value decreased faster and the final pH value was lower than the process in which Gemlik variety was used (Borcakli et al., 1993). In general, olives used in table olive production are required to have a high sugar content (Kara ve Özbaş, 2013).

CONCLUSION

Table olive, which is an industrial and high added value product, is a product that is consumed all over the world and has an important place in the food industry with its functional properties and nutritive value. However, the production of table olives is a process that progress under the influence of different parameters and usually takes time. Understanding the basic parameters that determine the progress of the process in table olive production and the effects of these parameters on the process is of key importance for process control, acceleration and development of new production methods in table olive production.

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