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**DESARROLLO DE BEBIDAS FERMENTADAS A PARTIR DE FRUTAS NO
CONVENCIONALES RICAS EN ANTIOXIDANTES (GRANADA Y CAPULÍN)**

Tesis presentada en cumplimiento parcial de los requisitos para obtener
el grado de Doctora en Ciencia de Alimentos

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Hacen constar:

Que la tesis titulada **“Desarrollo de bebidas fermentadas a partir de frutas no convencionales ricas en antioxidantes (granada y capulín)”**, presentada por Gabriela Rios Corripio, para optar por el grado de Doctora en Ciencia de Alimentos por la Universidad de las Américas Puebla, ha sido realizada en dicha universidad, bajo su dirección y que reúne las condiciones necesarias para ser defendida por su autora.

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UNIVERSIDAD DE LAS AMÉRICAS PUEBLA
SCHOOL OF ENGINEERING, DEPARTMENT OF CHEMICAL ENGINEERING AND
FOOD



**DEVELOPMENT OF FERMENTED BEVERAGES OF NO COMMON FRUITS WITH
ANTIOXIDANTS (POMEGRANATE AND BLACK CHERRY)**

Thesis presented in partial fulfillment of the requirements for the
degree of Doctor of Food Science

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FOREWORD

This doctoral thesis developed two fermented beverages of pomegranate and black cherry fruits, this research evaluates the physicochemical, antioxidant, and sensory changes before and after fermentation process. Likewise, fermented pomegranate and black cherry fermented beverages are processing by two novel technologies, high hydrostatic pressures (HHP) and pulsed electric field (PEF).

Doctoral thesis was divided in nine chapters. The first chapter is an introduction of the work done. The second chapter talks about the justification of this research, why these fruits were used, why they were fermented and why they used of emerging technologies for processing. The third chapter are the research aims, general and specific that were raised for all the research work of the thesis. The fourth chapter is the hypothesis of what was predicted to occur in this investigation. The fifth chapter is the theoretical framework, in this a bibliographic review was made of the most important information related to the topic of work, we talk about the fruits used, the antioxidants, the fermentation process, the thermal processing and the subsequent analysis of the beverages.

In the sixth chapter, the methodology of the research is explained, talk about each method. In the seventh chapter the results are presented for each part of this work of the thesis. Tables and figures of the results are presented. The results are discussed with comparative information from other authors. This part is divided in the results of pomegranate beverage, fresh juice, fermented beverage, and beverage processing by HHP and PEF. Subsequently, the results of the black cherry fruit, are presented. The analysis of the fresh juice and once fermented, as well as the application of the processing by HHP and its analysis along of the storage. In the eighth chapter the general conclusions are presented about the results. In the last chapter present a list of general recommendations. What could be applicable in others researches. In the final part of the doctoral thesis are the bibliography used, books, journals, pages of Internet, as well as the annexes including the evidences of participation in congresses, formats used for sensory evaluation and other resources generated during the doctorate studies.

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RESUMEN GENERAL

La granada y el capulín son frutas de temporada que crecen en cantidades mayores a las que son aprovechadas. Diversos estudios demuestran que estas frutas tienen propiedades benéficas para la salud debido a los compuestos antioxidantes con los que cuentan. Dentro de estos compuestos se encuentran principalmente, los compuestos fenólicos y los flavonoides, los cuales le atribuyen a las frutas una alta capacidad antioxidante. La granada y el capulín se consumen en su mayoría en forma fresca y de menor forma como productos procesados. Para incrementar su valor estas frutas pueden aprovecharse como materia prima de un producto alimenticio. Actualmente las personas demandan productos más naturales, siendo esto un incentivo para el diseño de alimentos que se generan y conservan utilizando otras técnicas de conservación como la fermentación y las tecnologías emergentes. Por lo cual, el objetivo de esta investigación fue desarrollar bebidas fermentadas de granada y capulín, utilizando nuevas tecnologías como las altas presiones hidrostáticas (HHP) y los pulsos eléctricos (PEF), para su estabilidad microbiológica, antioxidante, fisicoquímica y sensorial. Para el procesamiento por HHP, se usó un equipo de planta piloto MINI FOODLAB FPG5620 de alta presión (HHP) para tratar las bebidas fermentadas de granada a 200, 400, 550, 500 MPa (10 min) y 600 MPa (5 min). En la metodología de PEF, se usó un sistema de flujo continuo, utilizando el equipo ELCRACK-HVP 5 (Quakenbrück, Alemania), se aplicaron dos tratamientos de PEF a las bebidas fermentadas. La intensidad de campo eléctrico aplicada fue: 11.7 (50%, 15 μ s) y 18.8 (80%, 20 μ s) kV/cm (frecuencia de 200 Hz). Las bebidas también fueron pasteurizadas mediante las tecnologías VAT y HTST. Después de los tratamientos las bebidas fermentadas fueron almacenadas a 4°C y se analizaron a lo largo de 42, 49 y 56 d. La actividad antioxidante, fenoles totales, flavonoides y antocianinas se analizaron de acuerdo con los métodos de DPPH, fenol Folin-Ciocalteu, Dewanto et al., y pH diferencial, respectivamente. Los parámetros fisicoquímicos (sólidos solubles totales, pH y acidez total, fija y volátil y concentración de etanol) mostraron ligeros cambios, en las muestras procesadas por HHP y PEF de granada y capulín. El color de las muestras mostró cambios significativos ($p \leq 0.05$) en todas las muestras procesadas y pasteurizadas con HHP al final del almacenamiento, aumentando los parámetros de L^* . La actividad antioxidante, compuestos fenólicos, flavonoides y antocianinas aumentaron ligeramente, después de la aplicación de HHP y PEF. Además, los compuestos antioxidantes disminuyeron durante el almacenamiento en todos los tratamientos y muestras usadas. La evaluación sensorial mostró que las muestras de tratamiento

con HHP y PEF no fueron afectadas sensorialmente. Tanto las bebidas procesadas por HHP y PEF como las pasteurizadas fueron bien aceptadas por los jueces. La aplicación de HHP y PEF podría ser una buena alternativa para el procesamiento de bebidas de granada y capulín fermentadas para garantizar las características de seguridad antioxidante, sensorial y microbiana.

GENERAL ABSTRACT

Pomegranate and black cherry are fruits seasonal that grow in larger quantities than those that are consumed. Some researches show that these fruits have beneficial properties for health due to their antioxidant compounds. These compounds are mainly phenolic and flavonoids, which attribute to fruits a high antioxidant capacity. Pomegranate and black cherry fruits are consumed mostly in fresh and in a lesser way as processed products. To increase their value, these fruits can be used as raw material for a food product. Currently people demand more natural products, which is an incentive to design foodstuffs that are produced and preserved using other conservation techniques such as fermentation and emerging technologies. Therefore, the aim of this research was to develop pomegranate and black cherry fermented beverage, using novel technologies such as high hydrostatic pressures (HHP) and pulsed electric field (PEF), for their microbiological, antioxidant, physicochemical and sensory stability. A MINI FOODLAB FPG5620 high pressure (HP) pilot plant equipment was used to treat pomegranate fermented beverages at 200, 400, 500, 550 MPa (10 min) and 600 MPa (5 min). To PEF methodology a continuous-flow, bench-scale system ELCRACK-HVP 5 (Quakenbrück, Germany). The applied electric field strength was: 11.7 (50 %, 15 μ s) and 18.8 (80%, 20 μ s) kV/cm (frequency of 200 Hz). The pomegranate and black cherry beverages were also pasteurized using the VAT and HTST technologies. After the treatments the fermented beverages were stored at 4°C and analyzed throughout 42, 49 and 56 d of storage. The antioxidant activity, total phenols, flavonoids and anthocyanins were analyzed according to the methods of DPPH, phenol Folin-Ciocalteu, Dewanto et al., and differential pH, respectively. The physicochemical parameters (total soluble solids, pH and total acidity, fixed and volatile and ethanol concentration) showed slight changes in the samples processed by HHP and PEF. The color of the samples showed significant changes ($p \leq 0.05$) in all the samples processed and pasteurized with HHP and PEF at the end of storage, increasing the parameters of L^* . The antioxidant activity, phenolic compounds, flavonoids and anthocyanins increased slightly, after the application of HHP and PEF. In addition, antioxidant compounds decreased during storage in all treatments and samples used. The sensory evaluation showed that the samples of treatment with HHP and PEF were not affected sensory. Both beverages processed by HHP and PEF and the pasteurized ones were well accepted by the judges. The application of HHP and PEF could be a good alternative for the processing of fermented black cherry and pomegranate to guarantee the antioxidant, sensory and microbial safety characteristics.

I. INTRODUCTION

In Mexico, there are little known fruits, these fruits are generally consumed sporadically and seasonally, and are not so commercially exploited. They are sown and grown in quantities greater than they are consumed, which results in significant post-harvest losses. They represent a valuable agricultural resource, which has been poorly used and should be exploited, due to the antioxidant compounds they have.

The pomegranate fruit (*Punica granatum* L.) comes from a shrub origin to the region from Iran to the north of India. It was introduced to America by the Spaniards. Today it is widely distributed in the tropical and subtropical regions of the world. In Mexico, the production of pomegranate fruit is concentrated mainly in seven states: Oaxaca, Hidalgo, Guanajuato, Morelos, Sonora, State of Mexico and Puebla (Cintora-Martínez et al., 2017). Nowadays, this fruit has gained importance for its beneficial health properties. Several studies suggested that pomegranate juice can exert antiatherogenic, antioxidant, antihypertensive, and anti-inflammatory effects (Sahebkar et al., 2017). These benefits are attributed to the antioxidant properties of the fruit, due to its polyphenols, such as flavonoids and phenolic compounds (Bektas and Ozturk, 2007).

The black cherry (*Prunus serotina* subsp. *Capuli*) is another fruit cultivated in Mexico. It belongs to the *Rosaceae* family. It is a tree native to America (Mc Vaugh, 1951). In Mexico, it grows mainly in the states of Guanajuato, Querétaro and Veracruz (Hurtado and Pérez, 2014). The fruit of the black cherry is a globose drupe, of reddish black color in the maturity, of 12 to 20 mm of diameter, the fruit has a bittersweet and astringent flavor; black cherry contains only one seed (Rodríguez, 2011). The black cherry is distinguished as a fruit with a high content of nutrients and antioxidant compounds. The black cherry fruit has a wide variety of phenolic compounds, such as flavonoids, and tannins (Jiménez, Castillo, Azuara and Beristain, 2011).

Due to the nutritional and antioxidant properties in pomegranate and black cherry fruits, these fruits should be exploited as a raw material for a food product. The fermentation process is a method of food preservation, which may improve the content of the bioactive and nutritional compounds of a food, also provides products with very attractive sensory attributes and health-promoting properties. In many regions of the world, fermented beverages have been known for these beneficial attributes

to health. Fermented beverages have been a fundamental part in the culture and dietary habits of the population of many Western countries. An example of this is the red wine.

The commercial industry of fermented beverages uses methods such as pasteurization, the addition of chemical compounds such as nitrates, filtration, cold storage, freezing and heating, to inactivate microorganisms and maintain the physicochemical and organoleptic quality of beverages. However, the use of these methods may change some of the above-described quality characteristics of the beverages. Particularly important disadvantages of conventional thermal processing technologies are chemical reactions leading to off flavors, destruction of nutrients, and other losses of product quality (Mújica-Paz et al., 2011). Unfortunately, most phenolic compounds in fruits and vegetables are destroyed during traditional thermal treatment and subsequent storage due to their high instability.

Novel-technologies such as high hydrostatic pressures (HHP) and pulsed electric field (PEF) may have the potential to change the heating method and reduce or even eliminate heat treatment. High hydrostatic pressure (HHP) processing and pulsed electric field has been developed as an innovative nonthermal preservation technologies, which offers many advantages over thermal processing. HHP and PEF processing can inhibit microorganisms and inactivate enzymes with limited impact on the sensory and nutritional qualities of food materials ensuring food safety of products as well as maintaining maximum freshness of them.

High hydrostatic pressures (HHP) subject food to pressures of 100-1000 MPa using water as a pressure transmitting medium at moderate or refrigerated room temperature (Zhao, Zhang, & Zhang, 2016). PEF utilizes very short pulses (in milliseconds, or even in microseconds) of a high voltage (depending on purpose ranging from 1 to 80 kV/cm), on foods placed between the two electrodes. PEF processing involves very short, but strong pulses thus shortening total processing time (TPT) with a high efficiency (Gabrić et al., 2017). Recently, the effects of HHP and PEF processing on the retention of health-promoting phenolic compounds and antioxidant activity of fruit and vegetable products have gained widespread attention from researchers.

II. JUSTIFICATION

Pomegranate and black cherry have become an important fruits because of its considerable nutritional and antioxidant characteristics. They contain sugars, organic acids, amino acids, polysaccharides, vitamins, minerals, and polyphenols. The pomegranate and black cherry polyphenols include flavonoids (flavonols, flavanols and anthocyanins among others), condensed tannins (proanthocyanidins), and hydrolysable tannins (Sepúlveda et al. 2010). High antioxidant activity has been reported in pomegranate arils and black cherry pulp (Tehranifar et al. 2010; Gil et al. 2000; Tzulker et al. 2007). Studies of pomegranate and black cherry polyphenols have shown anticarcinogenic, anti-cardiovascular, antimicrobial, and anti-inflammatory properties (Sepúlveda et al. 2010; Tehranifar et al. 2010; Gil et al. 2000). Pomegranate juice has shown a threefold higher antioxidant activity than red wine or green tea (Sepúlveda et al. 2010) and 2-, 6- and 8-fold higher antioxidant activity than that detected in grape/cranberry, grapefruit, and orange juices, respectively (Gil et al. 2000). Pomegranate juice are a source of anthocyanins (red color), mostly anthocyanin-3-glucoside, 3,5-diglucoside, and derivatives of delphinidin, cyanidin, and pelargonidin (Gil et al. 2000). Products of pomegranate and black cherry fruit have great benefits to the human health due to the high content of polyphenols. Pomegranate and black cherry are mostly consumed in a fresh fashion; however, it is also consumed as juice and processed products such as jams, syrups, jellies, among others (Gumienna et al. 2016). Also, black cherry, as most of the fruits, is rich in nutrients and antioxidants. It possesses a great variety of total phenolic compounds, such as flavonoids and tannins. Flavonoids in the fruit belongs to the anthocyanins group, mainly cyanidin-3-O-glucoside and cyanidin-3-O-rutinocide (Jiménez et al., 2011). These compounds also provide a great antioxidant activity to the fruit (694 mg Trolox/100 g), even greater antioxidant activity than other fruits such as blackberries, guavas, and grapes: 279.18, 324.53 and 363.17 mg Trolox/100 g, respectively (Jiménez et al., 2011; Hurtado and Perez, 2014).

Changes to pomegranate and black cherry juices might be done to modify its flavor by fermentation; therefore, to increase its shelf life. Fermented products, such as wines, probiotic beverages, yogurts, and plant products have shown benefits to the human health, being documented elsewhere by scientific researches (Gumienna et al. 2016). Due to its high content of sugars, pomegranate and black cherry juices may be a good raw material to carry out an alcoholic fermentation (Zhuang et al. 2011). The fermentation process might provide new potential applications for pomegranate and black

cherry; the process may yield a liquid beverage that may keep its nutritional properties and be a functional beverage. Some researchers have suggested that this process increases the digestibility and bioavailability of nutrients and bioactive compounds found in the pomegranate juice (Gumienna et al. 2016). During fermentation, the changes taking place along the process, and until the end, modifies the sensory (flavor, aroma, color, and texture) and physicochemical characteristics of the pomegranate juice, improving its content in bioactive compounds. Some researchers have suggested that the chemical reactions taking place during fermentation generate different compounds for producing a completely different product with many metabolites (Gumienna et al. 2016), responsible of flavor, aroma, and textural characteristics.

Thermal treatments are the most used preservation methods for liquid foods to inactivate microorganisms and/or enzymes. However, when processing with high temperatures, irreversible losses of nutritional compounds may occur as well as unwanted changes in physicochemical and sensory properties (Sánchez-Moreno, Plaza, Elez-Martinez, De Ancos, Martín-Belloso & Cano, 2005). A disadvantage of the thermal processing is the slow conduction and convection of heat. The effectiveness of heat treatments can also be affected by the complexity of the product and type and load of microorganisms. Another, negative effect of overheat processing is that the sensory attributes of a food may change. The preservation of the sensory characteristics is also very important in a food product (González & Barrett, 2010). In addition to the conventional thermal processing, there are other treatments or novel-technologies that do not use heat (non-thermal technologies), for processing foods. Among these emerging non-thermal technologies are high intensity pulsed electric fields (PEF). PEF is one of the most attractive technologies due to its short treatment times and the reduction of heating effects with respect to other technologies. High-intensity electric pulse fields are highly valued as a non-thermal food preservation technology that involves the discharge of short, high-voltage electric pulses through food (Barbosa-Cánovas & Altunaka, 2006). PEF processing offers potential applications for the winemaking process, since it could improve wine quality, inactivating microorganisms, maintaining bioactive compounds and maintaining adequate organoleptic characteristics (Puértolas, López, Condón, Álvarez, & Raso, 2010). Successful inactivations of about 4 cycles \log_{10} in yeasts and more resistant bacteria have been observed, after a treatment with PEF at 35 kV/cm for 1 ms at a frequency of 303 Hz (Marsellés-Fontanet, Puig, Olmos, Mínguez-Sanz, & Martín-Belloso, 2009). The application of PEF before fermentation or after fermentation could be enough to avoid contamination of the wine and control the development of physical, antioxidant and sensory alterations, by having the finished product or during its subsequent storage (Puértolas et al., 2010). For other hand, processing by high hydrostatic pressures (HHP) is a novel technology that is increasingly used in the food industry due to its favorable application in the processing of acidic foods such as jams, juices and other fruit products. The main advantages of the high hydrostatic pressures for food preservation are the elimination or significant reduction of heating, thus avoiding the thermal degradation of food components (Cheftel, 1995, Welti-Chanes,

Ochoa-Velasco, & Guerrero-Beltrán, 2009; et al., 2012). Researches has shown that the processing with HHP may efficiently improve the microbial stability of food products (Simpson, & Gilmour, 1997; Ritz, Minet, Laclie, & Federighi, 2000; Pérez, Grande, Gálvez, & Lucas, 2017; et al., 2019). In addition, changes in low molecular weight compounds such as micronutrients, phenolic compounds and sensory input agents are minimal because covalent bonds do not break during the high-pressure process (Cheftel, 1992; Ramírez, Saraiva, Pérez, & Torres, 2009).

III. RESEARCH AIMS

3.1. General

- ❖ Develop fermented beverages from unconventional fruits with antioxidants (pomegranate and black cherry).

3.2. Specific

- ❖ Determine the yield of pomegranate and black cherry juice.
- ❖ Evaluate three adjustment conditions of total soluble solids in the pomegranate and black cherry juice for the fermentation process to choose the most effective condition.
- ❖ Make the physicochemical, microbiological and antioxidant characterization of the juices and the fermented beverages of pomegranate and black cherry.
- ❖ Evaluate two emerging technologies as methods of conservation of fermented beverages.
- ❖ Determine the physicochemical, microbiological, sensory and antioxidant stability of fermented beverages during storage.
- ❖ Sensory evaluate fermented beverages of pomegranate and black cherry at the end of storage.

IV. HYPOTHESIS

The fermentation process modifies the antioxidant, physicochemical and sensory composition of pomegranate and black cherry juices. The use of processing by emerging technologies, such as high hydrostatic pressures and pulsed electric field, provide a fermented pomegranate and black cherry beverages microbiologically safe, sensorially acceptable and with amounts of antioxidants after of a storage time in refrigeration.

V. THEORETICAL FRAMEWORK

5.1. Antioxidants

5.1.1. Polyphenols

There is a wide variety of compounds in nature, which have a molecular structure characterized by the presence of one or several phenolic rings. These compounds are called polyphenols. They originate mainly in plants, which synthesize them in great quantity, as a product of their secondary metabolism. Some are essential for plant physiological functions. Others participate in defense functions in situations of stress and various stimuli (water, light, etc.) (Manach et al., 2004).

There are several classes and subclasses of polyphenols that are defined according to the number of phenolic rings they have and the structural elements that these rings present; Its structure can vary from simple molecules to highly polymerized compounds. The phenolic compounds in foods usually do not appear free but in glycosylated form. The main polyphenols are classified into three groups: simple phenols and phenolic acids (derivatives of hydroxybenzoic acid or hydroxycinnamic acid), flavonoids and tannins (Manach et al., 2004, Quiñones et al., 2012).

Likewise, polyphenols present antioxidant activity in foods, so obtaining and preparing products with a high content of these compounds suppose health benefits. The antioxidant behavior of polyphenols seems to be related to their ability to chelate metals, either by maintaining or increasing their catalytic activity or by reducing them (Vauzour et al., 2010).

5.1.2. Phenols and phenolics acids

Simple phenols and phenolic acids have an aromatic ring with one or more hydroxyl substituents, include the hydroxybenzoic acid and hydroxycinnamic acid derivatives (Fig. 1). Two main groups of phenolic acids are distinguished: benzoic acids and cinnamic acids (Meillón, 2010).

The benzoic acids or hydroxybenzoic acid derivatives have a basic molecular structure (C6-C1), their derivatives are: gallic, salicylic, p-hydroxybenzoic, protocatéuico, vanílico and syrinic acid; its content is generally very low in the edible parts of the plants except for some red fruits. Gallic acid can be conjugated as such or with its dimers (ellagic acid), trimers (tergalic acid) or tetramers (galágic

acid), gallic and ellagic acid are found in high amounts in red fruits (Scalbert and Williamson, 2000; Manach et al., 2004; Meillón, 2010).

Cinnamic acids or derivatives of hydroxycinnamic acid are widely distributed in plants in conjugated form since they are rarely found in free form. Its derivatives are: caffeic acid (3,4-hydroxycinnamic), ferulic acid (4-hydroxy-3-methoxycinnamic), p-coumaric acid (4-hydroxycinnamic), synampic acid (4-hydroxy-3,5-dimethoxycinnamic) and chlorogenic acid (5 caffeoyl quinic). Red fruits are a significant source of these acids. Caffeic acid is the most abundant in many fruits (Manach et al., 2004; Meillón, 2010).

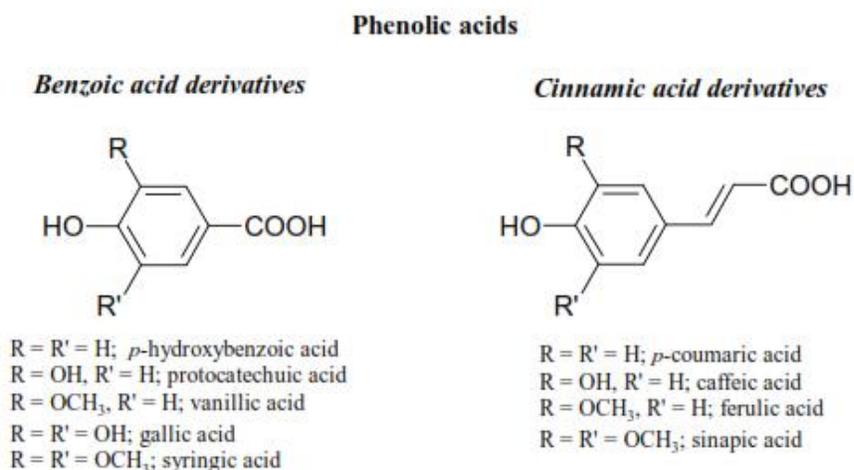


Figure 1. Molecular structure of phenolic acids (Pereira *et al.*, 2009).

5.1.3. Flavonoids

They are characterized by having two aromatic benzene rings joined by a bridge of three carbon atoms, with the general structure C₆-C₃-C₆, which may or may not form a third ring. The rings are called A, B and C; the individual carbon atoms are referred by a number system, which uses ordinary numbers for rings A and C and prime numbers for ring B. Natural flavonoids usually have at least three phenolic hydroxyl and are usually combined with sugars in glycosylated form, although they also occur relatively frequently as free aglycones (Fig. 2) (Cartaya and Reynaldo, 2001).

Flavonoids are grouped into anthocyanins and anthoxanthines. Anthocyanins are orange, blue and purple red pigments present in plants, whose phenolic structure gives them a marked antioxidant activity by the donation of electrons or transfer of hydrogen atoms to free radicals. The anthoxanthines include flavonols, flavones, flavanols and isoflavones that are colorless or colored molecules that range from white to yellow (Gimeno, 2004, Meillón, 2010).

On the other hand, several subgroups of flavonoids are classified according to the substitution of the C ring. This basic structure allows them to present a multitude of substitutions and variations giving

rise to flavonols, flavones, flavanones, flavanolols, isoflavonoids, catechins, calconas, dihydrochalcone, anthocyanidins and leucoanthocyanidins. Among them, flavones (epigenina, luteolina and diosmetina) and flavonols (quercetin, mirecitina and kampferol) are the most abundant compounds in vegetables. In this classification, the oxidation state of the heterocyclic ring and the position of the B ring are very important (Cartaya and Reynaldo, 2001; Meillón, 2010).

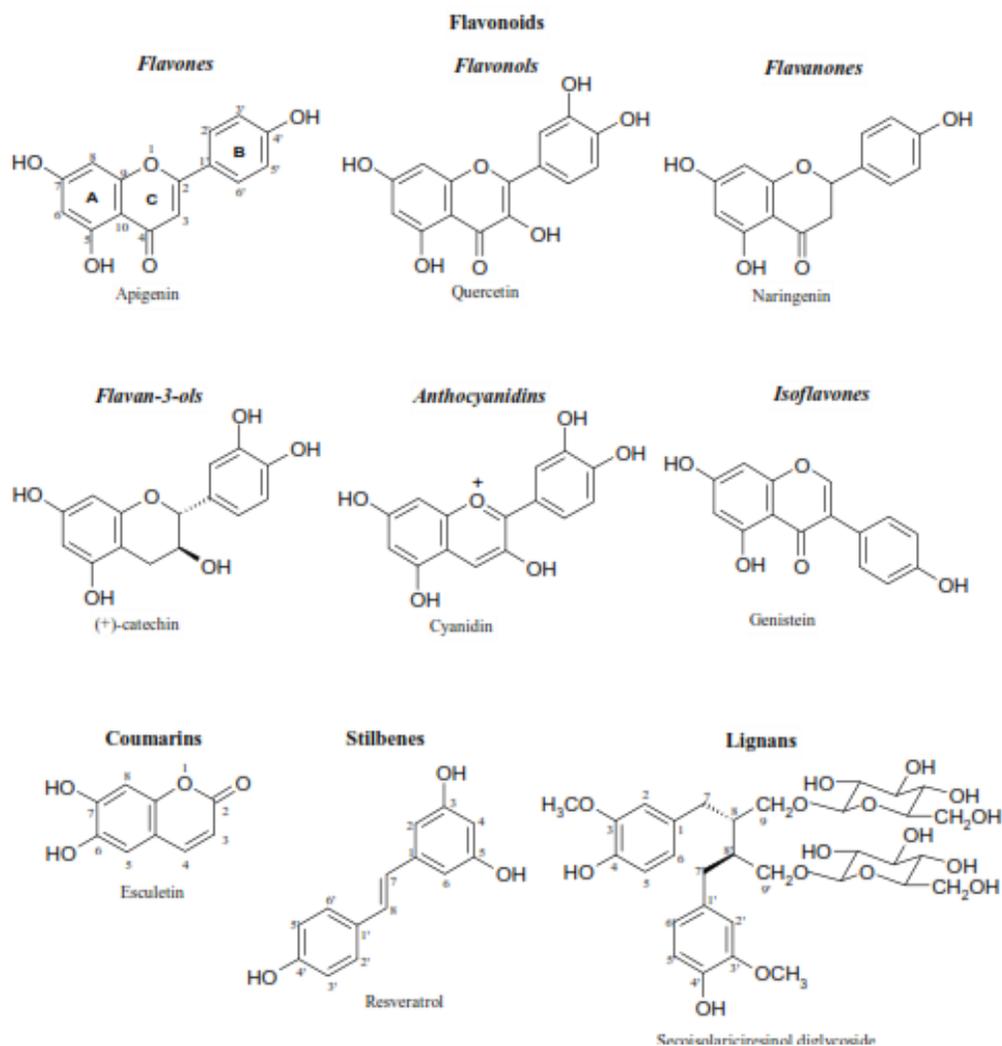


Figure 2. Molecular structure of phenolic acids (Pereira et al., 2009)

5.1.4. Tannins

Tannins may belong to the class of flavonoids; both flavonoid and non-flavonoid groups can be found forming very high molecular weight compounds (> 500 UMA). They have enough hydroxyl groups, linked to phenolic structures that give them the characteristic of forming complexes with proteins, minerals and other macromolecules (Olivas-Aguirre et al., 2015).

Each group of flavonoids originates a specific type of tannins: non-flavonoids, which polymerize to form hydrolysable tannins and certain flavonoids that, when polymerized, form condensed tannins. Hydrolysable tannins, such as gallotannins or ellagitannins, come from the esterification of non-flavonoid polyphenolic compounds, such as gallic or ellagic acid, respectively. On the other hand, condensed tannins or proanthocyanidins come from the esterification of flavonoid polyphenolic compounds, such as catechins or flavan-3-oles (Vázquez-Flores et al., 2012). However, other authors classify tannins into two more groups: the florotannins (derived from brown algae) and the complex tannins (Olivas-Aguirre et al., 2015).

The hydrolysable tannins contain a central core of polyhydric alcohol in which glucose and hydroxyl groups are partially or fully esterified with phenolic groups, after hydrolysis with acids, bases or enzymes, the gallotannins produce glucose and gallic or hexahydroxydiphenic acids and the polyol of origin. On the other hand, condensed tannins are very abundant colorful substances in fruits. The condensed tannins can be oxidatively degraded to anthocyanins (Millón, 2010, Olivas-Aguirre et al., 2015).

5.2. Pomegranate (*Punica granatum* L.)

5.2.1. Generalities of pomegranate

The pomegranate or pomegranate is a plant that belongs to the family *Punicaceae*, is native to Asia from a region that extends from Iran to the north of the Himalayas in India; It is a shrub of 3 to 6 m in height, its flowers can be scarlet, white or shaded, bell-shaped substrata, with 5-8 petals and sepals. The flowers are of two types large or hermaphrodite (fertile) and small that are male flowers (sterile) (Meillón, 2010). The pomegranate fruit is a large berry with leathery and globose thick skin 10 to 15 cm in diameter. This encloses inside arils, grains or seeds that correspond to the edible part of this fruit. Grains or seeds are a food with important nutritional properties; these have a woody, fleshy or pulpy consistency, have a prismatic shape, their color varies from an intense red to a light red, and bittersweet flavor (García and Pérez, 2004).

There are several varieties of pomegranates in the world, however, the main variety marketed worldwide is the Wonderful (weight of 200 to 700 g), which has a weight of 200 to 700 g, has a uniform red vermilion rind, its seeds or arils are large, dark red and semi-acid flavor. In Mexico the main varieties are: Apaseo (approximate weight 300 g, thick yellow to orange peel, red at maturity, bright red seeds and sweet flavor, harvest from July to September), Late Apaseo (approximate weight 400 g), thin skin of green to yellow, dark red seeds of sweet flavor, harvest from August to October) and Tecozautla (approximate weight more than 250 g, yellow to orange peel and pink shade at maturity, dark red seeds) sweet taste, harvest from June to August) (Mondragón, 2012; Díaz, 2014).

5.2.2. Geographical distribution, climatic conditions of growth and production

The pomegranate is a species native to South Asia, the area of Iran and Afghanistan. It has been cultivated for years in many countries of the Mediterranean basin and is found in almost all the warm regions of the world, especially in the subtropical ones. The Arabs introduced them to Spain and it was taken by the Spaniards to America (FAO, 2006). In Mexico it is found mainly in the states of Guanajuato, Hidalgo, Oaxaca and Puebla (SIAP, 2014).

It usually grows in subtropical or tropical warm climates, has the characteristic of an excessive requirement of water. It develops in deep, permeable and light soil. It is sensitive to frost and resistant to drought. It can be cultivated from sea level up to 1800 m in height. It develops practically in all soils and tolerates well the alkalinity and salinity of the same (FAO, 2006).

The main producing countries of Granada are: Israel, Lebanon, Egypt, Tunisia, Spain, Italy and the United States. The best regions with export markets for pomegranate and pomegranate products are Pakistan and India (FAO, 2006). Since the eighties the production of pomegranate has been stable, being between 800 000 and 1 million tons; However, since the beginning of the 21st century, crops have increased to a production of more than 2 million tons. The export of pomegranate in the world is not as widespread as other fruits, so it is included in the category "other exotic fruits", so there is no net data regarding the volumes that are marketed (FAO, 2006 ; Díaz, 2014).

In Mexico, pomegranate cultivation is minimal compared to the main producing countries. In the year 2014, the production of pomegranate was 4,362.82 tons, being the states of Oaxaca, Hidalgo and Guanajuato, the main producers, which contributed 77% of the national production. However, since 2002 Mexico has imported fruit from the Wonderful variety produced in California, to satisfy the demands of consumers (Díaz, 2014, SIAP, 2014).

5.2.3. Chemical composition

The pomegranate is mostly made up of water and sugars, with a lower content of fats (1.17 g/100 g of fresh fruit (FF)) and fatty proteins (1.67 g/100 g of FF), which gives it a low value caloric (approximately 83 kcal/100 g). Likewise, it is important to highlight its high micronutrient content, such as vitamin C, phosphorus, magnesium and potassium (USDA, 2009, López-Mejía et al., 2010). On the other hand, Calín-Sánchez et al. (2008) analyzed the content of organic acids in pomegranate fruit. The most commonly found organic acids were, phytic (10.50 g/100 g), malic (2.49 g/100 g) and citric (0.52 g/100 g). Citric acid has been reported as the most abundant organic acid in pomegranate; however, in this work it was reported, as the majority acid to phytic acid. Other acids detected in lesser amounts were oxalic, tartaric, and ascorbic acid.

Currently, the consumption of pomegranates has become important, since it is rich in antioxidants (Rajan et al., 2011, Mena et al., 2011, Shiban, et al., 2012). Within its antioxidant compounds are phenols, flavonoids and tannins. Viuda-Martos et al., (2010) and Fischer et al., (2013) reported approximately fifty compounds in pomegranate juice, among the main ones were: anthocyanins, gallotannins, ellagitannins, catechins, quercetin, rutin, galagil esters, hydroxybenzoic acids, hyoxynamic acids and dihydroflavones.

Likewise, Gil et al. (2000), García and Pérez (2004), Mousavinejad, et al. (2009) and Moghaddasi and Haddad (2011), reported that the pomegranate is characterized by the presence of pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside, mainly; in lesser amounts are cyanidin-3-glucoside and cyanidia-3,5-diglucoside. It is important to note that the composition, as well as the concentration of compounds found in pomegranate, is variable according to the varieties and cultivation conditions (Gorena, et al., 2010).

5.2.4. Pomegranate food products

The pomegranate is used in different ways. The main use of this fruit in different countries is its fresh consumption. The industrialization of this is done to obtain products of food, pharmaceutical or cosmetic interest: juices, syrups, jams, jams, jellies, dried seeds, dietary fiber, dried bark to prepare infusions, pomegranate oil and extracts from its different parts (Andreu et al., 2008).

Likewise, due to the difficulty of extracting pomegranate seeds for consumption as a fresh product, there is an interest in the food industry to process the seeds of the pomegranate through the production of minimally processed arils, which give the consumer a clean final product ready to be consumed (Sepúlveda et al., 2001). On the other hand, the content of pigments in pomegranate, makes it also used as a natural colorant in food (Gorena et al., 2010).

Currently, pomegranate juice is the most common form of use of this fruit, either naturally or processed. Some commercial brands of pomegranate juice are Jumex®, Del Valle®, Sonrisa®, OceanSpray®. However, most of the time, these juices are mixed with another, mainly apple and therefore its phenolic composition is different from natural juice (Gorena et al., 2010).

5.3. Black cherry

5.3.1. Generalities of black cherry

The black cherry belongs to the family *Rosaceae*. The tree of the black cherry has a height of 5 to 15 m. their inflorescences can present diverse forms, the flowers can be solitary or fasciculate, they are commonly hermaphroditic and rarely unisexual. The fruit is a drupe globose, has a smooth or rough bone, and usually contains a single seed with scarce or absent endosperm, the fruit is reddish-black

at maturity, from 12 to 20 mm in diameter, has a flavor bittersweet and astringent; It contains only one seed (Rodríguez, 2011). In Mexico and other countries of America, the black cherry is also known as the American black cherry tree (Hurtado and Pérez, 2014).

5.3.2. Geographical distribution, climatic conditions of growth and production

The black cherry is distributed from southeastern Canada, to Guatemala and Central America. In Mexico it is commonly found in the states of Guanajuato, Querétaro, Veracruz, Puebla, Sinaloa, San Luis Potosí, Hidalgo, Michoacán, Oaxaca and Chiapas. The black cherry tree grows generally in humid forests of encino and conifers, as well as in the mesófilos of mountains. It grows in heights of 1000 to 3200 m, it flowers in the months of March and April; its fruit ripens between May and August (Rodríguez, 2011).

Like the pomegranate, the black cherry is considered "other exotic fruits", so there is no net data regarding the volumes that are commercialized (FAO, 2006). In Mexico, pomegranate cultivation is minimal compared to the main producing countries. In 2014, the production of black cherry was 253.82 tons, with the state of Querétaro being the main producer. Being more sporadic in the south of Guanajuato and in the north of Michoacán (SIAP, 2014).

5.3.3. Chemical composition

The black cherry distinguishes like a fruit with big content of nutrients, is composed mainly by water (81.18%), stand out his content of fiber, protein, vitamin C and of minerals like the calcium, potassium, magnesium and phosphorus (Guijarro, 2013, Luna-Vázquez et al., 2013).

Luna-Vázquez et al. (2013) compared the chemical composition of the black cherry with plum and grape, because they are fruits similar to the black cherry and of great consumption. The black cherry had higher protein content (2.10 mg/100 FF) than plum and grape (0.49 and 0.46 mg/100 FF, respectively). The moisture content, fat, fiber and ash, was similar in the three fruits. Likewise, minerals such as K, Ca, P and Mg had higher values in black cherry, than in plum and grape. This indicates that the black cherry represents a good source of nutrients, similar and even higher in content than other fruits.

The black cherry also contains a great variety of phenolic compounds, such as flavonoids, and tannins. The type of flavonoids presents in the black cherry fruit belong to anthocyanins, mainly cyanidin-3-glucoside and cyanidin-3-rutinocide (Jiménez et al., 2011, Hurtado and Pérez, 2014). Villa

(2008), Rodríguez (2011), Luna-Vázquez et al. (2013) and Hurtado and Pérez (2014), determined the amount of total phenols in black cherry and reported a content of 316-818, 240.1, 362.2 and 242 mg EAG/100 g FF, respectively. The highest reported content was that of Villa (2008), which may be due to the solvent used to prepare the sample or the characteristics of the fruit (area of origin, maturity stage, temperature and time of extraction, etc.).

Rodríguez (2011), detected by HPLC and quantified the following antioxidant compounds in the black cherry: chlorogenic acid, epicatechin, and quercetin (8.52, 0.52, 3.0 mg/100 g FF, respectively). Likewise, García-Aguilar et al. (2015), also found chlorogenic acid, gallic acid, caffeic acid, catechin, epicatechin, quercetin, kaempferol and glycosides.

5.3.4. Food products of black cherry

The fruit of the black cherry is a little used fruit, it is generally consumed fresh in the places where it is distributed or found. The seeds are prepared toasted as a snack. This fruit is generally used as a raw material for jams, jellies and preserves, as well as tamales, fermented beverages, liqueurs and sweets. Also, it is used as an infusion for certain conditions, such as sore throat, stomach, inflammation, etc. (Hurtado and Pérez, 2014). In other countries (United States, Brazil, Canada, Chile and Spain) the black cherry is used as an ingredient in some foods and beverages and as a food supplement for its antioxidant properties (Villa, 2008).

5.4. Fermented beverages

Alcoholic beverages can be grouped into two broad categories: fermented beverages (wine, beer) and distilled beverages (whiskey, rum, brandy). Fermented drinks are those that are manufactured using only the fermentation process, in which a microorganism (yeast) is transformed into sugar alcohol. With this process only, beverages with a maximum alcohol content equivalent to the maximum tolerance of the microorganism are obtained (Freile, 2011).

5.4.1. Wines

The International Organization of Vine and Wine, defines wine in its International Code of Oenological Practices, as the drink that results from the complete or partial alcoholic fermentation of fresh grapes, crushed or not, or of grape must, and clarifies that its acquired alcoholic strength can not be less than 8.5. On the other hand, the European Union defines wine as the product obtained exclusively by alcoholic fermentation, total or partial, of fresh grapes, crushed or not, or of grape must (OIV, 2016, E. U., 2008).

There are also other types of wine, when another type of fruit is used, the product is always called wine, but followed by the name of the fruit. Although the vine wine comes from a fruit, the denomination of fruit wines is frequently applied to fermented beverages with a preparation very

similar to that of the traditional wine (of vine). Fruit wines are produced in countries where the climate makes it difficult or impossible for the natural production of vines and instead allows the production of vinifiable fruits. Depending on the concentration of alcohol in the final product, fruit wine can be classified as dry or sweet (Freile, 2011).

5.4.2. Process of making a fermented beverage

5.4.2.1. Fruit, reception, storage and juice extraction

The first stage of the wine making process is the reception of the fruit. It is important that it is in good condition to be processed. If not used at the time, it must be stored in refrigeration (6°C). For the preparation of the juice, the fruit should be washed, remove the peel and perform the extraction of the same. Extraction is the operation in which the separation of the pulp from other residues such as seeds, husks and others is achieved (Freile, 2011).

5.4.2.2. Fermentation process

In general terms, fermentation is described as an oxidation process in which the transformation of complex molecules into simple molecules leads to the generation of an organic final product, with the release of energy. Alcoholic fermentation is an anaerobic process performed by yeasts and some kinds of bacteria. Where the cellular substrate; Most mono and disaccharides are transformed mainly in ethyl alcohol and carbon dioxide; with the generation of reduction equivalents of the compounds NADH/NAD⁺ and NADHP/NADP and high energy phosphate bonds, ATP (Nielsen 2003). The energy is synthesized as ATP from a process of glycolysis followed by the metabolism of pyruvate. In this way, fermentation complements glycolysis and makes it possible to produce energy in the absence of oxygen (Nielsen 2003). Figure 3 shows the conversion of glucose to alcohol.

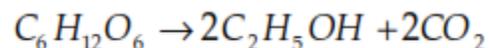


Figure 3. Products obtained from the fermentation of glucose

Yeasts are the main responsible for this transformation. *Saccharomyces cerevisiae*, is the most commonly used yeast species. Although it seems, at the stoichiometric level, a simple transformation, the sequence of transformations to degrade glucose to two molecules of alcohol and two molecules of carbon dioxide is a very complex process, because at the same time the yeast uses glucose and nutrients additional to reproduce (Freile, 2011).

5.4.2.3. Filtered and clarified

The clarification of the wine is done by adding bentonite from 50 to 100 g/Hl or pectic enzymes dissolved to 0.001%, to eliminate residual yeast and pulp. After one month a filtration is carried out again, a sample of the wine is taken to observe its transparency, if it is still cloudy, the clarification operation with bentonite is repeated. After this process the wine must be free of particles and clarified with the achieved alcohol content (Freile, 2011).

5.4.2.4. Packaging, sealing and storage

The bottles that will be used for the packaging of the wines must always be new and before being filled they are washed and sterilized. The current bottles have a standard volume of 750 mL. The necks of the bottles are filled with carbon dioxide to avoid any subsequent contamination of the wine. The wine is covered with corks, these are cylindrical and are made of natural cork which allows the wine to "breathe" and produce certain oxidation-reduction reactions that cause their aging (Freile, 2011).

Afterwards the bottles that require it are labeled and a collar is placed, where the month and year of the harvest appears, the label where you can see the commercial name, type of wine, alcoholic degree, emblem of the house and some related information with wine. Finally, the wine is stored at room temperature (Freile, 2011).

5.5. Preservation methods

5.5.1. Pasteurization

Pasteurization is a conservation method widely used in liquid foods. There are several methods for pasteurization of such foods. The first is known as a low temperature and long time method, LTLT (for its acronym in English), which involves applying temperatures of 63-66 ° C for 30 minutes. The second uses temperatures of 71-75 ° C for 15 seconds and is known as the high temperature method in a short time, HTST (for its acronym in English). In both cases the product requires refrigeration after treatment to ensure shelf life to the product. The third method is to apply temperatures of 135-140 ° C for 2 to 10 seconds, for its characteristics is named as ultrapasteurization, UHT (for its acronym in English). Although the word pasteurization appears in its name, it is a more severe process. Ultrapasteurized food (UHT) is aseptically packaged, does not require refrigeration for storage and its shelf life is 3-4 months (Badui, 2006; Pérez-Reyes, 2013).

5.5.2. High hydrostatic pressures

High pressure technology is a method used in food preservation, where food is subject to high pressures, with or without the addition of heat; with the purpose of inactivating microorganisms and

in deteriorating enzymes and creating desirable attributes in food (texture, smell, taste) for consumers (Téllez-Luis et al., 2001).

A high-pressure hydrostatic system consists of a high-pressure cylinder and its closure, a pressure generation system, a temperature control device and a support system for the material. Once loaded and closed, the vessel is filled with a pressure transmission medium (water or water / oil mixture). Air is removed from the vessel with a low-pressure, fast-filling, drained pump, in combination with an aeration valve, then high hydrostatic pressure is generated (Velázquez et al., 2005).

In this process the food product that is going to be treated is placed in a container capable of sustaining the pressure requirements, the product is submerged in water, which acts as a means of pressurization, or is placed in a hermetically sealed package that will transmit the Pressure. The product is generally treated in the primary final packaging, the main characteristic of which is that it is capable of watering changes in volume corresponding to the applied compression. Because foods experience a decrease in volume because of applied pressure and expansion of equal magnitude during decompression, the packaging used in high pressure processes must be able to withstand a 15% reduction in volume, as well as being able to return to its original volume without losing the integrity of the seal or its protective properties (Téllez-Luis et al., 2001).

High pressure treatments generally do not change the color, odor, taste or other characteristics of foods compared to conventional thermal treatments. It is known that high pressures cause an insignificant effect in low molecular weight food components, such as the compounds responsible for flavor, vitamins and pigments, compared to thermal processes. This effect is particularly important in vegetables or fruits, which have a high content of vitamins, antioxidants and pigments (LeBail et al., 2002).

Microbiological inactivation by high pressures can be the result of several factors. One of these factors is the change in the permeability of the cell membrane, including the denaturation of the proteins caused by the breaking of the bonds. The use of high pressures affects the morphology in several aspects: the structure of the vacuoles can collapse, elongation of the cell and affects the mobility of microorganisms. One of the main affected sites is the cell wall. It has been established that pressures greater than 300 MPa cause the irreversible denaturation of enzymes involved in DNA replication and transcription, so that the part of the mechanism of microbial inactivation can be attributed to the inactivation of enzymes (Téllez-Luis et al., 2001).

5.5.3. Pulsed electric field

Pulsed electric field are a non-thermal treatment for food preservation in which a fluid, semi-fluid or solid food is placed in an electrolytic solution between two electrodes for short periods of time (less than one second) and a certain number of High voltage pulses ranging from 20 to 80 kV/cm for the

inactivation of microorganisms, from 2.5 to 90 kV/cm for the inactivation of enzymes and from 0.5 to 1 kV/cm for the extraction of compounds from intracellular compounds . This technology has been widely used in the inactivation of molds, yeasts and bacteria. The benefit of this technology is to provide foods with characteristics like fresh ones and with an extended shelf life (Van Loey and Hendrickx, 2002, Cerón-Carrillo et al., 2010).

Another factor that is important to consider is temperature. The main reason for applying electric pulses is a characteristic as a non-thermal process to minimize food damage and loss of nutrients. The treatment time is defined as the effective time during which the microorganisms are subjected to the force field. It is expressed as the product of the number of pulses, the duration of these and the field strength, which are the factors that determine the lethal effect of the treatment (Cerón-Carrillo et al., 2010).

It is considered that the electrical pulses affect the cytoplasmic membrane of the microorganism, which leads to the formation of pores, filtration of cellular components and therefore death. This effect can be reversible or irreversible depending on the intensity of the treatment. The limit value of the field strength must be exceeded to induce a critical transmembrane potential of -1 V. A field strength of 30 kV/cm is required for most bacteria in a liquid medium (Heinz et al., 2002: Cerón-Carrillo et al., 2010).

VI. METHODOLOGY

6.1. Materials

Fruit Pomegranate (*P. granatum* L.) fruits, “Apaseo” variety, were purchased at a local market in Puebla, Mexico. Pomegranates were chosen free from physical and microbiological injuries; then, washed, and disinfected for 1 min with a 150 µL/L hypochlorite sodium solution.

Black cherry (*Prunus serotina* subsp. *capuli*) was obtained in June, at the beginning of the fruit season. Fruits were acquired from a local market in Cholula, Puebla, Mexico. Black cherries free from physical and microbiological injuries were chosen, washed with tap water and remaining water removed with absorbent paper before obtaining the juice.

Yeasts *Saccharomyces cerevisiae* strain Ysr128 for wine fermentation was obtained from the Laboratory of Food Microbiology at Universidad de las Americas Puebla (UDLAP), Puebla, Mexico. *S. cerevisiae* was grown in yeasts broth at 25 °C until reaching the early stationary phase.

6.2. Juices extractions

To pomegranate juice arils were separated by hand from the fruit. The juice extraction from arils was performed using a Standard Turmix extractor (Switzerland).

The black cherries juice was extracted using a laboratory Waring Commercial blender model 31BL40 (New Hartford, Connecticut, USA). Whole fruits were blended and then sieved throughout cheesecloth for eliminating all insoluble residues and pieces of seeds. The obtained turbid juice was centrifuged (HERMLE Z326K, Wehingen, Germany) at 6000 rpm for 30 min to eliminate tiny particles. Clarified juice was centrifuged and analyzed in physicochemical, antioxidant and sensory characteristics. The yield of juice was calculated using Eq. (1):

$$\text{yield (\%)} = \frac{\text{mL of juice}}{\text{g of whole fruit}} \times 100 \quad (1)$$

6.3. Fermentation

Three fermentation conditions were tested based on the total soluble solids (°Brix) in pomegranate and black cherry fresh juices (FRJ). The three conditions were fresh juice with its initial total soluble solids (TSS) (13.9 °Bx) (FEB1) and adjusted to 17.5 (FEB2) or 25 (FEB3) °Brix using standard sugar

(Zafra, S.A. de C.V., México). Each juice was filtered throughout cheesecloth and then centrifuged at 5500 rpm for 20 min. Finally, one liter of each juice was inoculated with five milliliters of *S. cerevisiae* inoculum [(2.60 ± 0.21) × 10⁶ CFU/mL]. The fermentation of juices was performed at 25 ± 1 °C in an incubator until reaching a constant TSS content. The physicochemical and antioxidant characteristics were analyzed immediately after the extraction of fresh juice and fermented products. All analyses were performed in triplicate.

6.4. Thermal treatments

Pomegranate and black cherry beverages were pasteurized at low (VAT) and at high (HTST) temperatures. The VAT process consisted in heating the beverages in glass beaker at 63±2°C for 30 min and the HTST pasteurization was carried out by heating the FP beverage to 72±2°C for 15 seconds; therefore, beverages were cooled down rapidly in ice water. Both pasteurized samples were placed in sterile commercial glass bottles for later analysis.

6.5. HHP processing

An AVURE HHP processing equipment (2 L Technologies® Inc., Middletown, Ohio, USA) was used. Three batches of fresh and pomegranate and fermented black cherries beverages were prepared to be treated at 200 MPa-10 min, 400 MPa-10 min, 500-550 MPa-10 min and 600 MPa-5 (Tejada-Ortigoza, Escobedo-Avellaneda, Valdez-Fragoso, Mújica-Paz, & Welti-Chanes, 2014; Leyva-Daniel, Escobedo-Avellaneda, Villalobos-Castillejos, Alamilla-Beltrán, & Welti-Chanes, 2017). After being treated, beverages were immediately analyzed or stored at 4±1°C for 42 and 56 days.

6.6. Pulsed electric field processing

A continuous-flow, bench-scale system ELCRACK-HVP 5 (Quakenbrück, Germany), which delivers square-wave pulses in bipolar mode, was used to treat the beverage sample. The PEF system is composed of two cells that have two stainless steel electrodes separated by a gap of 5 mm. The pomegranate fermented beverages were placed in the hopper of the PEF equipment and passed through the electrodes at a flow rate of 70 l h⁻¹. Two PEF treatments were applied to fermented beverages. The applied electric field strength was: 11.7 (50 %, 15 µs) and 18.8 (80%, 20 µs) kV/cm (frequency of 200 Hz); output temperature, 18.3 ± 1 °C. About 5 L of sample was collected at the outlet of the equipment, placed in polypropylene sterile tubes (Falcon®, NY, USA), and stored at 4 ± 2 °C during analysis (García-García et al., 2015; Vazquez-Cabral et al., 2016).

6.7. Infrared spectroscopy (FTIR)

Mid-Infrared (MIR) spectra were obtained using a Bruker Vertex 70 FTIR spectrometer (Bruker, Vertex, Wisconsin, USA). Attenuated Total Reflectance (ATR) accessory and a Deuterated Triglycine Sulfate (DTGS) detector, which operates with a resolution of 4 cm⁻¹, were used. A drop of sample

was used for obtaining the spectra based on the selection of optimal signal to noise ratios. Each spectrum was collected and rotated against the background spectrum of the clean crystal surface to present the spectra in absorbance. Two spectra of each sample were obtained at room temperature. After each measurement, the crystal surface was cleaned with demineralized water and dried with a soft paper. Therefore, the next sample was analyzed.

6.8. Shelf-life of beverages

Psychrophilic (P), mesophilic aerobic bacteria (MAB) and molds plus yeasts (MY) were evaluated using Plate Count Agar (PCA) and Dichloran Rose-Bengal Chloramphenicol (DIFCO, Sparks, MD, USA) methods. The inoculated petri dishes were incubated at 4, 25 and 37 °C, respectively. Psychrophilic and yeast plus molds were counted after 96-120 h and mesophilic after 24-48 h of incubation. Microbial counts were reported as log₁₀ (CFU/mL) reductions.

6.9. Antioxidant characteristics

6.9.1. Antioxidant capacity

The evaluation of the free radical-scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used to measure the antioxidant capacity (Brand-Williams, Cuvelier, & Berset, 1995). An aliquot of 10 µL of sample was mixed with 1990 µL of absolute ethanol and 2000 µL of DPPH (0.0039 g/100 mL of absolute ethanol). The mixture was totally homogenized and maintained in the dark for 45 min at room temperature (25 °C). Absorbances of the samples were measured at 517 nm using a JENWAY 6850 UV-Visible spectrophotometer (Stone, Staffordshire, UK). The antioxidant capacity was calculated using Eq. (2).

$$I (\%) = \frac{1-A_s}{A_c} \times 100 \quad (2)$$

where I is the inhibition (%), A_s is the absorbance of the sample and A_c is the absorbance of the control. A standard curve was prepared at various concentrations of Trolox (6-hydroxy-2,5,7,8 tetramethylchrome-2 carboxylic acid 97%): 0-0.030 mg (R² = 0.993). Results were calculated as mg of Trolox equivalents (TE)/100 mL of beverage using Eq. (3)

$$TE \left(\frac{mg}{100 mL} \right) = \left(\frac{A-b}{m} \right) * DF * 100 \quad (3)$$

where A is the absorbance of the sample, b is the intercept (1.2497), m is the slope (3233.7 1/mg), and DF is the dilution factor of the sample.

6.9.2. Total phenolic compounds

It was analyzed by the spectrophotometric Phenol Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). An aliquot of 10 μL of sample (FP diluted 1:9 mL distilled water) was mixed with 3990 μL of distilled water, 250 μL of Folin-Ciocalteu reagent and 750 μL of Na_2CO_3 (20%). Samples were mixed and maintained at room temperature (25 ± 1 $^\circ\text{C}$) in the dark for 2 h. The absorbance was measured at 765 nm using a JENWAY 6850 UV-Visible spectrophotometer (Stone, Staffordshire, UK). A standard curve was prepared with different concentrations of Gallic acid (GA): 0-0.064 mg ($R^2 = 0.990$). Total phenols content was calculated with Eq. (4). Results were reported as mg of Gallic acid per 100 mL of beverage.

$$GA \left(\frac{\text{mg}}{100 \text{ mL}} \right) = \left(\frac{A-b}{m} \right) * DF * 100 \quad (4)$$

where A is the absorbance of the sample, b is the intercept (0.0014), m is the slope (22.395 1/mg), and DF is the dilution factor of the sample.

6.9.3. Total flavonoids

Total flavonoids (TF) were analyzed according to the Dewanto, Wu, Adom & Liu (2002) method with some modifications. An aliquot of 10 μL of undiluted sample was mixed with 3265 μL of distilled water and 75 μL of NaNO_2 (5%), mixed and left in the dark. After 5 min, 150 μL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10%) were added. After 6 min, 500 μL of NaOH (1 M) was added and mixed. Immediately, the absorbance was measured at 510 nm in a JENWAY 6850 UV/Visible spectrophotometer (Stone, Staffordshire, UK). A standard curve was prepared with different concentrations of quercetin (QE 10 mg/100 mL of distilled water): 0-0.018 mg ($R^2 = 0.932$). Total flavonoids were calculated with Eq. (5). Results were reported as mg of quercetin (Q)/100 mL for pomegranate and catechin for black cherry of beverage.

$$Q/CAT \left(\frac{\text{mg}}{100 \text{ mL}} \right) = \left(\frac{ABS-b}{m} \right) * DF * 100 \quad (5)$$

where ABS is the absorbance of the sample, b is the intercept (0.0002), m is the slope (2.9303 1/mg), and DF is the dilution factor.

6.9.4. Total monomeric anthocyanins

The pH differential method was used to measure the total monomeric anthocyanins content (Giusti & Wrolstad, 2001). An aliquot of 1000 μL of sample was placed in an amber tube containing 3000 μL of potassium chloride buffer pH 1.0. In another amber tube, 3000 μL of sodium acetate buffer pH 4.5 and 1000 μL of sample was also placed. Tubes were prepared in triplicate. The blends were mixed

and allowed to stand at room temperature (25±1 °C) for 30 min. Absorbances were measured at 520 and 700 nm in a JENWAY 6850 UV/Visible spectrophotometer (Stone, Staffordshire, UK). Total monomeric anthocyanins (TMA) were calculated with equations (6) and (7).

$$Abs = (Abs_{520nm} - Abs_{700nm})_{pH1.0} - (Abs_{520nm} - Abs_{700nm})_{pH4.5} \quad (6)$$

$$C3OG \left(\frac{mg}{100 mL} \right) = \frac{Abs * MW * F * 1000}{\epsilon * 1} \quad (7)$$

where MW is the molecular weight (449.2 g/mole) of cyanidin-3-O-glucoside (C3OG), ϵ is the molar absorptivity coefficient (26,900 L/mole cm), F is the dilution factor, and 1 is the light pathway along the quartz cell (1 cm). Results were reported as mg of C3OG/100 mL of juice.

6.10. Physicochemical analysis

6.10.1. Total soluble solids, pH and ethanol concentration

The total soluble solids (TSS) were measured using an Atago refractometer (Atago Co. Ltd., Tokyo, Japan) according to 932.14C AOAC (2000) method and were expressed as °Brix (% w/w). A Conductronic pH-meter (Conductronic S. A., Puebla, Mexico) was used for measuring pH at 20±5 °C. The ethanol concentration was measured by specific gravity according to the 10.023 AOAC (2000) method.

6.10.2. Titratable acidity (TA), volatile acidity (VA) and fixed acidity (FA)

For TA, VA, and FA, around 5 g of sample were placed in conical flasks with 25 mL of distilled water and titrated with 0.1 M NaOH solution according to the 942.15, 11.047 and 981.12 AOAC (2000) methods, respectively. TA and FA were calculated as grams of citric acid (CA)/100 mL and VA as grams of acetic acid (AA)/100 mL.

6.11. Color characteristics

Ten milliliters of sample were placed in a small quartz cell (6 cm in height and 5 cm in width) for measuring color. The color parameters, in the transmittance mode, L*, a*, and b*, were measured using a tri-stimulus colorimeter Chroma Meter CR-400 (Konica Minolta Sensing Inc. Osaka, Japan) in the CIELab* scale. The total difference in color (ΔE^*), Chroma and hue were calculated using equations (8), (9), and (10).

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (8)$$

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (9)$$

$$hue = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (10)$$

where L^* , a^* , and b^* are the values of fresh juice; L^* , a^* , and b^* are values of the fermented pomegranate (FP) beverages.

6.12. Sensory analysis

All beverages were sensory evaluated using a nine-point structured hedonic scale (Wichchukit & O'Mahony, 2015). The sensory analysis was performed at the end of storage (42, 49 and 56 d) with 50 untrained judges. The following attributes were evaluated: appearance, color, aroma, sweetness, flavor, and general acceptability.

6.13. Statistical analysis

Experimental data were analyzed using a Minitab v. 17 Statistical Software (Minitab Inc., State College, PA, USA). Differences within means of treatments were considered statistically significant for $p \leq 0.05$ using ANOVA and Tukey tests. All measurements were completed in triplicate. The Pearson coefficients were calculated (Montgomery, 2017).

VII. RESULTS AND DISCUSSION

7.1. Pomegranate juice

7.1.1. Yield

A high yield is a desired characteristic for the food industry, since you want to get the most out of the fruit at a low cost (Díaz, 2014). The pomegranate fruit used in this study showed an important yield (65.53%). Díaz, 2014, obtained a yield of 63.99% pomegranate fruit (Wonderful variety) by means of processor extraction and using the complete fruit (c/cascara). The variation in the percentage of yield obtained may be due to the variety of pomegranate used, the ripeness index of the fruit and the extraction method used (Rajasekar et al., 2012).

7.1.2. Physicochemical characteristics

The physicochemical characteristics of pomegranate juice are shown in Table 1. These characteristics are important in pomegranate juice for the fermentation process, high-lighting the content of TSS.

TSS The TSS content found in this researched for pomegranate juice was $13.9 \pm 0.02\%$ (w/w). This TSS content in pomegranate juice was an adequate medium to carry out the alcoholic fermentation. Zarei et al. (2011) reported a TSS in pomegranate juice in the range 9.56–10.30% (w/w) and Sepúlveda et al. (2010) and Tehranifar et al. (2010) reported values of TSS in the range 11.37–15.07 °Bx. These changes in the TSS are mainly due to the variety and maturity of pomegranate.

pH. It is an important factor in the fermentation process due to its effect with the yeasts and quality attributes of the product such as flavor, color, and aroma. It is recommended that the pH in the fermentation wort be in the range 2.8-4.0 (Sepúlveda et al. 2010; Zarei et al. 2011).

TA. Total acidity calculated as percentage of citric acid (CA) was $0.35 \pm 0.02\%$ in FRJ. Tehranifar et al. (2010) reported a TA content in the range 0.33-2.44% CA in 20 samples of pomegranate from different Iranian cultivars. Results of this study showed a lower percentage of acidity than that reported by other researchers (Sepúlveda et al. 2010; Tehranifar et al. 2010; Zarei et al. 2011) which could be due to the variety and maturity of the fruit.

Table 1. Physicochemical characteristics of FRJ¹ (fresh juice) and FEB² (fermented pomegranate beverage) products.

	Beverage			
	FRJ	FEB1	FEB2	FEB3
TSS (% w/w)	13.90±0.02	4.30±0.01 ³	5.90±0.01 ³	11.70±0.02 ³
pH	3.30±0.02a	3.41±0.01b	3.60±0.02c	3.70±0.01d
TA (% citric acid)	0.35±0.02a	0.40±0.03b	0.62±0.01c	0.67±0.01d
FA (% citric acid)	-	0.30±0.02	0.52±0.03	0.57±0.05
VA (% acetic acid)	-	0.09±0.01	0.10±0.01	0.10±0.01
Ethanol concentration ⁴	-	6.82±0.01	9.73±0.01	12.88±0.01
<i>L</i> [*]	31.74±0.02	32.54±0.05	35.47±0.02	30.83±0.04
<i>a</i> [*]	34.02±0.05	32.04±0.01	31.62±0.01	31.98±0.06
<i>b</i> [*]	14.11±0.04	11.23±0.03	13.50±0.03	10.01±0.08
Hue (°)	22.50	19.30	23.10	17.40
Chroma	36.83	33.95	34.38	33.51
ΔE^*	-	3.59	4.48	4.67

¹FRJ: pomegranate fresh juice; ²FEB: pomegranate fermented beverage; ³At the end of fermentation;

⁴Percentage (v/v). Different letters within rows indicate significant differences ($p \leq 0.05$).

7.1.3. Color

The color parameters of pomegranate juice were $L^* = 31.74 \pm 0.02$, $a^* = 34.02 \pm 0.05$, $b^* = 14.11 \pm 0.04$, hue = 22.5° and C = 36.83 (Table 1). The L^* value indicate a dark pomegranate juice (being 0 = white and 100 = black). The a^* (red-green) value (34.02 ± 0.05) is characteristic of the red color which gives important information about the content of red natural pigments (anthocyanins) responsible for the typical color of pomegranate juice (Tzulker et al. 2007). Regarding the b^* color

parameter, the value (14.11 ± 0.04) was found in the red-yellow color segment of the color space; the value indicates a yellowish hue. The hue value, found in the red-yellow segment of the color space, had a tendency to an intense red color. The chroma parameter indicates a high saturation of red, which is mainly due to the content of anthocyanins. All values of the color parameters are located in the red-yellow segment of the color space (Baqueiro-Peña and Guerrero-Beltrán, 2017).

7.1.4. Antioxidant characteristics

The antioxidant characteristics of pomegranate juice are shown in Table 2.

Antioxidant activity. The antioxidant activity of pomegranate FRJ was 482.23 ± 0.20 mg TE/100 mL. Gil et al. (2000) reported that the antioxidant activity of commercial pomegranate juice was higher than that in red wine (354.89 mg TE/100 mL) and green tea (276.02 mg TE/100 mL). Çam et al. (2009), on the other hand, analyzed the antioxidant activity of eight cultivars of pomegranate from Turkey. They reported an antioxidant activity in the range 221.2-418.3 mg TE/100 mL of juice.

Total phenolic compounds. Pomegranate is rich in phenolic compounds such as ellagic acid, p-coumaric acid, tannins, anthocyanins and catechin. The total phenolic compounds content in this study was 393.78 ± 0.13 mg GA/100 mL. Poyrazoglu et al. (2002) reported Gallic acid as the major phenolic compound in pomegranate fruit. Sepúlveda et al. (2010) reported 123.6 mg GA/100 mL. Other researchers have reported lower amount of total phenolic compounds (275.0 mg GA/100 mL) from arils of pomegranate “Mollar” variety.

Flavonoids. The flavonoid content in FRJ was 172.10 ± 0.15 mg QU/100 mL. El Kar et al. (2011) analyzed different varieties of pomegranate juice and reported a total flavonoids content in the range 13.5-63.6 mg QU/100 mL. It has been reported kaempferol, luteolin, quercetin and catechin in pomegranate juice (Van Elswijk et al. 2004).

Total anthocyanins

The content of anthocyanins in pomegranate juice was 4.61 ± 0.38 mg of C3OG/100 mL (Table 2). Gil et al. (2000) reported that C3OG was the main anthocyanin in pomegranate juice (12.8 mg/100 mL). Sepúlveda et al. (2010) reported a total content of anthocyanins in pomegranate juice of ten different genotypes in the range 17.0-134.2 mg of C3OG /100 mL. Tzulker et al. (2007) reported 10-30 mg of C3OG/100 mL of juice from arils of 29 Israeli pomegranate cultivars.

7.2. Fermented pomegranate beverage

7.2.1. Physicochemical properties

Table 1 shows the physicochemical characteristics of fermented beverages.

TSS. Soluble solids were metabolized by yeasts to ethanol. Ethanol also has refractive properties; therefore, ethanol is detected as a “soluble solid” along with residual sugars in FEB beverages.

pH. It increased in the three FEB (fermented pomegranate beverage) beverages, being higher in the FEB3 (25°Brix) beverage. In fermented beverages, pH should be in a range 2.8-4.0; pH for the three FEBs was in this range. Berenguer et al. (2016) reported a pH of 3.4 at the beginning and 3.5 at the end of fermentation of pomegranate juice. Zhuang et al. (2011) reported 3.2 at the start and 3.3 at the end of the fermentation. All pH values were higher in FEBs than in FRJ. This increase was very probably due to the metabolites delivered during the fermentation. A significant difference ($p \leq 0.05$) in pH was observed in FEB beverages.

TA. TA of FEBs is shown in Table 1. Significant differences ($p \leq 0.05$) were observed in TA of pomegranate beverages. Due to the fermentation process, there are many changes in organic acids in the juice, which may increase, decrease, or be generated as new acids (citric, malic, lactic or acetic acids). Berenguer et al. (2016) reported TA contents of 0.28 ± 0.03 and $0.59 \pm 0.04\%$ of CA in fresh and fermented pomegranate beverages, respectively. Zhuang et al. (2011) reported TA values of 0.52 ± 0.05 and 0.73 ± 0.02 g/100 mL CA in fresh and fermented juices, respectively. Ordoudi et al. (2014) reported TA values of 0.36 ± 0.02 and $0.52 \pm 0.04\%$ CA in unfermented and fermented juices, respectively. Acidity has a decisive impact in aroma and flavor of fermented drinks. The increase in TA may be due to the production of α -ketoglutaric and succinic acids in the glyceropyruvic fermentation pathway and pyruvic acid in the glycolytic pathway.

VA and FA. There was a greater production of VA and FA in FEB3 than in FEB1 and FEB2 beverages. According to the International Code of Oenological Practices (2015), the maximum permitted limit of VA in wine is 0.12 g/100 mL (expressed as acetic acid) and the minimum permitted limit of FA is 0.4 g/100 mL (expressed as tartaric acid). The acidities found in this research were within the limits permitted by the International Code of Oenological Practices (2015).

Ethanol. The concentrations of ethanol in FEB1 (SSN), FEB2 (17.5°Brix), and FEB3 were 6.82 ± 0.01 , 9.73 ± 0.01 and $12.88 \pm 0.01\%$ (v/v), respectively. The Mexican Norm number NOM-142-SSA1/SCFI-2014 (DGN, 2014), for alcoholic beverages, classifies an alcoholic beverage when the alcohol content is in the range 6.1 to 20.0% by volume; therefore, the three FEBs had an average alcoholic content within this range.

7.2.2. Color

Differences in the color parameters were observed within all fermented pomegranate beverages (Table 1). In general, the a^* and b^* color values were higher in FRJ than in FEBs. It is important to notice that the a^* value (red color) of the FEB1 was higher than the values for FEB2 and FEB3. The hue ($^\circ$) was lower in the FEB1. The FEB2 beverage presented a greater angle (23.1°), indicating a more intense red color, according to the red-yellow color space segment. The Chroma color parameter decreased during fermentation, showing a less saturated color. For the total change in

color (ΔE^*), the FEBs beverages had lighter changes, being barely clearer than FRJ. All ΔE^* values were similar. The color of a fermented beverage is an important parameter for the consumer's acceptance. Anthocyanins are labile to physical factors such as pH, temperature, metals among others. In this work, the pH in the three FEB beverages barely changed; therefore, the color barely changed in fermented products.

7.2.3. Antioxidant characteristics

The antioxidant characteristics for FEB beverages are shown in Table 2.

Antioxidant activity. The antioxidant activity decreased from 482.23 ± 0.20 (in FRJ) to 262.61 ± 0.12 (in FEB3) mg TE/100 mL. After fermentation, the antioxidant activity was reduced about 45%. The decrease in the antioxidant activity could be due to the oxidation reactions that occurs along the fermentation process. The pomegranate compounds may interact with each other and oxidize, decreasing the antioxidant activity. Each phenolic compound and their interaction in the pomegranate beverages might contribute differently to the change in the antioxidant activity. Zhuang et al. (2011) and Ordoudi et al. (2014) reported changes in antioxidant activity in the range 16-39% in pomegranate beverages.

Total phenolic compounds. The amount of these compounds was 393.78 ± 0.13 and 188.6 ± 0.20 mg GA/100 mL, before (FRJ) and after fermentation (FEB3), respectively (Table 2). Significant differences ($p \leq 0.05$) were observed in the total phenolic compounds content. The content of Gallic acid in FEBs decreased as the initial TSS increased in the initial fermentation system. Ordoui et al. (2014) studied the effect of fermentation on the total phenolic compounds; they reported 138.7 and 139.5 mg GA/100 mL in unfermented and fermented products, respectively. Lantzouraki et al. (2015) compared the total phenolic compounds content of pomegranate ("Wonderful" variety) fermented beverages with red wine; they pointed out that the total phenolic compounds content was significantly higher in the pomegranate beverage (383 mg GA/100 mL) than in red wine (296 mg GA/100 mL).

Flavonoids. The flavonoids content was reduced in FEBs (Table 2) by about 60%. In addition to the significant differences ($p \leq 0.05$) of flavonoids within fresh juice (FRJ) and fermented beverages, the beverage with the lower flavonoids content was the FEB3 (64.35 ± 0.09 mg QU/100 mL). The amount of flavonoids in FEB3 was similar to that reported by El Kar et al. (2011) in pomegranate fresh juice (63.6 mg QU/100 mL).

Total anthocyanins. The total anthocyanins content in FEBs was in the range 2.22-1.92 mg of C3OG/100 mL (Table 2). Significant differences ($p \leq 0.05$) were observed within the anthocyanins content in FRJ and FEB beverages, being lower in FEB3. The amount of anthocyanins was barely

reduced in beverages that had the higher amount of initial TSS but produced the higher amount of ethanol. Therefore, the low change observed of anthocyanins was actually low; the reduction was about 13.5%. The reduction of anthocyanins in beverages, as the initial content of TSS increased, might be due to the reactions of polymerization with acetaldehyde, generated by the metabolic activity of yeasts, with the formation of complex compounds causing losses of the red color (Gil et al. 2000).

Table 2. Antioxidant profile of FRJ¹ and FEB² products.

	Beverage				Total
	FRJ	FEB1	FEB2	FEB3	
Antioxidant activity ³	482.23±0.20a	281.65±0.11b	273.35±0.09c	262.61±0.12d	
phenolics ⁴	393.78±0.13a	229.28±0.13b	191.14±0.14c	188.60±0.20d	
Flavonoids ⁵	172.10±0.15a	119.70±0.08b	86.80±0.06c	64.35±0.09d	
Anthocyanins ⁶	4.61±0.38a	2.22±0.06b	1.96±0.09c	1.92±0.15d	

¹FRJ: pomegranate fresh juice; ²FEB: pomegranate fermented beverage; ³mg ET/100 mL; ⁴mg GA/100 mL; ⁵ mg QU/100 mL; ⁶ mg C3OG /100 mL. Different letters within rows indicate significant differences ($p \leq 0.05$).

7.2.4. Sensory evaluation

In Table 3 are reported the results of the sensory evaluation of FRJ and FEBs. Significant differences ($p \leq 0.05$) were found within different samples. In general, the best-liked sample was the FRJ beverage. None of the samples obtained a score higher than 8 or less than 5. The FEB1 beverage was the best scored in appearance and color, this could be due to its red-bright color (a^* value of 32.04 ± 0.01), suitable for red color. For aroma, sweetness, taste, and general acceptability, the FEB3 beverage was very well accepted. FEB3 was the beverage with the highest content of TSS (11.7 ± 0.02 °Bx) and ethanol content ($12.88 \pm 0.01\%$ v/v). According to the International Code of Oenological Practices (2015), a beverage with more than 5 g sugars/100 mL is classified as a fermented sweet-beverage. The FEB3 of the three FEB beverages was the one with the highest content of ethanol and total acidity ($0.67 \pm 0.01\%$ citric acid). Ethanol is the most abundant volatile compound in fermented

beverages and it may modify both the sensory perception of aromatic attributes and detection of volatile compounds (Goldner et al. 2009). Ethanol also influence the body of the fermented beverage and the perception of astringency, acidity, sweetness, and flavor.

Table 3. Sensory evaluation of FRJ¹ and FEB² products.

	Beverage			
	FRJ	FEB1	FEB2	FEB3
Appearance	7.75±0.786ab	8.05±0.686a	7.45±1.432a	6.95±1.877b
Color	7.05±0.999b	8.15±0.933a	6.80±1.795b	7.20±1.240ab
Aroma	7.80±0.768a	6.45±1.986b	6.75±1.916b	6.85±1.461ab
Sweetness	7.50±0.827a	5.05±1.877b	5.70±2.296b	7.25±1.372a
Taste	7.80±0.834a	5.30±2.515b	6.05±2.395b	6.60±1.957ab
General acceptability	7.85±0.671a	5.25±2.403b	5.30±2.812b	6.90±1.971ab

¹FRJ: pomegranate fresh juice; ² FEB: pomegranate fermented juice. Different letters within rows indicate significant differences ($p \leq 0.05$).

7.3. HHP processing in pomegranate beverage

7.3.1. Inactivation of microorganisms

From day 0 until the end of storage (42 d), no growth of psychrophilic bacteria (<10 CFU/ mL) was observed in all processed samples (HHP, VAT and HTST), including control. Table 4 shows the aerobic mesophilic bacteria (AMB) and yeasts plus mold (YM) counts in all samples analyzed during the storage time. In both AMB and YM there was no growth (<10 CFU/mL) through the entire storage time for samples treated with HHP and VAT pasteurization. In the HTST pasteurization, a reduction of 3 logarithmic cycles of AMB and YM was observed immediately after HHP processing. A few growth of AMB (4 and 17 CFU/mL at 0 and 42 d, respectively) and YM (2.5 and 18 CFU/mL, 0 and 42 d,

respectively) was observed through the storage time. According to these results, the AMB and YM population increased one log cycle from day 0 to day 42 of storage. According to the results in this study, at day 0 the microbial population in HTST pasteurized beverages would be within the maximum permissible limits. This indicates that the HTST treatment of beverages may be enough for the inactivation of the microbial population.

Tonello, Largeteau, Demazeau & Lonvaud-Funel (1996) applied HHP (300 MPa/6 min) to inactivate yeasts, lactic acid bacteria and acetic acid bacteria in white, red and rosé wines; they reported a total inactivation of yeasts and bacteria in all wines. It has been observed complete inactivation of vegetative bacteria, yeasts and molds when pressures are above 200 MPa. However, in practice, pressures of up to 700 MPa and treatment times from seconds to minutes have been tested to inactivate microbial cells (Terefe, Buckow & Versteeg, 2013). The bacterial spores, on the other hand, are highly resistant to pressure, showing a remarkable tolerance to pressures above 1000 MPa. However, by combining other intrinsic factors in foods, such as fermentation, it is possible to inactivate bacterial spores at pressures in the range 500-900 MPa (Terefe et al., 2013). Regarding yeasts and molds, they are less resistant to pressure than bacteria; they can be inactivated with pressures between 200 and 400 MPa. However, it has also been observed that *Saccharomyces cerevisiae* could be more resistant than Gram-negative bacteria (Daher et al., 2017). Basak, Ramaswamy & Piette (2002) demonstrated that *S. cerevisiae* was not inactivated under 400 MPa in orange juice. Shahbaz et al. (2015) reported a reduction of 5.8 logarithmic cycles of *S. cerevisiae* in apple juice treated with HHP at 500 MPa for 1 min. The results obtained in this study demonstrated that bacteria and yeasts (*S. cerevisiae*, mainly) were inactivated in beverages processed at 500-600 MPa for 5 to 10 min.

Table 4. Aerobic mesophilic bacteria (AMB), yeasts plus mold (Y/M) in HHP and pasteurization (VAT/HTST) pomegranate fermented beverages throughout storage.

Time (d)	Control		HTST	
	AMB	Y/M	AMB	YM
0	1.0x10 ⁴	1.0x10 ⁴	4.0x10 ¹	2.5x10 ¹
3	1.4x10 ⁴	1.1x10 ⁴	4.1x10 ¹	2.6x10 ¹
7	1.9x10 ⁴	1.5x10 ⁴	4.8x10 ¹	4.0x10 ¹
14	2.0x10 ⁴	1.9x10 ⁴	6.3x10 ¹	5.5x10 ¹
21	4.6x10 ⁴	4.1x10 ⁴	8.8x10 ¹	5.8x10 ¹
28	5.6x10 ⁴	5.2x10 ⁴	9.1x10 ¹	6.0x10 ¹
35	7.1x10 ⁴	6.4x10 ⁴	9.9x10 ¹	1.0x10 ¹

7.3.2. Antioxidant capacity

The antioxidant capacity (AC) results are shown in Figure 4. A significant difference ($p \leq 0.05$) was observed in the AC content between the VAT pasteurized and HHP2 and HHP3 processed beverages. Control, HHP1 and HTST beverages were not statistically different ($p > 0.05$). The antioxidant capacity values at day 0 and 3 for control, HHP1, HHP2 and HHP3, VAT and HTST were 317.76 ± 1.262 and 313.27 ± 1.109 , 321.94 ± 1.076 and 320.01 ± 1.171 , 323.55 ± 1.119 and 323.10 ± 1.101 , 326.51 ± 1.199 and 324.88 ± 1.421 , 296.48 ± 1.181 and 293.41 ± 1.102 , 303.54 ± 1.233 and 300.73 ± 1.601 mg Trolox/100 mL of beverage, respectively. The beverages with the highest loss of AC was the VAT at day 0 (296.48 mg Trolox/100 mL) and day 42 (270.90 mg Trolox/100 mL) of storage; this could be due to the heat and the longer time used for pasteurization. The sample treated at 600 MPa for 5 minutes (HHP3) had the highest amount of AC. Queiroz et al. (2010) reported similar results to those found in this work. They report that the antioxidant activity of apple juice increased when it was treated at 250 MPa for 3 min. Plaza et al. (2006) found a greater reduction of the antioxidant capacity in pasteurized (70 °C-30 s) orange juice than in the samples processed for HHP at 400 MPa for 1 min and stored 40 days at 4 °C. Corrales, Butz, & Tauscher (2008) applied HHP to wine from the Dornfelder grape variety. When wine was subjected to 600 MPa, for 10 min, no significant differences in antioxidant activity were found. Some studies have shown that pasteurization and HHP processing may exert different effects on the total phenolic content and antioxidant activity in products under refrigeration (Zhao, Zhang & Zhang, 2016). In this research, the HHP1 (500 MPa-10 min), HHP2 (550 MPa-10 min), and HHP3 (600 MPa-5 min) beverages showed an increase of approximately 10% in the AC after the HHP processing, compared to the control. It is difficult to know the effect of the pressure on the antioxidant activity of the fermented beverage, because the effect of high hydrostatic pressures on antioxidant compounds is very dependent on the matrix, since each fruit, used as raw material, has a different composition in antioxidants and the overall effect depends on the stability of them to the HHP processing.

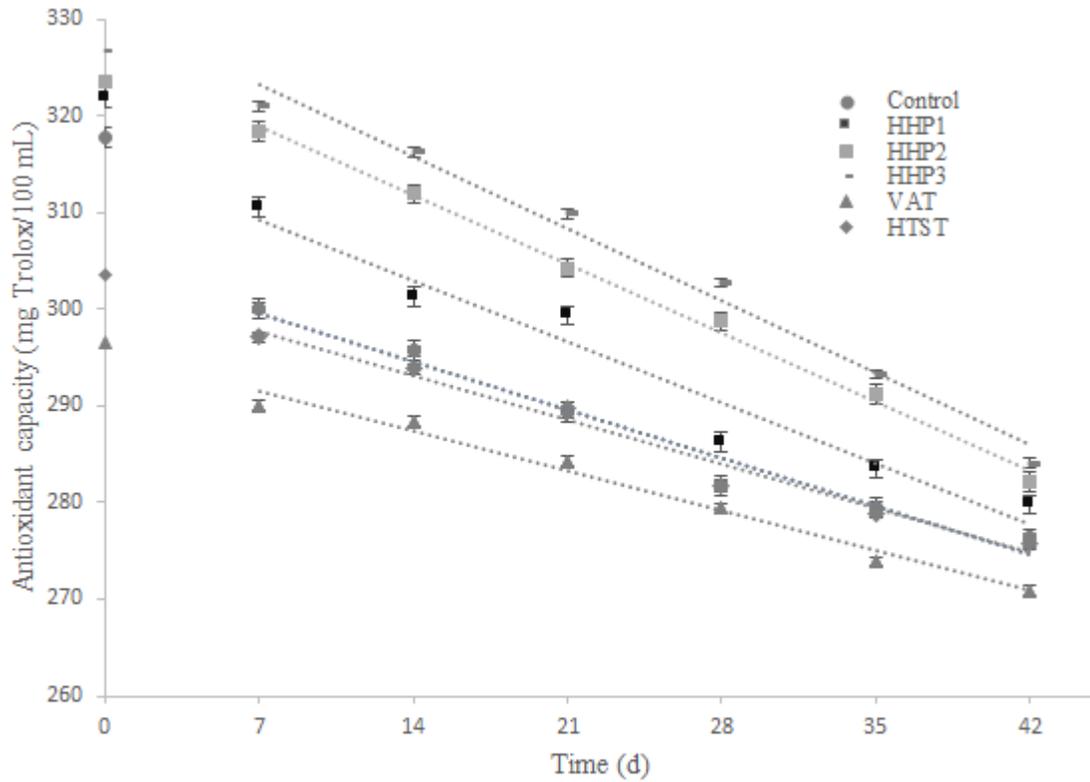


Figure 4. Effects of high hydrostatic pressure (HHP) processing and thermal pasteurization (VAT, HTST) on the antioxidant capacity of pomegranate beverages stored under refrigeration (4 ± 1 °C).

In Figure 1, from day 7, a linear (Table 5) reduction in antioxidant capacity was observed for control and all treatments; the lower correlation coefficient (0.955) was observed for the HHP1 beverage. The slope (m) indicates the reduction velocity along the storage. On the other hand, an increase in AC was observed in HHP1, HHP2 and HHP3 beverages in comparison with the control beverage.

Table 5. Antioxidant characteristics of fresh and processed pomegranate beverages.

		HHP/T ^a (MPa/min)				Pasteurization	
	LRP ^b	Cont. ^c	500/10	550/10	600/5	VAT ^d	HTST ^e
AC ^g	m ^f	-0.715	-0.898	-1.016	-1.064	-0.587	-0.654
	b	304.61	315.46	325.99	330.57	295.53	302.19
	R ²	0.971	0.955	0.996	0.985	0.982	0.978
TPC ^h	m ^f	-0.560	-0.822	-0.345	-0.228	-0.692	-0.999
	b	370.90	388.80	388.66	392.26	313.17	345.12
	R ²	0.929	0.968	0.950	0.924	0.983	0.970
TF ⁱ	m ^f	-0.401	-0.304	-0.350	-0.316	-0.843	-0.845
	b	97.57	100.96	104.97	107.49	99.52	101.74
	R ²	0.998	0.937	0.975	0.791	0.930	0.958
TMA ^j	m ^f	-0.016	-0.014	-0.021	-0.013	-0.016	-0.014
	b	4.537	4.552	4.935	4.899	4.396	4.451
	R ²	0.985	0.946	0.952	0.912	0.987	0.993
Eth ^k	m ^f	0.018	-0.004	-0.003	-0.004	-0.003	0.008
	b	11.65	11.64	11.75	11.74	11.51	11.56
	R ²	0.984	0.771	0.686	0.771	0.686	0.938

aHHP/T: High hydrostatic pressure/time, bLRP: Linear regression parameters, cCont.: Control, dVAT: Pasteriirization at 63°C for 30 min, eHTST: High Temperature-Shor Time pasteurization (72°C for 15 s), fm: slope, gAC: Antioxidant activity (mg Trolox/100 mL d), hTPC: Total phenolic compounds (mg Gallic acid/100 mL d), iTF: Total flavonoids (mg quercetin/100 mL d), jTMA: Total monomeric anthocyanins (mg C3OG/100 mL d), kEth: Ethanol (mL/100 mL d).

7.3.3. Total phenolic compounds

Figure 5 shows the total phenolic compounds throughout the storage time. The quantity of total phenolic compounds at day 0 and 3 for control and HHP1, HHP2, HHP3, VAT and HTST processed beverages were 389.71±2.00 and 380.43±1.02, 392.32±1.00 and 389.65±1.08, 395.02±1.22 and

391.83±1.06, 399.21±2.04 and 395.85±1.21, 324.37±1.08 and 319.42±1.20, 358.90±1.04 and 346.11±2.00 mg GA/100 mL, respectively. Significant changes ($p \leq 0.05$) were observed between pasteurized beverages (VAT and HTST) compared to control, HHP1, HHP2, HHP3. The HHP processed samples had higher phenolic compounds content at day 0 and 42 of storage. The HHP3 beverage showed significant differences ($p \leq 0.05$) in the content of total phenolic compounds compared to control, HHP1 and HHP2. HHP3 beverages was the one with the highest phenolic compounds content just after pressurization and at the end of the storage: 399.21 and 383.40 mg of GA/100 mL, respectively. Nevertheless, the total phenolic compounds decreased in control and all processed samples throughout the storage.

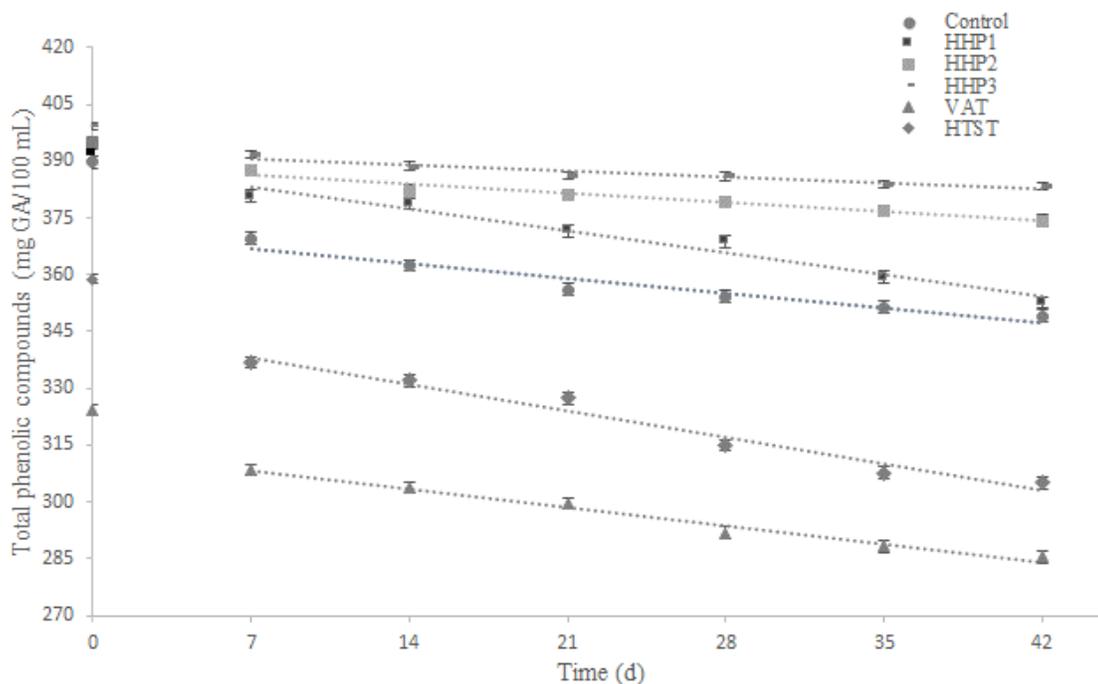


Figure 5. Effects of high hydrostatic pressure (HHP) and pasteurization (VAT, HTST) on total phenolic compounds of refrigerated (4 ± 1 °C) pomegranate beverages.

Chen et al. (2013) & Varela-Santos et al. (2012) studied the effect of HHP on phenolic compounds of pomegranate and its subsequent storage. They observed that the total phenolic compounds content in pomegranate juice increased significantly after the HHP processing at 350 and 500 MPa for 30-150 seconds. They observed an increase of phenolic compounds at day 3 of storage; however, at day 5 the total phenolic compounds began to decrease. Alpas (2013) analyzed pomegranate juices treated with HHP in the range 200-400 MPa for 5-10 min; he reported a significant decrease in total phenolic compounds. Ferrari, Maresca & Siccarone (2011) observed a significant lessening (30-60%) of total phenolic compounds in HHP processed pomegranate juice throughout the storage; they observed a stability until day 14; however, at the end of the storage, the phenolic content remained

stable or barely increased. They suggested that these changes were probably due to some differences of the pomegranate juice. In this study, the VAT pasteurized beverage was the one with the lowest content of phenolic compounds (389.71 mg of GA/100 mL at day 0); therefore, the reduction could be related directly to the temperature and time of processing. However, at day 42 of storage, the amount of total phenolic compounds was still high in the beverage (285.40 mg GA / 100 mL).

Throughout the storage, the losses velocity of TFC is reported in Table 5. The highest reduction was observed in the VAT and HTST pasteurized beverages and the lowest reduction was in the HHP2 and HHP3 beverages. However, little reduction was observed in control and all processed pomegranate beverages along the storage for 42 days.

7.3.4. Total flavonoids

Figure 6 shows the TF content in control and all processed pomegranate beverages. Total flavonoids content at day 0 and 3 for control and HHP1, HHP2, HHP3, VAT and HTST processed beverages were 103.27 ± 1.11 and 101.40 ± 1.27 , 108.67 ± 1.52 and 106.79 ± 1.95 , 114.96 ± 1.01 and 112.43 ± 1.98 , 121.54 ± 1.21 and 117.32 ± 1.00 , 97.82 ± 1.61 and 94.56 ± 1.42 , 99.01 ± 1.53 and 96.32 ± 1.02 mg QE/100 mL, respectively. The HHP3 processed beverage had the highest content of TF at day 0 (121.54 mg of QE/100 mL) and day 42 (95.78 mg of QE/100 mL). Even though an increase in flavonoids was observed after the HHP processing, this increase did not show significant differences ($p \geq 0.05$).

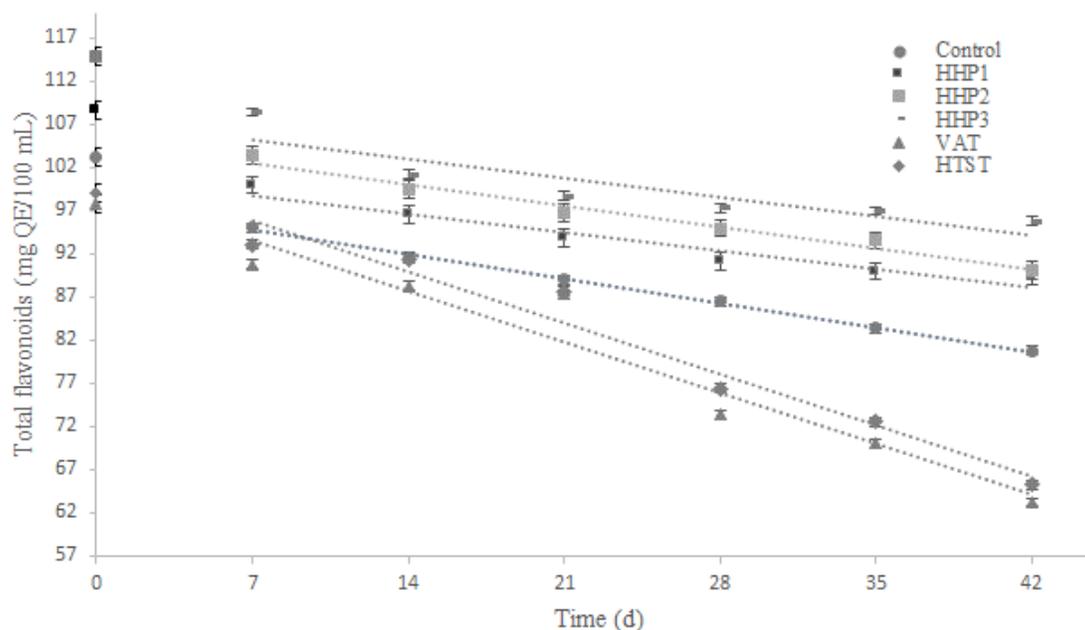


Figure 6. Effects of high hydrostatic pressure (HHP) and thermal treatments (VAT, HTST) on the total flavonoids content of pomegranate beverages during refrigeration (4 ± 1 °C).

Briones-Labarca et al. (2017) investigated the effect of HHP processing on the antioxidants and oenological quality characteristics of a young white wine (Sauvignon blanc). They did not find significant differences ($p > 0.05$) among the amount of the flavonoids content in all HHP processed wines in comparison with control. Significant differences ($p \leq 0.05$) were observed within the flavonoids content in the VAT and HTST pasteurized beverages, being they with the highest loss of flavonoids. The VAT beverage had 97.82 and 63.25 mg of QE/100 mL at day 0 and day 42, respectively. Approximately, 60% of flavonoids remained at day 42 of storage in comparison with the initial amount of control at day 0 (103.27 mg of QE/100 mL).

On the other hand, similar losses of TF were observed (Table 2) throughout the storage in control and HHP1, HHP2, and HHP3 beverages. The higher losses in TF were observed in the VAT and HTST pasteurized beverages.

7.3.5. Total monomeric anthocyanins

Figure 7 shows the effect of thermal and non-thermal treatments on the total monomeric anthocyanins content throughout the storage. It is known that anthocyanins are highly unstable to physical factors such as light, oxygen, pH, temperature, among others. The total anthocyanins content at day 0 for control and HHP1, HHP2, HHP3, VAT and HTST processed beverages were 4.68 ± 0.01 , 4.76 ± 0.03 , 4.79 ± 0.00 , 4.81 ± 0.02 , 4.35 ± 0.01 , 4.44 ± 0.00 mg C3OG/100 mL and at day 3 were 4.58 ± 0.01 , 4.74 ± 0.01 , 4.76 ± 0.02 , 4.77 ± 0.02 , 4.33 ± 0.01 , 4.41 ± 0.01 mg C3OG/100 mL. No significant differences were observed ($p > 0.05$) within control and thermal or non-thermal treated pomegranate beverages. A decrease in anthocyanins content was observed in all samples along the storage. Terefe, Matthies, Simons & Versteeg (2009) reported an insignificant change in the content of anthocyanins in strawberries treated with HHP at 600 MPa for 10 min. They evaluated the effect of storage for three months and reported a reduction of anthocyanins, starting at day 27. Varela-Santos et al. (2012) did not find changes in the anthocyanins content of HHP processed (350-500 MPa for 30-150 seconds) pomegranate juice before day 35 of storage; the control juice showed a decrease in these pigments. There are some hypotheses about the mechanism of anthocyanin degradation in HHP processed fruit products. One hypothesis say that the anthocyanins degradation could be due to the remaining enzymes; a relationship between the remaining enzymes (β -glucosidase, peroxidase, and PPO) and the stability of anthocyanins has been observed in several fruit products (Ferrari et al., 2010). The enzymatic degradation of anthocyanins by β -glucosidase is due to the loss of the glycosidic group that leads to the formation of anthocyanidin; consequently, this may affect the color (García-Palazon, Suthanthangjai, Kajda, & Zabetakis, 2004). However, it must be taken into account that pressure, temperature and time of processing as well as the physicochemical properties such as TSS, pH and acidity could have some effects on the enzymes responsible for the stability of anthocyanins in food products (García-Palazon et al., 2004).

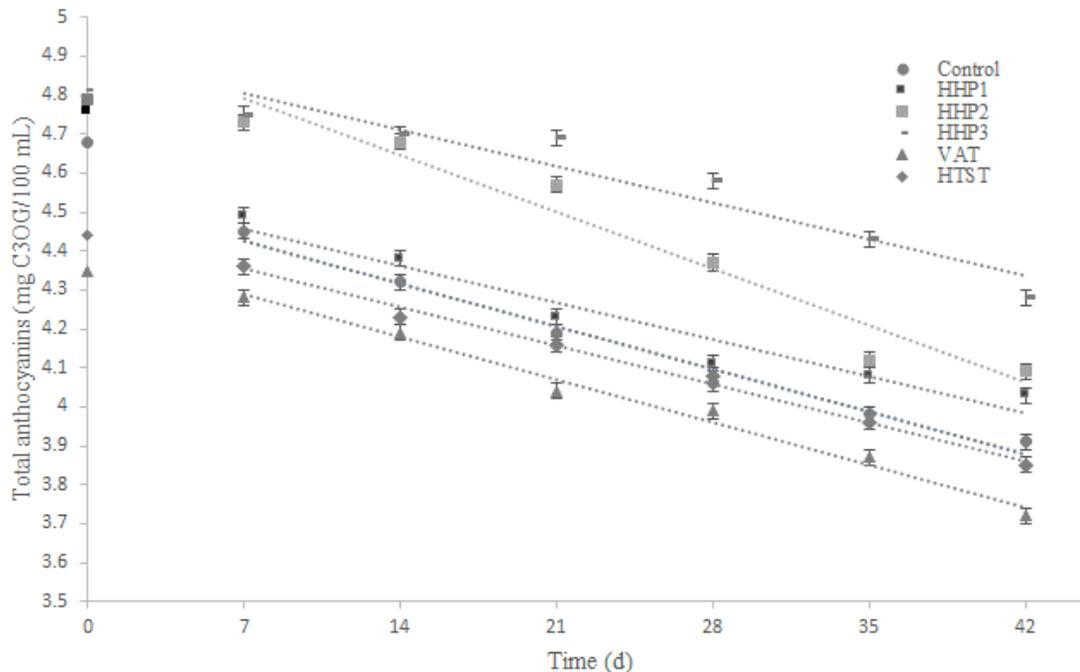


Figure 7. Effects of high hydrostatic pressure (HHP) and pasteurization (VAT, HTST) on total monomeric anthocyanins content in stored (4 ± 1 °C) pomegranate beverages.

Corrales et al. (2008) pointed out that the HHP processing conditions may affect the condensation reactions of anthocyanins in wines; therefore, they recommended using low pressures and short times for processing. In this work, the HHP processed beverages had a higher content of anthocyanins than untreated beverages; HHP3 for instance, had 4.81 and 4.28 mg of C3OG/100 mL at day 0 and 42, respectively. In the VAT pasteurization pomegranate beverage, the loss of anthocyanins was greater immediately upon pasteurization (4.35 mg of C3OG/100 mL). At the end of the storage time, the beverage had 3.72 mg of C3OG/100 mL.

Similar losses of TMS were observed (Table 5) throughout the storage in control and HHP1, HHP3, VAT and HTST processed beverages. The higher losses of TMA were observed in the HHP2 processed beverage (0.021 mg of C3OG/100 mL d).

7.3.6. Physicochemical analysis

7.3.6.1. Total soluble solids and ethanol

Figures 8 and 9 show the total soluble solids and the ethanol contents, respectively, of control and processed pomegranate beverages. No significant changes ($p \leq 0.05$) in the content of total soluble solids within processed and control beverages were observed. The storage time did not affect the total soluble solids content in beverages; however, on day 42, beverages that decreased in total

soluble solids were control (1.2°Bx) and HTST (0.7°Bx) pomegranate beverages; therefore, a light increase in ethanol was observed (Figure 6). Queiroz et al. (2010), Briones-Labarca et al. (2017) and Corrales et al. (2008) pointed out that no significant changes ($p \leq 0.05$) were observed in the TSS and pH of juices and wines treated with HHP. In this study, the observed changes in TSS in the control beverage could be due to more ethanol production. The same happened in the concentration of ethanol (Figure 6). There were no significant changes ($p \leq 0.05$) observed within treatments and no important changes were observed along the storage. The samples that most showed more changes in ethanol content (increase in 0.7 ml/100 mL) were the control and the HTST (increase in 0.3 ml/100 mL) pomegranate beverages.

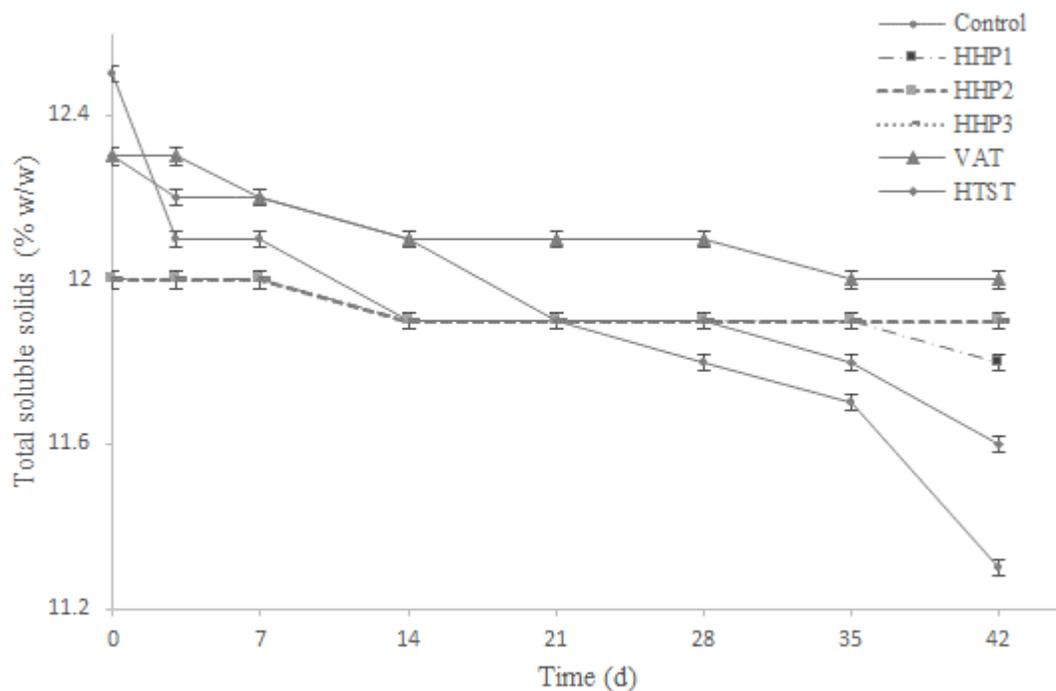


Figure 8. Effects of high hydrostatic pressure (HHP) and thermal pasteurization (VAT, HTST) on total soluble solids in pomegranate beverages through refrigeration (4 ± 1 °C).

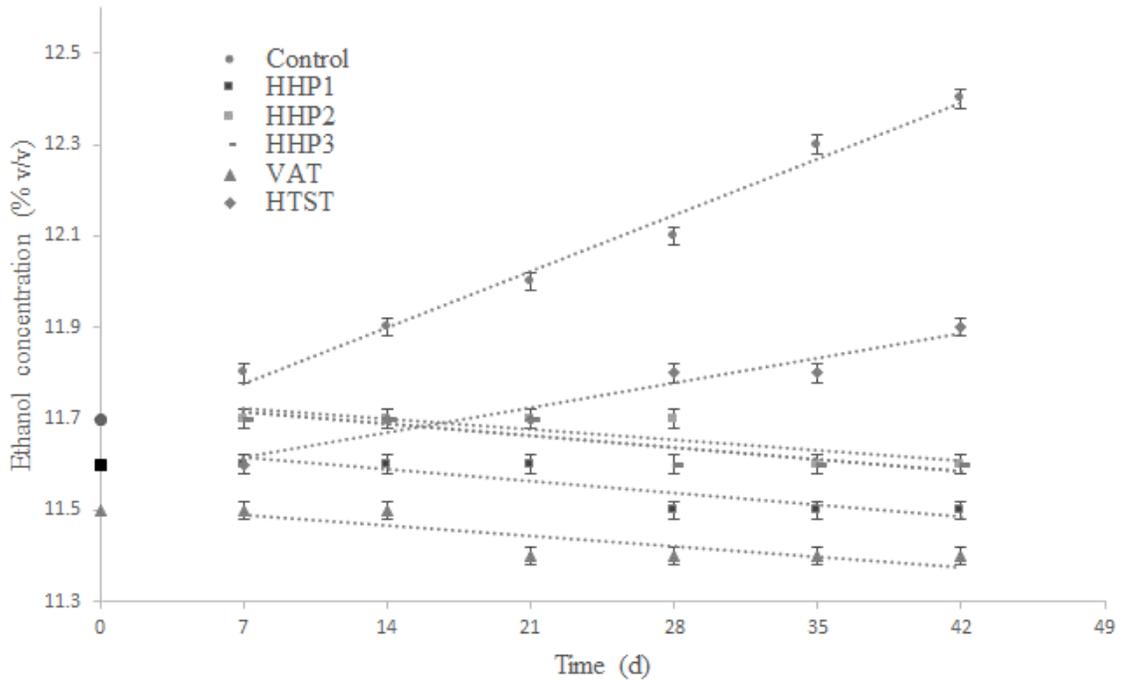


Figure 9. Effects of high hydrostatic pressure (HHP) and pasteurization on ethanol content in pomegranate beverages under low temperature (4 ± 1 °C).

7.3.6.2. pH and acidity

Table 6 shows the changes in pH and acidity (total, fixed and volatile) within means of control and all processed beverages at days 0 and 42. No differences ($P > 0.05$) were observed in pH, TA and FA within means of all samples at day 0 or 42. The volatile increased slightly in control and HTST pasteurized beverage. Corrales et al. (2008) pointed out that pH and acidity of the 2004 red wines (Dornfelder variety, Niederkirchen, Germany) were barely affected in HHP wines at pressures lower than 600 MPa. Briones-Labarca et al. (2017) did not find significant differences ($p > 0.05$) in pH and acidity in white wines (Sauvignon blanc) treated with HHP at 300-500 MPa for 5, 10 and 15 min.

Table 6. pH and total, volatile and fixed acidities of fermented HHP processed and pasteurized beverages at days 0 and 42 of storage (4 ± 1 °C)^a.

Charact.	Control	HHP/T ^b (MPa/min)			Pasteurization	
		500/10	550/10	600/5	VAT	HTST
0 day						
<i>pH</i>	3.09±0.01a	3.08±0.02a	3.09±0.02a	3.10±0.01a	3.10±0.02a	3.10±0.01a
<i>TA</i>	0.69±0.03a	0.68±0.03a	0.69±0.01a	0.69±0.03a	0.68±0.02a	0.69±0.02a
<i>VA</i>	0.06±0.01a	0.05±0.01b	0.05±0.01b	0.06±0.02b	0.05±0.01b	0.06±0.02b
<i>FA</i>	0.62±0.01a	0.63±0.02a	0.64±0.01a	0.63±0.01a	0.63±0.02a	0.63±0.01a
42 day						
<i>pH</i>	3.10±0.02a	3.07±0.02a	3.09±0.01a	3.09±0.01a	3.10±0.01a	3.10±0.03a
<i>TA</i>	0.74±0.03a	0.69±0.02a	0.68±0.01a	0.69±0.03a	0.69±0.02a	0.71±0.02a
<i>VA</i>	0.11±0.02a	0.06±0.01b	0.06±0.01b	0.05±0.02b	0.06±0.01b	0.08±0.01b
<i>FA</i>	0.63±0.10a	0.63±0.07a	0.62±0.11a	0.64±0.09a	0.63±0.10a	0.63±0.06a

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$). ^bPressure/time (MPa/min). *TA* (total acidity, percentage citric acid, w/v), *VA* (volatile acidity, percentage acetic acid, w/v), *FA* (fixed acidity, percentage citric acid, w/v).

7.3.7. Color characteristics

Table 7 shows the color parameters of HHP processed and pasteurized pomegranate beverages at day 0 and 42 days of storage. Color is one of the most important physical properties of a drink; it provides important information about the quality of the food. All pomegranate beverages had an average L^* value of 29.01 ± 0.37 , indicating a “dark” luminosity; however, no significant differences were observed ($p \leq 0.05$) within control and all processed samples. The VAT pomegranate beverage was the one with the lowest luminosity (28.50). At day 42 of storage, the L^* values barely decreased in all beverages. Tao et al. (2012) reported significant changes in the L^* , a^* , b^* , Chroma and hue color parameters of commercial red wine (Nero D'avola Syrah: 93% Nero D'avola, 7% Syrah) treated with HHP at 650 MPa for 5, 15, 60 and 120 min. L^* values were in the range 77.63 ± 0.02 - 78.68 ± 0.06 , a^* values were in the range 22.87 ± 0.06 - 22.08 ± 0.07 , b^* values were in the range 8.70 ± 0.0 - 8.69 ± 0.03 , C values were in the range 24.47 ± 0.05 - 23.73 ± 0.07 and H values were in the range 20.84 ± 0.04 - $21.48\pm 0.02^\circ$. The L^* values increased as the pressure holding time increased; the HHP-treated wines were brighter than untreated ones. Contrary to reported in this study, where the luminosity parameter

was darkening, which can be attributed to the type of fruit used in the fermented beverage and/or to the complexity of the food matrix and its behavior when exposed to different pressures and times.

Table 7. Color parameters of HHP processed and pasteurized fermented pomegranate beverages after 42 days of storage (4±1 °C).

Charact.	Control ^b	HHP/T (MPa/min)			Pasteurization	
		500/10	550/10	600/5	VAT	HTST
Day 0						
<i>L</i> *	29.43±0.02a ¹	29.37±0.03a	29.12±0.02a	28.99±0.01ab	28.50±0.02b	28.66±0.03ab
<i>a</i> *	23.87±0.10a ¹	23.97±0.07a	23.99±0.11a	24.51±0.09a	21.07±0.10b	21.23±0.06b
<i>b</i> *	10.45±0.04bc ¹	10.49±0.06b	10.56±0.04b	11.23±0.05a	9.81±0.06d	9.90±0.04cd
<i>H</i> (°)	23.6c	23.6bc	23.8bc	24.6a	25.0ab	25.0a
<i>C</i>	26.06b	26.16ab	26.21ab	26.96a	23.24c	23.42c
ΔE^*	NA	0.12b	0.35b	1.10b	3.02a	2.80 ^a
Day 42						
<i>L</i> *	28.03±0.04a	28.01±0.02a	27.98±0.01a	27.76±0.03ab	27.49±0.02b	27.65±0.02ab
<i>a</i> *	21.86±0.11a	22.05±0.09a	22.79±0.08a	23.03±0.10a	18.62±0.11b	19.08±0.10b
<i>b</i> *	9.12±0.05bc	10.10±0.06b	10.21±0.05b	10.95±0.03a	8.11±0.04d	8.36±0.04cd
<i>H</i> (°)	22.6c	24.6bc	24.1bc	25.4a	23.5ab	23.7a
<i>C</i>	23.69b	24.25ab	24.97ab	25.50a	20.31c	20.83c
ΔE^*	2.79b	2.33b	1.82b	1.94b	6.07a	5.52a

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$). ^bData taken into account as a reference for the calculation of the net color change (ΔE^*).

The *a** positive values were in the red-yellow segment of the color space in the range 18.62-29.43 at days 0 and 42 for both control and processed pomegranate beverages; however, at day 42 of storage the red color (*a**) decreased. High temperatures combined with a long times may degrade color-related pigments such as anthocyanins. These changes are mostly due to the conversion of monomeric anthocyanins to more condensed compounds during storage. This condensation reaction, induced by high pressure and/or temperatures, involves covalent association of anthocyanins with other flavonols or organic acids leading to the formation of a new pyran ring by cycloaddition. Anthocyanin condensation may be responsible for the changes in red color during storage, forming other complexes (Marszałek, Wozniak, Kruszewski & Skapska, 2017). The HHP3 processed

pomegranate beverage showed the highest a^* value, followed by HHP2 and HHP3. The beverages that were more modified in the a^* value were those pasteurized. Both VAT and HTST beverages were statistically different ($p < 0.05$) to the control and HHP1, HHP2 and HHP3 regarding the a^* value. Concerning the b^* color parameter, the highest values were for the HHP3 pomegranate beverage. The pasteurized beverages had the lower values ($p < 0.05$) in comparison to the b^* values of the HHP processed and control beverages. In general, the b^* value decreased lightly from day 0 to day 42. About H, no significant differences ($p \geq 0.05$) were observed within of all beverages at day 0 or day 42 of storage. The H or color angle for all pomegranate beverages was in the range 23.6-25.0°, indicating a red color, found in the red-yellow segment of the color space. The hue value decreased lightly at the end of storage in control and pasteurized pomegranate beverages. These changes were expected due to the values obtained for the a^* parameter. The low reduction in H was mainly due to the reduction of L^* . Significant differences ($p \leq 0.05$) were observed within the C values of control and processed beverages; however, no significant differences ($p \geq 0.05$) were observed within the three HHP processed beverages. The lower C values were for pasteurized beverages at day 0 and 42 days of storage. Therefore, it could be said that anthocyanins were degraded due to the exposition to high temperature. The ΔE^* values (total change in color) indicate how big is the color change of a material. The higher change in color was observed in the pasteurized beverages ($p \leq 0.05$) comparing with the HHP processed beverages. The ΔE^* values were the result of the changes occurred in the L^* , a^* and b^* color parameters. Puértolas, Saldaña, Álvarez & Raso (2010) have pointed out that the color differences can be perceived by the naked eye if the value of ΔE^* is greater than 3, value that was only observed in the pasteurized beverages at day 0 and 42 days of storage. Therefore, the HHP processing may maintain the anthocyanins that give color to the fermented pomegranate beverages. Keenan, Brunton, Gormley, & Butler (2011) reported ΔE^* values lower than 3 in HHP processed (450 MPa-1.3, 5 min) strawberry, banana, orange and apple smoothies in comparison with thermally pasteurized smoothies. According to Keenan et al. (2011), despite of changes in chromatic characteristics in fermented beverages treated with HHP, these changes could not be perceived by the naked eye.

7.3.8. Sensory analysis

Table 8 shows the sensory evaluation data of fermented pomegranate beverages at the end of storage (42 days). Significant differences ($p \leq 0.05$) were observed only within appearance and color of control and processed beverages. Sweetness, aroma, flavor and general acceptability did not have any statistically difference ($p \geq 0.05$).

Table 8. Sensory evaluation data of fermented beverages treated with HHP and pasteurization (VAT and HTST) at the end of storage (42 d) at low temperature.

Charact. Control ^b	HHP/T ^a (MPa/min)			Pasteurization		
	500/10	550/10	600/5	VAT	HTST	
Appearance	7.25±1.02ab	6.95±1.05b	7.15±1.31b	7.15±1.27b	7.90±0.72ab	8.15±0.74a
Color	7.40±1.19ab	7.25±1.21ab	7.05±1.43b	7.15±1.31b	8.20±0.69a	8.25±0.55a
Aroma	7.10±1.48a	7.00±1.26a	7.05±1.00a	6.80±1.44a	7.30±1.56a	7.25±1.41a
Sweetness	7.45±1.23a	7.20±1.54a	6.75±1.92a	6.75±1.48a	7.40±1.35a	7.00±1.62a
Flavor	7.40±1.54a	7.35±1.18a	6.75±2.05a	6.65±1.76a	7.45±1.43a	7.35±1.76a
Acceptability	7.55±1.39a	7.35±0.87a	6.80±1.70a	7.10±1.41a	7.65±1.35a	7.40±1.23a

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$); ^bData of control: untreated fermented beverage.

In most of the sensory attributes, a minimum average value of 7 (Like much) was given by judges for control and HHP processing and pasteurized beverages. In general, no significant difference ($p > 0.05$) was observed within treatments for sweetness, aroma, flavor and overall acceptability; however, significant differences ($P \leq 0.05$) were observed within treatments for appearance and color. Keenan et al. (2011) pointed out that no significant differences were observed about acceptability of HHP processed and pasteurized smoothies of fruits. Tao et al. (2012) reported differences in the appearance of wines treated with HHP (650 MPa). However, it should be taken into account that in general there are “likes” and “dislikes” within any type of food and beverage and within qualities of different types of foods and beverages. The results of the sensory evaluation are of paramount importance since samples treated with heat could lose color or be browning due to thermal caramelization (Tao et al., 2012). In this study, pomegranate beverages treated with heat were those with the highest loss of pigments and with the greatest color changes; however, they were still well accepted by the judges.

7.4. PEF processing in pomegranate beverage

7.4.1. Inactivation of microorganism

As shown in Fig. 10 there was a reduction of approximately 3.5 logarithmic cycles in the sample treated with PEF at 18.8 kV/cm and the heat-treated sample VAT, compared with the control at day 0, in addition there was a complete inactivation of aerobic mesophilic bacteria. In the treatment with PEF at 11.7 kV/cm the microbial load of aerobic mesophilic bacteria remained very similar to the control at day 0, slightly reducing the aerobic mesophilic bacteria, but without inactivating them. According to the microbiological criteria for citrus juices proposed by FDA, 5 log reductions of target microorganisms can be considered as a good benchmark to obtain safe products (Goodrich, 1999; Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín -Belloso, 2010). Likewise, for wine, the International Oenological Codex (updated in OIV Resolution No. 16/2003), recommends a load lower than 10^3 CFU/g of bacteria for wine. The samples 18.8 kV/cm and VAT pasteurization would be within the microbiologically safe limits. Puértolas et al. (2009), evaluated the use of pulsed electric field technology as an alternative system for microbiological safety in wines. They reported an optimum treatment at 186 kJ/kg at 29 kV/cm to reduce the 99.9% of the spoilage flora of must and wine, limiting the risk of alteration of these products by microorganisms of *Brettanomyces* and *Lactobacillus* genera. The sample with HTST pasteurization reduced the load of mesophilic bacteria (MB) relative to the control by 1 logarithmic cycle. During the storage time, the control sample, the PEF 11.7 kV/cm sample, and the HTST sample increased their microbial load. At day 56 of storage there was an increase in MB of 1 logarithmic cycle. PEF at 18.8 kV/cm and VAT did not show MB growth during the entire storage time. In addition, it is observed that the control sample and the sample PEF 11.7 kV/cm present a tendency like a greater growth of MB passing the days of storage. This indicates that the treatment of PEF at 11.7 kV/cm is not enough to completely inactivate the microbial load.

In Fig. 11 the growth of yeasts and molds is shown. The samples treated with PEF 18.8 kV/cm, VAT and HTST presented a total inactivation of these microorganisms, reducing the microbial load 3 logarithmic cycles, with respect to the control at day 0. None of the samples presented growth of yeasts and molds during the entire storage time.

Delsart et al. (2015), studied the effect of the application of PEF on sweet white wine must, obtaining a total inactivation of yeasts with the application of the treatment at 4-20 kV/cm, they reported that the highest rate of inactivation was obtained with the treatment at 20 kV/cm. The treatment of PEF at 11.7 kV/cm reduced the load of yeasts and molds almost 2 logarithmic cycles with respect to the control sample at day 0. In Fig. 2 it is observed that the control sample begins a greater phase of growth at day 35 of storage. In the sample treated with PEF 11.7 kV/cm, the same tendency was observed in the control sample of growth of yeasts and molds, during the days of storage, only that this growth is less marked than in the control sample. The heat treatment and the application of PEF

(18.8 kV/cm) to the fermented pomegranate beverage may inhibit the growth of yeasts and molds during 56 days of storage.

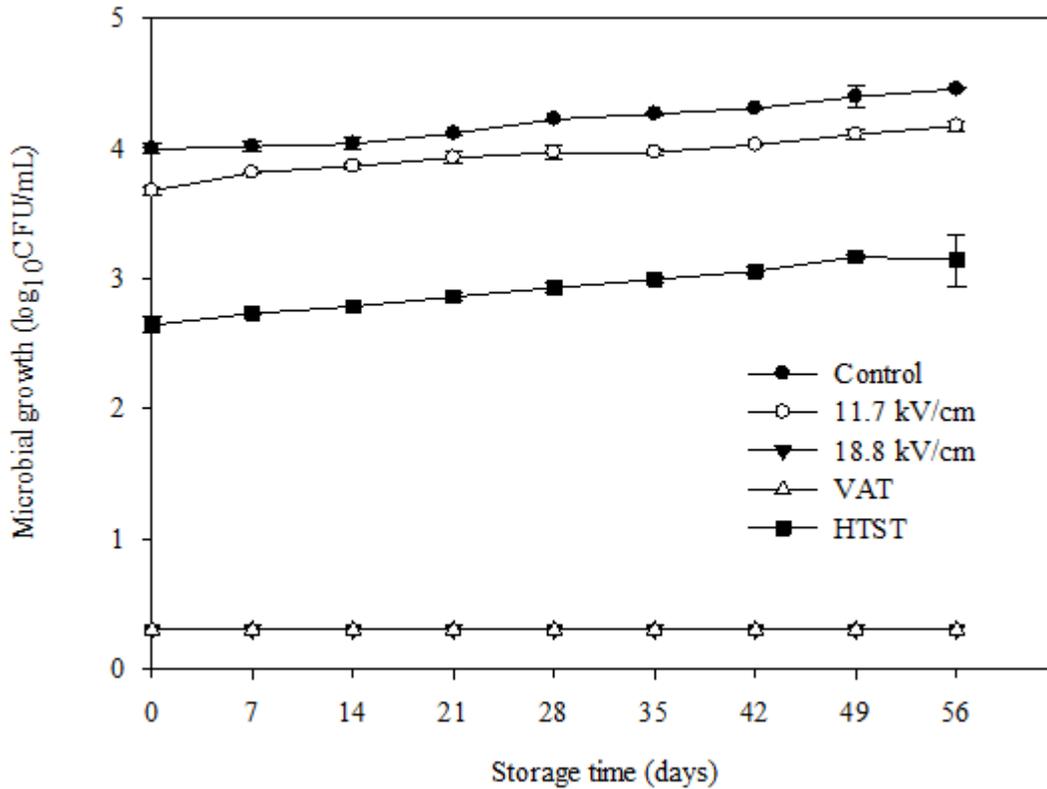


Figure 10. Growth curve of aerobic mesophilic in untreated, PEF-treated, and pasteurization-treated pomegranate fermented beverage during storage at 4°C for 56 days.

In this research bacteria were more resistant to processing by PEF than yeast. Puértolas, López, Condón, Raso, & Álvarez (2009), investigated the lethal effect of PEF in different microorganisms of deterioration, in must and wine. They reported that bacteria were more resistant on the processing by PEF on yeast. The size, shape, morphology, and biochemical characteristics of microbial cells are involved in cell resistance when PEF processing is applied (Puértolas et al., 2010). Additionally, other physicochemical factors such as pH, alcohol content, water activity, soluble solids, electrical conductivity or temperature should also be considered for the elimination of microorganisms (Toepfl, Heinz, & Knorr, 2007; Heinz & Knorr, 2000; El-Hag, Jayaram, Gonzalez, & Griffiths, 2011).

7.4.2. Changes in TSS, ethanol concentration and pH

Fig. 12 shows the effect of the application of PEF on TSS. Significant differences $p \leq 0.05$ were found in the change of total soluble solids. On day 0 after the application of the treatments, it can be

observed that the samples that most reduced the TSS content were PEF 18.8 kV/cm and the VAT pasteurization, the sample that had less changes was the HTST pasteurization. The control sample showed a greater change in the TSS content during and at the end of the storage time. Followed by the pasteurized sample HTST. Evrendilek (2017), study the effect of the application of pulsed electric field on quality properties of pomegranate juice, he did not report significant changes ($p \geq 0.05$), between his control and the pomegranate juice treated with PEF (0, 17, 23 and 30 kV/cm electric field strengths).

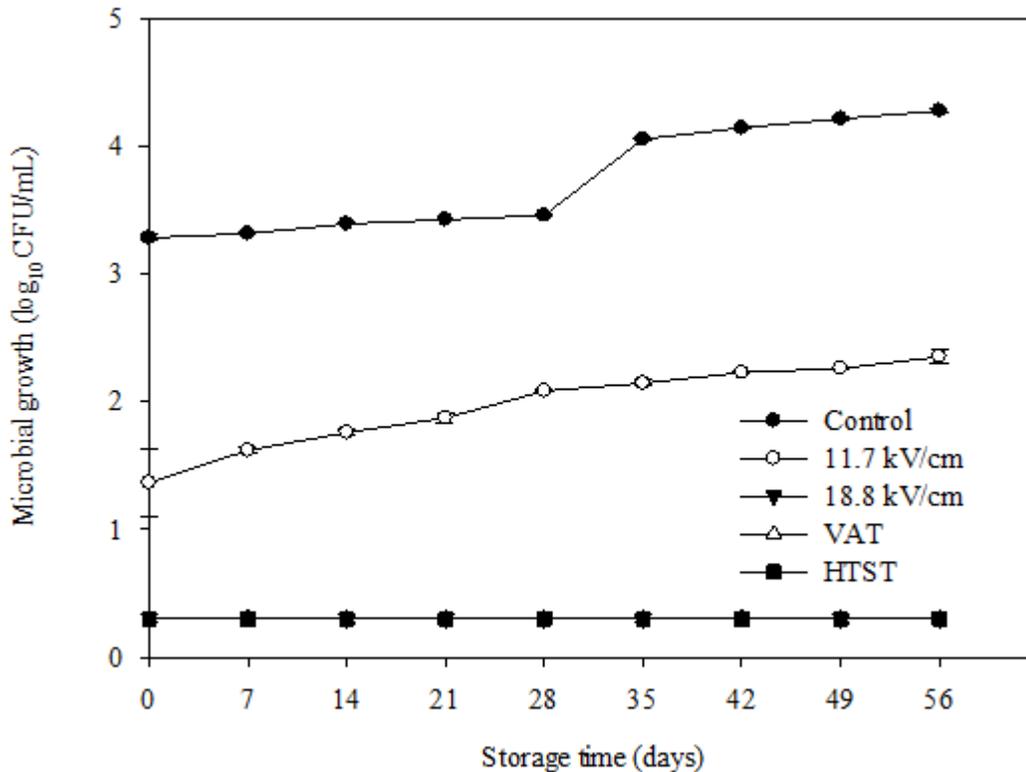


Figure 11. Growth curve of yeast and molds in untreated, PEF, and pasteurization treated pomegranate fermented beverage during storage at 4°C for 56 days.

Fig. 13 shows the change in the ethanol concentration of the samples treated with PEF and pasteurization during the storage time. Significant changes ($p \leq 0.05$) were observed between the control sample and the other samples treated with PEF and pasteurization. On day 0, the VAT sample showed the greatest decrease in ethanol concentration, from 12.4 to 12.3% v/v, compared to the control. The samples treated with PEF were very similar to the control and were more stable during the storage time. The untreated sample or control showed a slight increase in ethanol concentration from 12.3 to 12.9% v/v. The VAT pasteurized sample showed a slight decrease in the concentration of ethanol during storage (VAT: 12.2-11.9% v/v), which may be due to the high temperature and the long time that was used for pasteurization and that could cause a slight evaporation in the ethanol concentration. López, Puértolas, Hernández-Orte, Álvarez, & Raso (2009), applied the PEF treatment

(5 kV/cm) in Cabernet Sauvignon wine samples, they reported that PEF processing did not affect the concentration of ethanol in their samples.

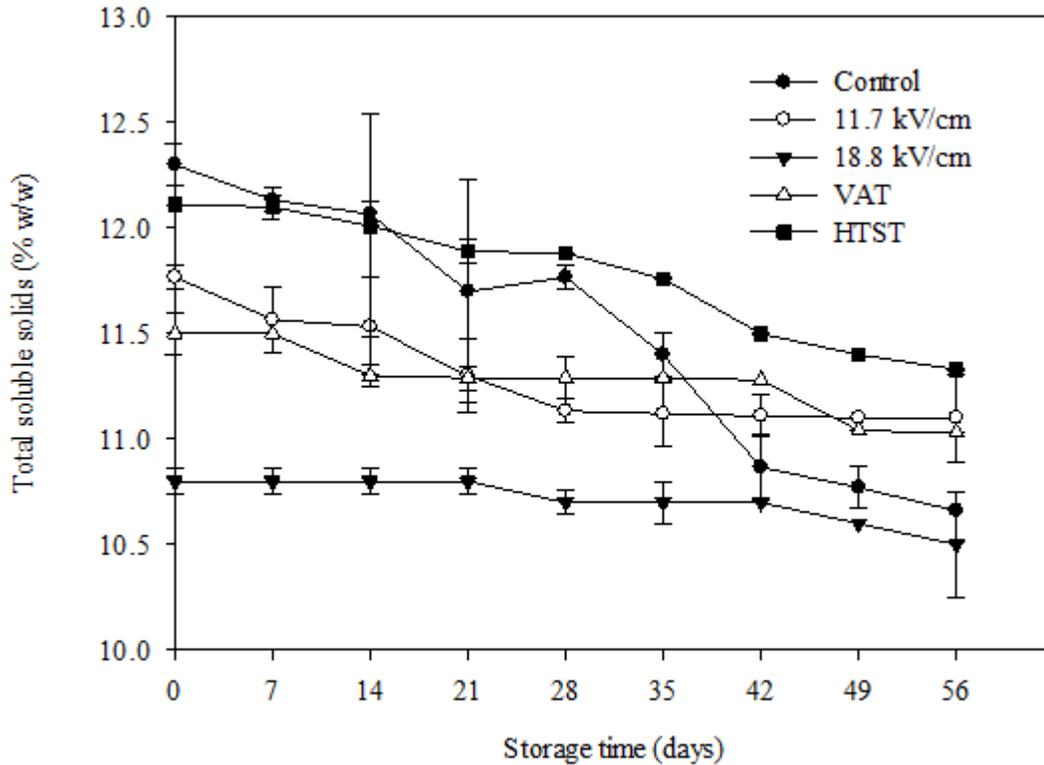


Figure 12. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on total soluble solids in pomegranate beverages through refrigeration (4 ± 1 °C).

In Fig. 13 the pH value in the treated (PEF and pasteurization) and untreated samples during the storage time is observed. Significant changes ($p \leq 0.05$) were observed between the control sample and the other treated samples. The pH decreased slightly to day 0 in the samples that were treated with respect to the control. During the storage time all the treated and untreated samples slightly decreased the pH, this change being more observable in the sample PEF 11.7 kV/cm. The pH value of all the samples was in a range of 3.25 to 3.34. Delsart et al. (2015), reported that the pH value only underwent a slight change when the PEF treatment was applied (sweet white wine, 4-20 kV/cm). Other authors who also evaluated the effect of PEF processing did not report significant changes ($p \geq 0.05$) in the pH value, in the sample of citrus juices, grape juice, carrot juice and pomegranate (Hartyáni et al., 2011; Cserhalmi et al. , 2006, Caminitni et al., 2012).

7.4.3. Changes in total, fixed, volatile acidities

Table 9 shows the change in the total, fixed and volatile acidity of the samples treated with PEF and pasteurization at day 0 and 56 of storage. Between the control sample and the PEF sample 18.8

kV/cm, no significant changes ($p \geq 0.05$) in the total acid content were observed. The VAT sample showed a significant reduction ($p \leq 0.05$) of total acidity with respect to the control at day 0. At the end of storage, the total acidity increased slightly in the control, PEF 11.7 kV/cm, VAT and HTST.

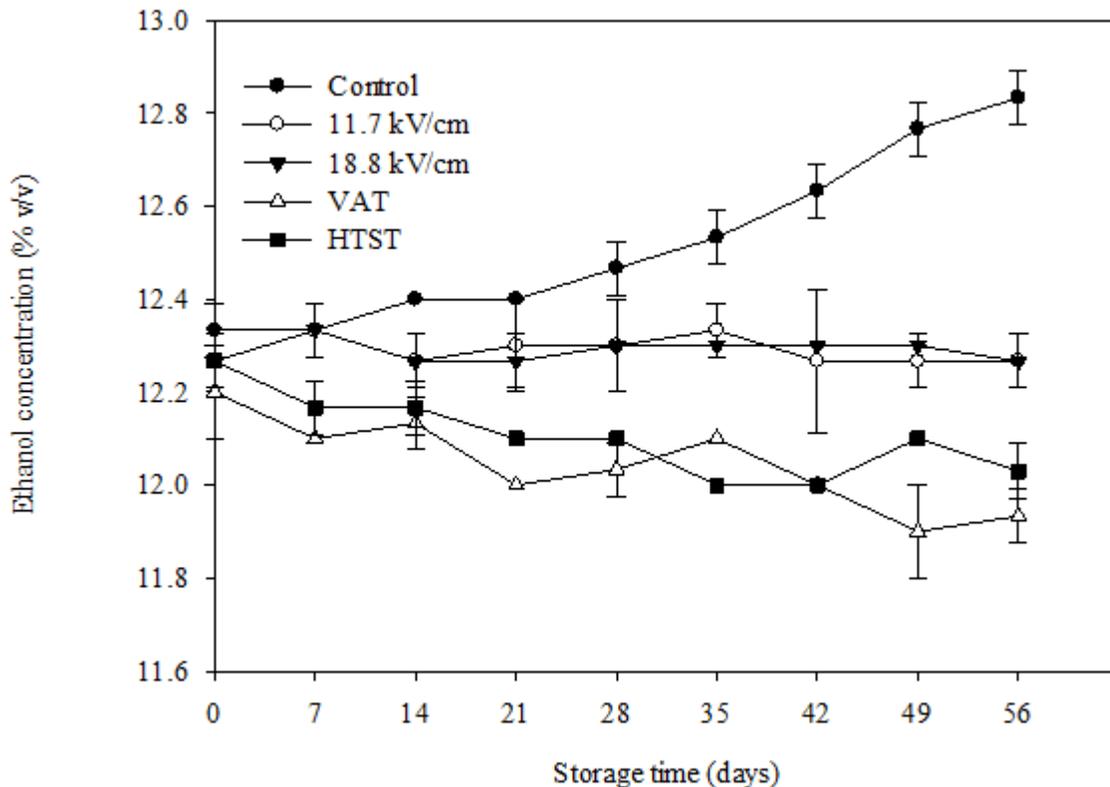


Figure 13. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on ethanol concentration (% v/v) in pomegranate beverages through refrigeration (4 ± 1 °C).

The fixed acidity showed significant changes ($p \leq 0.05$) of the untreated sample to the samples that were treated. The PEF sample 18.8 kV/cm had higher fixed acidity and the VAT sample had the lowest amount of fixed acidity. At day 56 of storage the fixed acidity increases for all samples except for PEF 18.8 kV/cm. Hartyáni et al. (2011), reported that the content of acids such as malic and citric did not decrease significantly after treatment with PEF in citrus juices (28 kV/cm). Other authors also reported that values such as total and fixed acidity are not influenced by PEF processing (López et al., 2009; Evrendilek, 2017). Volatile acidity at day 0 was different ($p \leq 0.05$) for all samples. The control sample and the VAT sample had a higher content of volatile acidity. Some research has reported that volatile acidity decreases after the application of PEF (González-Arenzana et al., 2016).

7.4.4. Color measurement

In Fig. 15 the changes in the luminosity parameter during the storage time are observed. Significant changes were found among all the samples ($p \leq 0.05$).

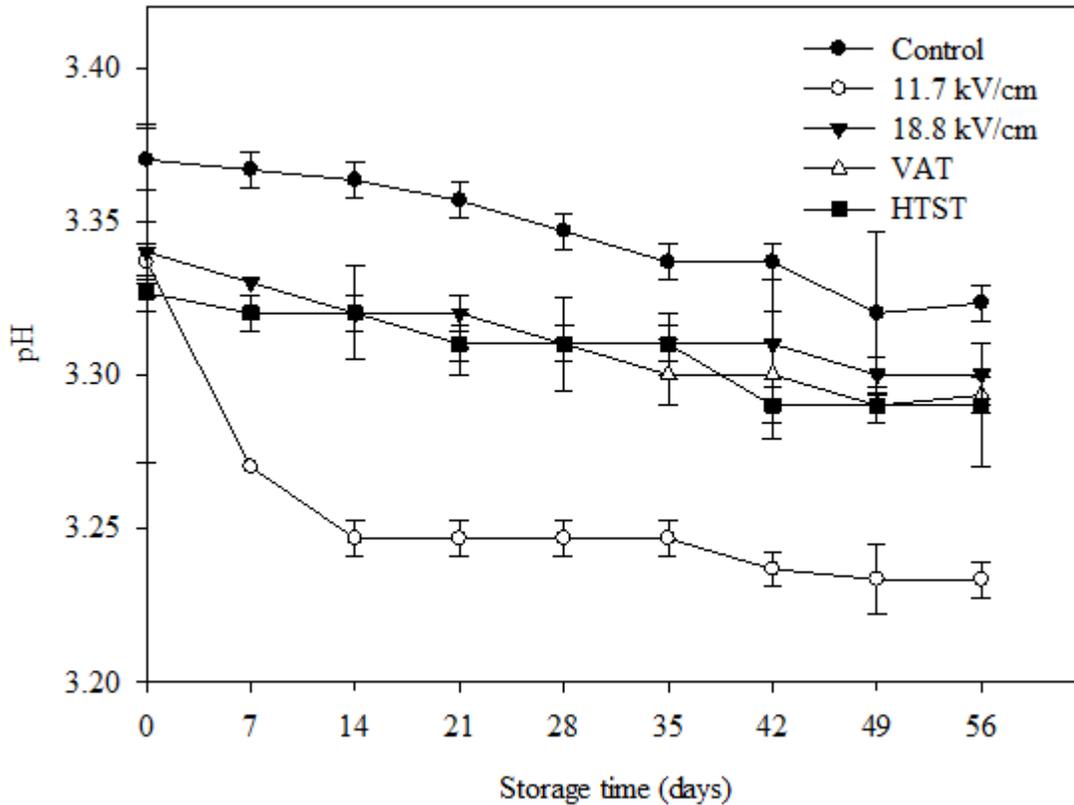


Figure 14. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on pH in pomegranate beverages through refrigeration (4 ± 1 °C).

The sample that had the highest luminosity at day 0 was PEF 18.8 kV/cm, followed by PEF 11.7 kV/cm. The VAT sample was the one that darkened, having a lower L^* value than the others (26.79). All samples decreased the luminosity parameter during the storage time. As seen in Fig. 6 the most stable sample was PEF 11.7 kV / cm. Other authors have reported an increase in luminosity after application by PEF (Morales-de la Peña et al., 2017; Li, Zhang, Lee, & Pham, 2003). Evrendilek (2017), reported an increase in L^* in a sample of pomegranate juice treated with PEF (control: 16.93 ± 0.30 , PEF: 17.04 ± 0.70 , 0-30 kV / cm). Vazquez-Cabral et al. (2016), also reported an increase in the value of L^* processed by PEF at 37.3 kV / cm, from the samples obtained the control values: 11.22 to PEF: 11.26.

In the parameter of a^* , there were no significant changes ($p \geq 0.05$) between the control sample and the samples treated with PEF 11.7 and 18.8 kV/cm. The pasteurized VAT sample was the one that obtained a value less than a^* at day 0 (14.89), followed by the sample HTST (a^* : 15.01). In Fig. 16 it is observed that all the treated and untreated samples gradually decreased this value during the storage time. Carbonell-Capella et al. (2017), also reported a slight decrease in the value of a^* of samples of fruit juices treated by PEF (20-40 kV/cm). The a^* values diminished when the fruit juice was treated by PEF, independently of the electric field strength and time treatment. On the other

hand, other authors have reported an increase in the value of a^* after processing by PEF (Guo et al., 2013; Vazquez-Cabral et al., 2016).

Table 9. Total fixed and volatile acidities of fermented PEF processed and pasteurized beverages at days 0 and 56 of storage (4 ± 1 °C)^a.

Charact.	PEF (kV/cm)			Pasteurization	
	Control ^b	11.7	18.8	VAT	HTST
<i>TA</i>	0.441±0.00a	0.415±0.00b	0.463±0.00a	0.399±0.00b	0.431±0.00b
<i>FA</i>	0.405±0.00a	0.404±0.00c	0.453±0.00d	0.366±0.00b	0.395±0.00c
<i>VA</i>	0.036±0.00d	0.011±0.00c	0.010±0.00a	0.033±0.00b	0.036±0.00c
56 day					
<i>TA</i>	0.528±0.00a	0.490±0.00b	0.407±0.00a	0.459±0.00b	0.511±0.00b
<i>FA</i>	0.467±0.00a	0.476±0.00c	0.397±0.00d	0.425±0.00b	0.473±0.00c
<i>VA</i>	0.061±0.00d	0.014±0.00c	0.010±0.00a	0.034±0.00b	0.038±0.00c

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$). *TA* (total acidity, percentage citric acid, w/v), *VA* (volatile acidity, percentage acetic acid, w/v), *FA* (fixed acidity, percentage citric acid, w/v).

The value of b^* at day 0 was different between the samples ($p \leq 0.05$). As shown in Table 10, the pasteurized samples had a lower value of b^* , similar to that reported by Vazquez-Cabral et al., 2016. The sample with the highest value of b^* was PEF 18.8 kV/cm.

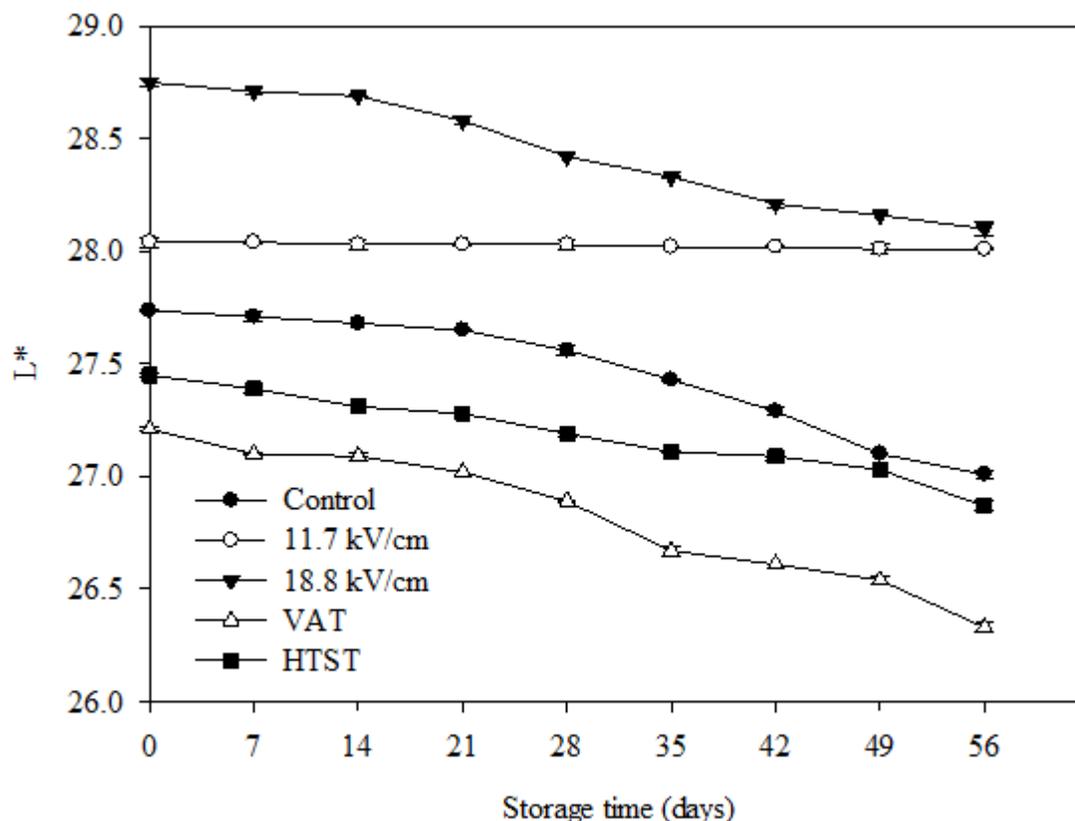


Figure 15. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on L* color parameter in pomegranate beverages through refrigeration (4 ± 1 °C).

At day 56 of storage this parameter of b^* decreased in all samples. Caminiti et al. (2012), reported a decrease in the value of b^* in citrus juices after being treated by PEF (24 kV/cm). On the other hand, other authors did not find changes in the value of b^* in samples treated with PEF (Guo et al., 2013). In the hue ($^\circ$), significant changes were observed ($p \leq 0.05$) between the control and the samples treated with PEF 11.7 and 18.8 kV / cm. The sample that had the largest hue ($^\circ$) was that of PEF at 18.8 kV/cm. At the end of storage, the hue ($^\circ$) decreased for all samples, treated and untreated. In color saturation or chroma, only significant changes ($p \leq 0.05$) were present between the control sample and the VAT sample. The sample with the highest chroma was PEF at 18.8. kV/cm. During the entire storage time this parameter was reduced, with a greater reduction in the VAT sample. López et al. (2009), reported a maximum value of chroma in the sample treated with PEF (68) (5 kV/cm), that in the control (63), on samples of must and Cabernet Sauvignon wine. However, in hue measurement ($^\circ$) they did not observe differences in this parameter.

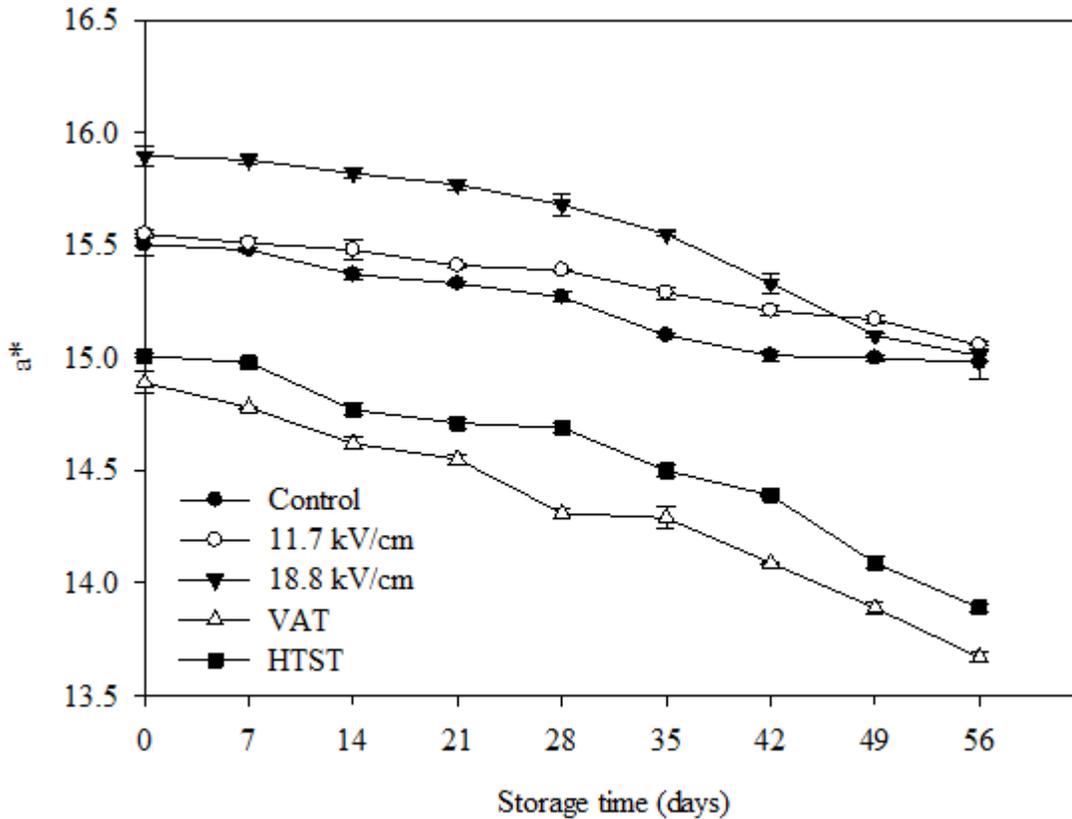


Figure 16. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on a^* color parameter in pomegranate beverages through refrigeration (4 ± 1 °C).

The net color change is an important value to observe the color differences between the samples. A value higher than 3 indicates that observers can easily detect a difference among samples (Gómez-Mínguez, González-Miret, & Heredia, 2007). The net color change ΔE^* at day 0 with respect to the control or untreated sample was greater in the PEF samples 18.8 kV/cm and VAT pasteurization. The sample with the lowest ΔE^* was PEF 11.7 kV/cm. The ΔE^* of each sample was also analyzed at day 0 and day 56 of storage (Table 10). The sample with the highest color change on storage days was pasteurized by VAT, followed by PEF 18.8 kV/cm and the control sample. The sample that had the least color changes was PEF 11.7 kV/cm.

7.4.5. Antioxidant characteristics

7.4.5.1. Antioxidant capacity

Fig. 17 shows the change in the antioxidant capacity of the treated and untreated samples during the storage time. Significant differences ($p \leq 0.05$) were found between the control and pasteurized samples with the samples treated with PEF 11.7 and 18.8 kV/cm.

Table 10. Color parameters of PEF processed and pasteurized fermented pomegranate beverages after 56 days of storage (4±1 °C)^a.

Charact.	PEF ^a (kV/cm)			Pasteurization	
	Control ^b	11.7	18.8	VAT	HTST
0 day					
<i>b</i> *	4.78±0.07b	4.69±0.02b	4.99±0.01a	4.58±0.01c	4.61±0.02bc
<i>H</i> (°)	17.12±0.23b	16.78±0.05c	17.43±0.03a	17.11±0.03b	17.09±0.05ab
<i>C</i>	16.22±0.02a	16.23±0.03a	16.66±0.11a	15.58±0.13b	15.70±0.04ab
ΔE^*	-	0.32±0.00 ^c	1.11±0.00 ^c	0.83±0.00 ^c	0.59±0.00 ^c
56 day					
<i>b</i> *	4.17±0.01b	4.11±0.01b	4.33±0.01a	4.01±0.02c	4.11±0.01bc
<i>H</i> (°)	16.53±0.03b	15.25±0.03c	16.95±0.03a	17.01±0.06b	16.50±0.01ab
<i>C</i>	14.66±0.01a	15.61±0.02a	14.84±0.02a	13.72±0.01b	14.49±0.01ab
ΔE^*	1.59±0.00 ^d	0.76±0.00 ^d	1.94±0.00 ^d	2.21±0.00 ^d	1.36±0.00 ^d

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$). ^bData taken into account as a reference for the calculation of the net color change (ΔE^*). ^cFrom day 0 with respect to the control sample. ^dFrom day 0 of the same sample with respect to day 56 of the same sample.

It can be observed that the sample that had the highest content of antioxidant capacity was PEF 18.8 kV/cm at day 0 (279.41 mg TE/100 mL). The VAT sample was the one that had a greater decrease in antioxidant capacity at day 0 (240.81 mg TE/100 mL).

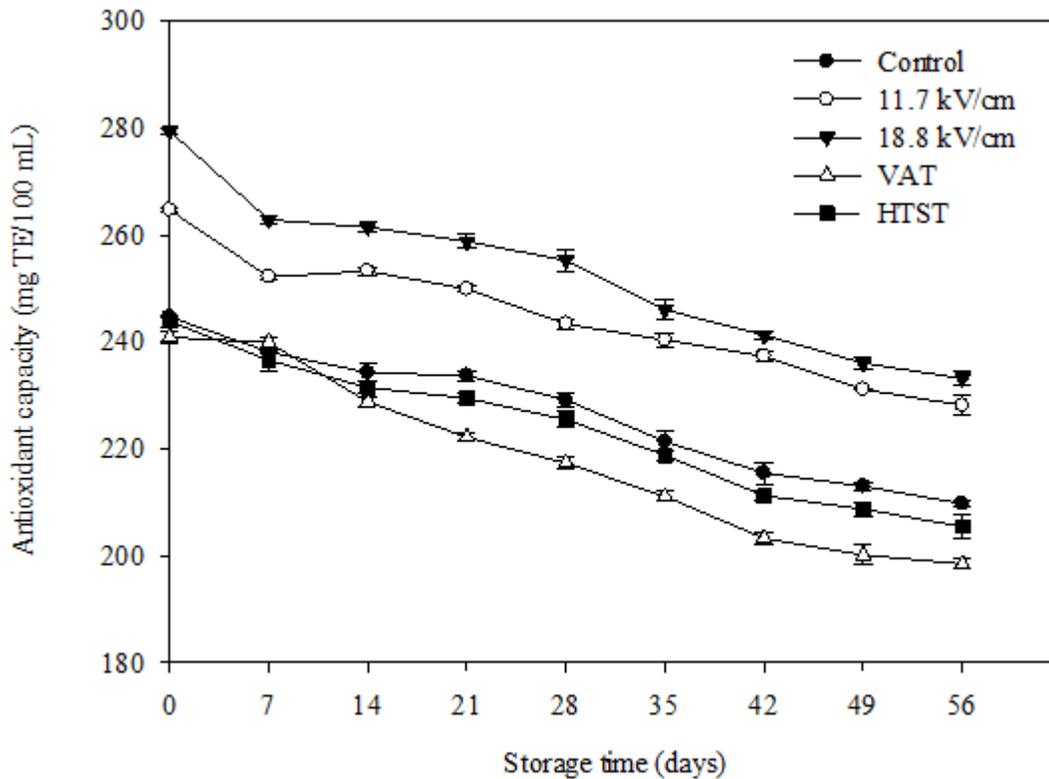


Figure 17. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on antioxidant capacity in pomegranate beverages through refrigeration (4 ± 1 °C).

Vicaș, Bandici, Teușdea, Turcin, Popa, & Bandici (2017), studied the effect of PEF processing (7 kV/cm) on the content of bioactive compounds and the antioxidant capacity, in wines derived from three grape varieties (Muscat Ottonel, Pinot Noir and Merlot), they reported higher values of antioxidant capacity in the samples that were treated with PEF compared to the controls in the Pinot Noir grape, likewise they report a high correlation related to other bioactive compounds such as phenols. López-Giral et al. (2015) have also obtained a good correlation between the total phenols index and antioxidant capacity. Non-antioxidant compounds in wine (amino acids, uronic acids) may cause interferences and influence the estimated values of antioxidant capacity. Donsi et al. (2011) have evaluated the effect of PEF treatment applied to four varieties of grapes in Italy in respect to antioxidant activity (by DPPH method) and have obtained a high antioxidant capacity (+ 40%). All samples decreased antioxidant capacity during storage time.

7.4.5.2. Total phenols

In Fig. 18 it is observed that at day 0, the sample of PEF 18.8 kV/cm increased significantly ($p \leq 0.05$) the content of total phenols with respect to the other samples.

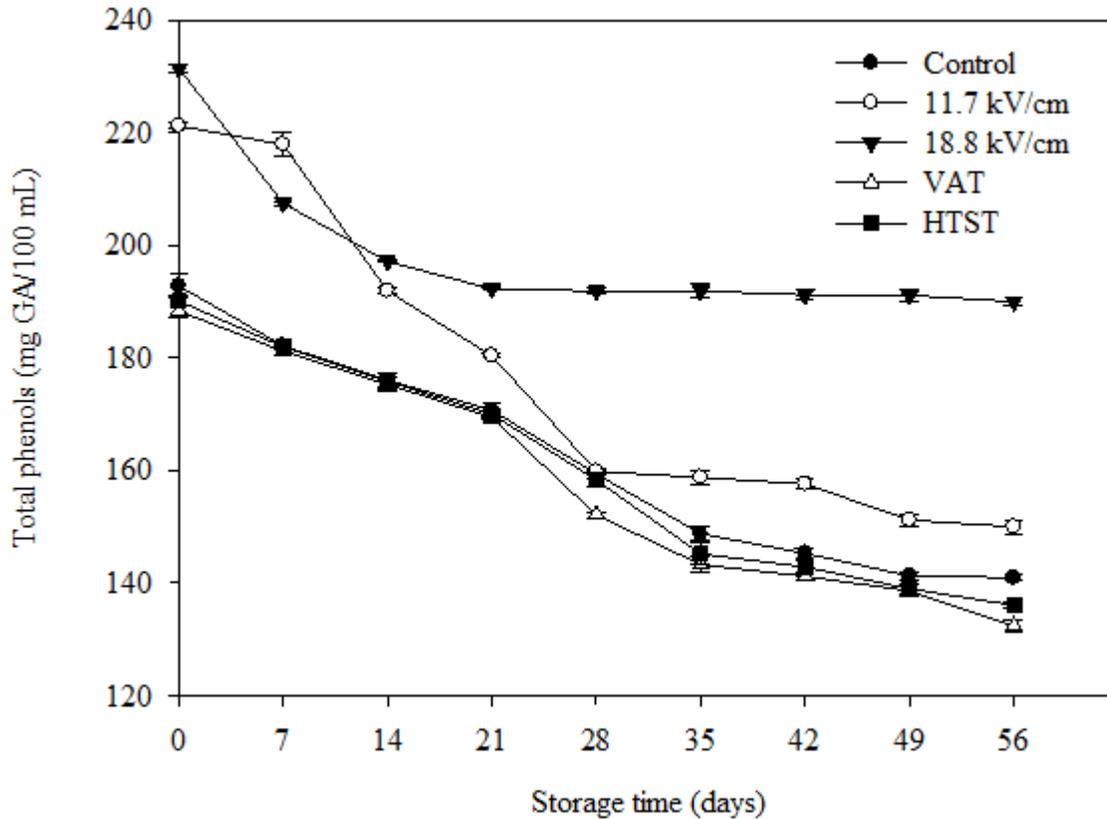


Figure 18. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on total phenols in pomegranate beverages through refrigeration (4 ± 1 °C).

The control samples, PEF 11.7 kV/cm, VAT and HTST were statistically equal ($p \geq 0.05$). Other authors have not reported significant changes in the content of total phenols after the application of PEF (Caminiti et al., 2012; Chen et al., 2014). Morales-de la Peña et al. (2010), reported the effect on the content of total phenols in samples of soy beverages processed by PEF (35 kV/cm), their results varied from 79.88 (in the untreated samples) to 83.09 mg GA/100 mL, but they were not significant. All samples decreased the content of total phenols during the storage time. However, this reduction was more marked in the pasteurized, control and 11.7 kV/cm samples. Researches mention that heat treatments are, in general, the main cause of depletion of natural antioxidants (Anese, Manzocco, Nicoli and Lericci, 1999, Roy, Takenaka, Isobe and Tsushida 2007), these researchers conclude that thermal temperature affects considerably the phenolic content in materials where the raw material is vegetable. Hence, the high temperatures applied during the thermal treatment may have affected more the decrease of phenolic compounds, making them easily degradable over time.

7.4.5.3. Flavonoids

In Fig. 19 the effect of PEF processing and pasteurization during the storage time is shown.

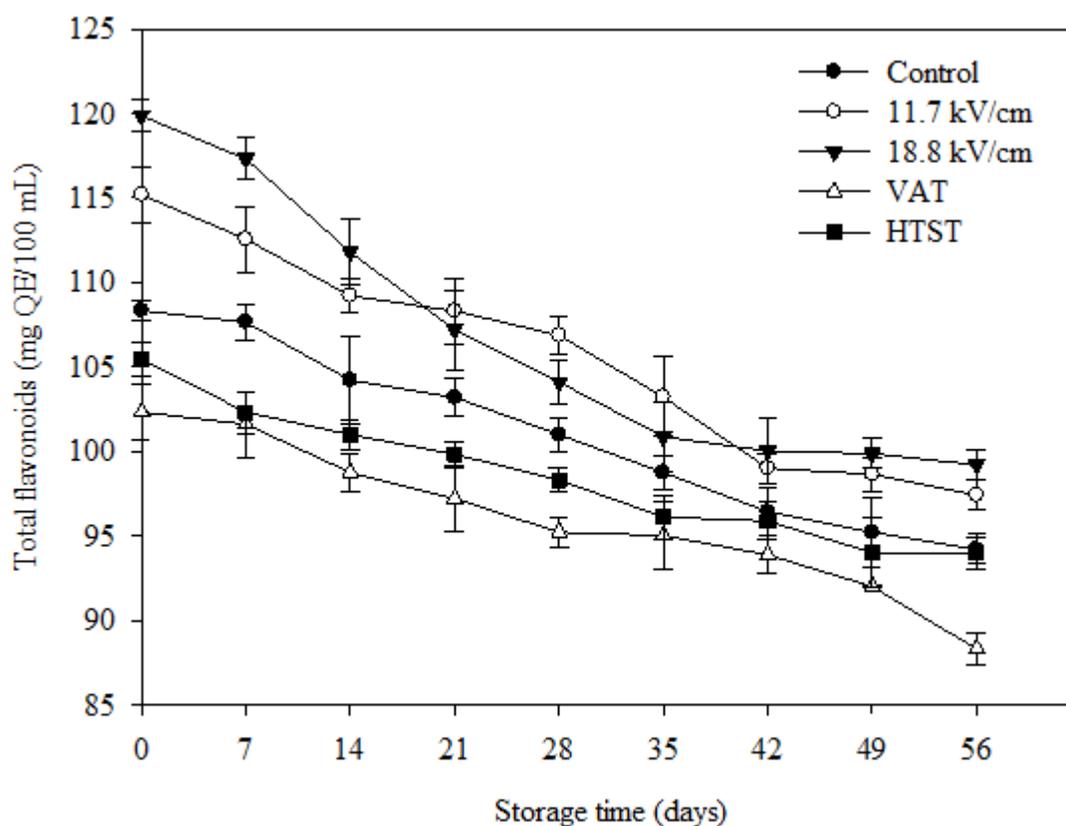


Figure 19. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on total flavonoids in pomegranate beverages through refrigeration (4 ± 1 °C).

Significant differences ($p \leq 0.05$) were observed in the samples treated with PEF 11.7 and 18.8 kV/cm compared with the control and pasteurization samples, VAT and HTST. The sample with the lowest flavonoid content was VAT at day 0 and day 56 of storage 102.32 ± 1.18 and 88.31 ± 1.29 mg QE/100 mL, respectively. The sample that retained the most flavonoid content was PEF 18.8 kV/cm 119.87 ± 1.08 and 99.21 ± 1.03 . Vazquez-Cabral et al. (2016), reported an increase in flavonoid content after treating kombucha tea samples by PEF (37.3-53.4 kV/cm). A similar behavior has been described for PEF-treated tomato juices, showing a significantly greater content of flavonoids (such as chlorogenic acid, caffeic acid and quercetin) than thermally treated juices during storage for 56 days at 4 °C (Odrizola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009). Sánchez-Moreno et al. (2005) showed that flavonone concentration of orange juice increased with PEF (35 kV cm) and thermal pasteurization at 70 °C for 30 s. However, its concentration decreased when orange juice was treated at a high thermal pasteurization (90 °C for 60 s).

7.4.5.4. Anthocyanins

Fig. 20 shows the change in anthocyanin content in the treated and untreated samples during the storage time. Significant differences ($p \leq 0.05$) were found between the control and the PEF treatment and pasteurization.

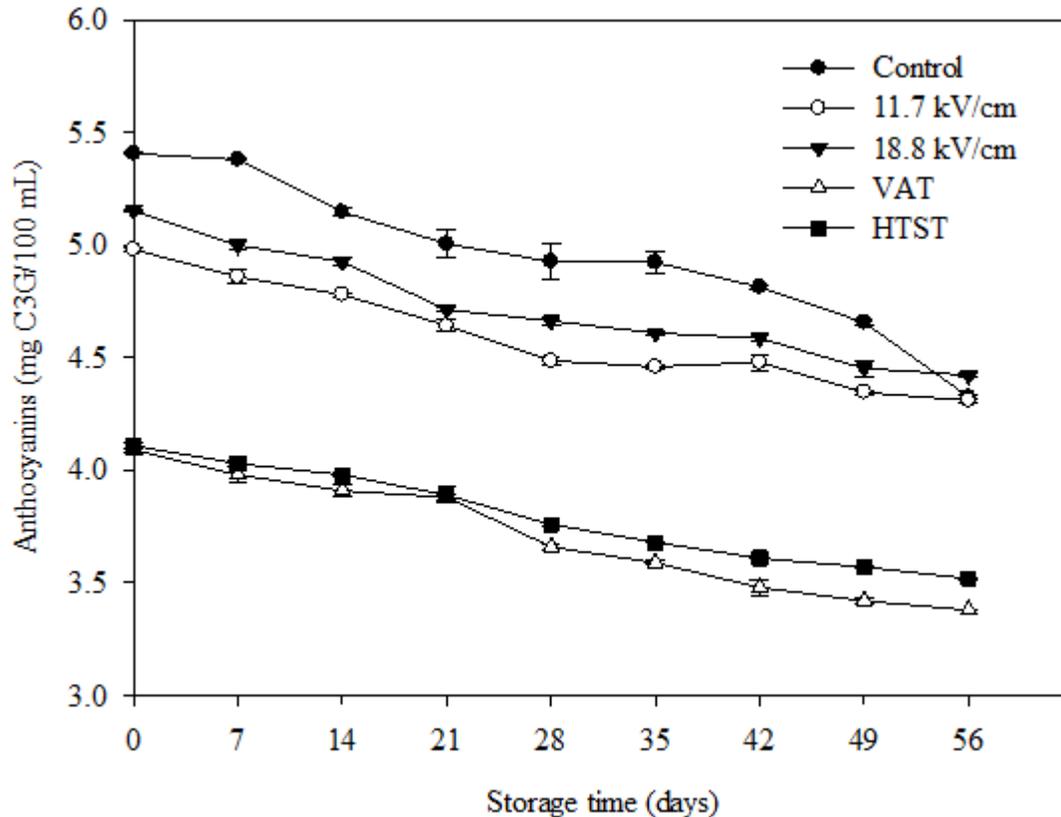


Figure 20. Effects of pulsed electric field (PEF) and thermal pasteurization (VAT, HTST) on anthocyanins in pomegranate beverages through refrigeration (4 ± 1 °C)

The sample with the highest anthocyanin content was the control sample at day 0 (5.41 ± 0.10 mg C3G / 100 mL). Klopotek et al. (2005) reported that pasteurization (85 C, 5 min) led to a 27% decrease in strawberry juice anthocyanins. The stability of anthocyanins is influenced by pH, storage temperature, presence of enzymes, light and processing conditions (Rein & Heinonen, 2004). In a fresh juice, an equilibrium exists between four anthocyanin species, the quinoidal base, the flavylium cation, the pseudobase or carbitol, and the chalcone. Unless, structural features or other factors are successful in reversing equilibrium towards the formation of the flavylium or quinoidal base, formation of chalcones (colorless) is favored by increasing temperature at the expense of quinoidal, flavylium and carbitol species (Brouillard, 1982). In addition, chalcones can be degraded to brown-colored polymeric structures when kept at a high temperature for excessive time (Jackman & Smith, 1997). Zhang et al. (2007) observed that after processing a cyanidin-3- glucoside methanolic solution by

PEF (at 1.2, 2.2 and 3.0 kV/cm, 300 number of pulses), the anthocyanin was degraded and the formation of the colorless anthocyanin species, particularly chalcones took place (Odrizola-Serrano et al., 2009). The content of anthocyanins is degraded for all samples during the storage time. At day 56 the PEF sample 18.8 kV/cm had the highest content of anthocyanins (4.42±0.9 mg C3G/100 mL). The VAT pasteurized sample at day 56 was the one that decreased the anthocyanin content more (3.38± 0.13 mg C3G/100 mL).

Table 11. Sensory evaluation data of fermented beverages treated with PEF and pasteurization (VAT and HTST) at the end of storage (56 d) at low temperature.

Charact.	PEF ^a (kV/cm)			Pasteurization	
	Control ^b	11.7	18.8	VAT	HTST
Appearance	7.64±0.98a	7.70±1.21a	7.68±1.25a	7.12±1.52a	7.28±1.72a
Color	7.58±1.07a	7.92±0.96a	7.94±1.18a	7.36±1.60a	7.28±1.48a
Aroma	6.82±1.80a	7.18±1.84a	7.24±1.43ba	6.46±2.15a	6.46±1.94a
Sweetness	7.14±1.32a	7.04±1.74a	6.88±1.49a	5.58±2.05b	5.80±1.64b
Flavor	7.04±1.51a	7.12±1.83a	6.76±1.79ab	5.88±2.27bc	5.60±2.18c
Acceptability	7.24±1.47a	7.12±1.93ab	7.06±1.62ab	6.20±2.18b	6.14±1.79b

^aDifferent letters within rows indicate significant differences ($p \leq 0.05$); ^bData of control: untreated fermented beverage.

7.4.6. Sensory evaluation

Table 11 shown the analysis of sensory evaluation of beverages treated by PEF and pasteurization at day 56 of storage. The maximum rating awarded in general was 7.92±0.96, which corresponds to a I like a lot to moderately. The minimum rating given in general for all the samples was 5.58±2.05, which corresponds to I do not like or dislike me. In the appearance attribute, the best-qualified samples were those treated with PEF 11.7 and 18.8 kV/cm. The lowest score in appearance was obtained for VAT. In the color attribute the most punctuated sample was PEF 18.8 kV/cm, the lowest was pasteurization by HTST. In the aroma attribute, no significant differences were found between the samples ($p \geq 0.05$), however, the sample treated with PEF at 18.8 kV/cm was the best rated. Those who disliked the most in aroma were the pasteurized VAT and HTST. In sweetness, the sample that was most liked was the control sample and the one that tasted least was VAT pasteurization. In flavor the highest sample was pasteurization PEF 11.7 kV/cm and the one with the least taste was

HTST pasteurization. In general acceptability the most accepted was the control, and the least accepted was HTST pasteurization. Evrendilek (2017), reported that the sensory attributes of flavor, taste, aftertaste, and overall acceptance were not significantly affected by the PEF treatments conducted at 30 kV/cm.

7.5. Black cherry

7.5.1. Yield

The yield of the juice obtained from whole black cherries was 31.66 mL/100 g. This percentage was lower than that reported for other fruits since the fruit is small and has a large stone. The fruits are 2.2 cm in diameter approximately and the stone is of 1.1 cm approximately. For orange and pineapple juices, the yield has been 45.07 and 36.5 mL/100 g fruit, respectively (Jiménez et al., 2011). The percentage of yield is important in the food industry and can vary depending on the type of fruit, maturity, and other physicochemical characteristics.

7.5.2. Physicochemical characteristics of unfermented and fermented

The physicochemical characteristics of FRJ and FEB black cherry beverages are shown in Table 12. The TSS, pH and total acidity values were characteristic of the FRJ fruit and similar to those reported by Jiménez et al. (2011): 10.30 ± 0.09 °Bx, 3.9 ± 0.25 and 0.31 ± 0.02 g citric acid/100 g, respectively. These values are very important for performing the fermentation process; values in the averages 2.8-4.0 for pH and 0.25-0.40 g citric acid/100 g for acidity are recommended by the EC (2009).

Table 12. Physicochemical characteristics of fresh and fermented black cherry beverages.

Characteristics	FRJ	Fermented beverages		
		FEB1 (10.9 °Brix)	FEB2 (17.5 °Brix)	FEB3 (25 °Brix)
TSS	10.90 ± 0.01	5.1 ± 0.02	6.0 ± 0.01	11.9 ± 0.01
pH	4.40 ± 0.02^a	3.73 ± 0.01^b	3.66 ± 0.02^c	3.70 ± 0.01^b
TA (g citric acid/100 mL)	0.26 ± 0.03^c	0.47 ± 0.03^b	0.48 ± 0.02^{ab}	0.49 ± 0.01^a
FA (g citric acid/100 mL)	-	0.46 ± 0.02^a	0.46 ± 0.02^a	0.45 ± 0.02^a
VA (g acetic acid/100 mL)	-	0.01 ± 0.0^b	0.02 ± 0.01^b	0.04 ± 0.00^a
Ethanol concentration (% v/v)	-	4.94 ± 0.02^a	8.66 ± 0.00^b	13.66 ± 0.00^c
L^*	24.00 ± 0.06^c	24.9 ± 0.03^b	34.6 ± 0.06^a	34.6 ± 0.03^a
a^*	10.90 ± 0.03^a	23.3 ± 0.05^b	17.0 ± 0.03^c	20.0 ± 0.06^d
b^*	5.50 ± 0.09^a	21.5 ± 0.02^b	36.3 ± 0.01^c	41.1 ± 0.02^d
Hue (°)	26.80 ^a	42.7 ^b	64.9 ^c	64.1 ^d
Chroma	12.24 ^a	31.7 ^b	40.0 ^c	45.7 ^d
ΔE^*	-	20.26 ^a	33.14 ^b	38.24 ^c

Different letters in the same row show indicate significant differences ($p \leq 0.05$).

The three initial musts with different total soluble solids content (10.9, 17.5 and 25 °Brix) had different fermentation times. Juice with the lower TSS content (FEB1) was the first in finishing the fermentation process: 7 d. FEB2 and FEB3 lasted 12 and 15 days for finishing the fermentation. Once fermented, the three beverages showed statistical differences ($p \leq 0.05$) in their physicochemical characteristics (Table 1). The TSS content decreased; however, the beverage that finished with greater amount of TSS was the FEB3 beverage. All characteristics of FEB were statistically different to those of fresh juice ($p < 0.05$). pH decreased (3.70-3.66) and TA increased at the end of the fermentation, this was expected since fermentation generate organic acids such as citric, malic, lactic, or acetic (Gumienna et al., 2016). The FA and VA were within the suggested ranges (0.45 ± 0.02 and 0.04 ± 0.00 g/100 mL) by the ICOP (2017) for fermented beverages. The maximum limit permitted for VA in wines is 0.12 g of acetic acid/L and the minimum limit permitted for FA is 0.4 g of tartaric acid/L. There was no statistical differences ($p > 0.05$) in FA within the fermented beverages. The VA in FEB3 was statistically different ($p < 0.05$) to FEB1 and FEB2. FEB 3 was the beverage with the highest volatile acidity.

At the end of fermentation, three different concentrations, statistically different ($p \leq 0.05$), of ethanol were obtained; however, the one with the highest concentration of ethanol was FEB3; this was expected since it started with a higher content of TSS (25 °Bx). According to the Official Mexican Norm No. NOM-142-SSA1/SCFI-2014 (Secretaría de Salud, 2014), for alcoholic beverages in Mexico, this beverage would be classified as a medium alcoholic beverage. Ethanol in FEB 3 (13.66% v/v) was in the range similar to the amount in wine (10-13% v/v) (Bozoglu et al., 2015). FEB1 and FEB2 had 4.9 and 8.7% (v/v) of ethanol. This indicate that to reach an ethanol concentration similar to wine, the black cherry juice have to be adjusted in TSS to about 25 °Bx.

7.5.3. Color

The color parameters of FRJ and FEBs are shown in Table 12. The color parameters for FRJ were characteristic to the color of the black cherry (light brown color). Significant statistical differences ($p \leq 0.05$) were found in all color parameters (L^* , a^* , b^* , hue and Chroma) for FRJ and FEBs. FRJ had lower color parameters than those for FEBs; this indicates, according to the color space, a pale brown color. The luminosity increased at the end of the fermentation in the three FEBs, showing a lighter luminosity. The value of a^* goes from green to red, as the lightness increases, generating less intense brown hues. The changes in hue could be due to the degradation of anthocyanins and flavonoids at the end of the fermentation process. The values obtained in this work were different to those reported by Jiménez et al. (2011): $L^* = 14.60 \pm 0.03$, $a^* = 4.54 \pm 0.06$, $b^* = 13.83 \pm 0.03$, hue = 71.8° and Chroma = 14.56. According to the total change in color (ΔE), the condition with the greatest ΔE change was the FEB3 beverage (38.24); the one with the least ΔE change was the FEB1 (20.26).

Some factors that influence the color change during the fermentation are anthocyanins, pigments found in black cherry. The conditions that may affect anthocyanins in the fermentation process are temperature, light, storage conditions, and oxygen, among others. Under the influence of these factors, the color change, in the range from orange to blue, could form yellow or colorless chalcones leading finally to brown polymers that may be an undesirable color from the point of view of the consumer (Gumienna et al., 2016).

7.5.4. Antioxidant characteristics

The antioxidant characteristics of FRJ and FEB black cherry beverages is shown in Table 13. The antioxidant activity and total phenolic compounds in FRJ were 196.95 ± 0.18 mg Trolox/100 mL and 198.40 mg GA/100 mL, respectively. The antioxidant activity and total phenolic compounds in black cherry FRJ were lower than that reported by Luna-Vázquez et al. (2013): 231.21 ± 0.15 mg Trolox, 362.2 ± 0.04 mg Gallic acid/100 mL, respectively. Rodríguez (2011) reported 240.00 ± 0.02 mg Gallic acid/100 mL for phenolic compounds. The total phenolic compounds content found in this work was higher than that reported for grapes (160.9 ± 0.12 mg Gallic acid/100 mL). The content of total flavonoids in black cherry FRJ was lower than that reported by Luna-Vázquez et al. (2013) (201.8 ± 5.2 mg catechin/100 mL) and higher than that reported by Rodríguez (2011) (88.9 ± 0.10 mg catechin/100 mL). A higher content of total flavonoids was obtained in this work than that reported for grapes (121.2 ± 0.49 mg catechin/100 mL) (Rodríguez, 2011). The total monomeric anthocyanins content was lower in black cherry FRJ than that reported by Jiménez et al. (2011) (3.08 ± 0.09 mg C3OG/100 mL) and higher than that reported by Cedillo-López et al. (2006) (1.24 ± 0.03 mg C3OG/100 mL). On the other hand, in Table 3 are shown the Pearson correlations within combinations of different antioxidant characteristics (antioxidant activity, phenolic compounds, total flavonoids, and total monomeric anthocyanin). The reported Pearson correlations were calculated taking or not taking into account black cherry juice antioxidant characteristics. It can be seen a correlation for antioxidant activity and the other antioxidant characteristics, as well as relationship combining the other antioxidant characteristics. However, it could not be a correlation between antioxidant activity and the other antioxidant characteristics. Not all phenolic compounds are antioxidants or are correlated with antioxidant activities (Kiselova, 2006; Conde-Hernández and Guerrero-Beltrán, 2014).

At the end of the fermentation process, there were significant statistical differences ($p \leq 0.05$) in antioxidant activity, total phenolic compounds, total flavonoids and total monomeric anthocyanins. The four characteristics were reduced, mainly in the FEB3 beverage. This could be due to the longer processing time and/or the more drastic biochemical changes occurred in it. Therefore, FEB3 had the lowest antioxidant activity: 150.4 ± 0.11 mg Trolox/100 mL. At the end of fermentation, FEB3 had 102.76 ± 0.10 mg Gallic acid/100 mL, 59.72 ± 0.06 mg CAT/100 mL and 0.72 ± 0.17 mg C3OG/100 mL contents of TPC, TF, and TMA, respectively. The reductions in the antioxidant characteristics at the end of fermentation were due to reactions of polymerization, condensation, oxidation, hydrolysis,

enzymatic activity and by interactions of the yeasts with the bioactive compounds. Some researchers have pointed out that the content of polyphenols, including phenols, and anthocyanins at the end of the fermentation may decrease (Gumienna et al., 2016); the same was observed in this research. The remaining antioxidant characteristics were in the range 34.90–76.40% after fermentation. Therefore, all beverages still kept good amounts of their antioxidant properties.

Table 13. Antioxidant characteristics of fresh and fermented black cherry beverages.

Antioxidants	FRJ	Fermented beverages		
		FEB1 (10.9 °Brix)	FEB2 (17.5 °Brix)	FEB3 (25 °Brix)
Antioxidant activity ¹	196.95 ± 0.18 ^a	179.92 ± 0.09 ^b	161.09 ± 0.07 ^c	150.41 ± 0.11 ^d
Total phenolic compounds ²	198.40 ± 0.10 ^a	179.04 ± 0.06 ^b	134.21 ± 0.14 ^c	102.76 ± 0.10 ^d
Total flavonoids ³	171.09 ± 0.02 ^a	94.21 ± 0.04 ^b	75.33 ± 0.05 ^c	59.72 ± 0.06 ^d
Total monomeric anthocyanins ⁴	1.64 ± 0.08 ^a	1.10 ± 0.04 ^b	1.08 ± 0.03 ^c	0.72 ± 0.17 ^d

¹mg ET/100 mL, ² mg GA/100 mL, ³ mg CAT/100 mL, ⁴ mg C3G/100 mL.

Different letters in the same row show significant differences ($p \leq 0.05$).

Table 14. Pearson correlation coefficients for antioxidant activity (AA), phenolic compounds (TP), total flavonoids (TF), and monomeric anthocyanins relationships of black cherry juice and alcoholic beverages.

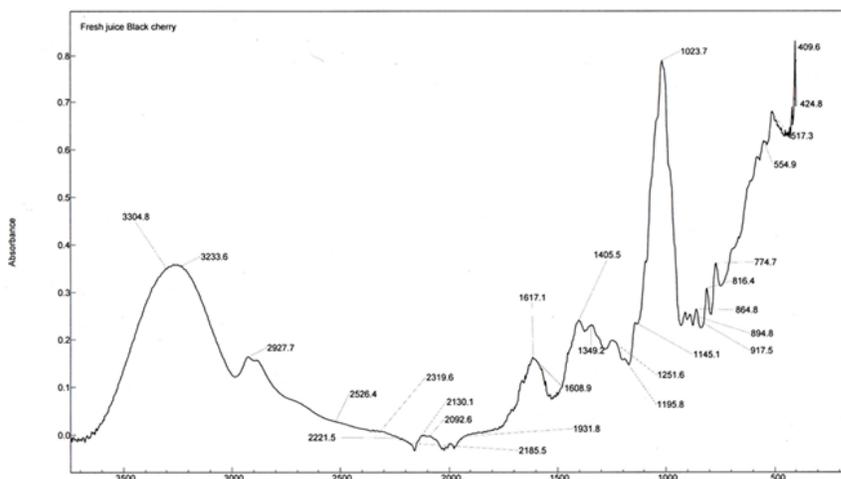
Antioxidant characteristic	Considering juice	Correlation coefficients		
		AA	TF	TMA
PC	Yes	0.984*	0.868	0.838
	No	0.998*	0.999*	0.892
TF	Yes	0.940**	1	0.964*
	No	0.995**	1	0.862
TMA	Yes	0.935**	0.964*	1
	No	0.805	0.862	1

*p < 0.05. **p<0.10

7.5.5. FTIR analysis

Figures 1, 2, 3 and 4 display the FTIR spectra of fresh and fermented black cherry beverages in the region 3500-500 cm^{-1} . The FTIR technique provided important information about changes in fresh juice after fermentation. The spectrum of the FRJ of black cherry (Figure 21) shows the characteristic peaks that allow to distinguish the presence of glucose, fructose, and sucrose in the range of 1400 to 950 cm^{-1} (Kelly et al., 2004). The bands between 1153 and 900 cm^{-1} are typical of C-O and C-C bonds, while those between 1400-1199 cm^{-1} are due to O-C-H, C-C-H and C-O-H bonds (Wiercigroch et al., 2017). The C-O-C stretching bond indicates the presence of glycosidic bonds. As for fructose, its bond is observed at 917 cm^{-1} , corresponding to the C-H stretching bond (Tewari and Irudayaraj, 2004). Sucrose is also observed in the band at 1349 cm^{-1} (Rosas and Fernández, 2012). The region of 1650-1500 cm^{-1} corresponds to the phenolic compounds, where two extra bands were presented, in 1617 and 1608 cm^{-1} . Fresh juices are mostly composed of water, therefore the presence of OH groups in FRJ black berry corresponding to the band observed at 3233 cm^{-1} (Plyler, 1952). In the FRJ black berry spectrum, the 1617 cm^{-1} band corresponds to a C = C double bond of the aromatic ring in the anthocyanin structure (Ortega et al., 2007) which is reduced in the FEBs (Figures 2, 3, and 4).

Figure 21. FTIR spectrum of fresh black cherry juice (FRJ).



The main changes in the FTIR spectra of fermented beverages are observed in the region 1850-1150 cm^{-1} . The FTIR spectrum of the FEB1 beverage (Figure 22) depicts differences in the baseline compared to the spectrum of FRJ black cherry. These differences lie mainly in the region of 2000 to 700 cm^{-1} that are mainly due to the fermentation process and the formation of new chemicals such

as alcohol, organic acids, phenols and glycerol. The bands observed in the spectrum of FEBs at 3307 cm^{-1} are due to the presence of the hydroxyl group that indicates the presence of water (Stuart, 2004); however, the band decreased due to the formation of ethanol in FEB beverages. As for FRJ, the presence of the bonds C=O, C-O, OH is observed (Ortega et al., 2007). Similarly, it is observed the presence of organic acids (C-OH and OH). The most relevant vibration band of organic acids corresponds to the C=O bond of the carboxylic acid at 1720 cm^{-1} for tartaric and malic acids. The OH bond of the carboxylic acid is at 1400 cm^{-1} (Bauer et al., 2008).

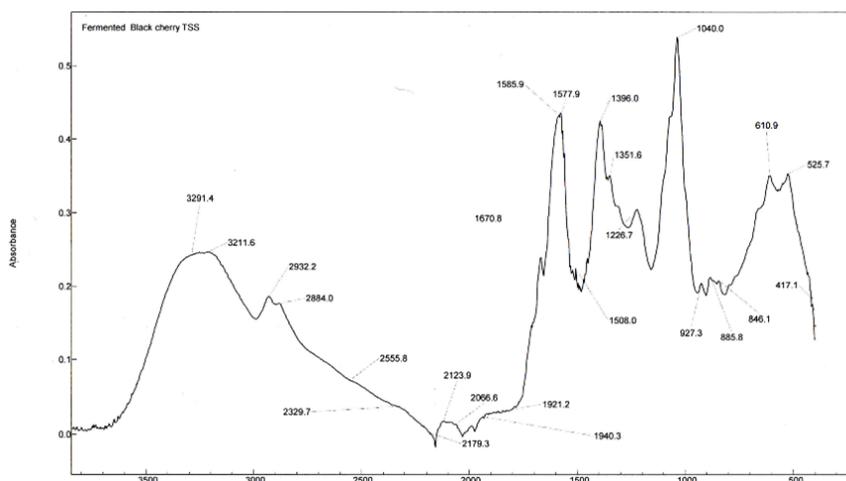


Figure 22. FTIR spectrum of fermented black cherry beverage (FRB1) from juice with 10.9% of total soluble solids.

The spectra of the FEB2 and FEB3 beverages are very similar to that for FEB1, mainly due to the formation of new compounds during the fermentation process such as ethanol, phenolic compounds and organic acids. The main bands showing similarity were found at 1670, 1716, 1595, 1398 and 1042 cm^{-1} . Both FEBs spectra are alike. This could indicate that the samples have a greater similarity of compounds formed during fermentation. Within these bands, the 1398 cm^{-1} band corresponds to the formation of citrate (Bauer et al., 2008). Regarding the spectrum of the sample FEB1, a new band is observed in the region 1716 cm^{-1} , corresponding to the C=O bond of carboxylic acids. Sometimes, due to the complexity of the spectrum, it is not possible to determine the position of certain bands since they are overlapped with adjacent bands.

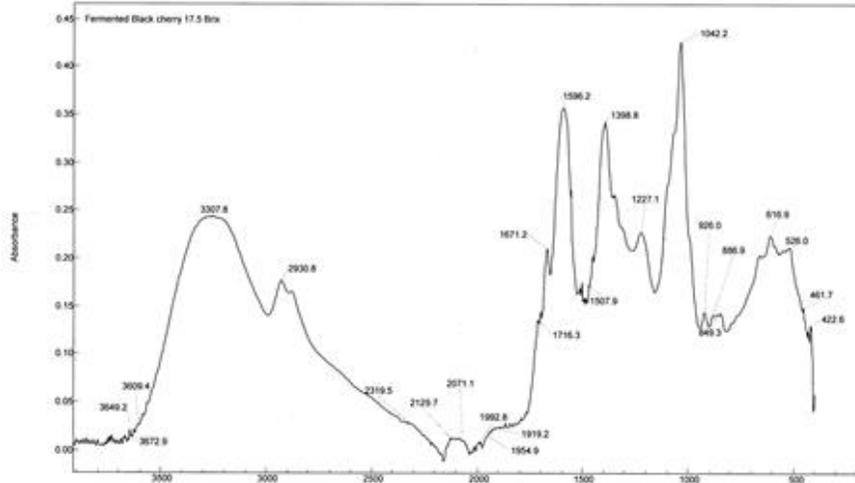


Figure 23. FTIR spectrum of fermented black cherry beverage (FRB2) from juice with 17.5% of total soluble solids.

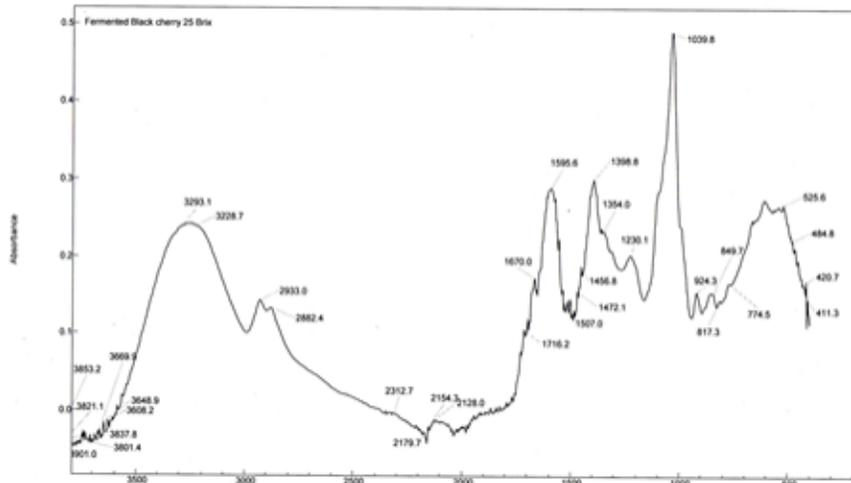


Figure 24. FTIR spectrum of fermented black cherry beverage (FRB3) from juice with 25% of total soluble solids

7.5.6. Sensory characteristics

Table 15 shows the results of the sensory evaluation using a structured nine points hedonic scale. For all attributes, a minimum of 5 and a maximum of 8 were observed. A number 5 corresponds to "neither like nor dislike" and number of 8 corresponds to "like very much". There were not statistical differences ($p > 0.05$) in appearance for FRJ and FEBs beverages; however, differences ($p \leq 0.05$)

were observed in the other attributes. As usual, the fermentation process affected all these parameters. Within FEBs, there were no significant statistical differences ($p > 0.05$) in appearance, color and sweetness; but differences ($p \leq 0.05$) were observed in aroma, flavor and general acceptability. In general, FRJ, FEB2 and FEB3 were very well accepted. It is important to say that the flavor of black cherry is very light, light sweet and have “grass” notes in flavor. In addition, few people use to “taste” it since it is not consumed regularly. However, FRJ black cherry was well accepted in sweetness, flavor and general acceptability. The higher the TSS content the higher the preference of beverages by the judges. In the attribute of aroma, sample FEB2 and FEB3 were well accepted; this may indicate that the volatile compounds generated in the fermentation (due to the generation of alcohol, acidity, phenols, among others.) liked to consumers. In flavor, the best qualified sample was FRJ beverage; however, there were no statistical differences ($p > 0.05$) within FRJ and FEB2 and FEB3 beverages. The sample that did not like too much was FEB1, it was some simple in flavor and aroma and has a low TSS content (Table 1).

Table 15. Sensory evaluation of fresh and fermented black cherry beverages.

<i>Characteristics</i>	FRJ	Fermented beverages		
		FEB1 (10.9 °Brix)	FEB2 (17.5 °Brix)	FEB3 (25 °Brix)
Appearance	6.55 ± 1.10 ^a	7.40 ± 1.10 ^a	7.45 ± 1.19 ^a	7.50 ± 1.23 ^a
Color	6.25 ± 1.62 ^b	7.65 ± 0.81 ^a	7.80 ± 0.77 ^a	7.35 ± 1.35 ^a
Sweetness	7.80 ± 0.83 ^a	5.85 ± 1.39 ^b	6.00 ± 2.00 ^b	6.30 ± 1.89 ^b
Aroma	6.65 ± 1.27 ^{ab}	5.55 ± 1.64 ^b	6.80 ± 1.24 ^a	6.90 ± 1.33 ^a
Flavor	7.75 ± 0.79 ^a	4.55 ± 1.28 ^b	6.65 ± 1.56 ^a	6.75 ± 1.71 ^a
General acceptability	7.10 ± 1.21 ^a	4.65 ± 1.23 ^b	6.75 ± 1.29 ^a	7.10 ± 1.52 ^a

Different letters in the same row show significant differences ($p \leq 0.05$).

It is also important to keep in mind that the concentration of ethanol has its own sensory characteristics such as sweetness (Mattes and DiMeglio, 2001). In wine, in addition to sweetness, astringency, due to tannins, acidity is also important; however, high acidity may make the wine non-pleasurable (Baker and Ross, 2014). In addition to the concentration of ethanol, the phenolic compounds have their own sensory attributes. They can produce a sensation of astringency (Baker and Ross, 2014). If their concentration is high, the acceptance in taste of the beverage may decrease. If their concentration is balanced, it is better accepted. However, it is very well known that the taste

of consumers, regarding alcoholic beverages, depends on many characteristics of the beverage. In red wines for instance, previous researching has shown that flavor compounds can interact with non-volatile and volatile components in fermented beverages, including alcohol and phenolic compounds, affecting aromatic compounds in wine and, therefore, sensitivity (Villamor et al. 2013; Baker and Ross, 2014).

7.6. HHP processing in black cherry fresh juice and fermented beverage

7.6.1. Microbial analysis

As shown in Fig. 25, the high pressure treatment completely eliminated the charge of mesophilic aerobic (MAB) bacteria during 49 days of storage in the samples of black cherry juice treated with HHP at 600 MPa-5 min and VAT pasteurization and in the samples of fermented black cherry beverages treated at HHP at 400 MPa-10 min, 600 MPa-5 min and VAT pasteurization, reducing the load by approximately 3 logarithmic cycles, compared to those not treated. The samples of black cherry juice treated at HHP 200 and 400 MPa-10 min, at day 0 reduced the microbial load below the limit of detection, however, on day 7 of storage the sample of treated juice 200 MPa-10 min began to show MAB growth ($2.56 \pm 0.03 \log_{10}$ CFU/mL), at day 28 we observed growth above the limit of 5 log ($5.28 \pm 0.03 \log_{10}$ CFU/mL), at 400 MPa- 10 min growth began to be observed on day 14 of storage ($1.01 \pm 0.01 \log_{10}$ CFU/mL), at the last day of storage (49 days), a growth of ($5.98 \pm 0.02 \log_{10}$ CFU/mL) was observed. In the fermented sample treated with HHP 200 MPa-10 min, growth was seen until day 35 of storage ($2.48 \pm 0.04 \log_{10}$ CFU / mL).

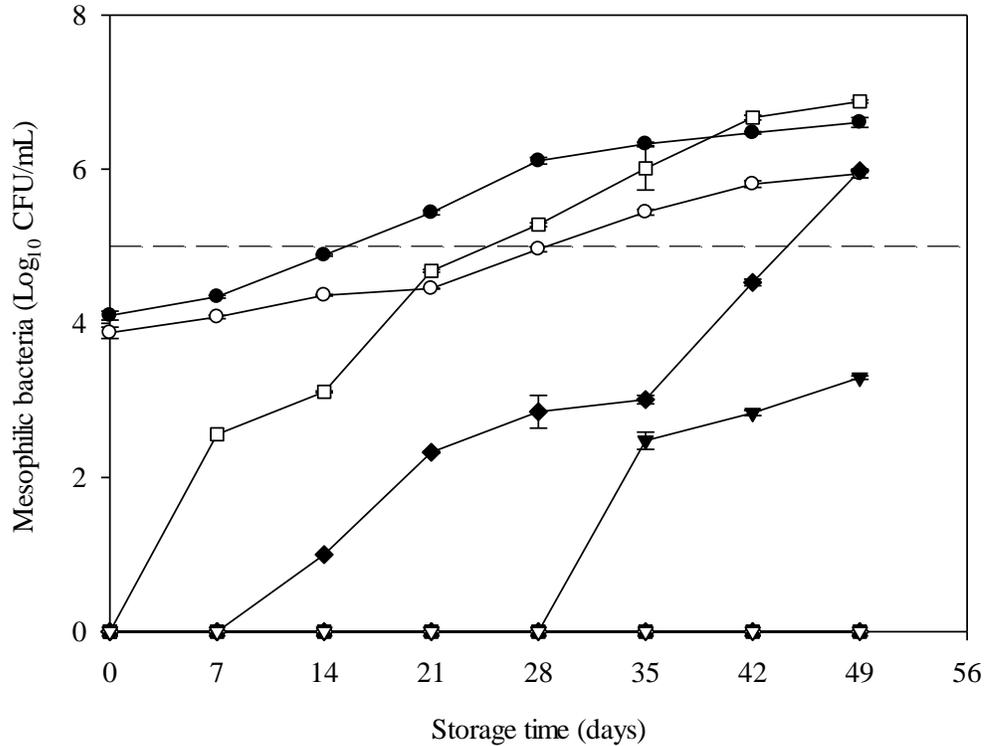


Figure 25. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on the microbial counts (mesophilic bacteria) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Log 0 CFU/mL corresponding to values under the limit of detection (<log 30 CFU/mL and <log 15 CFU/mL for mesophilic bacteria and mold and yeast, respectively). Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (∇). (--) upper acceptable limit. Symbols are a mean \pm standard deviation.

Other researchers have reported the inactivation of microorganisms applying the processing by HHP. Mok et al. (2006), studied the effect of processing by pasteurization of low-alcohol using a high hydrostatic pressure (HHP: 100-355 MPa 0-30 min). They reported that the original microbial count, 4.15×10^5 CFU/mL, decreased to 2.41×10^3 CFU/mL after 5 min treatment at 253 MPa and to 1.17×10^3 CFU/mL, 7.10×10^2 CFU/mL, 2.00×10^2 CFU/mL after 10, 20, and 30 min treatments, respectively. At pressures higher than 300 MPa, the aerobic bacteria decreased below the detection limits after 20 min treatment at 300 MPa and after 10 min at 355 MPa. Other researchers reported that HHP treatment at 500 MPa for 5 min resulted in a 99.99% reduction in bacterial count without resulting in changes in the chemical or organoleptic properties of wine (Puig et al., 2008).

In Fig.26, the growth of yeasts and molds during the storage time is observed. Samples of black cherry juice treated with HHP at 400-10, 600-5 MPa, VAT pasteurization and fermented black cherry

samples treated with HHP at 200, 400-10 and 600-5 MPa and VAT pasteurization did not show yeast growth and molds during the entire storage time, reducing the population 3 logarithmic cycles. Some investigations mention that pressures between 415 MPa and 460 MPa induces mechanical cell wall damage (Hartmann & Delgado, 2004). The sample of black cherry juice treated with HHP at 200 MPa-10 min, began to show growth of molds and yeasts at day 28 of storage ($2.45 \pm 0.07 \log_{10}$ CFU/mL). The sample of fermented beverage treated by HHP at 200 MPa-10 min started to microbial yeast grow at day 42 of storage ($2.47 \pm 0.05 \log_{10}$ CFU / mL). Sokolowska et al. (2013) reported an inactivation of *Saccharomyces cerevisiae* (NCFB 3191) using a high hydrostatic pressure of 300 MPa with a retention time of 0, 1, 5 and 10 min. The reduction of *S. cerevisiae* in the sample of beet juice was approximately 5 log after 10 min of pressurization.

7.6.2. Physicochemical parameters

Table 16 shows the changes in the physicochemical parameters at day 0 and 49 of storage. The TSS were higher in the juice samples than in the fermented beverages, the treatment with high pressures and pasteurization did not affect this parameter. No significant differences ($p \geq 0.05$) between the control samples and the samples treated with HHP was observed. The storage time did not reduce the TSS, the only observable reduction of this parameter was seen in the controls on day 49, which could be related to the microbial load that these samples had and that may be attributed to the fact that a slight process of fermentation in the juice or that this process is still maintained in the fermented beverage. Torres-Ossandón et al. (2015), report that the TSS content of Cape gooseberry Pulp on the soluble solids content was significantly lower in samples treated at 300, 400 and 500 MPa compared with the untreated sample. They said that this decrease may be due to degradation during pressure treatment of sucrose, identified as the main sugar in Cape gooseberry pulp.

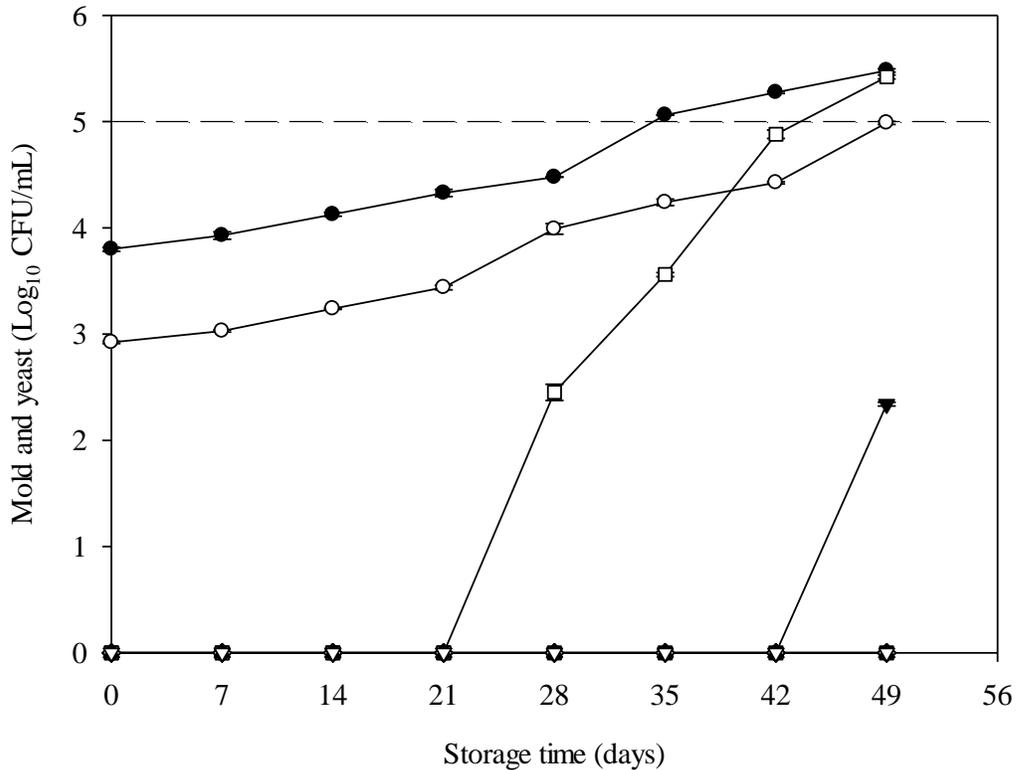


Figure 26. E Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on the microbial counts (mold and yeast) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Log 0 CFU/mL corresponding to values under the limit of detection (<log 30 CFU/mL and <log 15 CFU/mL for mesophilic bacteria and mold and yeast, respectively). Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (△), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (∇). (--) upper acceptable limit. Symbols are a mean \pm standard deviation.

Subasi & Alpas (2016), did not report significant changes in the TSS content in samples of pomegranate juice processed by HHP (400 MPa-10 min). The pH value showed no significant changes with any of the treatments applied or during the storage time. Likewise, Torres-Ossandón et al. (2015), reported the initial pH of the untreated sample of Cape Gooseberry Pulp of 3.93 ± 0.06 on day 0. Their sample treated at 300 MPa showed no significant difference compared with untreated sample on day 0. However, their pH value increased significantly for samples treated at 400 MPa which they said that may be due to conformational changes associated with the unfolding and denaturation of proteins and exposure of basic amino acids to the medium (Kaur et al. 2013).

Table 16. Physicochemical parameters of HHP processed and pasteurized fresh juice and fermented black cherry beverages after 49 days of storage (4±1 °C).

	Untreated		HHP/T (MPa/min)						Pasteurization	
	Juice	Fermented	Juice			Fermented			VAT J	VAT F
			200/10	400/10	600/5	200/10	400/10	600/5		
Day 0										
TSS	16.2±0.02	8.9±0.02	16.1±0.05	16.3±0.06	16.1±0.03	8.8±0.05	8.7±0.03	8.7±0.05	16.03±0.01	9.13±0.05
pH	4.57±0.01	4.19±0.01	4.51±0.05	4.52±0.02	4.54±0.02	4.18±0.01	4.18±0.0	4.17±0.0	4.56±0.01	4.28±0.03
TA	0.57±0.01	1.02±0.01	0.50±0.0	0.50±0.00	0.50±0.01	0.94±0.01	0.94±0.02	0.94±0.0	0.57±0.00	1.01±0.01
FA	-	0.99±0.01	-	-	-	0.93±0.01	0.92±0.01	0.94±0.02	-	0.99±0.00
VA	-	0.02±0.0	-	-	-	0.01±0.00	0.01±0.01	0.01±0.00	-	0.01±0.00
Ethanol	-	11.83±0.02	-	-	-	11.80±0.01	11.86±0.05	11.86±0.01	-	11.86±0.05
Day 49										
TSS	14.9±0.00	8.7±0.01	16.0±0.02	16.0±0.02	16.1±0.03	8.6±0.05	8.6±0.05	8.7±0.05	16.0±0.02	9.03±0.05
pH	4.40±0.00	4.06±0.00	4.48±0.03	4.49±0.03	4.51±0.00	4.14±0.00	4.13±0.00	4.15±0.00	4.54±0.01	4.24±0.01
TA	0.58±0.01	1.30±0.03	0.52±0.00	0.51±0.30	0.50±0.02	0.95±0.01	0.94±0.02	0.93±0.01	0.57±0.00	1.02±0.02
FA	-	0.96±0.02	-	-	-	0.94±0.00	0.94±0.02	0.92±0.01	-	0.99±0.01
VA	-	0.06±0.00	-	-	-	0.01±0.00	0.01±0.00	0.01±0.00	-	0.02±0.00
Ethanol	-	12.38±0.01	-	-	-	11.86±0.01	11.87±0.00	11.86±0.03	-	11.81±0.05

TSS (total soluble solids °Brix w/w). TA (total acidity, percentage citric acid, w/v), VA (volatile acidity, percentage acetic acid, w/v), FA (fixed acidity, percentage citric acid, w/v). Ethanol concentration (% v/v)

The total, fixed, volatile acidity and ethanol concentration was not affected by the HHP and pasteurization treatments. Comparable results were reported by other authors working with carrot and tomato juice pressurized samples at 250 MPa-15 min (Dede et al., 2007). The total acidity was higher for the fermented samples than for the juices. The control of juice and fermented beverage showed greater total acidity, with respect to all the treated samples. The storage time increased this value for the control samples as shown in Table 16. The fixed acidity, the volatile acidity and the ethanol concentration were greater for the control of fermented drink. From day 0 to day 49 of storage the volatile acidity and the ethanol concentration increased (0.02±0.0 to 0.06±0.00% acetic acid w/v, 11.83±0.02 to 12.38±0.00). Mok et al. (2006), reported that the physicochemical (alcohol, pH, acidity, total sugar) properties exhibited little change during the HHP treatments at 100-350 MPa-0-30 min, on red wine samples.

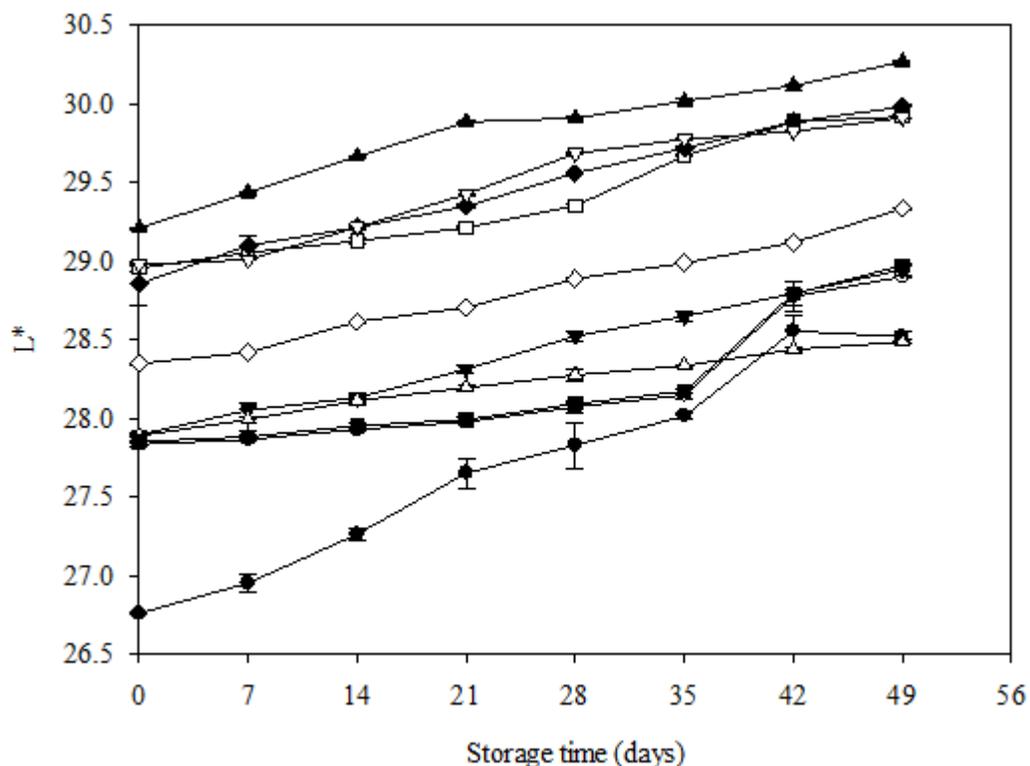


Figure 27. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on color parameter (L^*) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (◊). Symbols are a mean \pm standard deviation.

7.6.3. Color characteristics

As shown in Fig. 27, the luminosity value (L^*) increases in the samples treated with HHP and pasteurization, after the application of the treatments and during the storage time. Significant differences ($p \leq 0.05$) were found between the control samples and those treated with HHP. The samples with the highest increase in the L^* value were fresh juice and fermented beverage treated with VAT pasteurization at day 0 (29.20 ± 0.05 and 28.97 ± 0.04 , respectively) and 49 (30.26 ± 0.03 and 29.91 ± 0.02 , respectively) of storage. Samples of juice treated by high pressures and pasteurization have a higher value of L^* compared to samples of fermented beverages. Similar results were reported by Argyri et al. (2014), in samples of apricot, peach, and pear fruits treated by HHP at 600 MPa-5 min.

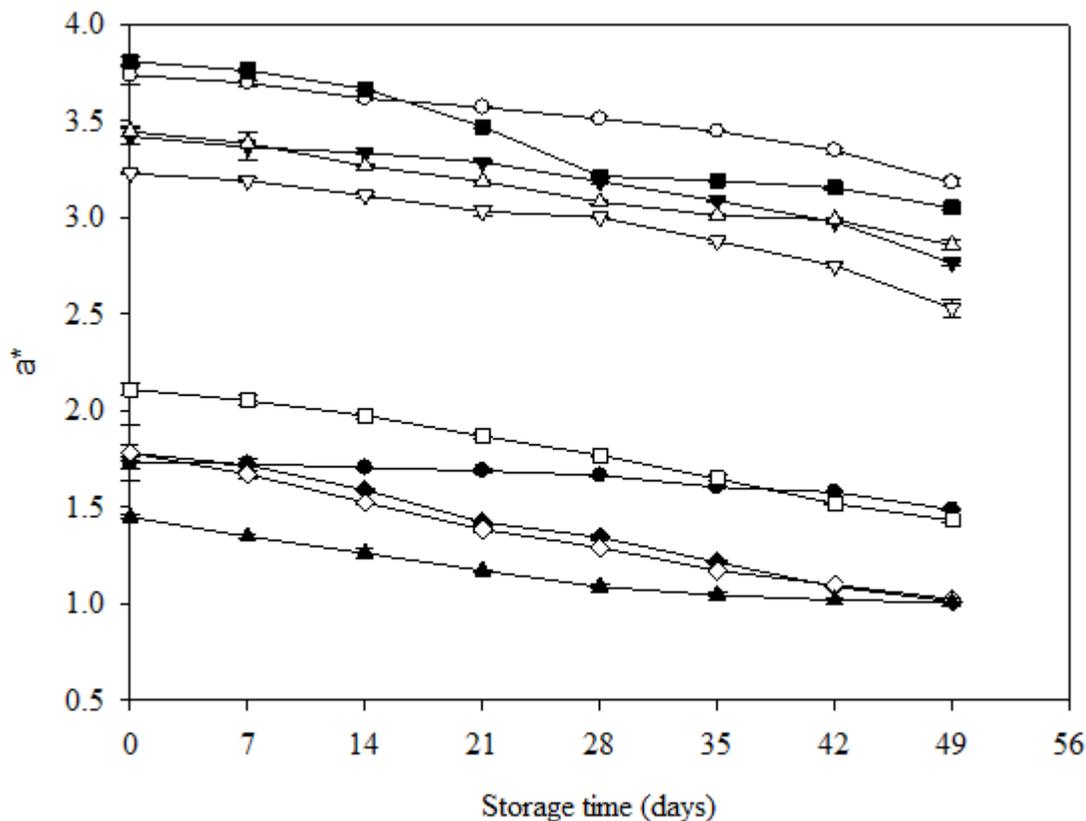


Figure 28. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on color parameter (a^*) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (∅). Symbols are a mean $a \pm$ standard deviation.

They observed an increase in lightness (L^*) of apricot samples during storage in all treatments, with changes found to be more intense in control samples. In this study, the less luminous sample was the control of fresh black cherry juice at day 0 (27.83 ± 0.01 and 28.90 ± 0.03 , respectively) and 49 day of storage. From the samples treated with high pressures it was observed that when applying higher pressure, the value of L^* was lower.

In the a^* color parameter, the fermented beverages had a higher value than the fresh juice beverages. As seen in Fig. 28, treatment with high pressures decreased the value of a^* , at higher pressure lower value, however, the greatest reduction in the parameter of a^* was observed in the samples that were fermented with pasteurization VAT. These results were similar to other authors where the value of a^* decreased after treating wine samples with high pressures (350 MPa-10 min) (Santos et al., 2016, Christofi et al., 2019). The storage time caused the parameter of a^* decreased for all samples. The value of b^* was higher in the juice samples than in the fermented samples. Changes in the value of

b* were observed in the samples treated with HHP, observing that at higher pressure the value of b* decreases. In the samples treated with VAT pasteurization, the b* value was higher than in the samples by HHP and the control. Argyri et al. (2014), also reported an increase in the value of b*, with the processing of high pressures. The storage time decreased the value of b* in all the samples. The hue (°) showed changes in the beverages. It was observed that the value of hue (°) was greater in the samples treated with HHP and VAT pasteurization. The hue (°) was greater in the juice samples than in the fermented samples. The storage time increased mostly the hue value (°), only the juice control samples, 200 and 400 MPa-10 min and fermented VAT pasteurization showed a reduction in the hue value (°) at day 49 of storage. Cebola-Lidon et al. (2015), reported an increase in hue (°), in samples treated with HHP (460 MPa), with respect to the control sample in apple juice. The chroma showed changes in the samples treated. The chroma was higher in the samples treated with HHP, it was observed that the pressure decreased the value of the chroma, at 600 MPa-5 min this value was lower (Table 17). A greater chroma was also shown in the juice samples than in the fermented samples. Chroma value decreased in all samples during storage time. The net color change ΔE^* was compared with the control samples at day 0, with a greater net change in color in the juice VAT and fermented VAT samples. The samples that showed the least change in color at day 0 were the fermented samples, compared to the juice samples, with the sample HHP 200 MPa-10 min having the least color change (Table 17). These results were similar to those reported by Dede et al. (2007), they mentioned that the application of HHP (250 MPa-15 min) had a smaller effect on color change than did thermal treatment throughout their storage period in carrot juices. The net change in color between the same samples from day 0 to day 49 was also compared, with a greater ΔE^* observed in the juice control samples, fermented VAT, 400 MPa-10 min juice, VAT juice, 200 MPa-10 min fermented and 200 MPa-10 min juice. The minor color changes were observed in the fermented samples.

Table 17. Color parameters of HHP processed and pasteurized fermented pomegranate beverages after 42 days of storage (4±1 °C)^a.

	Untreated		HHP/T (MPa/min)						Pasteurization	
			Juice			Fermented				
	Juice	Fermented	200/10	400/10	600/5	200/10	400/10	600/5	VAT J	VAT F
Day 0										
b*	4.24±0.07 ^{bd}	3.05±0.05 ^{cd}	4.25±0.03 ^d	4.20±0.01 ^d	3.29±0.06 ^d	3.13±0.07 ^d	3.03±0.05 ^d	3.01±0.02 ^d	4.30±0.03 ^d	3.14±0.03 ^d
H (°)	67.85±0.65	39.22±0.15	63.59±0.48	67.05±1.39	66.30±0.88	42.46±1.08	43.51±0.48	44.64±0.30	67.52±0.15	39.54±0.23
C	4.58±0.05	4.82±0.06	4.74±0.01	4.56±0.30	3.60±0.04	4.63±0.02	4.74±0.04	4.53±0.02	4.65±0.02	4.93±0.02

ΔE^*	-	-	2.23 ^b	2.10 ^b	1.93 ^b	0.33 ^c	0.37 ^c	0.73 ^c	2.49 ^b	1.15 ^c
Day 49										
b^*	3.51±0.01	2.60±0.02	3.64±0.02	3.48±0.01	2.52±0.02	2.54±0.00	2.65±0.02	2.52±0.02	3.65±0.00	2.15±0.03
$H(^{\circ})$	67.04±0.13	39.34±0.12	68.57±0.04	73.83±0.08	68.22±0.31	42.66±0.16	42.88±0.17	44.96±0.13	74.36±0.28	35.18±0.73
C	3.81±0.00	4.11±0.01	3.91±0.02	3.62±0.01	2.71±0.02	3.75±0.00	3.89±0.01	3.57±0.02	3.79±0.11	3.73±0.02
ΔE^*	1.92 ^d	0.76 ^d	1.33 ^d	1.55 ^d	1.33 ^d	1.37 ^d	1.05 ^d	1.11 ^d	1.46 ^d	1.56 ^d

^{bc}Data taken into account as a reference for the calculation of the net color change (ΔE^*). ^dFrom day 0 of the same sample with respect to day 56 of the same sample.

7.6.4. Antioxidants compounds

7.6.4.1. Antioxidant capacity

In Fig. 29, the change in antioxidant capacity during the storage time is observed. The sample that had lower antioxidant capacity was black cherry fermented beverage (0: 165.56 ± 2.38 and 59: 117.26±2.74, mg TE/100 mL).

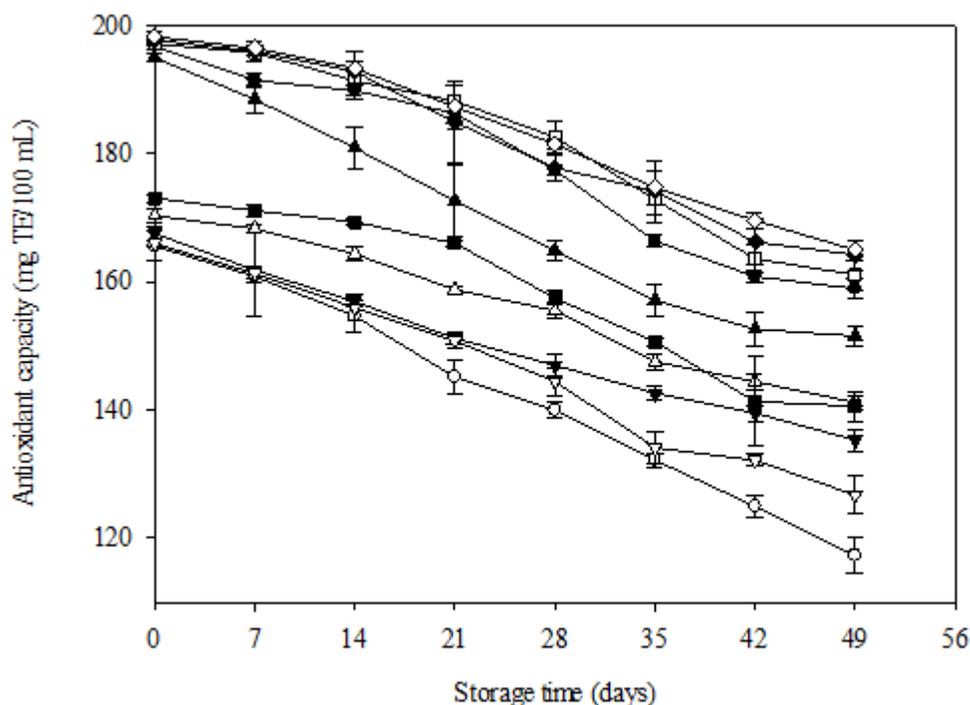


Figure 29. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on antioxidant compounds (antioxidant capacity) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600

MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (). Symbols are a mean \pm standard deviation

The samples with greater antioxidant capacity were the samples of fresh juice compared with the fermented beverages. Of the samples treated, those of VAT pasteurization had lower amount of antioxidant capacity compared with the HHP samples. Results similar to the Dede et al. (2007), they reported that the antioxidant capacity of orange juice decreased more quickly than that of carrot juice as pressure is increased from 100 to 800 MPa, however, for thermal treatments the activity decreases as the treatment becomes harsher (longer processing time, higher processing temperature). In the samples treated with HHP, the samples at 600 MPa-5 and 400 MPa-10 min showed a greater amount of antioxidant capacity. The storage time reduced the antioxidant capacity in all the samples. The sample with the highest antioxidant capacity at day 49 was fresh juice treated with HHP at 600 MPa-5 min (day 49: 164.95 ± 1.58 , mg TE/100 mL).

7.6.4.2. Total phenols

In Fig. 30, the change in total phenols is observed during the storage time. The samples with the least amount of total phenols were the samples of fermented black cherry beverage and the pasteurized samples. The samples with the greatest amount of total phenols were those treated with high pressures at 600 MPa-5 min at day 0 and 49 storage (juice, day 0: 195.29 ± 0.89 , day 49: 165.06 ± 1.69 , fermented, day 0: 99.03 ± 0.16 , day 49: 71.48 ± 0.75 , mg GA/100 mL). As seen in Fig 3 B. all samples had a decrease in the value of total phenols during the storage time.

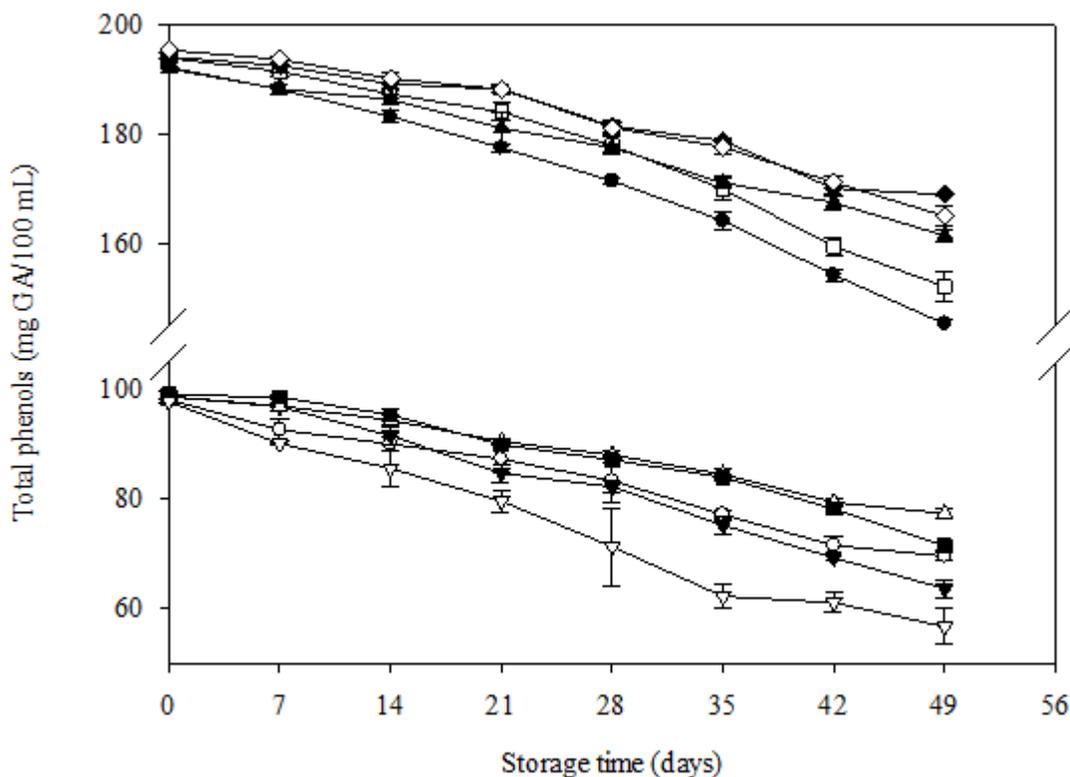


Figure 30. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on antioxidant compounds (total phenols) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (∩). Symbols are a mean \pm standard deviation.

7.6.4.3. Flavonoids

Changes in the content of total flavonoids were shown in Fig. 31. The VAT pasteurization treatment was the one that most affected this parameter, decreasing it in comparison with the samples treated with high pressures. The samples of fresh juice had a higher quantity of flavonoids compared to the samples of fermented beverages. Treatment with high pressures at 600 MPa-5 min slightly increased the flavonoid content at day 0 (juice, day 0: 171.95 ± 0.64 , day 49: 149.37 ± 0.57 , fermented, day 0: 70.39 ± 0.71 , day 49: 50.26 ± 0.55 , mg CAT/100 mL). The treatment of HHP at 200 MPa-10 min in fresh juice and fermented black cherry beverage was very similar to control, which may indicate that the treatment of HHP at a low pressure does not generate a significant effect in the samples.

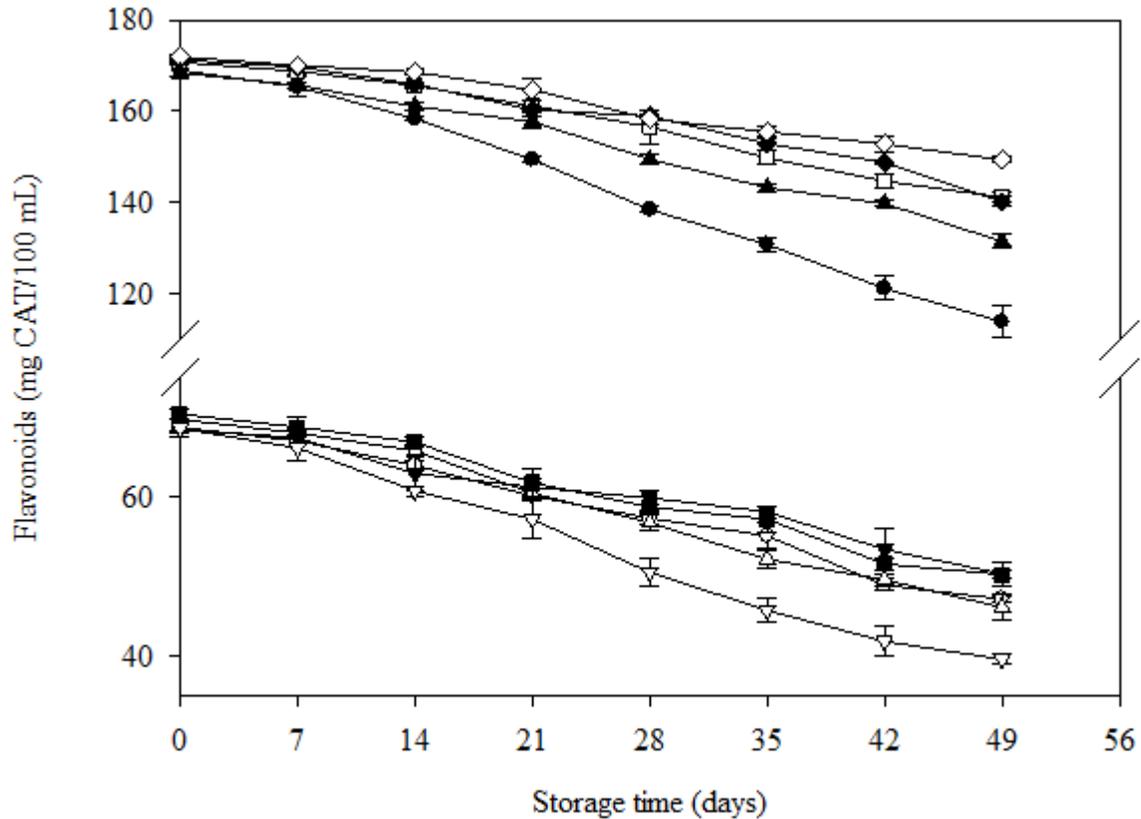


Figure 31. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on antioxidant compounds (flavonoids) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (). Symbols are a mean \pm standard deviation.

7.6.4.4. Anthocyanins

In Fig. 32, a greater amount of total anthocyanin content is observed in the samples treated with HHP of fresh black cherry juice. Significant changes ($p \leq 0.05$) were observed in the content of total anthocyanins in the samples treated with HHP with respect to the control and VAT pasteurization samples. The storage time decreased the total anthocyanin content in all the samples. The sample with the highest number of anthocyanins was fresh juice 600 MPa-5 min (day 0: 0.78 ± 0.96 , day 49: 0.66 ± 0.88 , mg C3G/100 mL).

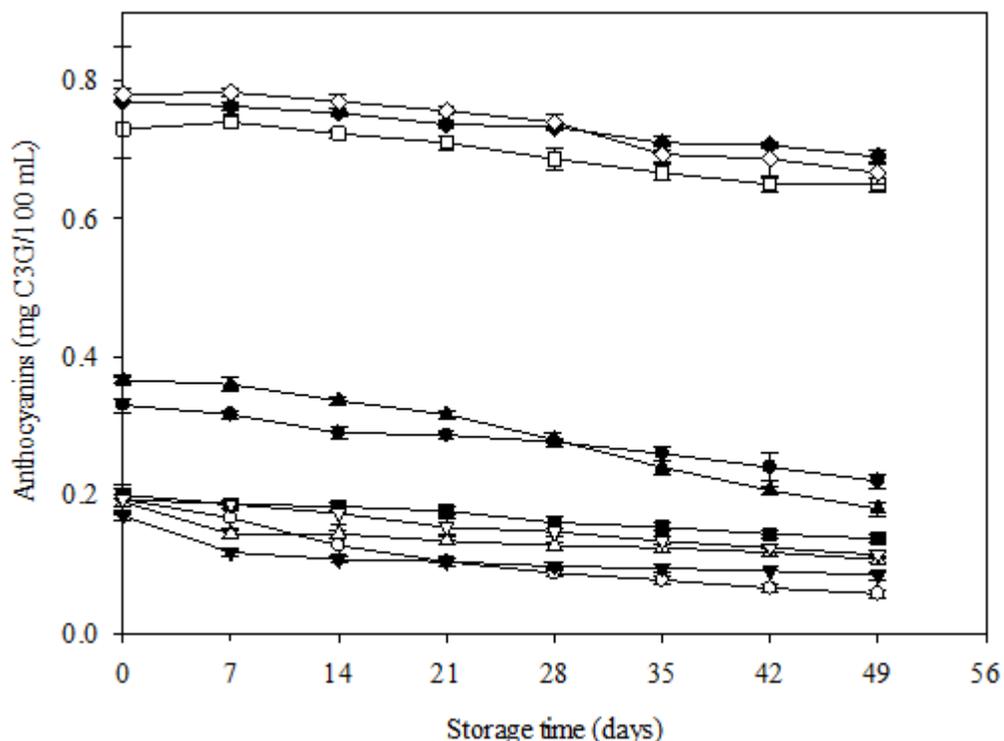


Figure 32. Effects of high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments on antioxidant compounds (anthocyanins) of a black cherry fresh juice and fermented beverage throughout storage at 4°C. Control black cherry fresh juice (●), control black cherry fermented beverage (○), 200 MPa-10 min fermented (▼), 400 MPa-10 min fermented (Δ), 600 MPa-5 min fermented (■), 200 MPa-10 min juice (□), 400 MPa-10 min juice (◆), 600 MPa-5 min juice (◇), VAT juice (▲), VAT fermented beverage (◊). Symbols are a mean \pm standard deviation.

Anthocyanins were usually reported to be unstable, especially at high and abuse temperatures during processing and storage (Berkel Kaşıkçı, 2015). Researches have reported that HHP processing is effective retained anthocyanins, phenolic compounds and color of pomegranate juice for treated samples with 350 MPa and 550 MPa at room temperature (Varela-Santos et al., 2012). Alpas (2013) also observed no significant decrease in monomeric anthocyanin pigment concentrations for HHP samples, but thermal treatment (85°C/10min.) decreased significantly monomeric anthocyanin pigment in pomegranate juices. Greater retention of HHP samples is found compared to HTST (110°C/8.6s) samples in cloudy pomegranate juice (Chen et al., 2013). In another study, 63% of pomegranate juice anthocyanins are retained after 400 MPa HHP process. At the end of 21- and 72-days storage, 65% and 63% of anthocyanins is retained at 4°C (Ferrari et al., 2011).

7.6.5. Sensory evaluation

In Fig. 33. The sensory evaluation analysis is observed for samples of fresh black cherry juice. The samples evaluated had a maximum score of 7, which corresponds to moderately I like and a minimum score of 6 which means I like it slightly, for which the samples in general had a good acceptance by the judges.

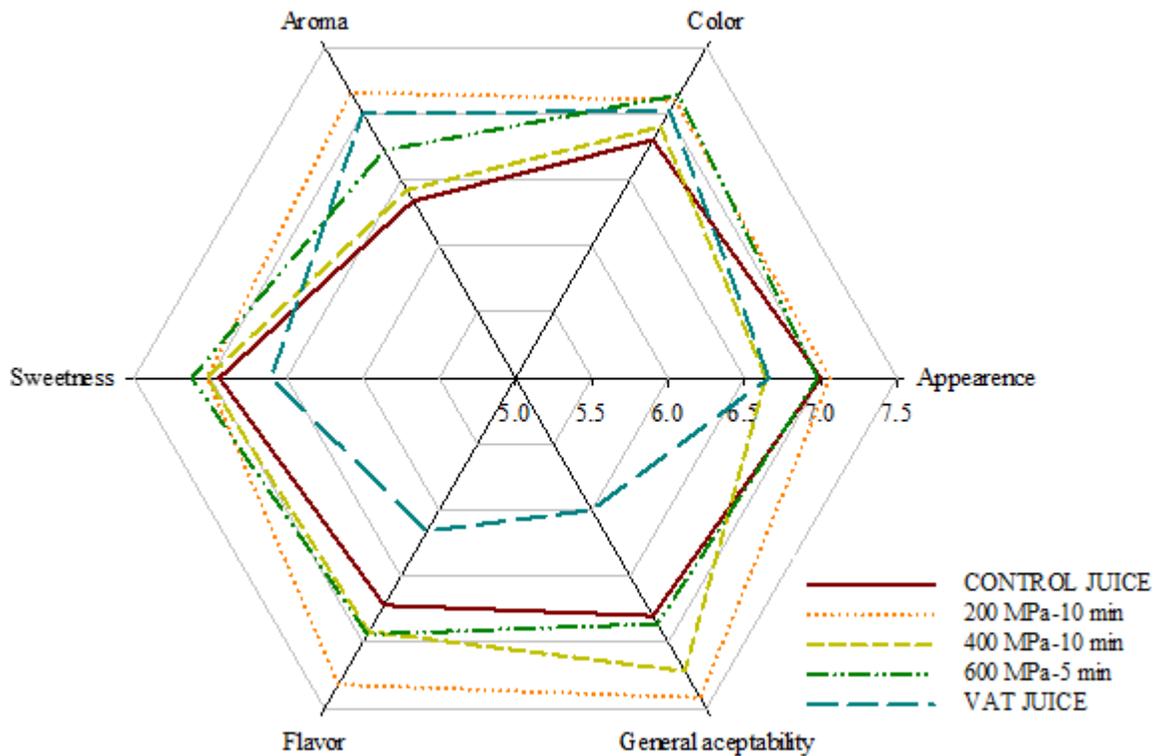


Figure 33. Sensory evaluation of black cherry juice processing by high hydrostatic pressure (200 y 400 MPa-10 min, 600 MPa-5 min) and thermal (VAT) treatments at the end of storage.

The attributes of appearance, color, smell and sweetness did not show significant changes ($p \geq 0.05$) among the qualified samples. The attribute of taste and general acceptability was better qualified for the sample HHP 200 MPa-10 min and lower qualified for the sample pasteurization VAT, showing significant changes ($p \leq 0.05$) in this attribute. This indicates that the sample that was rejected the most on Thursdays was the pasteurized sample. Likewise, when applying a lower pressure in the HHP treatment, the samples are more sensorially accepted. Argyri et al (2014), sensory evaluation of fruit juice samples of apricots, peaches, and pears treated by HHP, they mention in their research that the panelists marked with better scores the HHP-treated products compared to their respective controls, according to taste and total evaluation during storage.

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8. CONCLUSIONS

- ❖ The adjustment of the total soluble solids is important in the fermentation process to reach a specific amount in ethanol concentration of the fermented pomegranate and black cherry beverages.
- ❖ The fermentation process changes the physicochemical, antioxidant and sensory characteristics of fermented pomegranate and black cherry beverages.
- ❖ At the end of the fermentation process, the antioxidant activity, phenols, flavonoids and anthocyanins decrease in quantity, however, these compounds are still maintained in fermented pomegranate and black cherry beverages.
- ❖ The processing by high hydrostatic pressures at 400, 500, 550 and 600 MPa for 10 and 5 minutes may be effective to maintain the microbiological, physicochemical, antioxidant and sensory stability of the fermented pomegranate and black cherry beverages during 56 days of chilled storage
- ❖ The processing by pulsed electric field at 11.7 (50%, 15 μ s) and 18.8 (80%, 20 μ s) kV/cm (frequency of 200 Hz), proved to be effective maintaining microbiological, physicochemical, antioxidant and sensorial stability of the fermented pomegranate beverage during 56 days of chilled storage
- ❖ Thermal processing: VAT and HTST pasteurization showed more loss in color attributes compared to non-thermal treatments. The microbiological stability was affected with the HTST treatment.
- ❖ All treatments, thermal and non-thermal applied to fermented pomegranate and black cherry beverages were sensorially acceptable.
- ❖ Processing by HHP and PEF could be an alternative as a preservation method for fermented beverages, especially pomegranate and black cherry, generating harmless, sensory-acceptable beverages and with important antioxidant compounds.

9. GENERAL SUGGESTIONS

- ❖ Is necessary to control and study more fermentation conditions: yeast, time, temperature, light and oxygen conditions.
- ❖ It is recommended that the change in the antioxidant compounds after the fermentation and after processing by high pressures and pulsed electric field, be analyzed in a specific way and not only the total compounds.
- ❖ It would be important to evaluate other antioxidant compounds such as tannins that have pomegranate and black cherry fruits.
- ❖ It would be convenient to test the application of high hydrostatic pressure processing and the processing of pulsed electric field before the fermentation process, to see if this application improves the quality of the must.
- ❖ There is a need to generate more specific studies on the effects of HHP and PEF processing on different antioxidant compounds, sensory attributes and physicochemical characteristics of fermented pomegranate and black cherry beverages and optimum shelf life conditions.

RESEARCH PRODUCTS

Research papers published

1. Rios-Corripio, G. and Guerrero-Beltrán, J.Á. 2019. Antioxidant and physicochemical characteristics of unfermented and fermented pomegranate (*Punica granatum* L.) juices. *Journal of Food Science & Technology*, 56(1), 132-139. doi:10.1007/s13197-018-3466-6.

Research papers under review

2. Rios-Corripio, G. and Guerrero-Beltrán, J.Á. Physicochemical, antioxidant and sensory characteristics of black cherry (*Prunus serotina* subsp. *capuli*) fermented juice

International Journal of Fruit Science

3. Rios-Corripio, G, Guerrero-Beltrán, J.Á., Welti-Chanes, J., and Martínez, V. High hydrostatic pressure processing of pomegranate (*Punica granatum* L.) fermented beverages

Innovative Food Science and Emerging Technologies

4. Rios-Corripio, G, and Guerrero-Beltrán, J. Á. Antioxidants, pigments and products of black cherry (*Prunus serotina*), bilberry cactus (*Myrtillocactus geometrizans*), elderberry (*Sambucus nigra*), and pomegranate (*Punica granatum*): a review

Fruits

Research papers on hold

5. Pulsed electric field processing of pomegranate (*Punica granatum* L.) fermented beverage: effects on microbial inactivation and antioxidants compounds
6. High hydrostatic pressure processing of black cherry (*Prunus serotina* subsp. *capuli*) juice and fermented beverage

ANNEXES

ANNEX I. SENSORY EVALUATION

CUESTIONARIO DE ESCALA HEDÓNICA

Nombre: _____ Fecha: 02/Mayo/2019 Producto: **Bebida fermentada de capulín y/0 granada**

Instrucciones: Pruebe la muestra a evaluar y asígnele una puntuación, de acuerdo con su criterio, según la tabla que se le presenta.

Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						
Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						
Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						

Observaciones: _____

Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						
Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						
Código:	APARIENCIA	COLOR	OLOR	SABOR	DULZOR	ACEPTABILIDAD GENERAL
Me gusta extremadamente						
Me gusta mucho						
Me gusta moderadamente						
Me gusta levemente						
No me gusta ni me disgusta						
Me disgusta levemente						
Me disgusta moderadamente						
Me disgusta mucho						
Me disgusta extremadamente						

Observaciones: _____

FEATURE II. MEETINGS





SECRETARÍA DE
EDUCACIÓN PÚBLICA
GOBIERNO DE PROGRESO



INSTITUTO TECNOLÓGICO SUPERIOR DE ATLIXCO

Otorga el presente

Reconocimiento

a la:

M.C. Gabriela Ríos Corripio

Por su valiosa participación con la conferencia:

**“Propiedades del vino
y la granada”**

en el marco del “2do. Congreso de Ingeniería Bioquímica”
celebrado del 22 al 24 de marzo de 2017 en la
Ciudad de Atlixco.



SECRETARÍA DE EDUCACIÓN
PÚBLICA
INSTITUTO TECNOLÓGICO
SUPERIOR DE ATLIXCO

M.A.P. José Guillermo Velázquez Gutiérrez
Director General

AMIDIQ

La Academia Mexicana de Investigación y Docencia en Ingeniería Química A.C.
La Ingeniería Química en el Desarrollo Sostenible de Nuevos Procesos y Productos

Otorga el presente

RECONOCIMIENTO

A:

Mónica Dávila Rodríguez, Ana Cecilia Lorenzo Leal, Gabriela Ríos Corripio, María Teresa
Jiménez Munguía

Por la presentación del trabajo:

EVALUACIÓN DE LAS PROPIEDADES FÍSICAS, QUÍMICAS Y FUNCIONALES DE CLARA DE
HUEVO FRESCA Y SECADAS POR ASPERSIÓN

ID: 402

XXXVIII Encuentro Nacional de la AMIDIQ
Ixtapa Zihuatanejo, Gro., México, del 9 al 12 de mayo de 2017


Dr. Maiguel Sales Cruz
PRESIDENTE DE AMIDIQ




Dr. Jesús Alberto Ochoa Tapia
PRESIDENTE DEL COMITÉ TÉCNICO



Certificate of Participation

This certifies that

Gabriela Rios

POSTER ATTENDED

High Hydrostatic Pressures Application for Stabilizing Pomegranate (*Punica Granatum L.*) Fermented Beverages

Attended IFT18: Where Science Feeds Innovation® July 15-18, 2018 held in Chicago, IL USA

The IFT18 scientific and applied sessions qualify for Certified Food Scientist (CFS) recertification contact hours (CH). CFS Certificants may claim a maximum of 22 CH for their participation in scientific and technical symposia and poster sessions related to the CFS Content Domains.

IFT is a Continuing Professional Education (CPE) accredited provider (IN141) with the Academy of Nutrition and Dietetics' Commission on Dietetic Registration (CDR).

Daniel Gullicksen
Daniel Gullicksen
IFT Meeting Planner



El Consorcio Nacional de Recursos de Información Científica y Tecnológica
Otorga el presente

RECONOCIMIENTO

a

Gabriela Rios Corripio

Por su participación en el
Séptimo Seminario Entre Pares
con una duración de 16 hrs.

Puebla, Pue.
10 y 11 de septiembre de 2018

Mtra. Margarita Ontiveros y Sánchez de la Barquera
Coordinadora General
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AWARDS THE PRESENT CERTIFICATE TO:

Rios-Corripio, G., Guerrero-Beltrán, J. A.

IN RECOGNITION FOR THEIR PARTICIPATION AS
AUTHORS OF THE WORK: *Physicochemical and antioxidant characteristics of fresh and fermented beverages of black cherry (Prunus serotina subsp. capuli) (POSTER)*

DR. GUSTAVO DEL GUTIÉRREZ LÓPEZ
PRESIDENT OF THE AMECA
DIRECTING COUNCIL 2017-2019

DR. NICOLÁS OSCAR SOTO CRUZ
VICE PRESIDENT OF THE AMECA
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THE ORGANIZING DIRECTING
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DRA. LILIA ARELY PRADO BARRAGÁN
SECRETARY OF THE DIRECTING
COUNCIL PRESIDENT OF THE
SCIENTIFIC COMMITTEE

DR. SANTOS GARCÍA
PRESIDENT OF FOOD FOR HEALTH

Puerto Vallarta, Jalisco, México, 14-16 November 2018

