DEVELOPMENT AND CHARACTERIZATION OF EQUILIBRIUM MODIFIED ATMOSPHERE BIO-BASED PACKAGING SYSTEMS FOR BLUEBERRIES (*VACCINIUM CORYMBOSUM* L., BLUECROP)

By

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ABSTRACT

DEVELOPMENT AND CHARACTERIZATION OF EQUILIBRIUM MODIFIED ATMOSPHERE BIO-BASED PACKAGING SYSTEMS FOR BLUEBERRIES (*VACCINIUM CORYMBOSUM* L., BLUECROP)

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Equilibrium Modified Atmosphere Packaging (EMAP) is an effective technology for delaying senescence and prolonging the shelf life of fresh produce. An EMA that meets the fresh produce requirements can be achieved by using microperforated materials. So far, EMAP technology has been used only with petroleum-based materials. The goals of this research were: 1) to develop the first bio-based (poly(lactic acid), PLA) microperforated packaging systems for blueberries (*Vaccinium corymbosum* L., Bluecrop), 2) to assess the effect of the number of microperforations (0, 3, and 15 perforations) and temperatures (3, 10, and 23°C) on the physico-chemical, microbiological, and sensorial properties of blueberries, and 3) to characterize barrier properties of the packaging systems. Petroleum-based (poly(ethylene terephthalate), PET) microperforated packaging systems were used as controls. Blueberry weight loss was found to be material dependent regardless of number of perforations. Non-perforated PLA and PET packages showed the highest CO_2 and the lowest O_2 levels, and therefore, exhibited less fungal growth but a development of fermentative metabolites at all temperatures. The results of headspace analysis and weight loss were supported by the permeation rate of O_2, and water vapor permeance, respectively. Based on the outcomes of this research, PLA and PET packages with 3 perforations have demonstrated potential for maintaining the quality and prolonging the shelf life of blueberries for 19 days at 3°C.

I dedicate this thesis to my lovely parents and sisters

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Hayati Binti Samsudin

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KEY TO SYMBOLS

A	Surface area
Atm	Atmosphere
c.a	Chromatographic area
CO_2	Carbon dioxide
C_2H_4	Ethylene
Ep	Activation energy of respiration
hr	hour
kg	kilogram
m	meter
NL	Needles
N_2	Nitrogen
O_2	Oxygen
O_2 atm / CO_2 atm	O_2 / CO_2 in the atmosphere
O_2 pkg /CO_2 pkg	O_2 / CO_2 in the package headspace
pkg	package
P	microperforation
P	Permeability coefficient
P^0	Permeability pre-exponential factor
R,RR	Respiration rate (consumption/production)
R^0	Respiration pre-exponential factor

R	Universal gas constant
R_S	Ratio of short ellipse
R_L	Ratio of long ellipse
T	Temperature
s	seconds
t	Time
W	Mass
B	Permselectivity
μm	microlitre
μL	microlitre

KEY TO ABBREVIATIONS

AAT	Enzyme alcohol acetyltransferase
ADH	Enzyme alcohol dehydrogenase
ADP	Adenosine diphosphate
ALDH	Enzyme aldehyde dehydrogenase
ASTM	American Society for Testing and Materials
ATP	Adenosine- 5'- triphosphate
CoA	Acetyl coenzyme A
EMA	Equilibrium modified atmosphere
EMAP	Equilibrium modified atmosphere packaging
EPA	Enviromental Protection Agency
FID	Flame ionization detector
HB	Hydroxybutyrate
HV	Hydroxyvalerate
IQF	Individually quick frozen
LDPE	Linear density poly(ethylene)
MAP	Modified atmosphere packaging
MSW	Municipal Solid Waste
NAD	Nicotinamide adenine dinucleotide (oxidizing agent)
NADH	Nicotinamide adenine dinucleotide (reducing agent)
PDC	Pyruvate decarboxylase enzyme
PET	Poly(ethylene terephthalate)

PHA	Poly (hydroxyl alkanoate)
PLA	Poly(lactic acid)
RH	Relative humidity
RQ	Respiratory quotient
SPME	Solid phase microextraction
SSC	Soluble solid content

Chapter 1

1. Introduction

1.1 Background

Fresh produce consumption has been growing gradually in the last 20 years as a result of the consumers' health-awareness. This is reflected in the per capita consumption of fresh fruits and vegetables in the U.S., which has increased by 9.5 and 14.3%, respectively, from 1987 to 1997 (Kaufman, Handy, McLaughlin, Park & Green, 2000) . This trend has continued since it has been reported that fresh produce supermarket sales topped $ 43 billion as of August 2006, an increase of 5% over the previous year (Sloan, 2007). While fresh vegetable consumption continues to rise every year, the consumption of fresh fruit has been fluctuating from 2000 to 2007. For example, in 2007, fresh fruit consumption in the U.S., dropped by 4% from the previous year, with an average of 97.5 lbs consumed per person. According to the United States Department of Agriculture (USDA), the cause for this decrease is low fruit production particularly citrus-fruits, as a result of reduced bearing acreage. However, strong demand has been reported for non-citrus fruits like banana, grapes, blueberries, cranberries, strawberries, cherries, apricots and papayas (Pollack & Perez, 2008).

In order to maintain a healthy lifestyle, it is crucial to encourage consumers, regardless of age, to continue eating fruits. One of the best options is to offer fresh fruits that have a long shelf life and are pathogen-free. Fruits are a challenging food product to keep fresh, considering the complexity of their nature. They continue to respire by producing carbon dioxide (CO_2) and consuming oxygen (O_2). Their physico-chemical properties changes over time and vary from the moment they are harvested to the time

they are marketed and sold, as the enzymes, organic acids, pigments, and sugars continue to reacts. Once fruit senescence takes place, it results in unfavorable attributes. For instance, wilting and shriveling, off-flavor development, and fungal growth cause fruits to become less appealing. When consumers do not like what they sense (sight and smell), the fruits become unsalable, which causes profit losses to retailers.

Many efforts have been made to ensure that the fruit quality is acceptable for an extended time. Irradiation, coating, modified atmosphere packaging (MAP), and equilibrium modified atmosphere packaging (EMAP), are some of the technologies used for fruit preservation. EMAP is a commonly used technology for delaying senescence and prolonging the shelf life of fresh fruits (Almenar, Del-Valle, Hernandez-Munoz, Lagaron, Catala & Gavara, 2007a). By manipulating the packaging material permeability, this technology optimizes the respiration rate of fruits. An equilibrium modified atmosphere (EMA) can be achieved by initially flushed a single gas or a mixture of gases into the package before sealed or sealed without modification (Phillips, 1996a). Current understanding of the principles and applications of EMAP is mainly empirical. However, a systematic approach is being developed for creating optimal EMAP system (Almenar et al., 2007a; Chiesa, Seija & Moccia, 2004; Exama, Arul, Lencki, Lee & Toupin, 1993; Jacxsens, Devlieghere & Debevere, 1999; Jacxsens, Devlieghere, Falcato & Debevere, 1999).

1.2 Motivation

Blueberry is one of the fruits that have been in increasing demand by consumers (Pollack et al., 2008). However, there are marketing difficulties with blueberry fruit because it is highly perishable. Improper control of distribution and storage conditions

increase its vulnerability to post-harvest diseases caused by microorganisms such as *Colletotrichum acutatum*, *Alternaria alternata*, and *Botrytis cinerea* (Almenar, Samsudin, Auras, Harte & Rubino, 2008b; Smith, Magee & Gupton, 1996). Besides, the use of vented poly(ethylene terephthalate), PET, or poly(styrene), PS, clamshells for blueberry marketing raises other concerns. The design of clamshell containers does not allow the development of EMA, which would be an effective tool for prolonging blueberry shelf life (Almenar et al., 2008b). As a result, blueberry is subjected to physiological changes such as wilting, and shriveling. Almenar et al. (2008) reported that blueberries packed in clamshells lost 5% weight after 3 days at 10°C and 66% relative humidity (RH), making them unmarketable (Almenar et al., 2008b).

In addition, clamshells are produced from non renewable petroleum resources. The fact that clamshells are generally landfilled has contributed to environmental burdens such as land, air and water pollution. According to the U.S Environmental Protection Agency (EPA), 30% of plastic were introduced to the Municipal Solid Waste (MSW) stream as containers and packaging in 2008. This number accounts for almost half of the total plastics in the MSW streams (2008).

To help address these problems, EMAP technology could be used for improving blueberry shelf life. An EMA can be reached slowly or rapidly depending on package permeability and fruit respiration rate. This process requires some time before achieving an equilibrium atmosphere, while the fruits continue to deteriorate as a result of exposure to a non-optimal atmosphere. Microperforation can allow rapid gas exchange through the internal/external atmosphere of the product/package system. This technique has been

employed widely for preservation of fruits (Del-Valle, Almenar, Lagarón, Catalá & Gavara, 2003). Almenar et al. (2007), Del Valle et al. (2003), Sanz et al. (1999), and Rodov et al (1997), have utilized this technique for difference produces such as strawberry, mandarin, wild strawberry, and mango (Almenar et al., 2007a; Del-Valle et al., 2003; Rodov, Fishman, de La Asunción, Peretz & Ben-Yehoshua, 1996; Sanz, Pérez, Olias & Olias, 1999). To date, EMAP technology with microperforation has been only used with petroleum-based materials. Therefore, there is a need to develop a microperforated bio-based material and investigate its potential as an alternative to a microperforated petroleum-based material for fruit preservation. Emphasis on the different number of microperforations and its effect on physico-chemical, microbiological, organoleptic properties of fruits and barrier properties to water and gases should also be quantified.

1.3 Objectives

The objectives of this study are to:

- Develop the first bio-based microperforated packaging systems for fresh product.
- Develop the first bio-based microperforated packaging systems for blueberries.
- Assess the effect of the number of microperforations, and temperatures on the physico-chemical properties of blueberries.
- Characterize the microbiological, barrier and sensorial properties of the packaged systems.

Chapter 2

2. Literature Review

2.1.1 Blueberry: Brief history and introduction

Blueberries are native to North America. Their cultivation begun when the wild stands of native lowbush blueberry (*Vaccinium angustifolium* Ait. and *Vaccinium myrtilloides* Michx.) were burned by Native Americans in an effort to increase their production. The cultivation of northern highbush blueberry (*Vaccinium corymbosum* L.) started in the early 19th century by Elizabeth White and F.V. Coville. Then, at the end of the 19th century, the cultivation of rabbiteye blueberries (*Vaccinium ashei* Reade) was initiated (Strik, 2004). This crop has not only increased in acreage and production but also in demand. The popularity of this fruit has made it become the second most important berry, after strawberry.

Three species of blueberries are cultivated today (Table 2-1) and are commercially harvested and sold in the United States (Pollack & Perez, 2003). Highbush and rabbiteye species are the cultivated blueberries while the lowbush specie is often marketed as wild blueberry (Strik, 2007).

Table 2-1: Blueberry species cultivated in the United States

Blueberry Species	Profiles
Highbush	-thrives in the cooler climates of the northern temperate areas -the major type grown in North America
Rabbiteye	-more tolerant of the relatively warmer temperatures in the Southern USA
Lowbush	-harvested from managed wild stands in the northeastern USA (mainly in Maine) and eastern provinces of Canada

Note: Information adapted from Pollack and Perez (2003) (Pollack et al., 2003).

Blueberries, like other berry crops, are sold as fresh or processed fruits. For fresh consumption, they can be sold either through 1) U-pick (customer harvested) or on-farm sales (grower harvested) and 2) fresh sale through local stores or distributed to distant locations (Strik, 2007). The market for processed blueberry can be classified into three groups: 1) frozen (bulk frozen or individually quick frozen (IQF)); 2) dried; and 3) processed food in bakery products, juice/concentrate, baby food, etc (Pollack et al., 2003). Most of the lowbush blueberry (more than 97% of the total production) is channeled into the processed market (Strik, 2007).

2.1.2 Blueberry: Production and consumption

In 2005, the highbush blueberry world total production was 194, 830 tons, 78.2% of which was produced by North American with the United States as the leading country (Strik, 2007). Michigan, the largest producer of highbush blueberries, generates over 20% of the U.S. annual blueberry crop (Pollack et al., 2003). The most common varieties of blueberries grown in Michigan are Bluecrop, Jersey, Elliot, Duke, Rubel and Bluejay (Hancock, Hanson & Trinka, 2001). The market for these blueberries (fresh and

processed) was estimated at $139.7 million of the state's economy in 2006 (Pollack et al., 2003).

Over the last 7 years, market demand for blueberries has increased (Pollack et al., 2003). Blueberries, which are rich of antioxidants and vitamins, offer great health benefits to consumers. In addition, they have great flavors and are easy to consume (do not have to be peeled). So, they can be enjoyed in many ways: fresh, frozen, dried, or as liquid, depending on one's preferences. Fresh blueberry market has been increasingly favored by US consumers over frozen blueberry (Figure 2-1) (Pollack et al., 2008).

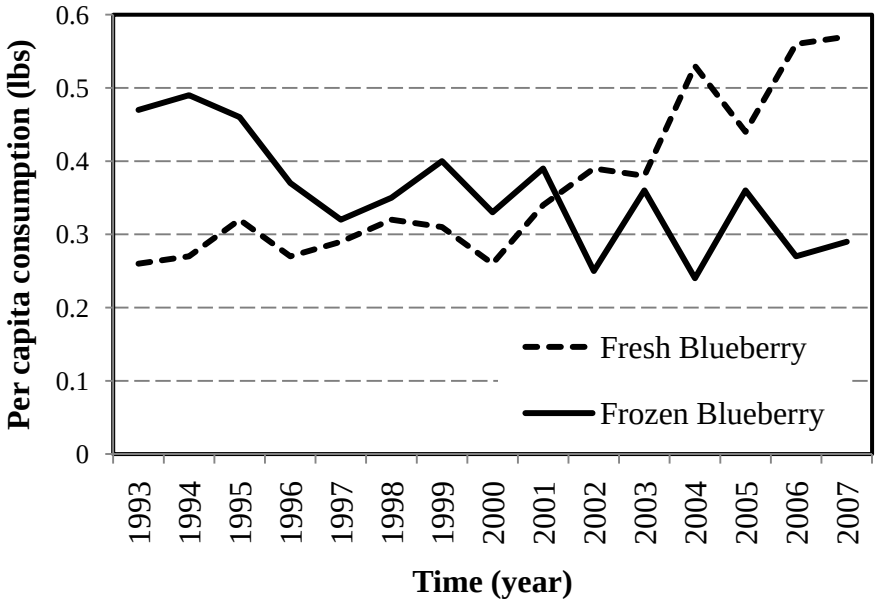


Figure 2-1: Per capita consumption of fresh and frozen blueberry from 1993 to 2007

Note: Information adapted from Pollack and Perez (2008) (Pollack et al., 2008).

2.2.1 Physicochemical properties of blueberry

2.2.1.1 Color

The color of blueberry varies from blue to blue-black or purple (Strik, 2007) depending on the fruit's variety, maturity, and postharvest handling such as storage

condition, and the duration of storage. In Michigan, blueberry colors are graded as light, medium or dark blue (Table 2-2) (Hancock et al., 2001). Anthocyanin, a phenolic-based pigment, is the compound responsible for blueberry color. This component is water soluble and highly pH sensitive, and it will reversibly undergo structural changes as the pH changes (Wrolstad, 2007). Blueberry has a waxy coating known as 'bloom,' which causes berries to appear lighter in color (Strik, 2007).

2.2.1.2 Size

The size of blueberry also varies based on fruit variety, growing conditions, and stage of ripeness. The descriptive scale that is used to grade the fruit size is: very small, small, medium, large, and very large. The Bluecrop variety is known to have a medium to large size, while Elliot and Duke are medium and large in size, respectively. Other examples are given in Table 2-2 (Hancock et al., 2001).

Table 2-2: The profile of selected Michigan blueberry varieties

	Characteristics		
Blueberry Variety	**Size**	**Color**	**Flavor**
Berkeley	Large	Light blue	Fair, low acid
Bonus	Very large	Light blue	Good
Bluecrop	Medium to large	Light blue	Good, tart
Bluejay	Medium	Light blue	Mild, slightly tart
Brigitta	Large	Light blue	Good
Burlington	Medium	Light blue	Good
Coville	Very large	Medium blue	Good, tart
Duke	Large	Medium blue	Good
Elliot	Medium	Light blue	Good
Jersey	Medium	Light blue	Fair
Little Giant	Very small	Medium blue	Good
North Blue	Medium	Dark blue	Fair, acid
Rubel	Small to medium	Medium blue	Fair
Weymouth	Medium to small	Dark blue	Poor

Note: Information is extracted and adapted from Hancock et al. (2001) (Hancock et al., 2001).

2.2.1.3 Flavor

The Society of Flavor Chemists defines flavor as "the sensation caused by those properties of any substance taken into the mouth which stimulates one or both senses of taste and smell, and also the general pain, tactile and temperature receptors in the mouth" (Chen, Wang, Chung & Ma, 2007). However, this term can be simplified as an overall

impression perceived when the sensations of smell, taste and feeling combine during the moment food are consumed. Thus, flavor consists of two broad categories, which are taste and aroma.

2.2.1.3.1 Taste

The taste of blueberry is the results of the interactions between its chemical constituents such as water, sugar content and organic acids. Blueberry is reported to have 84.2 g/100 g (wet basis) water content (Talcott, 2007). Besides these two factors, the unique combination of tart and sweet flavors has made blueberry desirable. The tartness of the ripe fruit is attributed by the presence of organic acids such as quinic, citric and malic acids. These acids can be found predominantly in ripe blueberry fruit. Aside from affecting the fruit's taste, the organic acids also serve as the stabilizing agents for ascorbic acid and anthocyanin (Talcott, 2007). As mentioned earlier, the anthocyanin structure will reversibly transform with changing pH (Wrolstad, 2007). Sucrose, glucose and fructose in the fruit help to balance acidic flavors. Generally, glucose and fructose are present in equal concentration when the berries are ripe (Shaw, 1998). Shaw (1988) reported the value of glucose and fructose for blueberries as 3.28-3.87 and 3.34-3.88 % (w/w), respectively. Sucrose content value was found to be 0.12-1.14% (w/w). These values can vary depending on the degree of ripeness, and period of postharvest storage (Shaw, 1998).

2.2.1.3.2 Aroma

Aroma is the odor of a food perceived by consumers. Consumer would identify a food as desirable or undesirable (i.e spoiled food) based on the volatile compounds

produced by it. These compounds are known as aroma substances, which are detected by the olfactory system in the nasal cavity (Belitz, Grosch & Schieberle, 2004).

Aroma of fruits can be derived from fatty acid, amino acid or carbohydrate metabolism. In fresh fruits, aroma production takes place during the ripening process as a result of biochemical activity. For instance, in fatty acid metabolism, the fruit experience chloroplast degradation where linoleic and linolenic acid undergo oxidation through lypoxigenase. This enzyme reacts by promoting oxidative breakdown of unsaturated fatty acid chains, forming hydroperoxide species. Some of the hydroperoxides produced are unstable and continue to degrade to desirable or undesirable low molecular weight compounds such aldehydes, alcohols, and acids (Reineccius, 2006).

Amino acid metabolism serves as an important pathway in the production of compounds responsible for the aroma of ripe bananas and apples. This pathway is denoted by non-enzymatic browning reaction known as the Strecker degradation. The degradation of amino acids (i.e. valine and leucine in bananas) by reacting with α-dicarbonyls contributes to volatile products such as aldehyde, pyrazines, pyrazinone, and others (Reineccius, 2006).

Some flavor precursors are produced directly from carbohydrate metabolism, such as furanones, and terpenes, which are also a product of lipid metabolism. Terpenes can be grouped according to the number of isoprene units they contained. The oxygenated monoterpenes play a major role in the aroma of citrus fruits. While furanones such as 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 2,5-dimethyl-4-methoxy-3(2H)-furanone are considered as the most important flavor constituents of strawberries (Reineccius, 2006).

Aroma such as hexanal, (E)-2-hexenal, 1-hexanol, benzaldehyde, nonanal, and linalool creates a unique aroma profile for blueberry. Some common fruits and their typical aroma substances are listed in Table 2-3.

Table 2-3: Typical aroma compounds found in selected fruits

Fruits	**Typical aroma compounds**
Apricot	myrcene, limonene, p-cymene, terpinolene, α-terpineol geranial, geraniol, linalool, acetic and 2-methyl-butyric acids trans-2-hexenol γ-caprolactone,γ-octalactone, γ-decalactone,γ-dodecalactone δ-octalactone, δ-decalactone
Banana	Isopentyl acetate Isoamyl acetate Acetic, propionic and butyric acids
Bartlett pear	Ethyl trans-2-cis-4-decadienoate
Blackberry	2-heptanol, p-cymen-8-ol
Cherry	benzaldehyde, linalool, hexanal, (E)-2-hexenal, phenylacetaldehyde, (E,Z)-2,6-nonadienal, eugenol
Concord grape	Methyl anthranilate
Delicious apple	Ethyl-2-methylbutyrate
Grape	2-aminobenzoic acid methylester (methyl anthranilate)
Grapefruit	Nootketone + 1-p-menthenthiol
Highbush blueberry	Hexanal, (E)-2-hexenal, 1-hexanol, 2-ethyl-1-hexanol benzaldehyde, linalool, nonanal
Lemon	Citral (geranial + neral)
Lowbush blueberry	methyl-3-methylbutanoate
Peach	γ-decalactone

Table 2-3 (cont'd)

Pineapple	2,5-dimethyl-4-hydroxy-3(2H)-furanone
Raspberry	1-p-hydroxyphenol-3-butanone
	5-hydroxyoctanoic and 5-hydroxydecanoic acids
Strawberry	methyl butanoate, butyl butanoate
Wild strawberry	methyl butyrate, ethyl butyrate, ethyl hexanoate, hexanal, 2-heptanone, 2-nonanal, 2,5-dimethyl-4-hydroxy-3(2H) furanone, acetic acid, hexanoic acid, methanol, benzyl alcohol, 1-hexanol,cis-3-hexen-1-ol, β-citronellol

Note: Information adapted from Chen et al. (2007), Belitz et al. (2004), Ibáñez et al. (1998), Almenar et al. (2006) (Almenar, Hernández-Muñoz, Lagarón, Catalá & Gavara, 2006; Belitz et al., 2004; Chen et al., 2007; Ibáñez, López-Sebastián, Ramos, Tabera & Reglero, 1998).

2.2.1.4 Off-flavor

Off-flavor is one of the common criteria used to indicate poor fruit quality. Its formation is similar to typical flavors thereof. Fruits would develop off- flavor by either undergo an abusive environment or extensive storage as a result of fermentative metabolites accumulation. Acetaldehyde, ethanol and/or ethyl acetate are the three major compounds that usually tie to off-flavor notes when presence beyond their threshold concentrations (Kader, 2008).

Storage environment (e.g temperature and relative humidity), fruit nature (e.g respiration rate and maturity), and packaging material are some of the vital elements that may favor an off-flavor formation. Research in banana flavor production at prolonged storage demonstrated that at a temperature lower than 5°C, no aroma compounds were developed, while at temperature ranged from 10 to 12°C, aromas were found reduced

considerably of about 60%. At temperature higher than 27°C, anaerobic aroma development was detected (Reineccius, 2006).

Fruits with moderate and high respiration rates are more likely to develop an off-flavor than fruits with low respiration rates when they are kept in a closed package system. The higher the respiration rate the greater the production of CO_2 and consumption of O_2 will be, thus creating faster an anaerobic condition. When O_2 concentration decreases, fruits lose their final electron acceptor in respiration, causing a shifting of electrons from one organic intermediate of sugar breakdown to other organic metabolites. This process later results in the formation of lactate, acetaldehyde, and ethanol, of which govern off-flavor notes (Wang, 1990). In details, this process started with sugars' breakdown via glycolysis, in which as soon as O_2 is deprived, pyruvate is transformed to lactate by lactate dehydrogenase by means of the reducing factor NADH and producing NAD. Acidification could occur as the amount of lactate accumulates in the cytosol increases, thus resulted in inhibition of lactate dehydrogenase. Due to this acidification, a low pH condition is established in cytosol, in which the decarboxylation of pyruvate is stimulated by the activation of pyruvate decarboxylase. As a result, acetaldehyde is formed. The increase of pyruvate concentration in the cytoplasm may also favor a direct stimulation of pyruvate decarboxylase. From acetaldehyde, ethanol is then produced through the reaction of enzyme alcohol dehydrogenase (ADH) by utilizing the reducing factor, NADH. Under normal condition (aerobic respiration), pyruvate is transformed to acetyl Co-A before entering the citric acid cycle (Figure 2-2) (Paliyath & Murr, 2006).

However, for such an anaerobic condition to occur, permeability of gases through packaging material/systems needs to be taken into account. For example, material that has a low barrier to gases could maintain an aerobic respiration for some time before undergoing an anaerobic respiration since the O_2 from atmosphere can easily permeate through the material.

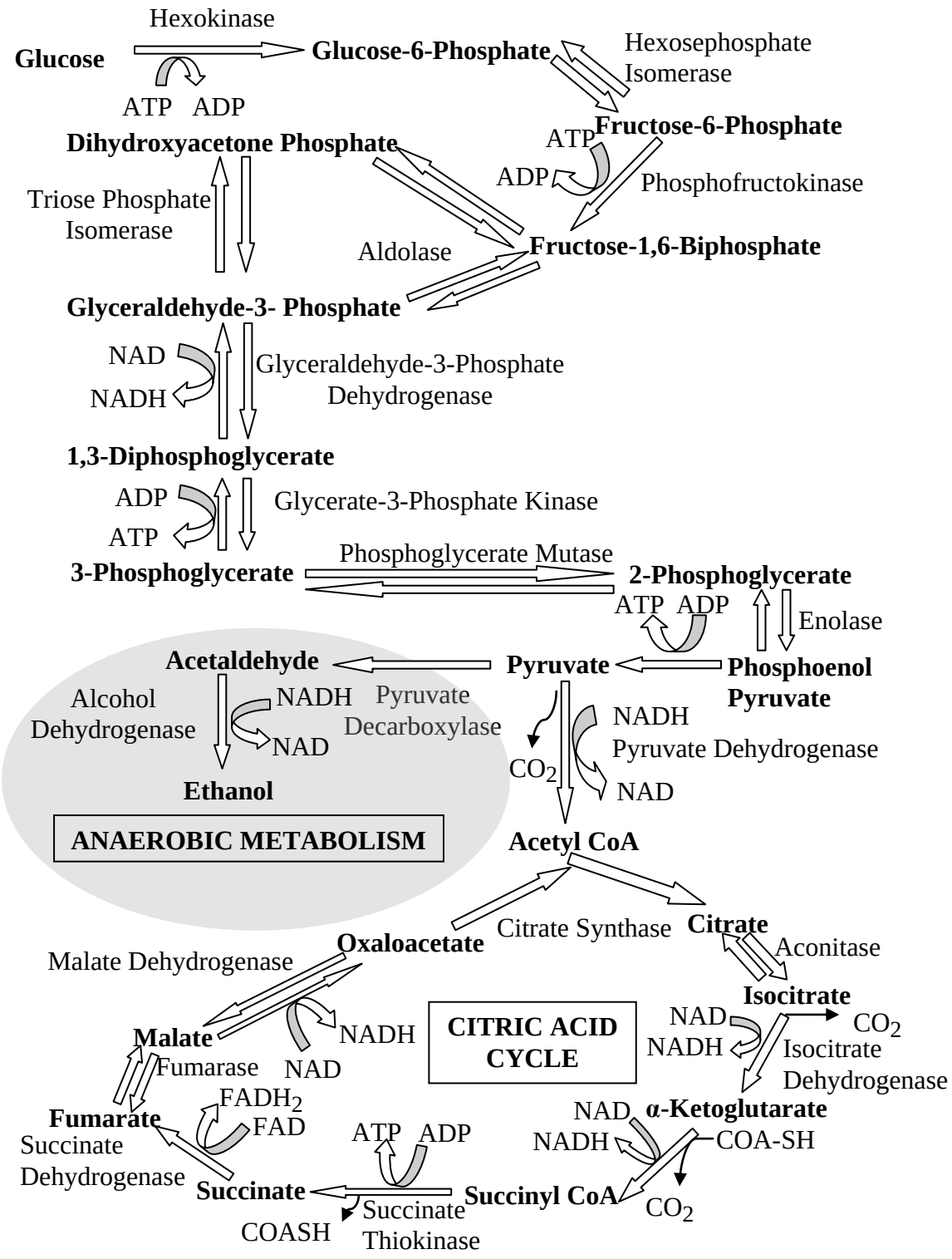


Figure 2-2: Glycolytic pathway and citric acid cycle for sugar's breakdown.

Note: Figure was adapted from Paliyath and Murr (2006) (Paliyath et al., 2006).

2.3 Microbiological quality

Fresh berry fruits are vulnerable to postharvest diseases caused by microorganisms, resulting in berries losses. Berries, normally kept in high humidity storage, can be easily infected by fungi. The most common post harvest microorganisms for berry fruits are *Botrytis cineria*, *Alternaria alternata*, and *Colletotrichum acutatum.* Among these three, *Botrytis cineria* is difficult to control since it can grow at a low temperature. Berry fruits can also be infected at field or during handling. Late season berries are generally more susceptible to decay than early harvested berries. Susceptibility of berries to decay varies from one to another (Table 2-4). Many efforts have been made to control this problem; some of them are the application of antimicrobial edible films on fruit, and the use of controlled atmosphere during storage and distribution.

Table 2-4: Common postharvest diseases of berry fruits.

Disease	Causative agent	Fruits
Gray mold	*Botrytis cineria*	Strawberry, blackberry, blueberry, raspberry and grapes.
Anthracnose fruit rot	*Colletotrichum acutatum* and *Colletotrichum gloesosporiodes*	Strawberry, blueberry, cranberry and grapes.
Botryosphaeria fruit rot	*Botryosphaeria dothidea*	Cranberry and grapes
Alternaria rot	*Alternaria sp.*	Occasionally occur on berries and grapes.

Note: Adapted from Ellis et al. (1991), Jenning (1988), Milholland (1995), Eck (1990), Prange and DeEll (1997), Tournas and Katsoudas (2005) (Eck, 1990; Ellis, Converse, Williams & Williamson, 1991; Jennings, 1988; Milholland, 1995; Prange & DeEll, 1997; Tournas & Katsoudas, 2005).

2.4 Factors affecting fruits quality and shelf stability

The most important determinant aspects for fruits marketability are their quality and shelf stability. Fruits are perishable products that are susceptible to physiological changes and decay. Poor quality control of fruits would result in a short shelf life. Therefore, it is crucial to understand the parameters that affect fruits quality and shelf stability, which are extrinsic and intrinsic factors.

2.4.1 Extrinsic factors

2.4.1.1.1 Temperature

Temperature plays an important role in fruits' quality. Temperature has a great impact to the respiration rate of fruits. For an increase of 10°C, the respiration rate would be increased by two to three folds, thereby reduce the shelf life (Brecht, Ritenour, Haard

& Chism, 1996). When a low temperature at an optimal tolerance is applied on the fruits, their respiration rate reduces, hence slow the metabolic process and extend the shelf life. Some of the fruits such as cranberry, tomatoes, and pepper are chilling sensitive. When this happen, they would experience chilling injury symptoms such as surface lesions, increased susceptibility to decay, and compositional changes as related to flavor and taste, to name a few (Tabil, Jr. & Sokhansanj, 2001). Most of berry fruits are not chilling sensitive. This includes blueberry, strawberry, raspberry, and blackberry. However, all berries can experience tissue damage if they had a long contact with ice, thus increase their susceptibility to decay (Bower, 2007).

2.4.1.2 Relative humidity

A relatively high humidity (90 - 95%) is recommended to maintain fruits' quality by preventing a moisture loss. However, this humid condition increases the susceptibility of the fruits to fungal growth (Bower, 2007). In a high humidity atmosphere, condensation may also occur on the inside surface of packaging material if the storage temperature are fluctuated. Consequently, the product becomes less appealing, thus limit its market.

2.4.1.3 Gases

2.4.1.3.1 Carbon dioxide (CO_2)

The evolution of CO_2 at higher level (greater than 10%) can inhibit the growth of fungal. This gas also has a positive effect to the fruits firmness. Almenar et al. (2007) reported a significant increase from 1.9 to 3.3 N/cm^2 on wild strawberry firmness, as a result of an exposure to high CO_2 concentration (Almenar et al., 2007a). It is also known

to suppress the respiration rate of fruits and at level greater than 1%, and to reduce fruits sensitivity to ethylene (Zagory, 1995). However, this gas is associated with the development of the off-flavor. Discoloration, softening, and off-flavor in raspberry are associated by riched-CO_2 atmosphere (> 20%) (Agar, Streif & Bangerth, 1997).

2.4.1.3.2 Oxygen (O_2)

Reduced O_2 atmosphere cause a delay in compositional changes such as softening and pigment development. O_2 at a concentration below 1-2% induces off-flavor and off-aroma production of fruits. This happens due to the absence of O_2 molecules to serve as the final electron acceptor in the glycolysis, which causing a shift of the electron to anaerobic pathway (Zagory, 1995).

2.4.2 Intrinsic factors

2.4.2.1 Respiration rate

Respiration is a metabolic process that supplies energy to a plant to carry out biochemical activities (Fonseca, Oliveira & Brecht, 2001). Different types of fruit have different level of respiration rate (low, moderate and high respiration). In general, the senescence of fruits is proportional to respiration rate. This parameter is temperature dependent and is markedly affected by modified atmosphere (Irtwange, 2006). Another term that is associated with respiration rate known as respiratory quotient (RQ). RQ is the ratio of CO_2 produced to O_2 consumed. RQ is normally assumed to be 1. However, it can be lesser than 1 when highly reduced lipids are used for respiration, and greater than 1 when organic acids are used for respiration (Brecht et al., 1996).

2.4.2.2 Transpiration

This process is a natural process encountered by fresh produce, and its rate dependant on different factors such as temperature, surrounding gases (type and level), relative humidity, etc. Without a proper control, this process may cause excessive loss of moisture, which would affect fruits appearance (i.e., shriveling), textural quality (i.e. loss of firmness) and ultimately nutritional quality (i.e. loss of vitamins). A water loss of less than 1 % (wt/wt) can result in poor fruits' quality and shelf stability, while a loss of 3 to 10% (w/w) of fruits' weight would reduce their marketability (Brecht et al., 1996).

2.4.2.3 Ethylene production

Physiological properties of fruits (i.e., climacteric fruits) are greatly affected by ethylene gas (C_2H_4). Ethylene is a gas which is produced in all parts of the plants. Ethylene is physiologically active even at a concentration of less than 0.1 ppm (Irtwange, 2006). The amount of ethylene produced by climacteric fruits is significantly large when compared to non-climacteric fruits during the development and ripening process of fruits (Brecht et al., 1996). Ethylene increases the rate of ripening process by stimulating the expression of the gene for chlorophyllase. This gas is used widely to ripen climacteric fruits (e.g., bananas, avocados, and apples) for a commercial market to obtain a uniform and faster ripening (reduce time between harvest and fruit consumption). Blueberries produced ethylene within a range of 0.1 to 1.0 µL C_2H_4/kg-hr at 20°C, while strawberries and cherries' ethylene production are between 0.01 and 0.1 µL C_2H_4/kg-hr at 20°C (Brecht et al., 1996).

2.5 Approaches used for fruits preservation

Fruits are susceptible to senescence as their biochemical reactivity continues until the end of shelf life. Temperature, relative humidity, and respiration rate are some of the most important factors that influence the quality of fruits. There are many approaches that have been applied to ensure fruits' shelf stability and reducing fruit loss, such as cold storage, coatings, irradiation, and packaging technology.

2.5.1 Cold storage

Cold storage has been used for a long time because of its efficiency in controlling fruits decay by reducing respiration rate and slowing down the ripening process. Fruits need to be cooled right after harvest to remove field heat. For this purpose, the most common refrigeration method used is forced-air ventilation. Donahue et al. (1999) reported that the cold moving air also removes excessive moisture that is present on berries surface, hence decreasing fruits susceptibility to decay (Donahue, Bushway, Moore & Hazen, 1999). This method also helps to retain berries' quality without causing weight loss or processing damage (Donahue et al., 1999). For this type of storage, relative humidity needs to be maintained especially since this forced-air ventilation method cause rapid moisture loss. A 95% relative humidity (RH) can be maintained with the help of a plastic-lined container. But, the presence of high humidity has some drawbacks such as that it may weaken the corrugated boxes and promote the development of post harvest microorganisms. The latter drawback can be controlled to a certain degree by maintaining sufficient air-circulation and using the coldest tolerated temperature to store the fruits without causing freezing them (Table 2-5) (Bower, 2007).

Cold storage could delay fruit senescence during distribution. However, once the fruit arrives at the stores, it depends on the retailers for how to store the product. Most of the time, the storage conditions vary from one store to another, which reduce the fruits quality and shelf life.

Table 2-5: Recommended cold storage for berry fruits

Berry fruits	Storage temperature (°C)	Optimum humidity (%)	Estimated postharvest shelf life
Blackberries and their hybrids	0.5-0	90-95	2-3 days
Blueberries	0.5-0	90-95	2-3 weeks
Cranberries	2.0-4.0	90-95	2-4 months
Grapes	0.5-0	85	2-6 weeks
Raspberries	0.5-0	90-95	5-7 days
Strawberries	0.5-0	90-95	5-7 days

Note: adapted from Salunkhe and Desai (1984), Kader (2001), Haffner et al. (2002), Bower (2007) (Bower, 2007; Haffner, Rosenfeld, Skrede & Laixin, 2002; Kader, 2001; Salunkhe & Desai, 1984).

2.5.2 Coatings

There are many research and developments working on the production of edible coatings for fruits preservation. This technology helps prevent moisture loss and slows the ripening process of fruit during storage. More novel, antimicrobial agents are some time incorporated into coatings to inhibit possible microbial growth. Some of the coatings are fortified with antioxidants, vitamins, and probiotics to enhance the nutritional value of the fruits. For instance, strawberry fruits coated with chitosan in combination with calcium gluconate showed an inhibition of fungal decay and maintained firmness at

10°C, 70 ± 5% RH (Hernández-Muñoz, Almenar, Del Valle, Verez & Gavara, 2008). Zivanovic et al. (2005) reported that chitosan combined with anise was efficient in inhibiting the growth of *Botrytis cineria* on strawberries (Zivanovic, Chi & Draughon, 2005).

However, to this day, the market demand has remained low for this technology in the fresh berry market since berry packing is often done right after harvest with as minimal handling as possible. There are also concerns regarding the application of this technology that limits its market, such as the possibility of film components migration and negative public perception of the use of chemical on fruits (Bower, 2007).

2.5.3 Irradiation

Irradiation technology is used to preserve food quality and prolong shelf life by killing spoilage organisms and slowing the ripening process. For fresh fruits and vegetables, a dose up to 1 kGy has been permitted to kill insects and extend shelf life. A study found that grapes decay causes by *Rhizopus* sp. and *Botrytis* sp. was inhibited with 2 kGy dose of gamma irradiation (Thomas, Bhusha & Joshi, 1995). While in the case of sensory quality, available studies indicated that no serious loss was found in flavor, texture, aroma and appearance for many of the tested fresh produce (Groth III, 2007).

Even though this technology is effective in extending produce shelf life, its market is limited due to some concerns. From an economic standpoint, irradiated produce is more costly than non-irradiated. While from a safety standpoint, there are concerns about possible chemical migration from packaging into produce or radiation-induced chemical reactions between packaging-fruits (Groth III, 2007) since the produce is irradiated after packaging.

2.5.4 Packaging technology

Fruits preservation through packaging is one of the most common approaches used when compared to all of the technology discussed earlier. Packaging technology is practical and economical. Packaging is needed for marketing and distribution purpose, thus the use of packaging as a preservation technique adding extra benefits to a product. Many technologies have evolved to effectively manipulate fresh produces respiration rate, together with gases concentration, and or film/ package permeability. Among these technologies are controlled atmosphere storage, modified atmosphere packaging, equilibrium modified atmosphere packaging, vacuum packaging, vacuum skin packaging and others. However, only the first three will be discussed, as it is often used in berry fruits preservation.

2.5.4.1 Controlled atmosphere storage

There are two terms associated with controlled atmosphere, which are controlled atmosphere packaging, and controlled atmosphere storage. Both terms are referred to a continuous control of atmosphere over a product to maintain a desired gas composition throughout storage. Blakistone (1998) discussed that the use of controlled atmosphere packaging term is incorrect, as it is impossible to control atmosphere, once the package is sealed (Blakiston, 1995). Controlled atmosphere storage is associated with storage facilities or sometimes-large bulk packages (Selke, 1997). In this case, the gas composition of which initially introduced into the atmosphere is maintained by constant monitoring and regulation (Blakiston, 1995). Controlled atmosphere technology is designed to preserve the quality of fresh produces during transportation and storage (Bower, 2007).

For different types of berry fruits, difference levels of atmospheric gases are introduced and maintained during transportation (Table 2-6). Bower (2007) cited that if this technology is used correctly, the shelf life of berry fruit can increase up to six weeks (Bower, 2007).

Table 2-6: Commercial use of controlled atmosphere for berry fruits

Berry fruit	Temperature (°C)	O_2 concentration (%)	CO_2 concentration (%)	Commercial use
Blackberry	0.5-0	5-10	10-20	Gases are sealed within pallet covers during transportation
Blueberry	0.5-0	1-10	10-15	Sometimes used during transportation
Cranberry	2.0-4.0	1-2	0-5	Not commercially used
Raspberry	0.5-0	5-10	15-20	Gases are sealed within pallet covers during transportation
Strawberry	0.5-0	5-10	15-20	Gases are sealed within pallet covers during transportation

Note: Adapted from Bower, Salunkhe and Desai, Kader, and Haffner et al. (Bower, 2007; Haffner, Rosenfeld, Skrede & Laixin, 2002; Kader, 2001; Salunkhe & Desai, 1984).

2.5.4.2 Modified atmosphere packaging (MAP)

This technology is designed by replacing the air with a single gas or a mixture of gases in the package headspace. There is no further control over the initially introduced gas composition (Phillips, 1996b). MAP technology is applied in variety of product such as, meats, fish, fruits and vegetables, and others. For different products, different gas composition is used depending on the amount of gas that the product can tolerate, and the quality of the products that is of concern. Table 2-7 lists some of the products with their recommended gas mixture for MAP application. Table 2-8 lists some of fresh fruits with their respective tolerance to gas concentration.

Table 2-7: Recommended gas mixture for selected products.

Product	O_2 (%)	CO_2 (%)	N_2 (%)
Red meat	60-85	15-40	-
Fish (white)	30	40	30
Fish (oily)	-	60	40
Fruits and vegetables	3-5	3-5	85-95

Note: Information sorted and adapted from Blakistone (1995) (Blakiston, 1995).

For fresh produces, the generation of modified atmosphere can be either active modification or passive modification. Active modified atmosphere is created by generating a slight vacuum and replacing the atmosphere inside the package with a desired mixture of gases. This gas mixture will be altered by the produce and the film permeability. In addition, this gas mixture can be modified by using absorbing or

desorbing substances such as carbon dioxide emitters, oxygen absorbents, and ethylene absorbents (Kader, 2002).

Passive atmosphere modification (commodity-generated atmosphere) is created by the interplay of the produce respiration and packaging material permeability. If the respiration characteristics are properly matched to the material permeability, the atmosphere can be passively generated within the package until an equilibrium atmosphere is reached. This modification is also known as equilibrium modified atmosphere (EMA) (Kader, 2002).

2.5.4.3 Equilibrium modified atmosphere packaging (EMAP)

Equilibrium Modified Atmosphere Packaging (EMAP) is a technology where equilibrium stage of gases (CO_2 and O_2) is reached in the headspace of the packaging system after going through passive atmosphere modification due to the respiration of the fresh produce and permeation of gases from inside to the outside of the package or vice versa. For EMAP application, two conditions can be established. Either a mixture of desired gas is flushed into the package/ product system, or the product is sealed without any modification to allow gases to reach equilibrium (Irtwange, 2006). During storage the product respires, by consuming O_2 and producing CO_2, which then permeates through the packaging films. This process continues until an equilibrium condition is reached between the internal and the external atmosphere. EMAP application is widely used for packaging fruits and vegetables. In the case of blueberries, recommended optimal modified atmosphere conditions are 0-10% of O_2 and 11-20% of CO_2 (Yam & Lee, 1995) (Table 2-8 and Figure 2-3). The atmosphere developed during storage helps in

inhibiting bacterial and fungi growth, reducing moisture loss (weight loss) and controlling biochemical and enzymatic activity to slow down senescence and ripening (Fellows, 2000).

Table 2-8: Selected fruits with their optimal tolerance to oxygen and carbon dioxide

Fruits	**O_2 (%)**	**CO_2 (%)**
Apple	2-3	1-2
Banana	2-5	2-5
Blueberry	0-10	11-20
Sweet cherry	3-10	10-12
Strawberry	5-10	15-20

Note: Information sorted and adapted from Yam and Lee (1995) (Yam et al., 1995).

EMAP is temperature dependent due to the respiration rate of fresh produce and the film permeability increment with temperature. For example, as temperature increases by 10°C, the reaction rate of fresh produces double. The Arrhenius model can be used to describe both parameters, respiration rate and gas permeability as follows (Equation 2.1 , 2.2, 2,3, and 2.4) (Yam et al., 1995);

Respiration rate:

CO_2 Evolution : $R\,CO_2 = R^0\,CO_2 \exp(-Ep\,CO_2/RT)$ ……..(Equation 2.1)

O_2 Consumption: $R\,O_2 = R^0\,O_2 \exp(-Ep\,O_2/RT)$……(Equation 2.2)

Gas permeability:

$P\,CO_2 = P^0\,CO_2 \exp(-Ep\,CO_2/RT)$……..(Equation 2.3)

$P\,O_2 = P^0\,O_2 \exp(-Ep\,O_2/RT)$……..(Equation 2.4)

R= Respiration rate (consumption/production) (mL $kg^{-1}hr^{-1}$)

R^0= Respiration pre-exponential factor (mL $kg^{-1}hr^{-1}$)

R= Universal gas constant (8.314 J mol^{-1} K^{-1})

T=Temperature (°K)

P= Permeability coefficient (mL mil cm^{-2} hr^{-1} atm^{-1})

P^0= Permeability pre-exponential factor (mL mil cm^{-2} hr^{-1} atm^{-1})

Ep=Activation energy of respiration (J mol^{-1})

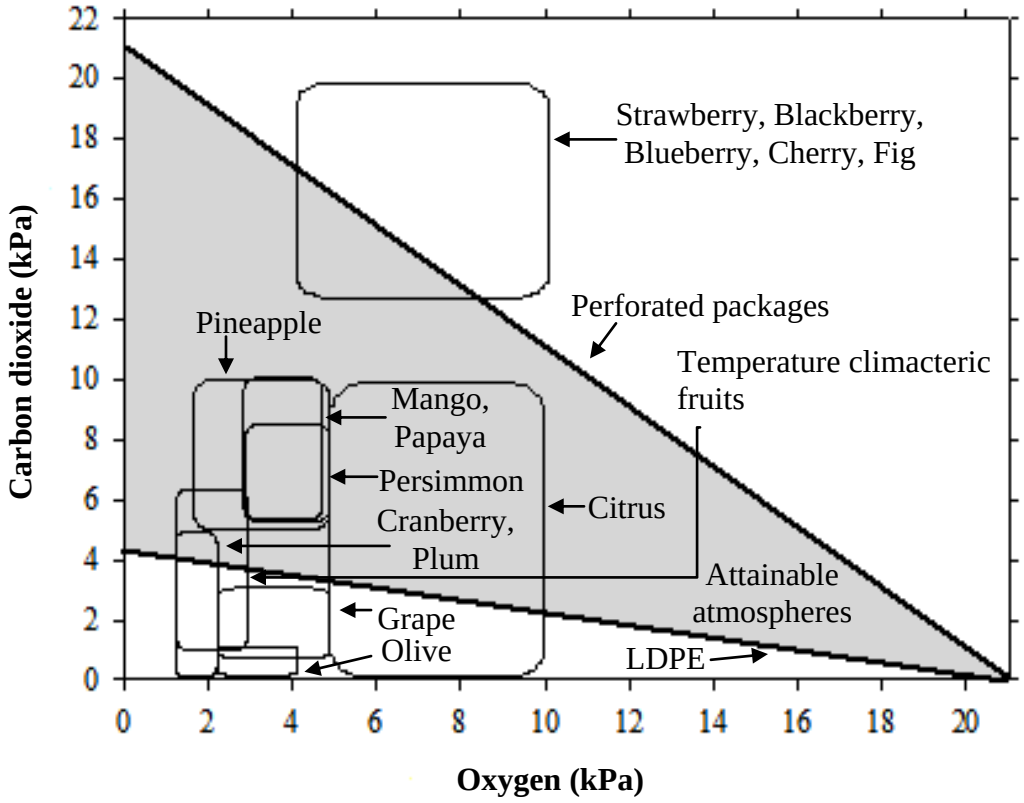


Figure 2-3: "Recommended O_2 and CO_2 combinations for the storage of fruit. The shaded area depicts atmospheres theoretically attainable by MAP by film permeation alone (low density polyethylene, LDPE, lower boundary) and via perforation alone (upper, dashed line) or their combination (shaded area)." Redrawn and adapted from Kader (1997a, b) (Beaudry, 1998).

EMAP can be achieved slowly or rapidly depending on the film permeability. Fruits with high and moderate respiration rate (e.g., strawberry and blueberry, respectively) require the use of films with an enhanced permeability and a wide range of CO_2 to O_2 permeability ratio to prevent the development of anaerobic atmosphere. CO_2 to O_2 permeability ratio is defined as β value or permselectivity. A β value of less than 3

is necessary for accurately matching the respiration characteristics of fruits. However, it is difficult to find films that meet this requirement. Most of the commercially available films have β value ranging from 3 to 6 (Table 2-9), and many fruits require β values outside of this range (Figure 2-4) (Yam et al., 1995).

Table 2-9: Permeability values of polymeric films at 10°C with their corresponding β value.

Polymeric films	**Permeabilities (ml mil/ m^2-hr –atm)**		**β value**
	CO_2	**O_2**	
Polybutadiene	1118	9892	8.8
Low density polyethylene	110	366	3.3
Ceramic-filled linear low density polyethylene	199	882	4.4
Linear low density polyethylene	257	1002	3.9
High density polyethylene	2.1	9.8	4.6
Cast polypropylene	53	151	2.9
Oriented polypropylene	34	105	3.1
Polyethylene terephthalate	1.8	6.1	3.3
Nylon laminated multilayer film	1.7	6.0	3.5
Ethylene vinyl acetate	166	985	5.9

Table 2-9 (cont'd)

Ceramic-filled polystyrene	116	630	5.4
Silicone rubber	11170	71300	6.4
Perforation (Air)	2.44×10^9	1.89×10^9	0.8
Microporous film	3.81×10^7	3.81×10^7	1.0

*Adapted from Exama et al. (1993); Lee et al. (1992); Lee et al. (1994); Ohta et al. (1991); Mannapperuma and Singh (1990); Anderson (1989); Shelekshin et al. (1992) (Anderson, 1989; Exama et al., 1993; Lee, Haggar, Lee & Yam, 1991; Lee, Haggar & Yam, 1992; Mannapperuma & Singh, 1990; Ohta, Nakatani, Saio, Nagota, Yoza & Ishitani, 1991; Shelekhin, Dixon & Ma, 1992).

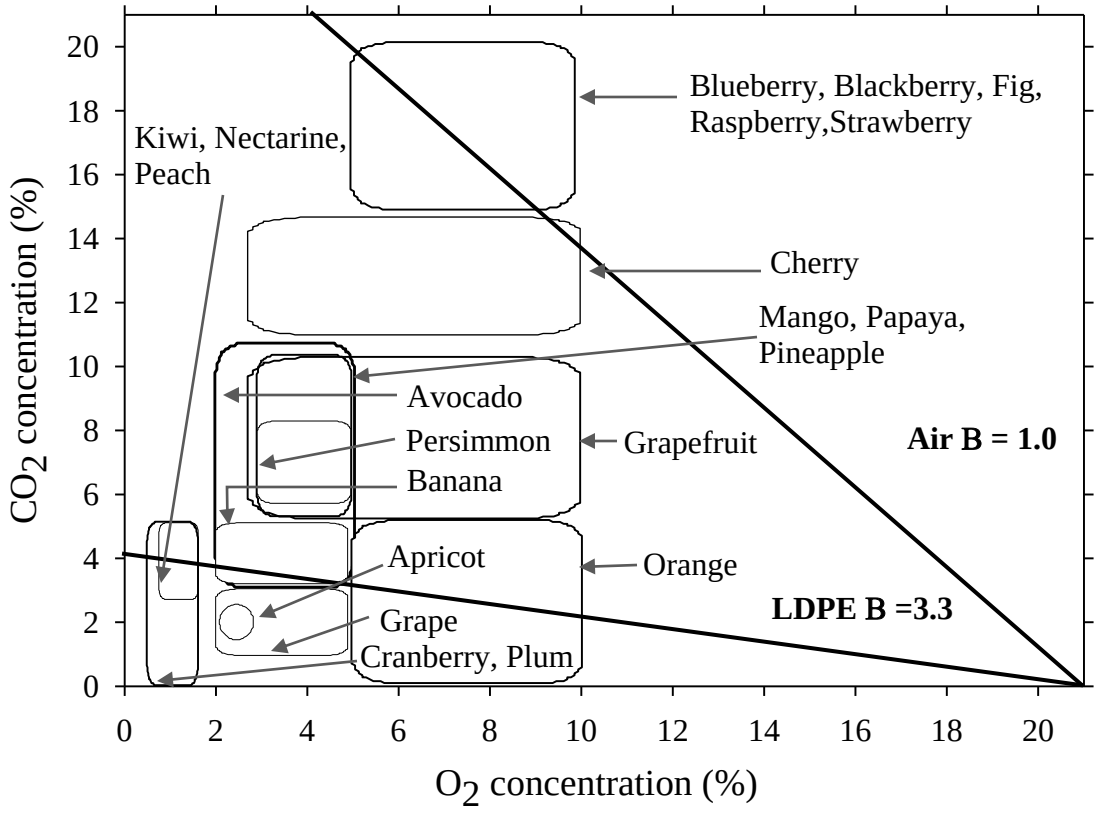


Figure 2-4: Recommended gas concentration of fruits for controlled atmosphere storage. Noted: Figure was adapted from Yam and Lee (1995) (Yam et al., 1995).

Alternatively, this requirement can be achieved by using a microperforation technique since the use of microperforated film would allow a more rapid gas exchange within the internal/ external product/ package system than that of normal film (Irtwange, 2006) (Figure 2-5). In the case of packaged fruits with continuous films, the package has high or medium barrier and the gas exchange process is limited to the sealed film surface. Microperforated films could be beneficial in achieving equivalent gas exchange at high rates (Irtwange, 2006) since the presence of microperforations on the film allows permeation of O_2 and CO_2 at equivalent rates resulting in limited ratio of gases (Yam et

al., 1995) at the headspace. As a consequences, microperforated film creates an equilibrium atmosphere of low O_2 (1-5%) and high CO_2 (15-20%) concentrations (Zagory, 1997). The β value for microperforated film was reported to range from 0.8 and 1 (Anderson, 1989). Since blueberries can tolerate high CO_2 concentration, this technology may help to reduce fungal growth, improve firmness (Yam et al., 1995) and prolong their shelf life.

Moreover, the thickness and surface area of the films are also important factors in adopting this technology since they involve directly with permeability and β values. A thick film has greater restriction over the gas diffusion through material than a thin film; hence, it can maintain a greater gas gradient within the outside air and atmosphere inside the package. By decreasing the surface area of the film, the gas diffusion would also be reduced. The quantitative relationship of these parameters, at a given temperature, can be expressed as follows (Equation 2.5, and 2.6) (Zagory, 1995):

$PO_2 = RR\ O_2 \times t \times W/A \times (O_2\ atm - O_2\ pkg)$……(Equation 2.5)

$PCO_2 = RR\ CO_2 \times t \times W/A \times (CO_2\ atm - CO_2\ pkg)$…….....(Equation 2.6)

P= Permeability coefficient ($m^2\ s^{-1}$)

RR= Respiration rate (consumption/production) ($m^3 kg^{-1} s^{-1}$)

t=time (s)

W= Mass (kg)

A=Surface area (m^2)

O_2 atm / CO_2 atm = O_2 / CO_2 in the atmosphere

O_2 pkg /CO_2 pkg = O_2 / CO_2 in the package headspace

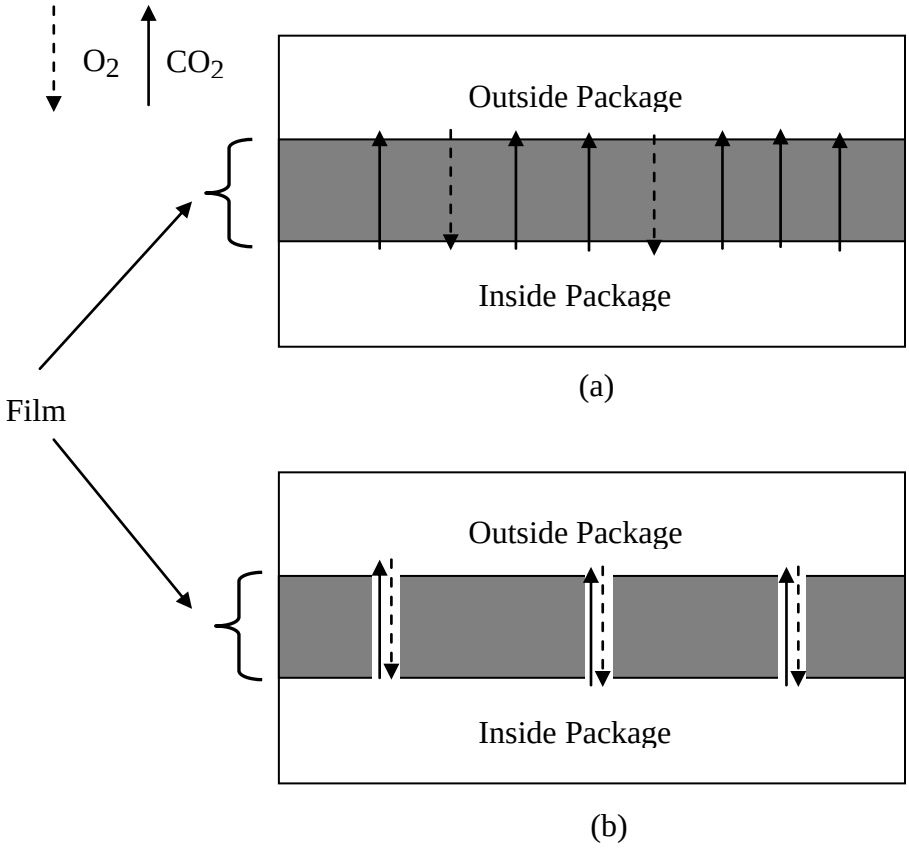


Figure 2-5: Flow of gases without (top) and with (bottom) perforations

2.6 Plastic packaging

Plastics are used widely in many industries all over the world. Plastics are high molecular weight polymers (i.e., repetition of a known constitutional unit) that can be processed and formed using a combination of heat, pressure and time. Plastics as packaging material started being used after World War II when polyethylene had been produced in huge quantities (Selke, Culter & Hernandez, 2004). The development of plastic has increased rapidly with the help of technology. Up to this day, there are many

types of plastic packaging like poly(ethylene), poly(styrene) and poly(vinyl chloride), to name a few. These plastics have been used in food and non-food packaging applications.

The wide ranges of applications with regard to these plastics are associated with their material properties and low cost. Weber et al. (2002) reported that even though in the earlier of the 20th century, most of the non-fuel industrial products like inks, dyes, paints, medicines, chemicals, clothing, synthetic fibres and also plastics were made from biologically derived resources, petroleum-derived chemicals replace those resources to a major extent 70 years later due to the reasons mentioned previously (Weber, Haugaard, Festersen & Bertelsen, 2002).

Then, at the beginning of the 21st century, growing attention on sustainability is being seen as more effort has been made to replace the non-renewable resources (especially for those derived from petroleum) to renewable resources, essentially plant-derived products and byproducts from their fermentation (Mohanty, Misra, Drzal, Selke, Harte & Hinrichsen, 2005). Due to oil crisis nowadays in which oil prices keep fluctuated and increasing, more markets are concerned with the usage of oil-based packaging material (2007). Petroleum-based packages are associated with environmental burdens such as land, water and air pollutions. Almost 30% wastes generated in the Municipal Solid Waste (MSW) stream in 2008 were container and packaging (2008). Even though, initiative such as recycling has been made to reduce these environmental associated issues, it may still not be sufficient. Not to mention, recycling is not an attractive option considering that the cost of the virgin resins is very low, and this option is limited to certain types of plastics with the vast majority being recycled is PET bottles

(Imam, Glenn, Chiou, Shey, Narayan & Orts, 2008). Moreover, about 500 million pounds of PET clamshell was estimated produced in 2003, however the amount that was recycled is negligible (King & Blue, 2006).

Therefore, with regards to an increase in awareness of the limitation of petrochemical resources and growing ecological awareness among consumer and industry, strong increase in research and development of bio-based polymer has been shown in recent years (Endres, Siebert & Kaneva, 2007).

2.7 Bio-based polymers

Bio-based polymers can be defined as "materials derived from primarily annually renewable resources" (Haugaard & Mortensen, 2003). This narrow definition is used to exclude paper-based materials which are also renewable. Generally, bio-based materials can be divided into three categories: 1. Polymers directly extracted from biomass, 2. Polymers synthesized from bioderived monomers, and 3. Polymers produced directly by natural or genetically modified organisms (Petersen et al., 1999) (Figure 2-6).

2.7.1 Polymers directly extracted from biomass

The polymers in this category are commonly extracted from marine and agricultural products such as chitin, cellulose, starch and others. Most of these polymers are hydrophilic in nature which resulted in limitation in processing and water barrier properties. However, they do pose excellent gas barrier.

2.7.2 Polymers synthesized from bioderived monomers

For this category, poly(lactic acid), PLA, has shown the highest potential for commercialization and is currently produced on a major scale. PLA, a biopolyester

polymerized from lactic acid monomers. The availability of PLA is projected to reach a production of 325, 000 tons per annum or more in 2010 (Endres et al., 2007).

2.7.3 Polymers produced directly by natural or genetically modified organisms

This category consists primarily of the microbial polyester poly (hydroxyl alkanoate)s, PHAs. This polymer is produced by many bacterial species in the form of intracellular particles which used as energy and carbon reserve materials. PHAs are accumulated intercellularly under growth-limiting conditions by different prokaryotic microorganisms. The manipulation of growth medium results in a random copolymer consists of 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV). One of the determining factors for the properties of final PHA material is the specific monomer composition. This factor is variable and the synthesis of this polymer can be controlled through selection of different substrates and bacteria strains.

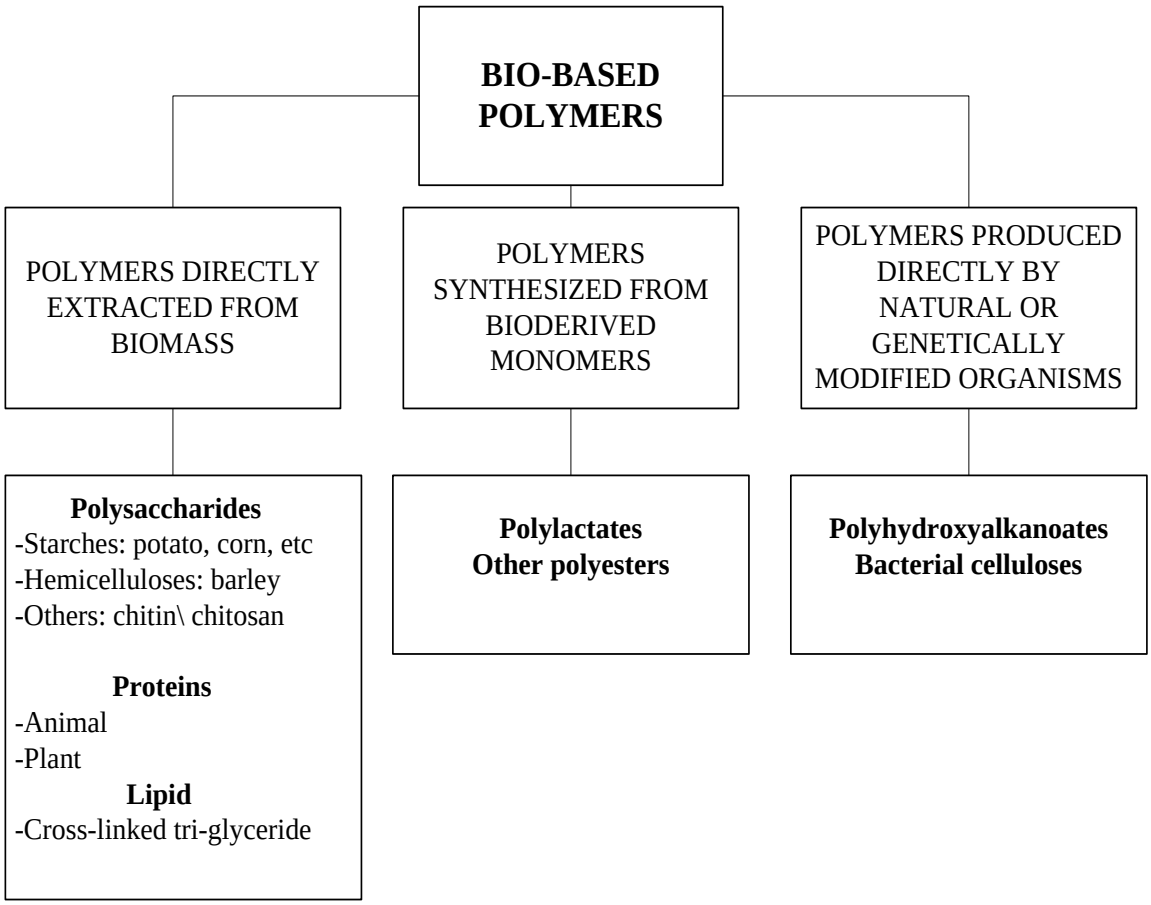


Figure 2-6: The classification of bio-based materials.

Note: Figure was adapted from van Tuil et al. (2000) (Tuil, Fowler, Lawther & Weber, 2000).

2.8 Green packaging as an alternative – Poly(lactic acid) (PLA)

Poly(lactic acid), PLA, is produced by Natureworks^{TM} in a plant built in Blair, NE, US. The plant has a capacity of 1.4 million tons per year and sold PLA under the trade name Ingeo^{TM} (Smith, 2005).

PLA is a bioplastic produced from polymerized lactic acid (Endres et al., 2007) that can be obtained from renewable resources like corn (Auras, Harte & Selke, 2003), sugar beet and sugarcane residues (Endres et al., 2007). In general, lactic acid which is

the basic building unit of PLA can be produced by carbohydrate fermentation or chemical synthesis. These days, carbohydrate fermentation is the most common route used to produce PLA. This fermentation process can be classified in accordance with the type of bacteria used: heterofermentative and homofermentative. Homofermentative bacteria such as a genus of *Lactobacilli* are used widely in the industry due to its high yield rate of lactic acid. As for the chemical synthesis, this route is used to obtain high molecular weight PLA. However, this method is not economically feasible. High molecular mass PLA of about 100,000 Daltons can be processed through three techniques: 1) direct condensation, 2) azeotropic dehydrative condensation, and 3) polymerization through lactide formation. The latter technique is employed by Cargill Dow LLC for commercial application (Auras, Harte & Selke, 2004).

2.8.1 PLA-Properties

PLA is a transparent material that can be formed into variety of containers, trays, film and other types of packaging. It has biodegradable, compostable and recyclable criteria that consumers and manufacturers are looking as end of life scenario for new green materials. Studies have also shown that has minimal lactic acid migration to simulated food (Conn et al., 1995); hence, it is suitable for food packaging application.

Lactic acid generally exists in two optical active configurations (Auras et al., 2004) or stereoisomers known as L (+) and D (-) isomers. Modification of PLA properties can be made by changing the content and distribution of these two isomers by copolymerization (Smith, 2005). PLA physical and mechanical properties are mostly related and can be modified by changing the the L/D lactic acid ratio, molecular weight, crystallinity, orientation and preparation methods. In general, PLA is comparable to

poly(ethylene terephthalate), PET and poly(styrene), PS in terms of its physical and mechanical properties. PLA is a rigid and brittle material, and at temperatures higher than its glass transition temperature, it has tendency to deform. Controlling the L/D lactic acid ratio or polymerizing PLA in a presence of specified catalyst helps in improving its mechanical properties (Auras et al., 2004).

PLA has low barrier to gases such as CO_2 and O_2. PLA has a CO_2 permeability coefficient higher than PET at 25°C (1.99-2.77 × 10^{-17} versus 1.73 × 10^{-18} kg-m/m^2-s-Pa). PLA also has higher O_2 permeability coefficient when compared to PET at the same temperature at 70%RH (1.21-1.39 × 10^{-18} versus 1.88 × 10^{-19} kg-m/m^2-s-Pa). In general, at 0% RH, O_2 permeability coefficient was reported to be ten times lower than that of CO_2 permeability coefficient. Meanwhile, the water vapor permeability coefficient of PLA was reported in the range of 1.79-1.89 × 10^{-14} and 1.61-1.65 × 10^{-14} kg-m/ m^2-s-Pa at 20, and 30°C, respectively between 40-90% RH. These values were comparatively higher than PET (1.1 × 10^{-15} kg-m/ m^2-s-Pa at 25°C between 40-90%RH) (Auras et al., 2003). As a result, PLA application in food packaging for instance, might be limited to certain type of food.

Chapter 3

3. Materials and Methods

3.1 Introduction

This project was divided into two phases. The first-phase was focused on the development and characterization of the packages. While in the second-phase, the effect of different packaging materials, numbers of microperforation, and the effect of temperatures on the physico-chemical properties, microbiological growth, and sensory properties of blueberries was investigated (Figure 3-1). For the second-phase, blueberries were packed at different time intervals for different temperatures.

Poly(ethylene terephthalate), PET, was used in this study because it is one of the most common materials used for fruit packaging. While the selection of poly(lactic acid), PLA, as a packaging material was due to its potential to become an alternative to petroleum-based material and to prolong blueberries' shelf life (Almenar et al., 2008b).

Three different numbers of microperforation (0, 3, and 15 porous) were chosen to investigate and compare the effect of the absence, and presence of microperforation, and the effect of the numbers of microperforation introduced in the packaging system on fruits' quality.

Three different temperatures (3, 10, and 23°C) were selected for this study. A low temperature of 3°C was chosen as it is the typical temperature used to store blueberries during transportation and distribution. The temperature of 10°C is the common temperature use to keep blueberries when they are marketed at retailer's store, and 23°C

was chosen to investigate fruits quality when they are exposed to the worst storage case-scenario.

3.2 Preliminary studies

3.2.1 Sealing condition

Since the package was designated to be a rigid container sealed with a flexible film, preliminary studies were done to determine the proper sealing condition for both PLA, and PET packaging system. Various condition (temperature, time, and pressure) combinations were applied to create a good sealing integrity of the packaging system. However, there was main issue occurred during these preliminary studies. This issue was the use of pressure had cause an error during the packaging development process. As a result, the pressure was deactivated to allow the process to run smoothly. Some of the conditions used to obtain a good sealing property are listed in Table 3-1.

Table 3-1: Preliminary results of the sealing conditions for the development of packaging system

Conditions (temperature (°C), time (s))	Observations
70, 20	The sealing was not enough
75, 20	The sealing was not enough
80, 20	3/4 packages was not sealed well
83, 20	3/4 packages was not sealed well
85, 20	2/4 packages was not sealed well
87, 20	The sealing was good for 4/4 packages
90, 20	The sealing was good but 3/4 packages had a leak
93, 20	The sealing was good but 4/4 packages had a leak

Based on this result, a condition of 87°C, 20 s was found sufficient for providing a good sealing property for both types of packages. More samples were run at this condition afterward, before finalize the appropriate sealing condition for both PLA, and PET packaging systems. The additional tests showed that there was no further problem, confirming that this condition was suitable for being used throughout this project.

3.2.2 Leakage testing

This test was performed in accordance to ASTM D 4991-94 (Standard Test Method for Leakage Testing of Empty Rigid Containers by Vacuum Method). A package was immersed in the ethylene glycol-water solution inside the transparent test vessel. The test vessel lid was sealed before the inlet tube was open and the outlet tube was closed.

The package was hold for two minutes while the vacuum pressure was applied up to 20 in Hg. The packages were found able to hold a vacuum pressure up to an average of 13 to 14 in Hg, which was considered good for the current process.

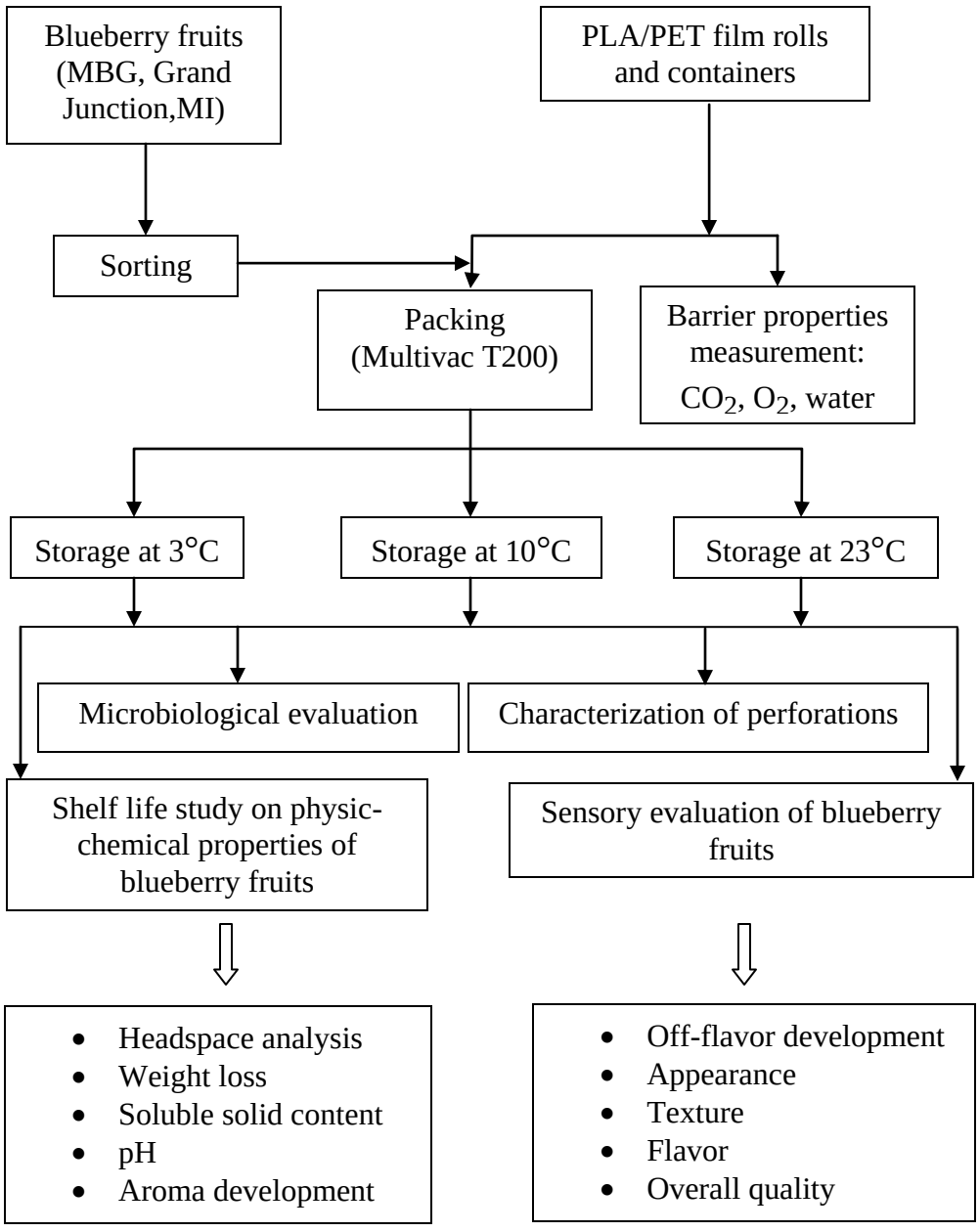


Figure 3-1: Flow chart of the project

3.3 Materials

Highbush blueberries (*Vaccinium corymbosum* L., Bluecrop) were obtained from Michigan Blueberries Growers (MBG) Marketing (Gran Junction, MI). Rigid poly (lactic acid), PLA, and poly(ethylene terephthalate), PET, containers, and PLA, and PET film rolls were provided by Clear Lam Inc. (Elk Grove Village, IL). The PLA and PET container thicknesses were both 11.24 ± 0.05 mil. While the films thickness were 1.00 ± 0.05 mil. This container has a volume of 250 cc. The dimension of the package is shown in Figure 3-4. Both PLA and PET films were coated with thin layer of ethylene vinyl acetate for better sealing properties.

The packaging system of PLA and PET were developed by using a Multivac T200 machine (Multivac Inc., Kansas City, MO). Rigid containers sealed with continuous film were used as controls for both types of materials. Stainless steel cylinder devices attached with different numbers of needles (NL) (Multivac Inc., Kansas City, MO) were used to microperforate both PLA and PET films. The numbers of needles and their corresponding number of microperforations on a 4.33 in diameter of film is shown in Table 3-2. The packaging systems developed and used in this project are summarized in Table 3-3.

Table 3-2: The numbers of microperforations obtained from two different numbers of needles

Number of needles (NL)	Number of microperforations obtained (P)
7	3
31	15

Table 3-3: Summary of packaging systems developed for this project.

Materials	Microperforations (P)
PLA	0
	3
	15
PET	0
	3
	15

3.4 Methods

3.4.1 Sample preparation

3.4.1.1 Sorting and filling

Blueberries were manually sorted to ensure uniformity of size and color, and to eliminate berries that showed sign of fungal growth. Samples weighing approximately 100g were then placed in either PLA or PET containers.

3.4.1.2 Packing process

PLA or PET containers filled with blueberries were sealed with PLA or PET films by using a Multivac T200 machine (Figure 3-2). This process started by placing containers into the molds located in the drawer part (Figure 3-3 (I)). As the drawer was pushed inside the machine, the film rolled and got ready for sealing (Figure 3-3 (II, III)). Any gases presence in the packages was removed before air was flushed in. The film was then sealed on the package, forming either continuous or microperforated packaging systems (Figure 3-3 (IV)). These packages were then removed after withdrew the drawer

(Figure 3-3 (V)). In the production of microperforated packaging system, the stainless steel cylinder devices attached with 7 NL, and 31 NL were placed at the cylinder devices terminal. In step II and III, as the film rolled, it passed through the needles tool, thus performing the microperforation. This process was then continued as previously described. Figure 3-4 (a, and b) show a close up illustration of packages before and after the packing process.

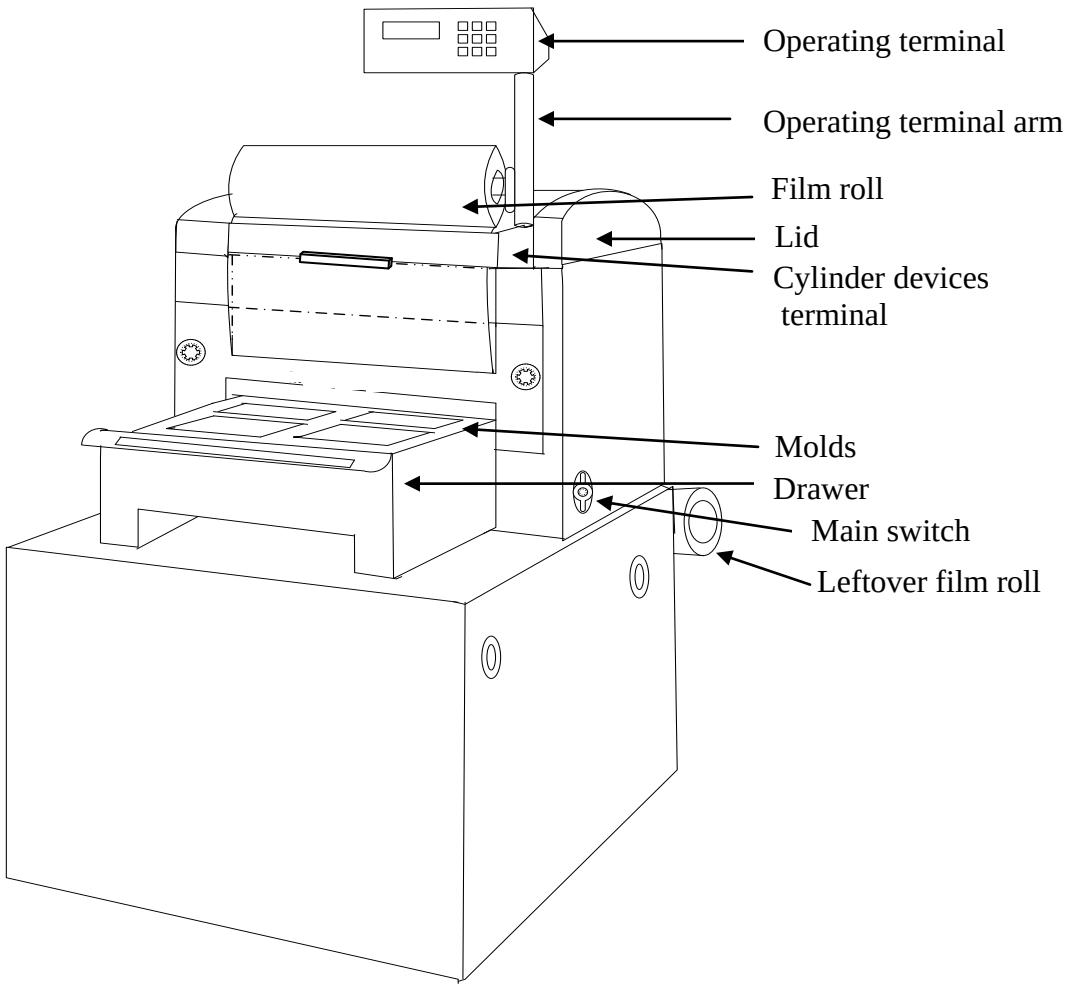


Figure 3-2: A Multivac T200 machine and its parts.

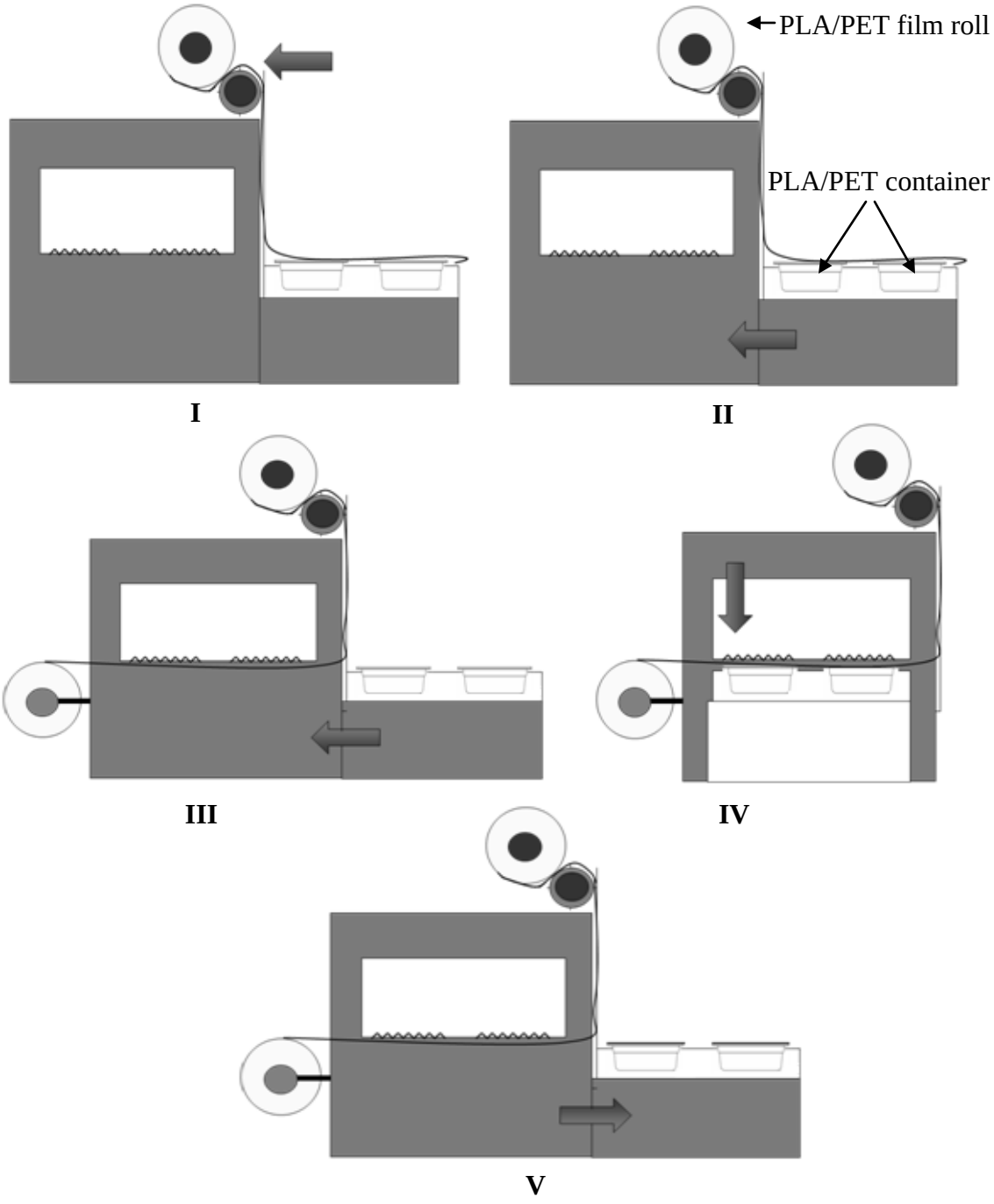


Figure 3-3: The sequence process of the development of continuous, and microperforated packages (I, II, III, IV, and V). Copy with permission from Almenar et al. (2008) (Almenar, Auras, Samsudin, Harte, Rubino & Harte, 2008a).

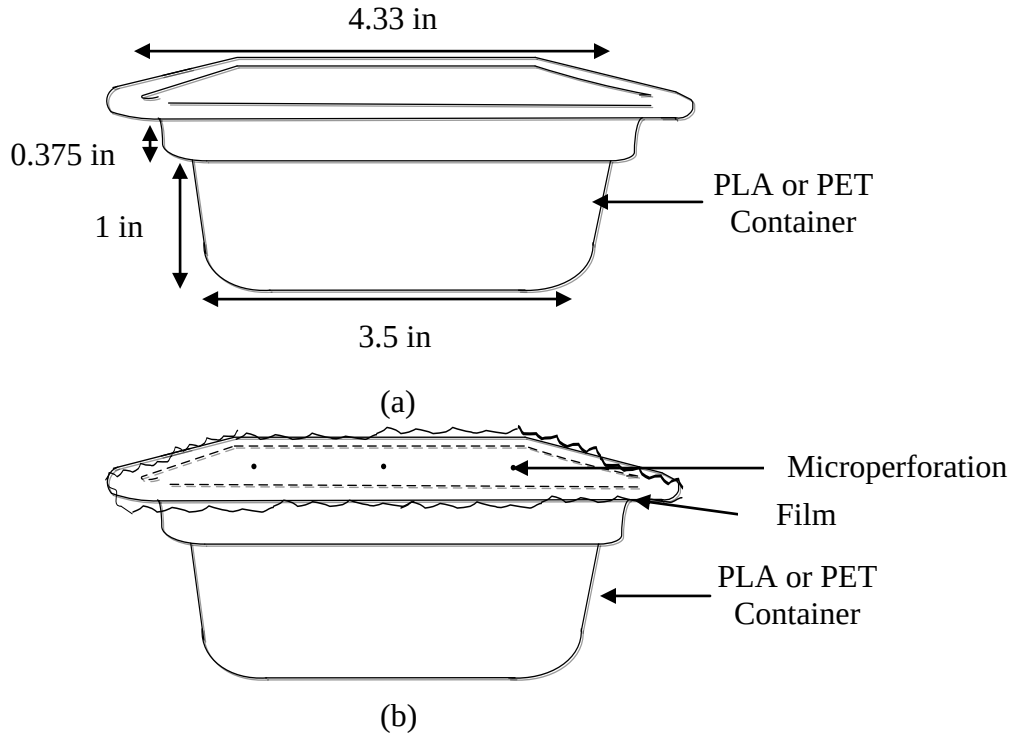


Figure 3-4 (a): Before process: PLA or PET container; (b) After process: Microperforated PLA or PET containers.

3.4.1.3 Storage

After packing, blueberries samples were stored in a conventional refrigerator (Whirpool Corp.,Benton Harbor, MI) at 3°C and 70% RH for 33 days (designated testing days: 0, 5, 12, 19, 26, 33) , and at 10°C and 80% RH for 20 days (designated testing days: 0, 5, 10, 15, 20). While for experiment at 23°C and 60% RH, samples were kept in a conditioning room for 9 days (designated testing days: 0, 3, 6, 9). Analyses were performed on four samples per treatment on every designated day.

3.4.2 Headspace analysis

The level of CO_2 and O_2 in the package's headspace was measured using Illinois Instrument 6600 headspace analyzer (Illinois Instrument Inc., Johnsburg, IL). Sampling needle attached to the sensor was inserted into package headspace via septum (Illinois Instrument Inc., Johnsburg, IL) patched on the lid. The values obtained were expressed in percentage.

3.4.3 Weight loss determination

Blueberries' weight was measured using an analytical balance (Ohaus Adventurer, Pine Brook, NJ). The amount of weight loss was calculated and expressed in percentage as follows:

$$Weight\ Loss\ (\%) = \frac{W_0 - W_t}{W_0} \times 100$$

W_0= Initial weight at 0 day (g)

W_t= Initial weight at t day (g)

3.4.4 Microbiological evaluation

Blueberries were visually inspected for any fruit rot presence (Figure 3-5). Infected blueberries were sorted and separated manually. The number of infected blueberries was divided by total number of blueberries originated in the package and was converted to percentage.

I II III

Figure 3-5: Typical fungi that infected blueberries. I) *Colletotrichum acutatum*, II) *Botrytis cineria*, and III) *Alternaria alternata* (Madival & De la Fuente, 2006; Wharton & Schilder, 2005).

3.4.5 Physicochemical analysis of blueberries

Blueberries were blended using a mixer (Hamilton Beach Brand Inc., Washington, NC) for 30 seconds. The blender was then placed into basin containing ice cooler to maintain blended blueberries at low temperature. This blend was used to determine pH, soluble solid content (SSC) and aroma evolution.

3.4.5.1 pH

pH of the blend was measured using a PHB-212 pH meter (OMEGA Engineering Inc., Stamford, CT).

3.4.5.2 Soluble solid content (SSC)

Soluble solid content of the blend was determined using a refractometer, Atago PAL-1 (Atago Co., Ltd., Tokyo, Japan). The values were expressed in °Brix unit.

3.4.5.3 Aroma and off-flavor evolution

Blueberries blend was weighted approximately 10 g into 20 ml vial, crimp sealed with aluminum/ silicon barrier septa (Sigma-Aldrich, St. Louis, MO) and kept in the freezer (Whirpool Corp., Benton Harbor, MI) at -20°C for further analysis. For analysis,

individual vial was thawed at 23°C for 10 min, heated at 75°C for another 10 min. Then the solid phase micro-extraction (SPME) fiber assembly Divinylbenzene/Carboxen/Polydimethylsiloxen attached to a SPME holder was used to extract aroma compounds in the vial headspace for 10 min. The evolution of the typical blueberry aroma; ethanol, ethyl acetate, hexanal, 2-E-hexanal, 1-hexanol, and linalool was measured using gas chromatography with a flame ionization detector (FID), HP 6890 Series GC System (Hewlett-Packard, Wilmington, DE). The column used for this procedure was a HP-5 (30 m × 0.32 mm × 0.25 μm, crosslinked 5% HP ME Siloxane) (Hewlett-Packard, Wilmington, DE). The fiber contained-aroma was desorbed in the splitless injection port at 220°C for 5 min. The oven was set up at 40°C for 5 min, heated up to 230°C at a rate of 5°C/ min and hold up for 10 min. The detector's temperature was set at 270°C. Hydrogen, air and helium flow were 30, 300 and 3.0 ml/min, respectively. The aroma evolution was also determined and expressed in chromatographic area, c.a.

3.4.8 Sensory analysis

Sensory analysis was performed on the final day of storage (day 33 for 3°C, day 20 for 10°C, and day 9 for 23°C) to evaluate the quality of the fruit. The testing method chosen for this evaluation was Quantitative Descriptive Analysis Method. This procedure was a modified version and was performed as has been described in Sensory Testing Methods-ASTM (Chamber IV & Wolf, 2005) and in Sensory Evaluation Technique (Meilgaard, Civille & Carr, 2007). The attributes tested in this test were off-flavor

development, appearance, texture, flavor (sweetness, tartness, and typical blueberry flavor), and overall quality. A question was also asked regarding the fruits' quality for consuming and purchasing purposes. A 15 universal spectrum scale was used to evaluate the samples.

3.4.8.1 Panelists' selection

The panelists selected for evaluating the sensory quality of fruits were screened based on their performance in a previous sensory analysis. During pre-screening process, panelists were given two sensory tests: i) triangle test, and ii) paired preference test (Almenar, Samsudin, Auras & Harte, 2010). Panelists' ability to distinguish the effect of packaging material on fruits was the main criteria being considered for selecting the trained panelists. After screening, 11 panelists were chosen for this project. The panelists were almost all students from Michigan State University.

3.4.8.2 Panelists' orientation and training

An orientation was performed to give an overview of the project. Panelists were explained on their rights before, during, and after testing, and were also given the consent forms (Appendix A). The completed consent forms were kept for record and future reference. Some of the topics discussed during this session were the nature of the samples, the attributes tested, and the grading scale based on the universal spectrum. The sample of questionnaire is attached in Appendix B.

Training sessions were conducted three times consecutively in every two weeks. Another three sessions were done a few days prior to the actual testing. The panelists performance to perform the sensory evaluation was evaluated two times (Appendix C). During training sessions, the panelists were provided with samples kept at different

temperature, and storage time, and exposed to descriptive words commonly used to describe the attributes, followed by discussions among them. The obstacles experienced by panelists (i.e appearance, and flavor (sweetness, and tartness) evaluation) were also addressed during these sessions.

3.4.8.3 Actual sensory testing

The testing was done at the Food Science Human Nutrition's sensory lab at Michigan State University at ambient temperature. Panelists were given separate booths during testing. For each testing, at least 9 panelists were present. A set of six samples weighted approximately 8 to 12 g of fruits, were presented to each panelists in a small container for all attributes tested except aroma. For aroma evaluation, another set of six samples was presented to panelists in the actual packaging system to avoid aroma loss. Since the supply of fruits and packages was limited, two panelists had to share one package per samples. A three random digits code was assigned to each sample.

A picture scale of blueberry was provided for each panelist to assist the panelists in the evaluation of samples' appearance (Appendix D). Reference solutions were also prepared and provided to panelists as a guide for evaluating the flavor attributes (sweetness, and tartness). Table 3-4 shows the summary of the reference solutions prepared. The reference solution for sweetness was established based on Amos (2007) with little modification (Amos, 2007), while for tartness, the reference solution was prepared based on panelists' feedback during training session. Sucrose and citric acid were used to prepare the reference solutions for sweetness and tartness, respectively.

Table 3-4: A summary of reference solutions prepared for flavor attributes.

Attributes	Concentration (g/L)	Reference value
Flavor: sweetness	60	11
Flavor: tartness	3	11

The test was done by using the Sensory Information Management System-SIMS 2000 version 6.0 (Sensory Computer Systems, LLC., Morristown, NJ).

3.4.9 Characterization of perforations

Image of the holes was captured by using a Q-Color 3 digital camera attached to a Olympus BX 40 microscope (Olympus America Inc., Center Valley, PA). The porous diameter was measured by using a QCapturePro 6 Software (QImaging, Surrey, Canada) (Figure 3-6). The void area of the film lid was measured by Adobe Photoshop CS3 (Adobe Systems Incorporated, Boston, MA). The area was selected by quick selection tool and the corresponded pixel value was obtained from the histogram. This value was then converted to the unit of μm^2 based on the extrapolation of 10 μm^2 standard bar scale.

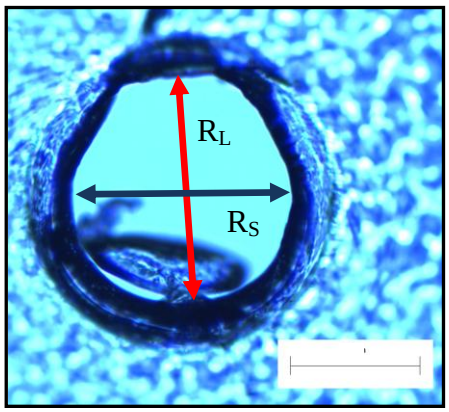


Figure 3-6: Image of porous

Note: Bar represent 10 μm

R_S= Ratio of short ellipse

R_L= Ratio of long ellipse

3.4.10 Permeation analysis

3.4.10.1 Water vapor permeance

PLA or PET container was drilled with thread milling tool of a 1/18 inch attached to a DeWalt cordless drill (DeWalt, Madison Heights, MI). Two holes were drilled on the bottom of a container. This container was later sealed with flexible lid as described earlier. One side of copper tubing was attached to the holes on the package and sealed with an epoxy and the other side was connected to a MoCON Permatran W3/33 machine (MOCON Inc., Minneapolis, MN). PET packages were then placed in a pouch made of low density polyethylene. Two sponges wetted with HPLC grade water were inserted into the pouch before sealing. These sponges were used to generate a 100% RH during analysis. The testing conditions were performed at 23°C and 100% RH based on ASTM F 1249-06 with minor modifications (ASTM, 2006). Since the transmission rate of water through PLA packages was beyond the sensitivity of the machine, a low RH environment

(15% RH) was prepared in a sealed bucket to run the test for these packages. Table 3-5 provided the information relevant to the test set up. The test was run until steady state was reached of at least 10 points. Three replications were performed for each sample.

Table 3-5: Test parameters and setting information for water vapor transmission rate

Parameters	Setting information
Test mode	Continuous
Module rezero	2
Exam minutes	30 min
Conditioning time	2 hrs
Flow of N_2 (cell A and B)	100 sccm

3.4.10.2 Carbon dioxide (CO_2) permeation rate

This test was done in accordance to ASTM F2476-05 by using a MoCON Permatran 4/41 Module C (MOCON Inc., Minneapolis, MN (ASTM, 2005b). The testing conditions were 23°C, and a relative humidity of 0% RH. Sample preparation was almost the same as previously discussed except that plastic tubing was inserted into the pouch instead of wet sponges. This tubing was connected to the machine to allowing the flow of CO_2 into the pouch for generating a 100% CO_2 atmosphere. The test was stop when an equilibrium state was reached of at least 10 points with a variation less than 5%. Three replicates were run for each sample. The test setting information is summarized in Table 3-6.

Table 3-6: Test parameters and setting information for CO_2 transmission rate

Parameters	Setting information
Test mode	Continuous
Module rezero	2
Exam minutes	30 min
Conditioning time	2 hrs
Flow of N_2 (cell A and B)	200 sccm
Flow of CO_2	400 sccm

3.4.10.3 Oxygen (O_2) permeation rate

O_2 transmission rate was determined using an Illinois 8001 machine (Illinois Instrument Inc., Johnsburg, IL), and the testing conditions were according to ASTM D3985-05. The testing temperature was 23 °C, 0% relative humidity and 21% permeant concentration (ASTM, 2005a). The samples were prepared as in the previous tests. The pouch was not used for this test since the required permeant concentration was 21%. The test was run continuously until the steady state is achieved with less than 5% variation for at least of 10 points. Three replicates were run per sample. A summary of setting information is listed in Table 3-7.

Table 3-7: Test parameters and setting information for O_2 transmission rate

Parameters	Setting information
Test duration	60 min
Bypass time	180 min
Purge level	30 cc/m^2-day
Bottom flow	10 cc/min
Top flow	0.5 cc/min
Sampling rate	15 min

3.5 Statistical analysis

A study was conducted to evaluate the effect over time of packaging material and storage temperature on physico-chemical, microbiological growth, sensory properties of blueberries, and on barrier properties of the packaging systems. For each of 3 storage temperatures (3, 10, and 23°C), blueberries were randomly assigned to 2 packaging materials (PLA and PET), different number of perforations (0, 3, and 15 perforations) and different storage time (9 days for 23°C, 20 days for 10°C, and 33 days for 3°C) in a completely randomized design. For each package, data of CO_2 and O_2 evolution, weight loss, fungal growth, soluble solid content (SSC), pH, flavor analysis, and barrier properties (CO_2 and O_2 permeation rates, water vapor transmission rate) were recorded. A separate linear mixed model was fitted to each response variable on each storage temperature. Each model was included with the fixed effects of packaging materials, number of perforations and storage time, as well as all possible 2 and 3-way interactions.

Model assumptions were evaluated using standardized residual plots and were found to be appropriately met for fitted models on the response variables SSC, pH, weight loss, and evolution of CO_2. In contrast, the response variables of weight loss at 23°C, concentration of CO_2 at 23 °C, fungal growth at 10 °C, evolution of fermentative metabolites and aroma compounds at all temperature, and barrier properties of packages needed to be log transformed prior to analysis in order to meet model assumptions. In addition, model assumptions for the responses weight loss at 3°C, concentrations of CO_2 at 3, 10 and 23 °C, and fungal growth at 3 and 23°C were met by fitting heterogeneous residual variances, as supported by Bayesian Information Criteria. All statistical models were fitted using the MIXED procedure of the statistical software SAS (Version 9.1, SAS Institute Inc., Cary, NC). Results are presented as estimated least square means and standard errors. Post-hoc pairwise comparisons were conducted by using Bonferroni adjustment to avoid inflation of type I error rate.

While for sensory evaluation, the data were analyzed by SIMS 2000 version 6.0 using Statistical Analysis Software version 6.0 (Sensory Computer Systems, LLC., Morristown, NJ) by using analysis of variance to evaluate overall differences with a significant level of 5%. Duncan's Multiple Range Test was used for means comparison. Results for this evaluation are presented as raw means and standard deviations.

Chapter 4

4. Results and Discussions

The results and discussions in this chapter are presented accordingly to the fruit and packages properties, and the data are provided based on each temperature.

4.1 Headspace analysis

During respiration, fresh produce consumes O_2 for its metabolic activities, and releases CO_2 and heat as by-products. In a close system (e.g sealed container) containing fruit, CO_2 and O_2 concentration in the headspace would be altered over time. This modification resulted from the interplay among the fruit respiration rate, gas transmission rate, permeability of the material, and storage conditions (i.e. temperature, and relative humidity). In general, PLA and PET packages showed different CO_2, and O_2 levels depending on the number of microperforation and the storage conditions.

4.1.1 CO_2 level

4.1.1.1 CO_2 level at 3 °C

Blueberries in both PLA, and PET packages sealed with continuous materials showed a higher development of CO_2 in the headspace than that of perforated packages (Figure 4-1a). After days 5, CO_2 concentration was found to be 39.5 and 44.5% for non-perforated PLA and PET, respectively. However, a sudden drop in the CO_2 level was observed after day 26. This could possibly due to packages' collapse as a result of imbalance internal and external partial pressure. All perforated PLA and PET packages

reached the equilibrium after day 5. Packages with 15 perforation had the lowest CO_2 level in the headspace, followed by 3 perforation and non-perforated packages. The presence of perforation induces gas exchange rates since the gas transportation through porous material is due to the collision of the gas molecules. On the contrary, the gas transportation via non-porous material is the result of solubility and diffusion process. From Figure 4-1a, the material has shown to have no effect on the CO_2 evolution, instead the interaction of perforation and days was found. CO_2 evolution in non-perforated packages was found to be significantly difference than perforated packages at day 5, 12, and 26 (Figure 4-1b). There were no differences ($p<0.05$) in CO_2 development between packages with 3 and 15 perforations during storage.

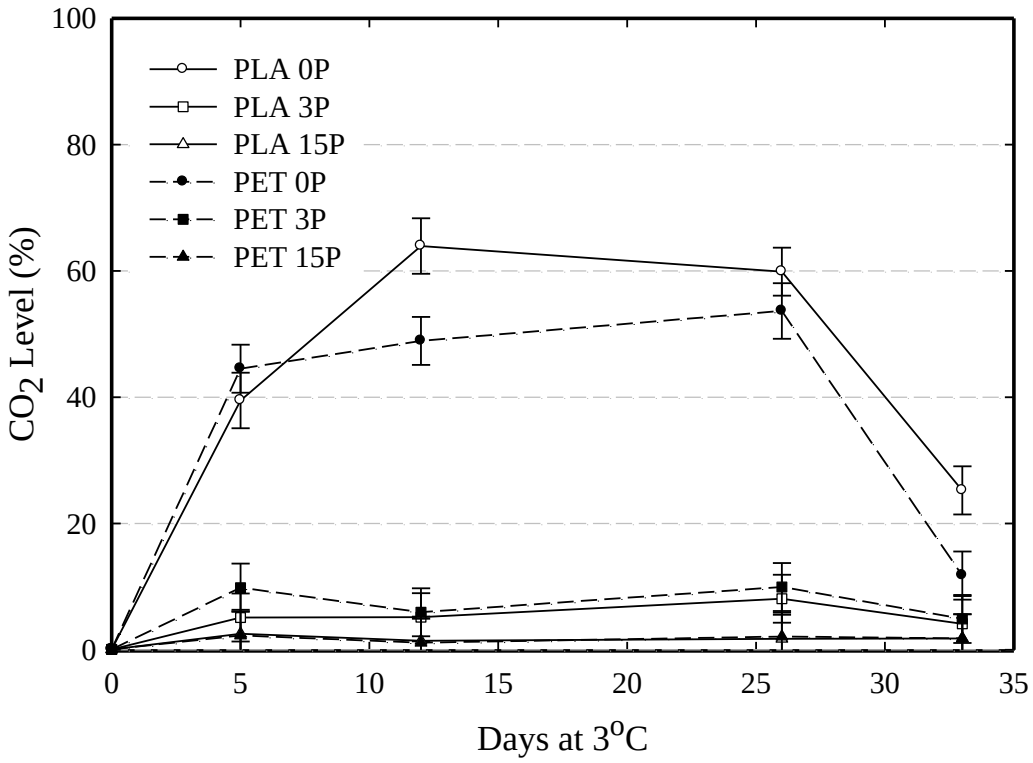


Figure 4-1a: The level of CO_2 in the packages at 3°C during 33 days of storage.

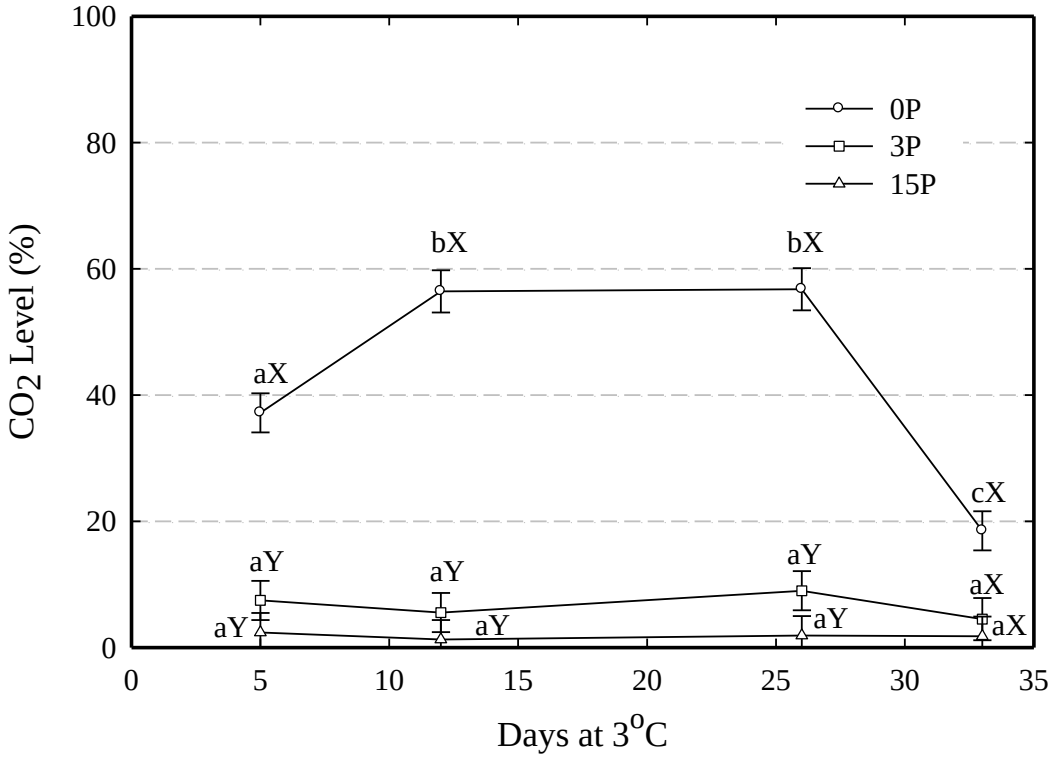


Figure 4-1b: The interaction effect of perforations and days on CO_2 level of blueberry stored at 3°C during 33 days. (a, b, c) Differences between days within perforations ($p < 0.05$); (X, Y) Differences between perforations within days ($p< 0.05$).

4.1.1.2 CO_2 level at 10°C

Figure 4-2a and Figure 4-2b showed the evolution of CO_2 in the PLA and PET packages, with different number of perforation during 20 days at 10°C. The equilibrium was reached after day 5 for CO_2 evolution in PLA and PET packages with 15 perforations, and after day 10 for packages with 3 perforations. The order of CO_2 evolution in terms of perforation at 10°C was similar to those shown at 3°C. As

expected, the higher the number of perforations, the lower the amount of CO_2 was observed.

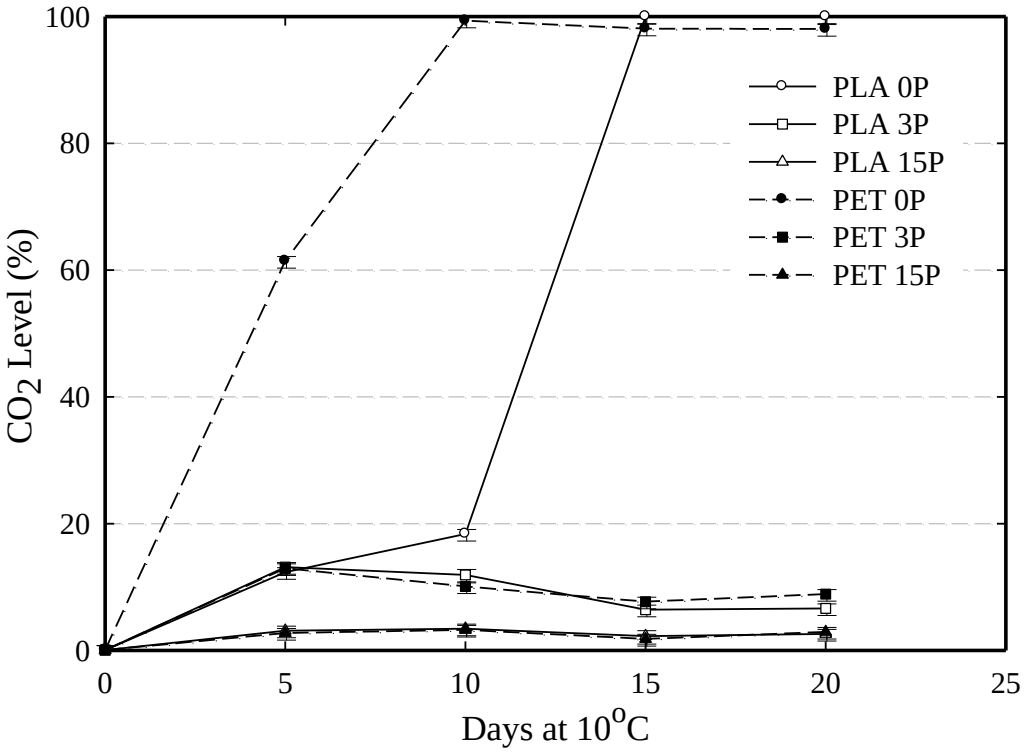


Figure 4-2a: The level of CO_2 in the packages headspace at 10°C during 20 days of storage.

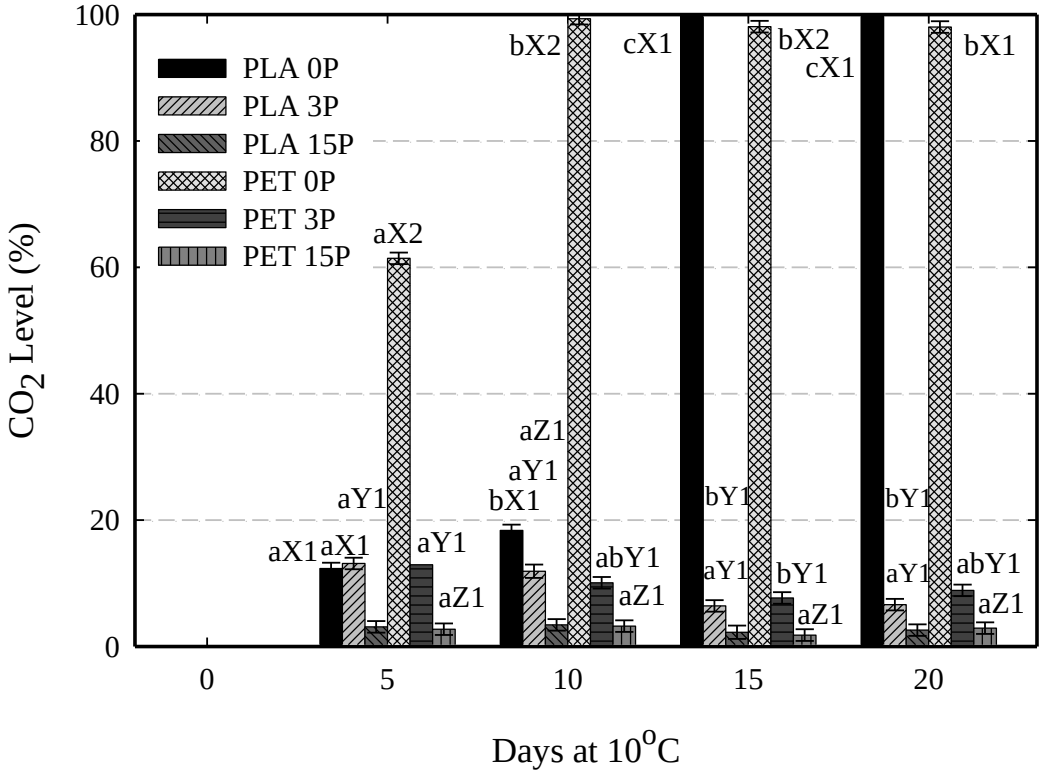


Figure 4-2b: The interaction effect of materials, perforations and days in CO_2 level of blueberries stored at 10°C during 20 days. (a, b, c) Differences between days within perforations and materials ($p<0.05$); (X, Y, Z) Differences between perforations within days and materials ($p<0.05$); (1, 2) Differences between materials within perforations and days ($p<0.05$).

Figure 4-2c indicated the two-way interactions effect of materials and perforations. CO_2 contents in PLA and PET packages were significantly ($p<0.05$) different than each other for a 0 perforation. All of the perforations differed statistically within each materials. Perforations and days interaction effects regardless of materials are shown in Figure 4-2d. CO_2 evolution with regards to perforations was significantly different within each analyzed day. No differences were observed for packages with 15

perforations during storage. The materials and days were also found statistically significant at $p<0.05$.

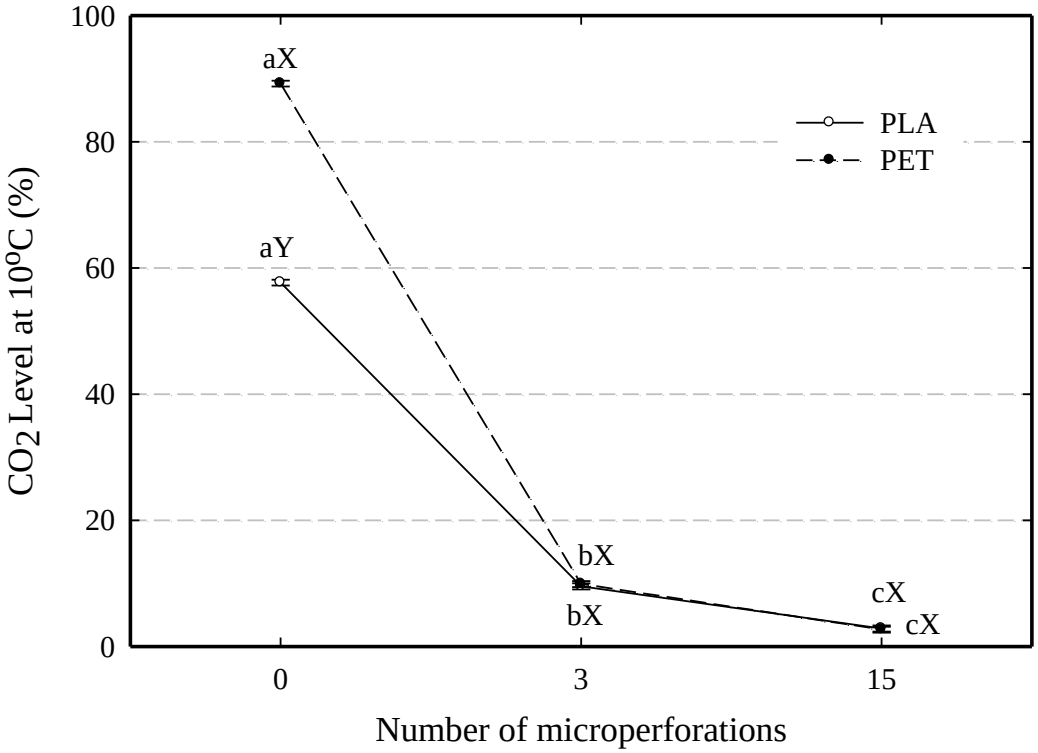


Figure 4-2c: The interaction effect of materials and perforations on CO_2 level of blueberries stored at 10°C during 20 days. (a, b, c) Differences between perforations within materials ($p<0.05$) ; (X, Y) Differences between materials within perforations ($p<0.05$).

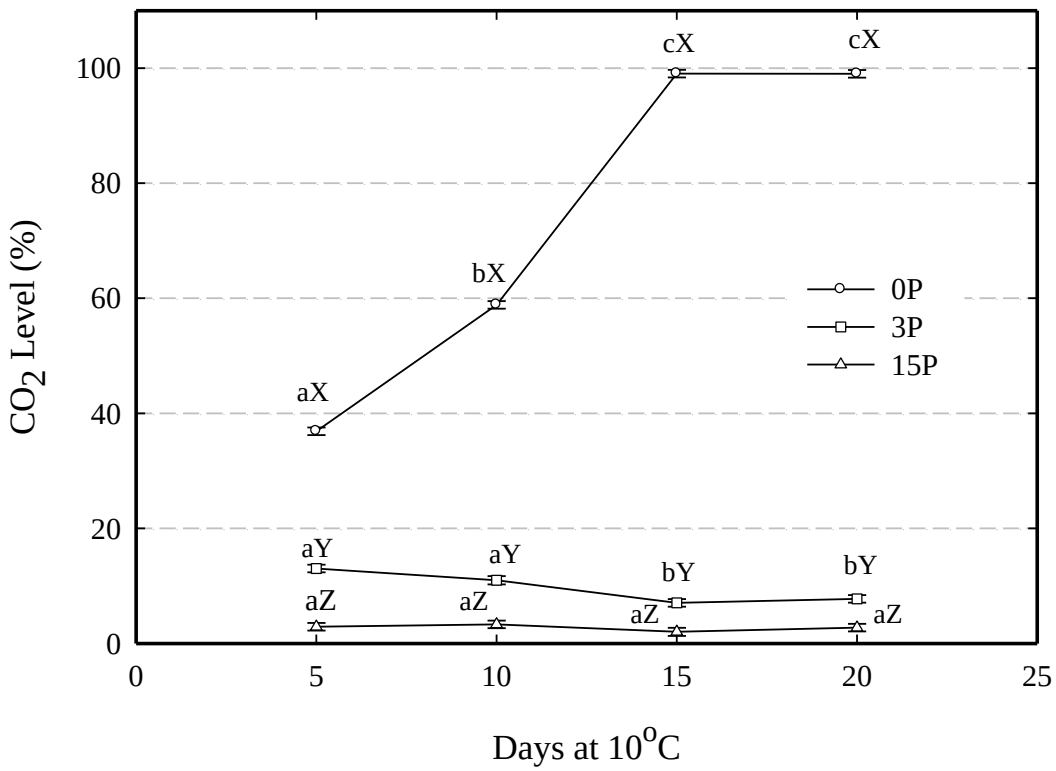


Figure 4-2d: The interaction effect of perforations and days on CO_2 level of blueberries stored at 10°C during 20 days. (a, b, c) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.1.1.3 CO_2 level at 23°C

The results of CO_2 level when stored at 23°C during 9 days are presented in Figure 4-3a in terms of perforations, the same order that was observed at 3 and 10°C was also exhibited at 23°C. The equilibrium was not reached at the end of the storage for any of the different packages due to a short testing time. The blueberries could not be analyzed after day 9 because of uncontrollable fungal growth. The interaction effect of perforations and days (Figure 4-3b), and materials and perforations was found

statistically significant at $\alpha = 0.05$. The values obtained for three perforations were highly significant ($p<0.001$) within the same day during storage (Figure 4-3b). Comparison between two materials within the same perforation (3, and 15) exhibited distinctive evolution of CO_2. There was no difference observed between non-perforated PLA, and PET. In addition, CO_2 had higher level at this temperature in comparison to 3 and 10°C. This trend is expected to behave as the Van's Hoff Rule which stated that every 10°C increases in temperature causes an increment of 2 to 3-fold of the velocity of biological reaction. The data obtained was compared at day 5 for 3 and 10°C and day 6 for 23°C. The results were mostly in agreement with the Van's Hoff Rule (e.g. PLA with 3 perforations showed 5.1% CO_2 evolution at 3°C, 13.1% at 10°C and 46.7 at 23°C) (Haffner et al., 2002). While, for the results that were not as they were expected to be, this could be associated with the differences in maturity stages, and possible variation in perforation size that may affect the respiration rate of blueberries. Almenar et al. (2008) also reported that blueberries packed in PLA containers experience small change in CO_2 level from 10 to 23°C (Almenar et al., 2008b).

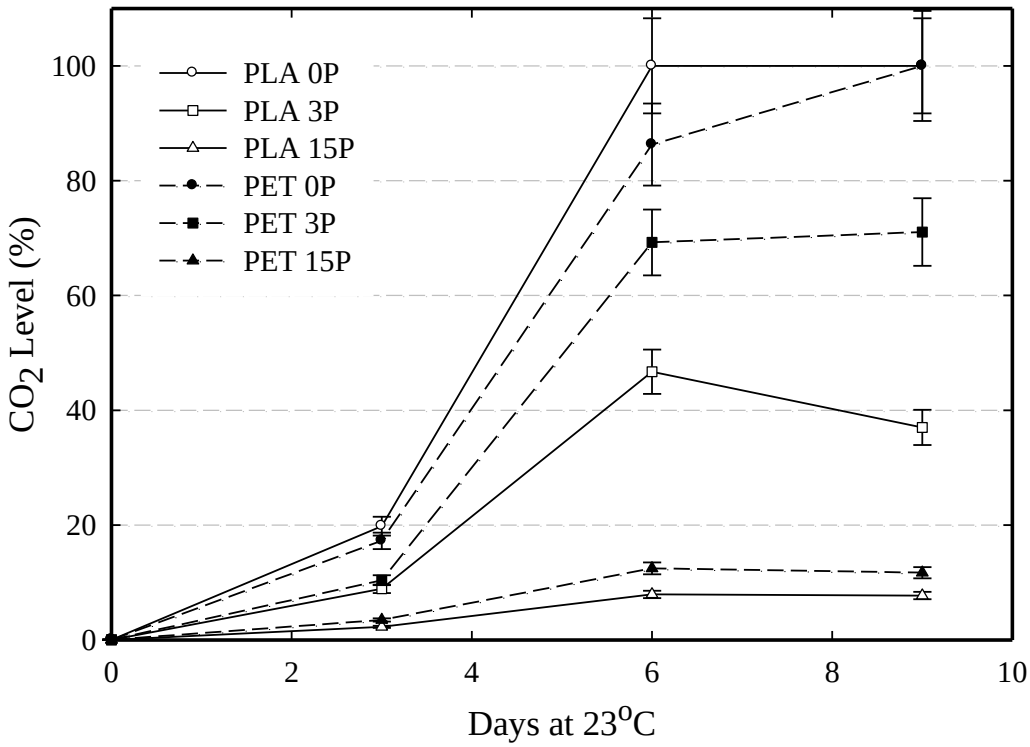


Figure 4-3a: The level of CO_2 in the packages stored at 23°C during 9 days of storage.

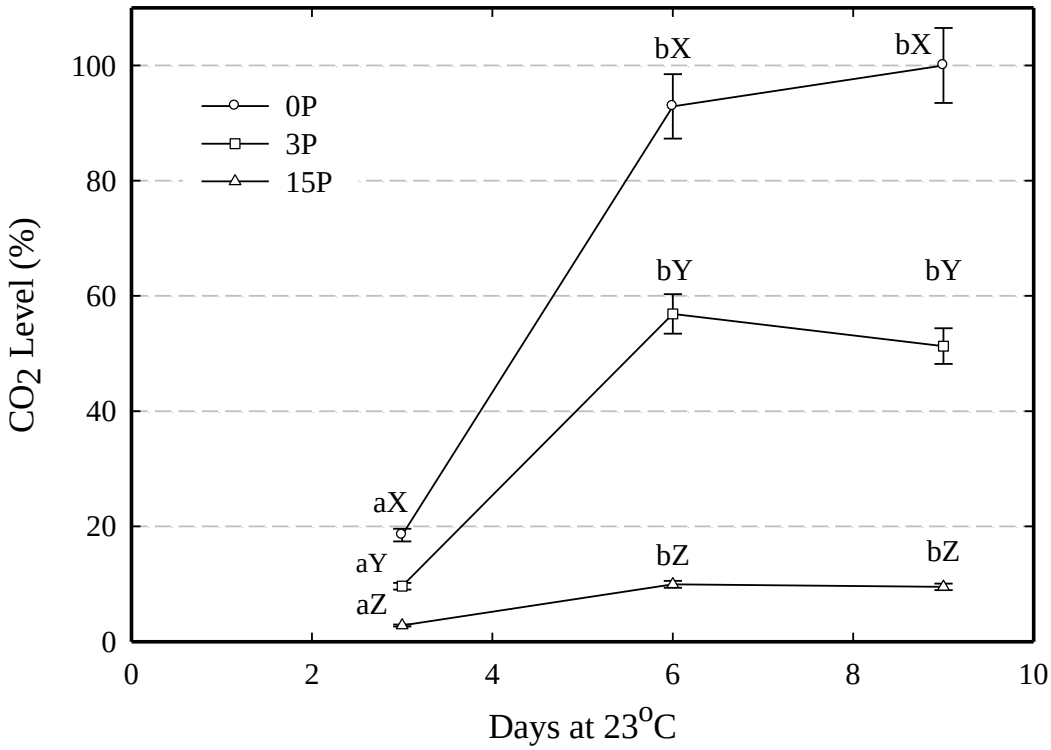


Figure 4-3b: The interaction effect of perforation and days in CO_2 level of blueberries stored at 23°C during 9 days. (a, b) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.1.2 O_2 level

4.1.2.1 O_2 level at 3°C

Due to respiration of the blueberries, the initial level of O_2 (20.9%) in the headspace is expected to be reduced over time. Figure 4-4a shows the O_2 levels reached in different packages during 33 days at 3°C. The O_2 content in non-perforated packages was reduced significantly after day 5, while O_2 in perforated packages decreased slightly

in the order of 15, and 3 perforations. The level of O_2 in non-perforated packages dropped due to the O_2 consumption by blueberry as it is also correlated to an increased CO_2 level in the package headspace. Besides, O_2 has two to six times less diffusivity through materials when compared to CO_2, resulted in low O_2 level in the headspace (Zagory, 1997). The permeation rates of CO_2 and O_2 via non-perforated materials are often below product respiration rates, thus the initial level of O_2 in the headspace tend towards zero-oxygen atmosphere (Catala & Gavara, 2000). On the other hand, the O_2 level in the perforated container was slightly reduced from the initial value because the presence of porous allows O_2 and CO_2 to enter and exit the headspace, respectively, at similar rates as previously described in section 2.5.4.3.2.

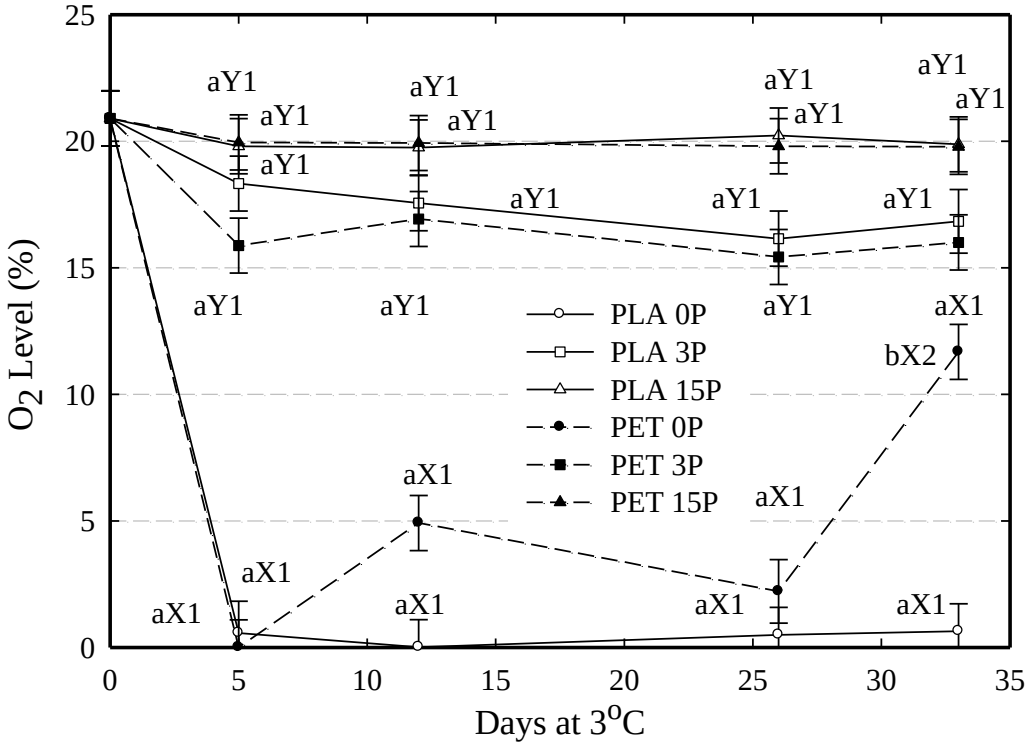


Figure 4-4a: The level of O_2 in the packages stored at 3°C during 33 days of storage**.**

(a, b) Differences between days within perforations and materials ($p<0.05$)**;** (X, Y) Differences between perforations within days and materials ($p<0.05$)**;** (1, 2) Differences between materials within days and materials ($p<0.05$).

The interaction between materials and perforations on O_2 level at 3°C is shown in Figure 4.4b. Materials act as a permeable medium in a presence of perforation. As can be seen from Figure 4-4b, the O_2 level in the headspace of non-perforated PET packages was higher than non-perforated PLA packages. This result was unexpected since non-perforated PET packages was expected to retain lower O_2 level than that of non-perforated PLA packages due to its high barrier to this gas that limits O_2 permeation into the headspace.

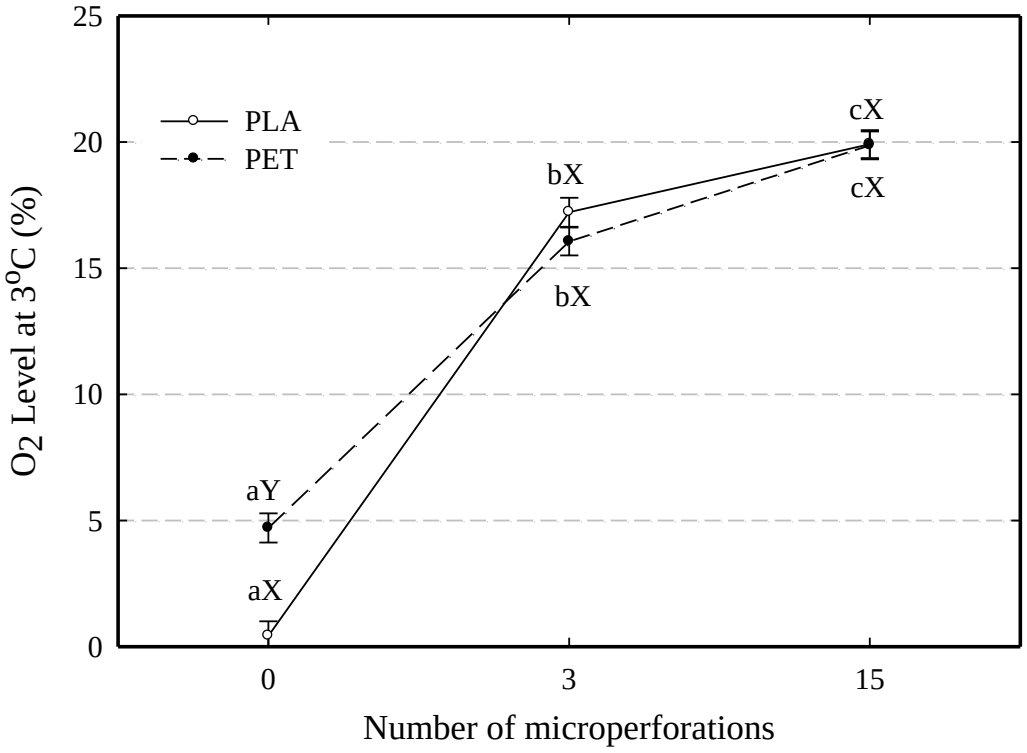


Figure 4-4b: The interaction effect of materials and perforations in O_2 level of blueberries stored at 3°C during 33 days.(a, b, c) Differences between perforations within materials ($p<0.05$);(X, Y) Differences between materials within perforations ($p<0.05$).

Figure 4-4c shows the perforations-days interaction effect in O_2 level during storage at 3°C. All non-perforated and perforated packages reached steady-state after day 5. The O_2 level in packages with 3 perforation when compared to that of packages with 15 perforations were not significantly difference ($p>0.05$) throughout storage except for day 26. In terms of materials-days interaction, there only difference observed was at the last day of storage between two materials.

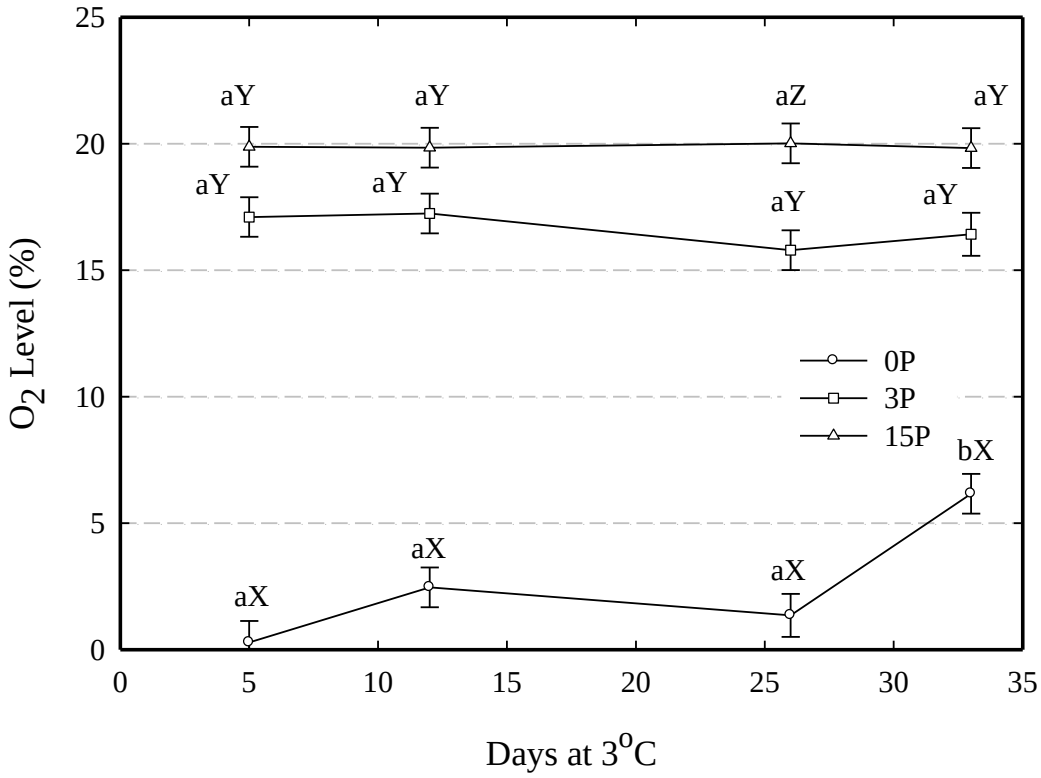


Figure 4-4c: The interaction effect of perforation and days in O_2 level of blueberries stored at 3°C during 33 days. (a, b) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.1.2.2 O_2 level at 10°C

The same order of O_2 level as at 3°C according to the number of perforations was observed at 10°C. The three-way interaction of materials, perforations, and days was significant at $p<0.05$ (Figure 4-5a). Figure 4-5b shows the effect of materials and perforations on O_2 level throughout storage. The effect of material for non-perforated packages at this temperature was different than that of discussed at 3°C, in which non-

perforated PET packages had a lower O_2 level in comparison with non-perforated PLA packages. This result is expected due to the continuous respiration of blueberries, and also due to the high barrier of this material (PET) to O_2. The effect of perforation on each analyzed day is shown in Figure 4-5c. All packages with 0, 3 and 15 perforations were significantly different ($p<0.05$) at day 5, and 10, while at day 15 and 20, there was no significant different found between packages with 3, and 15 perforations ($p>0.05$).

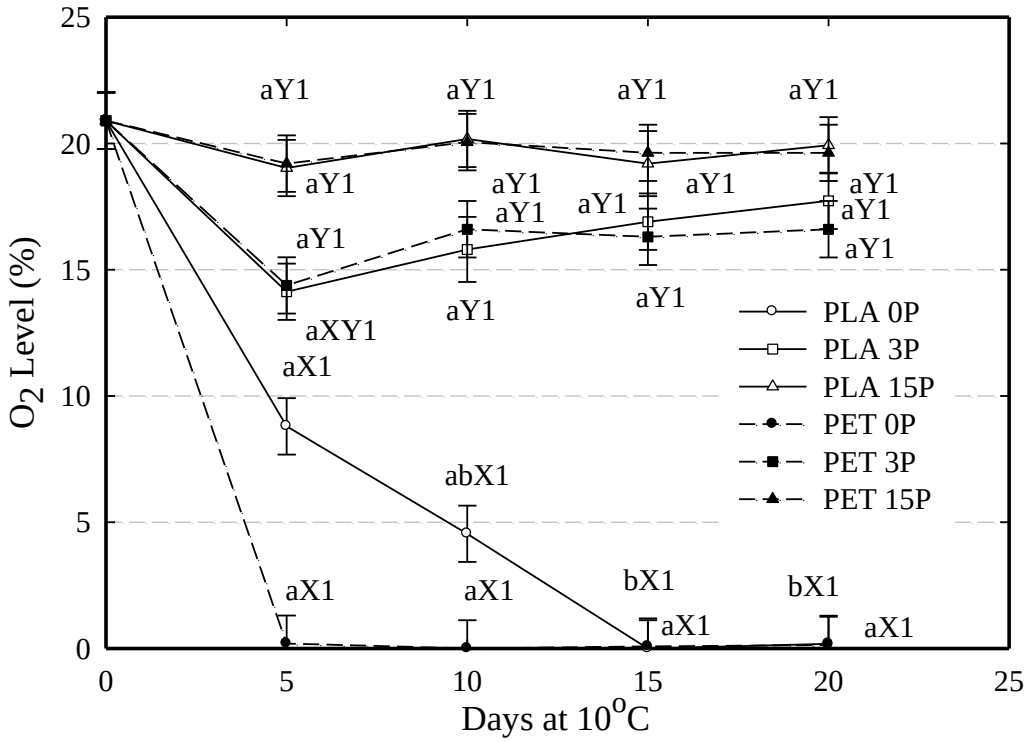


Figure 4-5a: The level of O_2 in the packages stored at 10°C during 20 days of storage.

(a, b) Differences between days within perforations and materials ($p<0.05$); (X, Y) Differences between perforations within materials and days ($p<0.05$); (1, 2) Differences between materials within perforations and days ($p<0.05$).

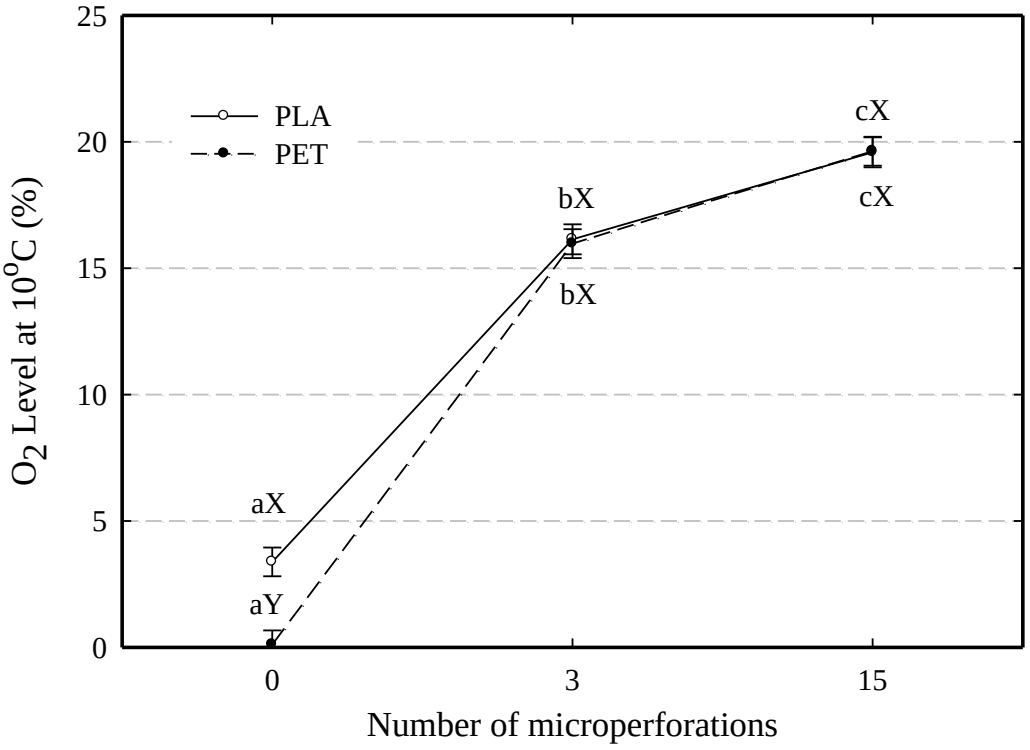


Figure 4-5b: The interaction effect of materials and perforations in O_2 level of blueberries stored at 10°C during 20 days. (a, b, c) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

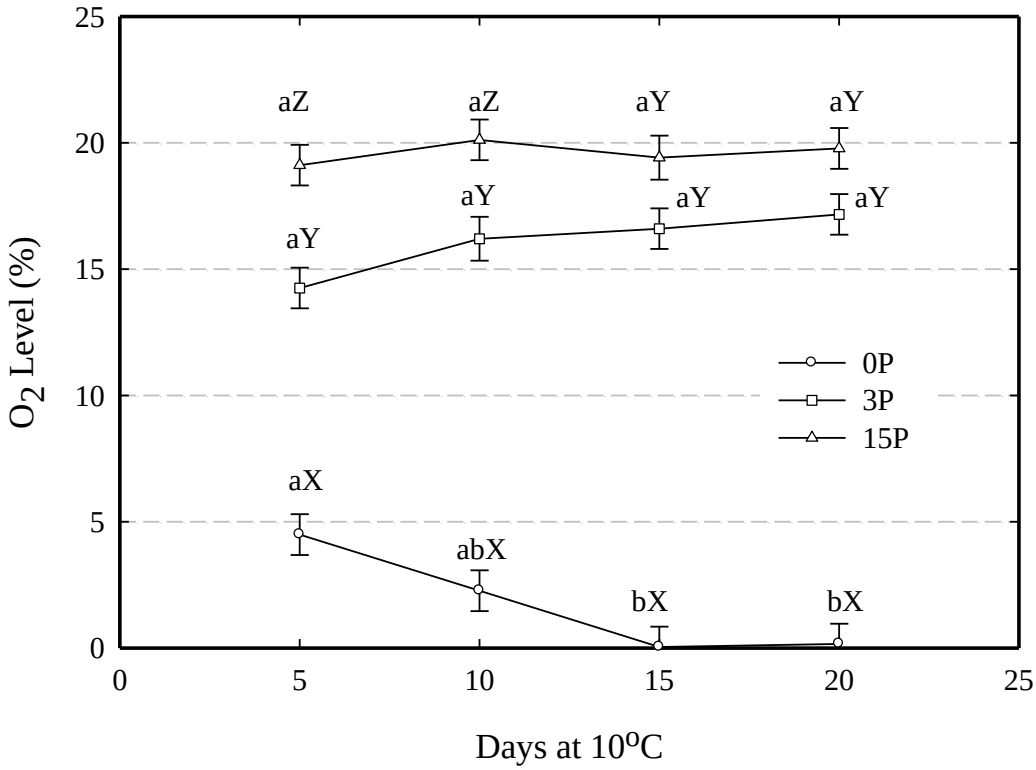


Figure 4-5c: The interaction effect of perforation and days in O_2 level of blueberries stored at 10°C during 20 days. (a, b) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.1.2.3 O_2 level at 23°C

O_2 level of all packages at 23°C was not at steady-state at day 9 due to the same reason discussed for CO_2 level at 23°C. The same trend of O_2 evolution that was observed at 3 and 10°C with regards to the presence, and absence of perforation was also revealed at this temperature (Figure 4-6a). The interaction effect of materials, and perforations at 23°C (Figure 4-6b) followed the pattern of the result obtained at 3°C

(Figure 4-4b). No marked influence of temperature was seen for O_2 composition of blueberries in this study. Almenar et al. (2008) stated that blueberries dependence of chemical attributes on the temperature is not as strong as the other fruits.

Overall, in this study, the CO_2 level found in non-perforated PLA, and PET packages at equilibrium at all temperature were greater than 25%. PLA, and PET packages with 3 perforations had CO_2 level of approximately 5-6% (3°C), 6-8% (10°C), and 40-70% (23°C), while those packages with 15 perforations had lower level of CO_2; 1-1.5% (3°C), 2% (10°C), and 8-12% (23°C). The level of O_2 found for non-perforated packages at three temperatures was between 0-2%, while for packages with 3 perforations, the O_2 level was 16-18% (3, and 10°C), and 3-7% (23°C). O_2 content in packages with 15 perforations was 19-20% (3, and 10°C), and 13.8-16% (23°C).

Optimum gas composition for extending 'Coville' blueberries shelf life was reported to be 17-18% CO_2, and ≤ 9% O_2 by Kim et al. (1995). Yam and Lee (1995) recommended a gas composition of 0-10% O_2, and 11-20% CO_2 for keeping blueberries fresh (Yam et al., 1995). However, Harb and Streif (2004) reported that the presence of CO_2 at a level greater than 12% changed the flavor, firmness, and acid content of 'Duke' blueberries (Harb & Streif, 2004). Thus, packages with 3 perforations at 10°C had shown to have CO_2, and O_2 levels close to those recommended gas composition.

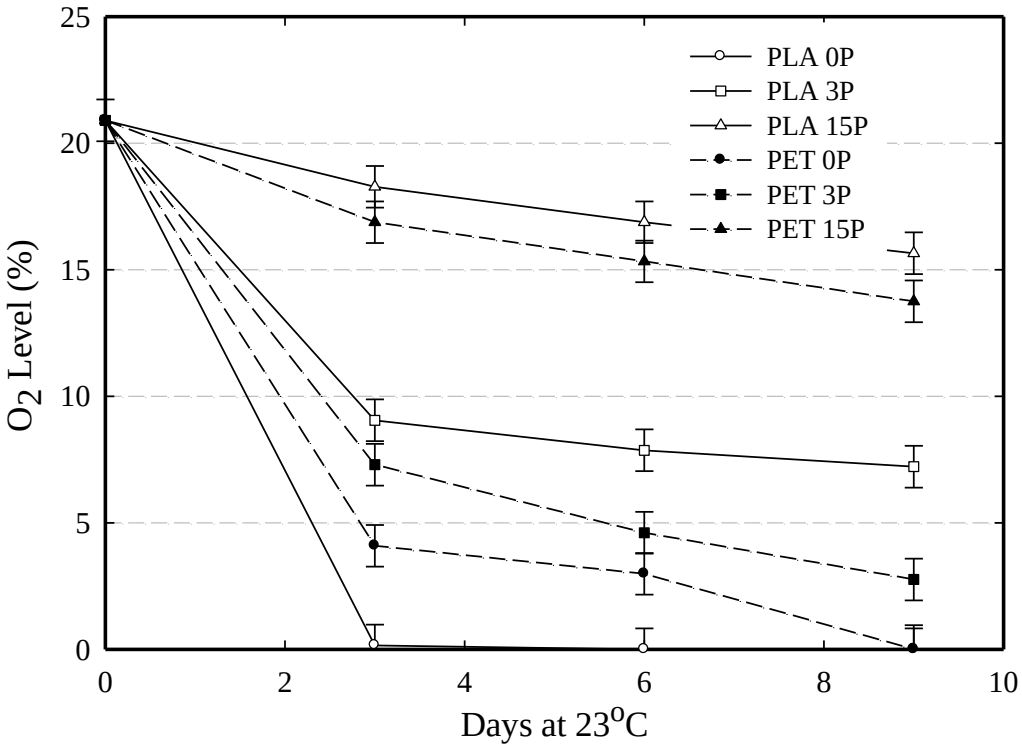


Figure 4-6a: The level of O_2 in the packages stored at 23°C during 9 days of storage.

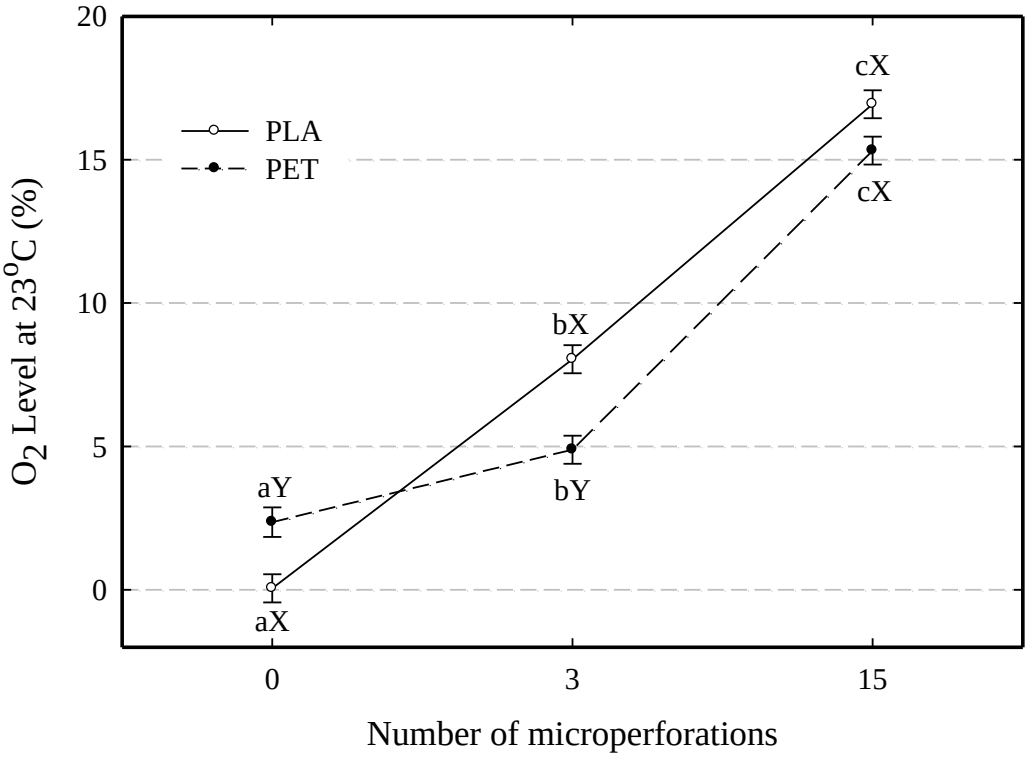


Figure 4-6b: The interaction effect of materials and perforations in O_2 level of blueberries stored at 23°C during 9 days. (a, b, c) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

4.2 Weight loss

The quality of blueberries as well as other produce is closely related to moisture loss. Significant loss of water would result in unappealing appearance, change in flavor, texture, and others.

4.2.1 Weight loss at 3°C

Blueberries weight loss during 33 days of storage at 3°C is shown in Figure 4-7a. Weight loss in non-perforated and perforated PLA was seen greater than non-perforated

and perforated PET packages. For both materials, packages with 15 perforations showed significant weight loss. No significant differences in blueberries weight loss were found for packages with 3 perforations, and non-perforated packages. The interaction effect between materials, and days was found significant at $p<0.05$ (Figure 4-7b). Weight loss of blueberries in PLA packages was statistically difference among each analyzed days.

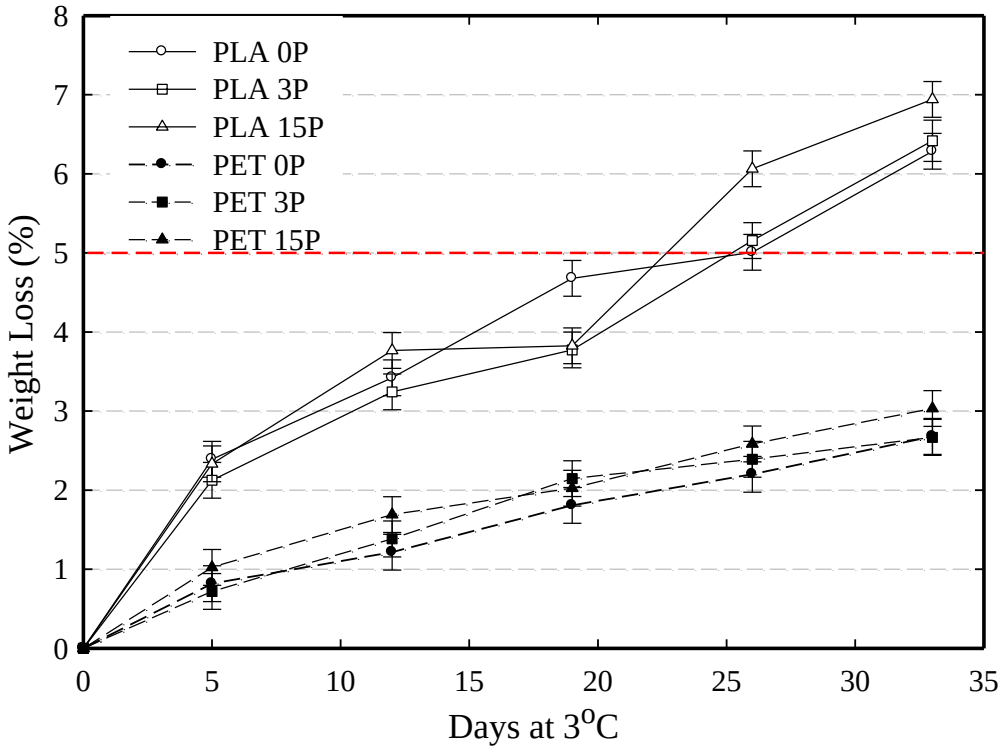


Figure 4-7a: Weight loss of blueberries in six different packages stored at 3°C during 33 days of storage. Note: Red short dash line indicates the minimum level of weight loss that would cause the reduction in retail value. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

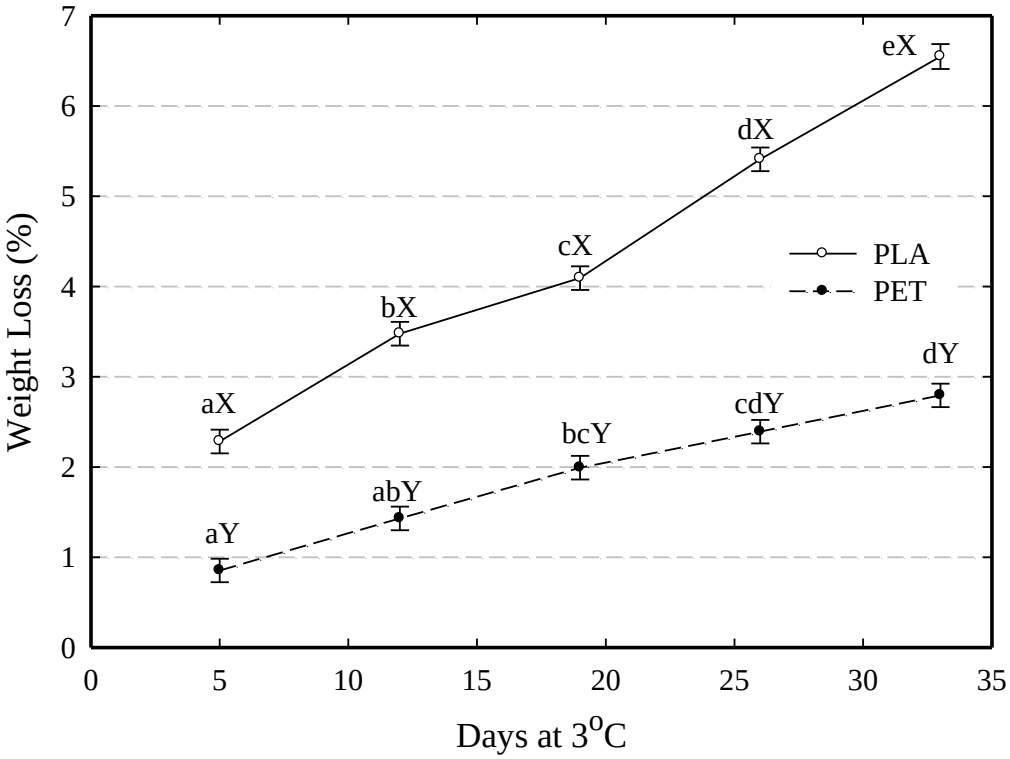


Figure 4-7b: The interaction effect of materials, and days in weight loss of blueberries stored at 3°C during 33 days. (a, b, c, d, e) Differences between days within materials ($p < 0.05$); (X, Y) Differences between materials within days ($p < 0.05$).

4.2.2 Weight loss at 10°C

Weight loss of blueberries packaged in PLA containers at 10°C was noticeably different when compared to those at 3°C. At the end of the storage, non-perforated PLA had a higher weight loss, followed by 15 and 3 perforated PLA packages. PET packages with 3 perforations showed the least weight loss at this temperature (Figure 4-8b). No significant different was found in blueberries weight loss between non-perforated PET, and perforated PET packages. Perforated PET packages, however, were significantly different to each other (Figure 4-8b). Weight loss comparison between materials for each

analyzed days was significant at $p<0.05$ (Figure 4-8c). Blueberries weight loss in all PLA packages differed greatly from day 5 to 10, 15, and 20. On the other hand, a gradual increase in weight loss of blueberries was observed for all PET packages throughout storage (Figure 4-8c).

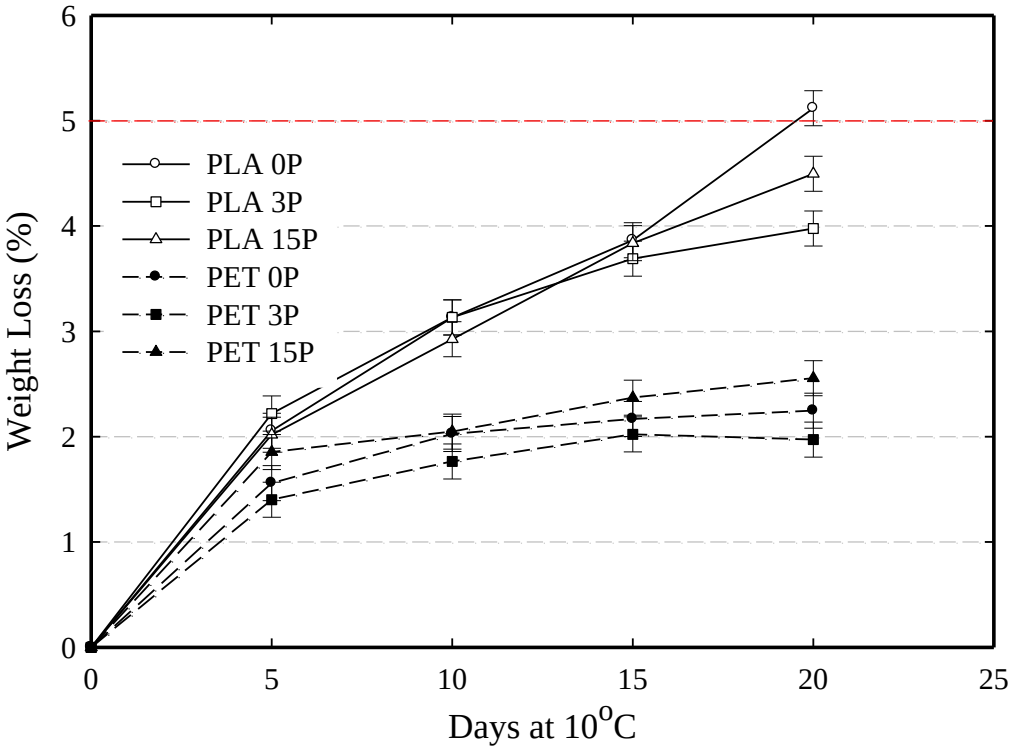


Figure 4-8a: Weight loss of blueberries in six different packages stored at 10°C during 20 days of storage. Note: Red short dash line indicates the minimum level of weight loss that would cause the reduction in retail value.

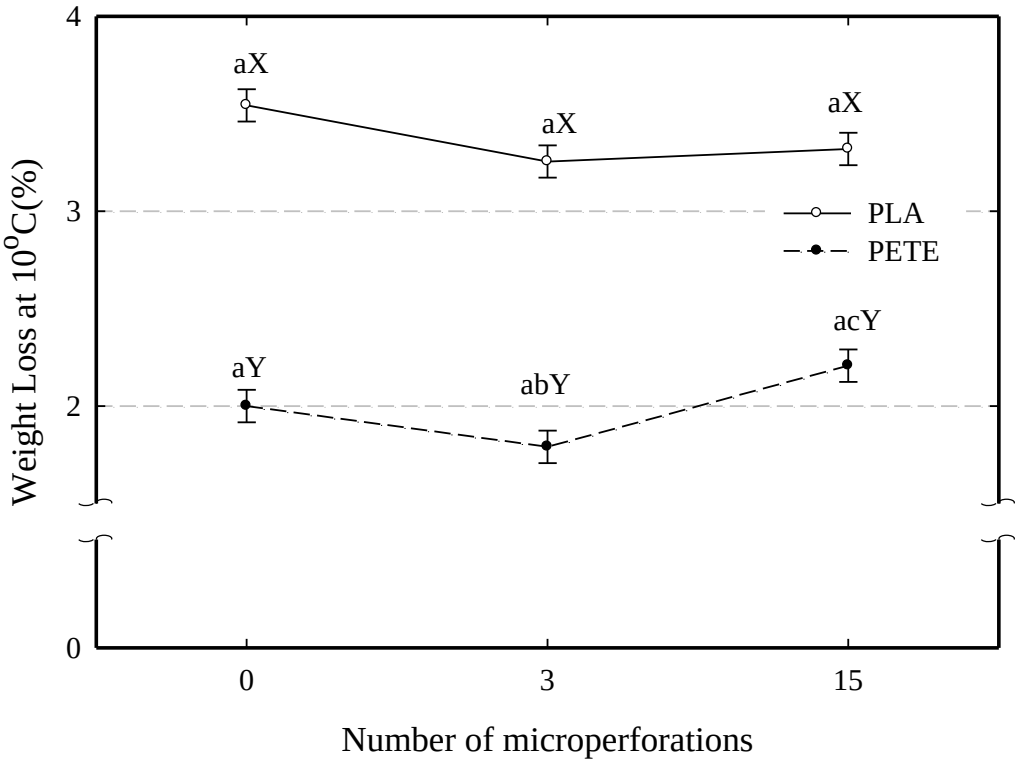


Figure 4-8b: The interaction effect of materials, and perforations in weight loss of blueberries stored at 10°C during 20 days.(a, b, c) Differences between perforations within materials ($p < 0.05$); (X, Y) Differences between materials within perforations ($p < 0.05$).

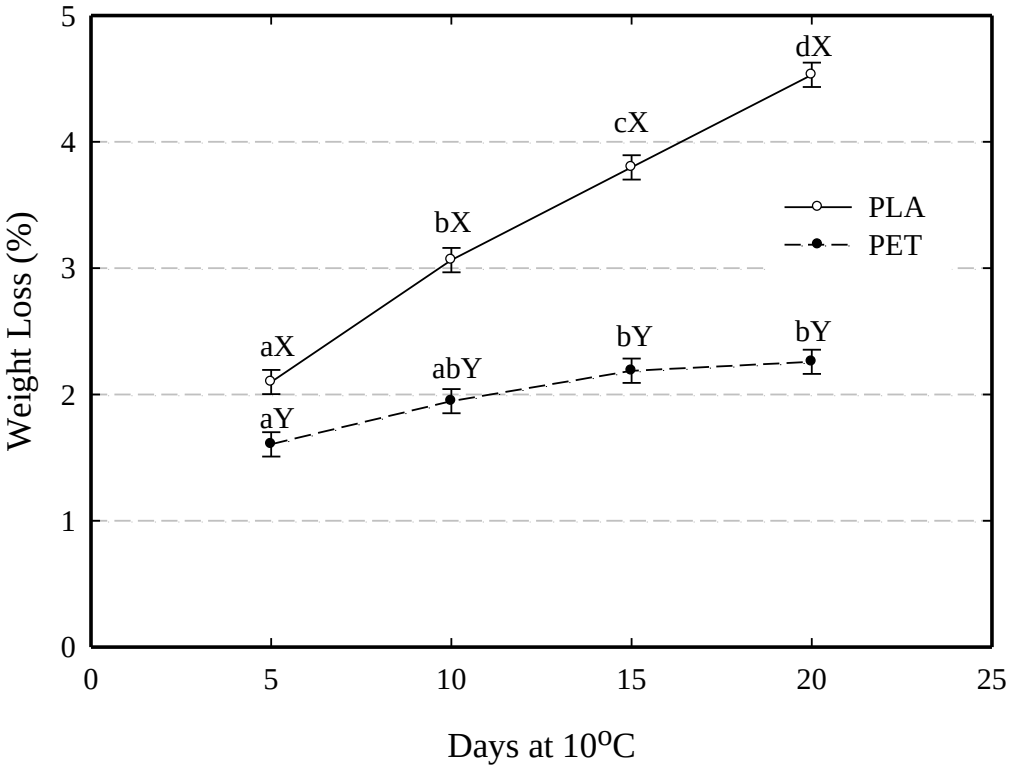


Figure 4-8c: The interaction effect of materials, and days in weight loss of blueberries stored at 10°C during 20 days. (a, b, c) Differences between days within materials ($p < 0.05$); (X, Y) Differences between materials within days ($p < 0.05$).

4.2.3 Weight loss at 23°C

PLA packages with or without perforations showed higher weight loss in comparison to those PET packages at 23°C, which is also the same pattern that was seen at 3 and 10°C. The presence of perforations was clearly seen to have a reduction effect on weight loss at this temperature (Figure 4-9a). The weight loss of blueberries packed in both materials decreased with increasing number of perforations. Blueberries weight loss was significantly different among perforations for PLA packages. In the case of PET, perforated packages were statistically different than non-perforated ones (Figure 4-9b).

Differences in weight loss of blueberries among days within perforation were remarkable. At day 3, the weight loss differ markedly among perforations, however later, the differences in weight loss between 3 and 15 perforation was not significant except for 0 perforation (Figure 4-9c).

The effect of a high temperature (23°C) (0-5.8% during 9 days of storage) was more obvious on blueberries weight loss than at 3 (0-7% during 33 days of storage), and 10°C (0-5% during 20 days of storage). This trend is expected because as the temperature increases, the free energy of water molecules also increases, thus enhances the molecules movement, and potential for exchange (Shamaila, 2005). The increase of storage temperature results in incidence of shriveled, decayed blueberry, and fruit breakdown (Sanford et al., 1991). Therefore, blueberries are best kept at a low temperature as to avoid significant moisture loss, thus maintain an acceptable, and preferable fruit quality.

According to Tabil et al. (2001), a weight loss of 5-10% experienced by fresh produces contributes to significant wilting, shriveling, poor texture and taste (Tabil et al., 2001). The signs of freshness loss of a produce reveals with a 3-10% weight loss (Ben-Yehoshua, 1987). In addition, a weight loss of more than 5% was reported to cause a reduction in fresh produces retail value (Ohta, Shiina & Sasaki, 2002). Considering this criterion solely, blueberries in all packages would be marketable up to three weeks at 3, and 10°C, and one week at 23°C. Almenar et al. (2008b) reported a weight loss of 4% at day 9 for 'Elliot' blueberries packaged in rigid PLA container, and stored at 23°C (Almenar et al., 2008b). In our findings, non-perforated PLA had a greater weight loss compared to the previously reported data possibly because of the differences in thickness

of materials (film versus sheet), and the use of film as a lidding material. This reported data, however, was comparable to those perforated PLA packages stored at the same condition. Moreover, maximum weight loss of blueberries in this study was significantly less than that reported for the ones packed in traditional petroleum-based clamshells. At 23°C, day 9, weight loss reported for 'Elliot' blueberries in PET clamshells was greater than that weight loss reported for 'Bluecrop' blueberries in this study (32 versus 6%).

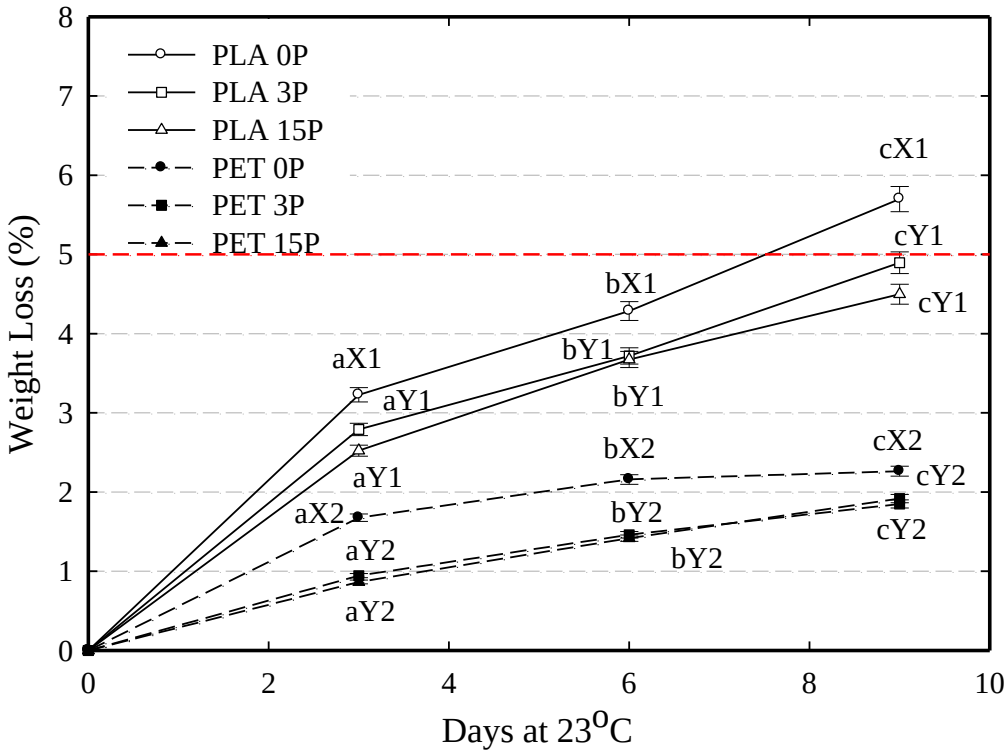


Figure 4-9a: Weight loss of blueberries in six different packages stored at 23°C during 9 days of storage. Note: Red short dash line indicates the minimum level of weight loss that would cause the reduction in retail value. (a, b, c) Differences between days within materials and perforations ($p < 0.05$); (X, Y) Differences between perforations within materials and days ($p < 0.05$); (1, 2) Differences between materials within perforations and days ($p < 0.05$).

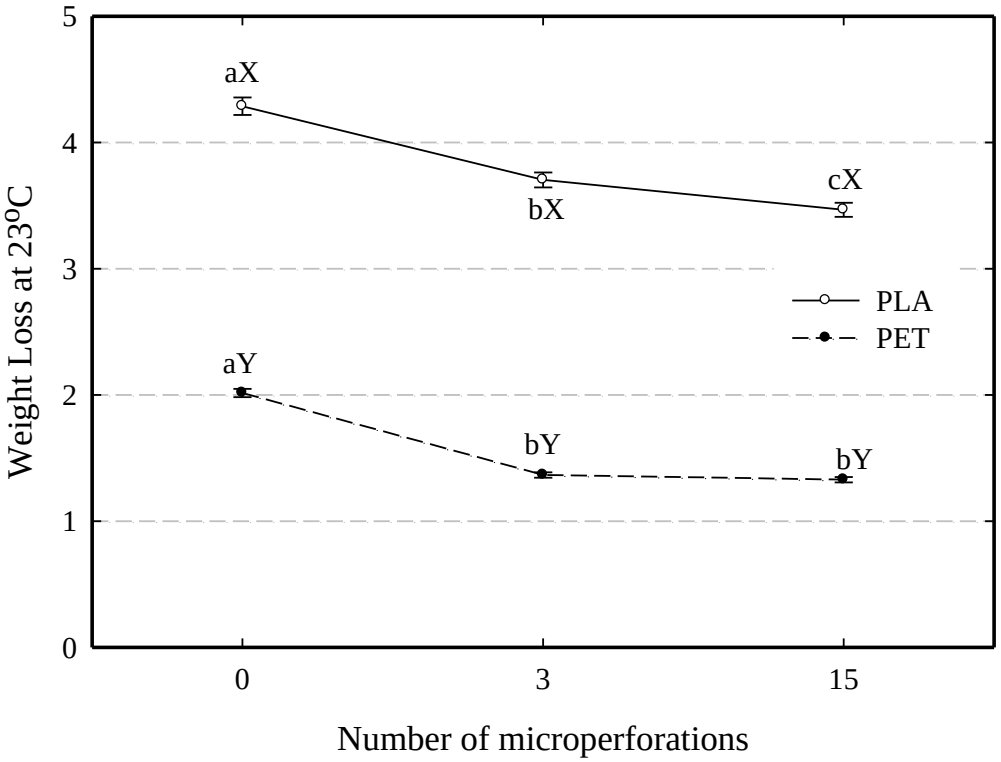


Figure 4-9b: The interaction effect of materials, and perforations on weight loss of blueberries stored at 23°C during 9 days. (a, b, c) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

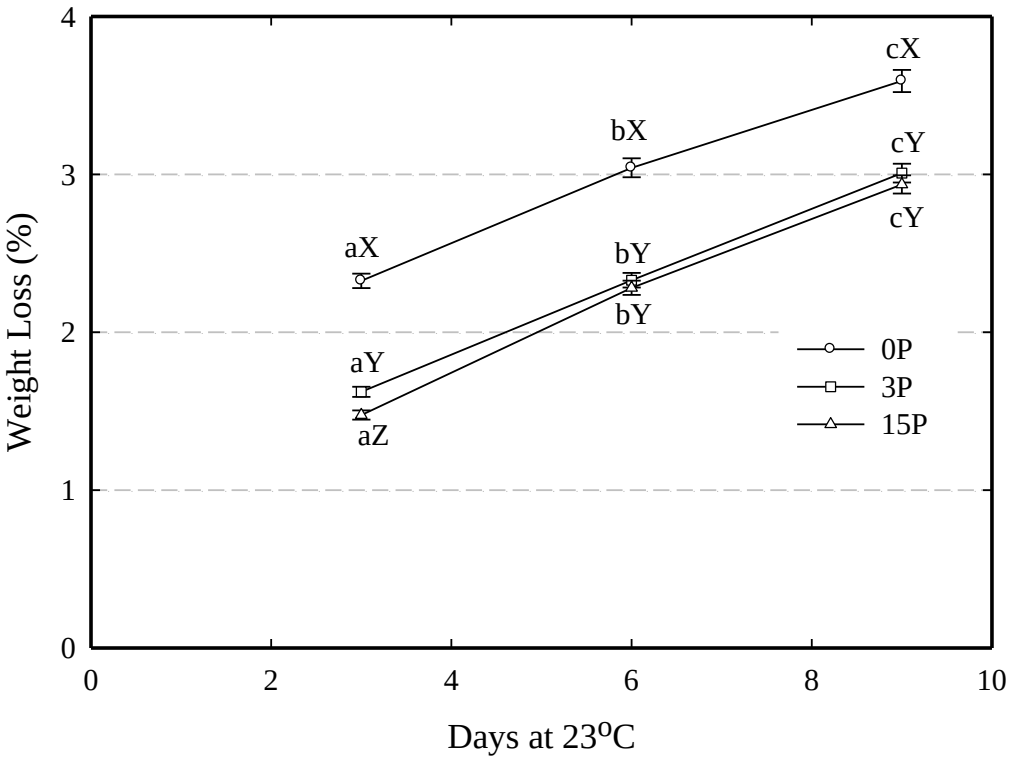


Figure 4-9c: The interaction effect of perforations, and days in weight loss of blueberries stored at 23°C during 9 days. (a, b, c) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.3 Microbiological evaluation

Fruit decay is one of the most critical parameters that need to be controlled since it affects the quality, safety, and shelf life of fruits. Blueberries are vulnerable to postharvest microorganisms mainly *Colletotrichum acutatum*, *Botrytis cineria*, and *Alternaria alternata*. However, blueberries susceptibility to decay has been reported as cultivars dependant. Throughout this study, *Alternaria alternata* was the commonly found fungal.

4.3.1 Fungal growth at 3°C

Figure 4-10a showed the percentage of fungal growth found on blueberries packaged in non-perforated and perforated PLA, and PET container kept at 3°C during 33 days. Non-perforated packages showed no sign of fungal growth until day 26. This is because of the riched-CO_2 atmosphere in the package headspace, 59.9 and 53.6% for non-perforated PLA and PET packages, respectively. CO_2 has been known as one of the effective tool to inhibit fungal growth when presence at relatively high concentration (Almenar, Del Valle, Catala & Gavara, 2007b; Talbot & Chau, 1991). Blueberries in perforated PLA packages showed more incidence of fungal growth than those fruits in perforated PET. This could be related to the level of CO_2 of which was slightly higher in perforated PET packages (8%) than in perforated PLA packages (6%) (i.e. packages with three perforations). There was no significant effect between 3 and 15 perforations within PLA packages in fungal growth. However, materials had a significant effect on the fungal growth within perforations (Figure 4-10b). Fungal growth among days within the same perforation was not significant except for days 26 and 33 (Figure 4-10c). The interaction effect between materials and days was also significant at $p<0.05$ (Figure not shown). Percentage of infected blueberries was found increased significantly from day 5 to 33 for PLA at 3°C. No significant differences were found of fungal growth incidence in PET packages throughout storage time.

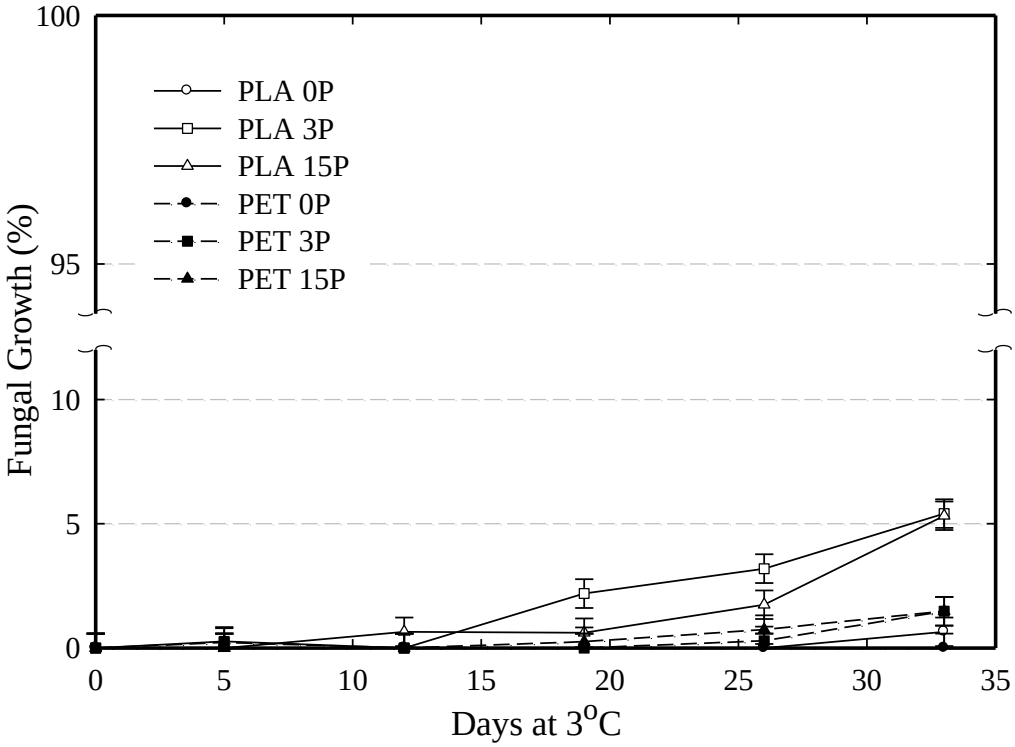


Figure 4-10a: Percentage of infected blueberries in six different packages when stored at 3°C during 33 days of storage.

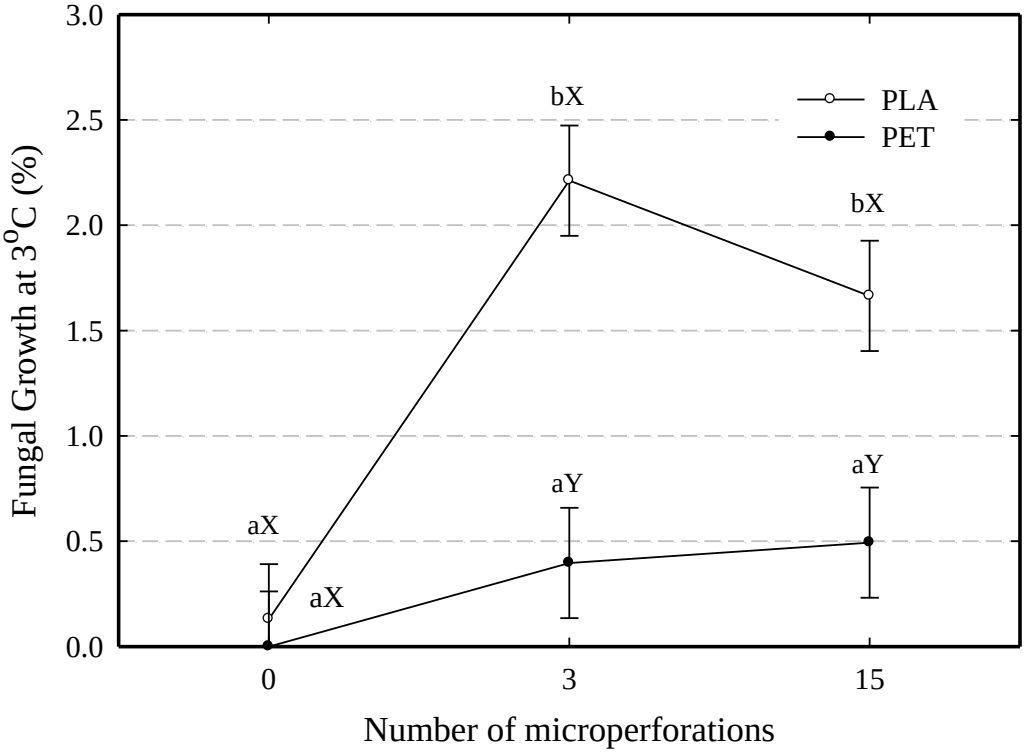


Figure 4-10b: The interaction effect of materials, and perforations on fungal growth of blueberries stored at 3°C during 33 days. (a, b) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

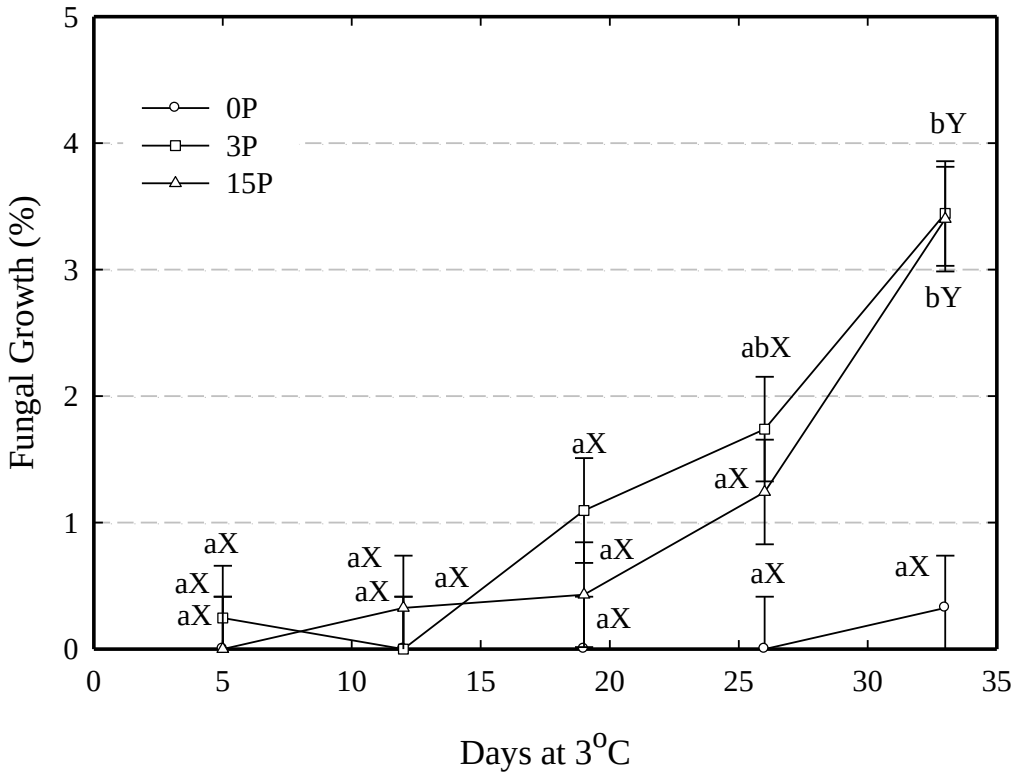


Figure 4-10c: The interaction effect of perforations, and days on fungal growth of blueberries stored at 3°C during 33 days. (a, b) Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.3.2 Fungal growth at 10°C

In general, fungal growth in all packages was not significantly different in comparison among days except at early of the storage time. Even though the level of CO_2 presence in these packages headspace increased during storage depending on the number of perforation, the effect of this gas on fungal growth was not as it was expected (Figure 4-11a). It seems the levels of CO_2 achieved during storage were not sufficient to inhibit fungal growth due to the increase of the temperature that increased the growth rate of the

fungal. Percentage of infected blueberries in relation to perforation indicated no differences between the last two analyzed days (Figure 4-11b).

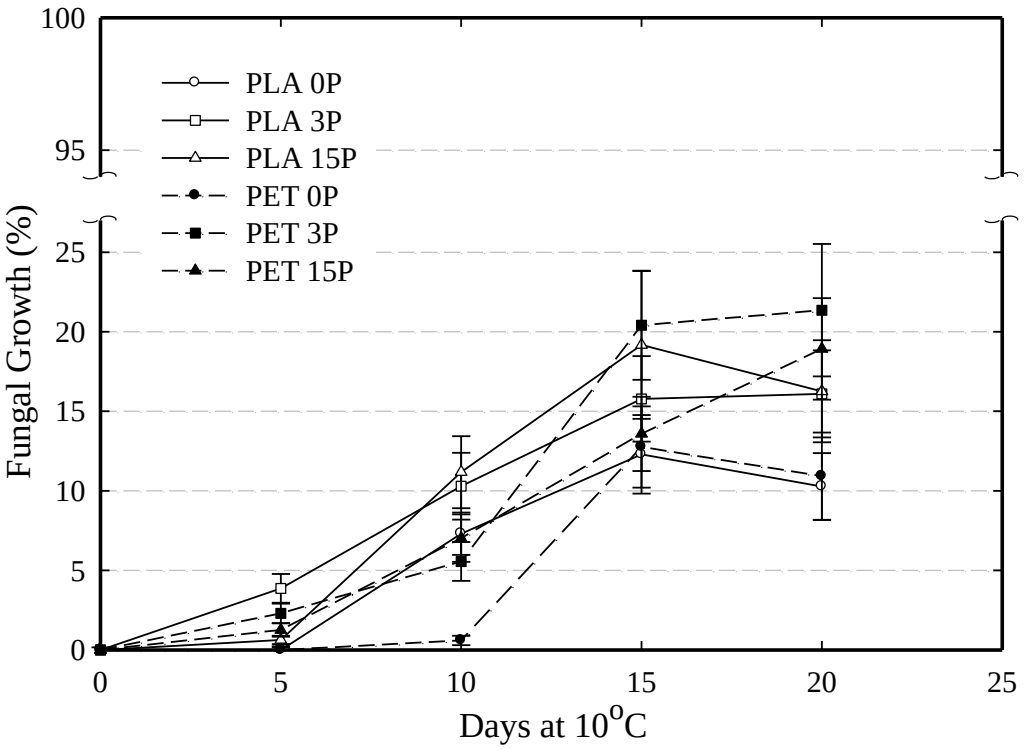


Figure 4-11a: Percentage of infected blueberries in six different packages when stored at 10°C during 20 days of storage.

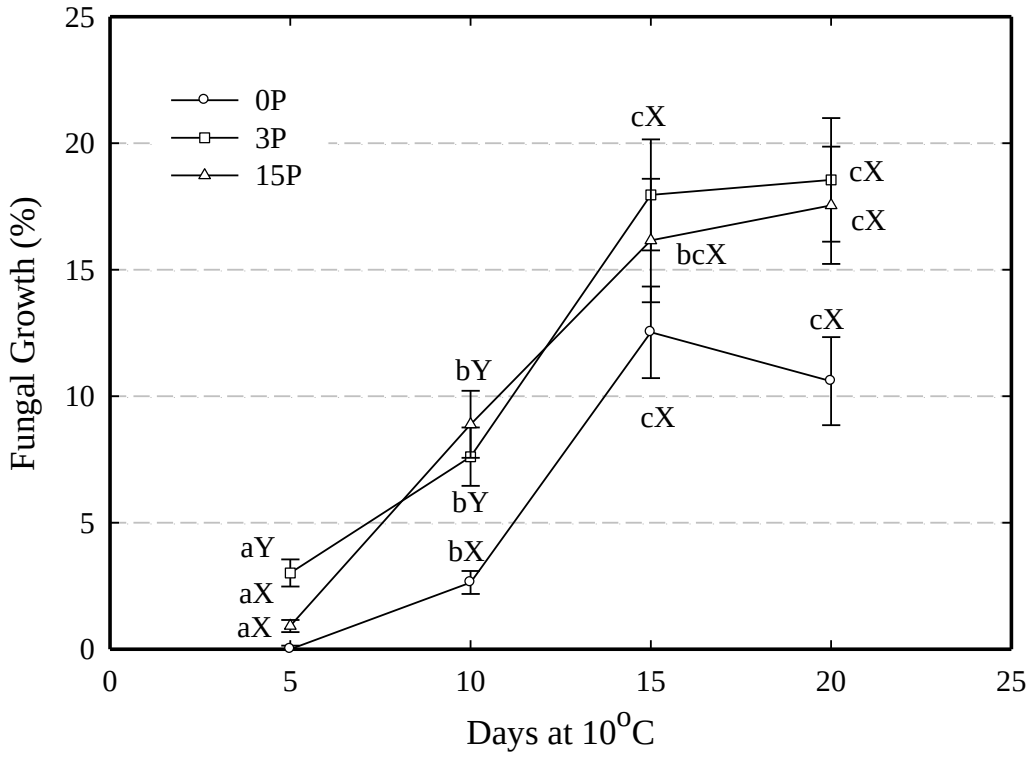


Figure 4-11b: The interaction effect of perforations, and days on fungal growth of blueberries stored at 3°C during 33 days. (a, b, c) Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.3.3 Fungal growth at 23°C

Non-perforated packages showed the least growth of fungal (12-7 and 17.1% for PLA and PET packages, respectively) and this could be due to the high level of CO_2 in the package headspace. Fungal growth was seen the greatest in packages with 15 perforations (65.8 and 66.1% for PLA and PET packages, respectively (Figure 4-12a). Fungal growth in non-perforated packages was not significantly different among days. The increase in infected number of blueberries was significant in packages with 3, and 15 perforations for every analyzed day (Figure 4-12b).

Fungal growth of blueberries increased with increases in temperature since high temperatures promote greater fungal growth. The concentration of CO_2 in all packages headspace at three temperatures was not effective to reduce fungal growth, except for non-perforated packages, and packages with 3 perforations at 3°C. The higher the CO_2 level, the lower the fungal growth and this was observed in this study. Based on these results, it could be suggested that temperature has more pronounced effect in controlling decay than that of CO_2 concentration. However, Smith et al. (1990) reported that CO_2 is a bacteriostatic, and fungistatic agent with its activity is temperature dependent (Smith, Ramaswany & Simpson, 1990). This gas has more efficient inhibitory effect at low than at high temperature due to its greater capacity to dissolve in the product's aqueous phase, thus increasing the product acidity (Brody, 1989; Gill & Tan, 1980). In addition, synergic effect of high CO_2, and low temperature was found to be effective to reduce, and even stop the growth of *Botrytis cinerea* (Agar, Garcia, Miedtke & Streif, 1990). CO_2 concentration greater than 6% was required to inhibit the growth of *Botrytis cinerea* at a temperature close to 0°C (Harb et al., 2004). However, *Alternaria alternata* was the commonly found fungal in this study. There is a possibility that this fungal can tolerate riched-CO_2 atmosphere, thus led to higher fungal growth although the blueberries were kept at relatively low temperature such as 10°C.

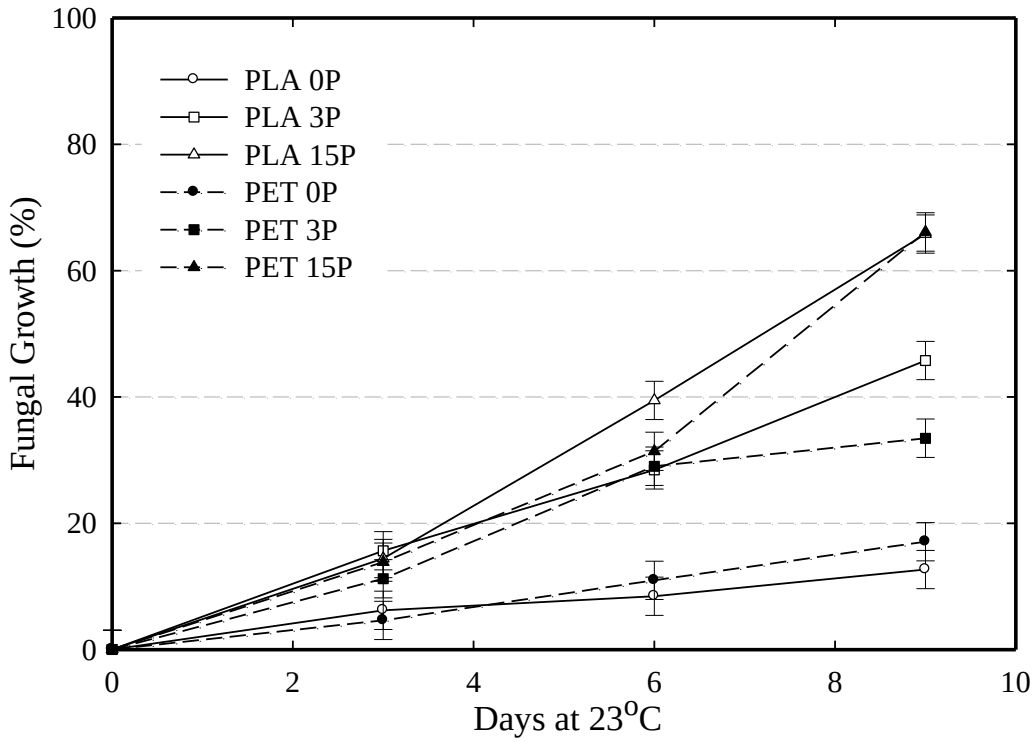


Figure 4-12a: Percentage of infected blueberries in six different packages when stored at 23°C during 9 days of storage.

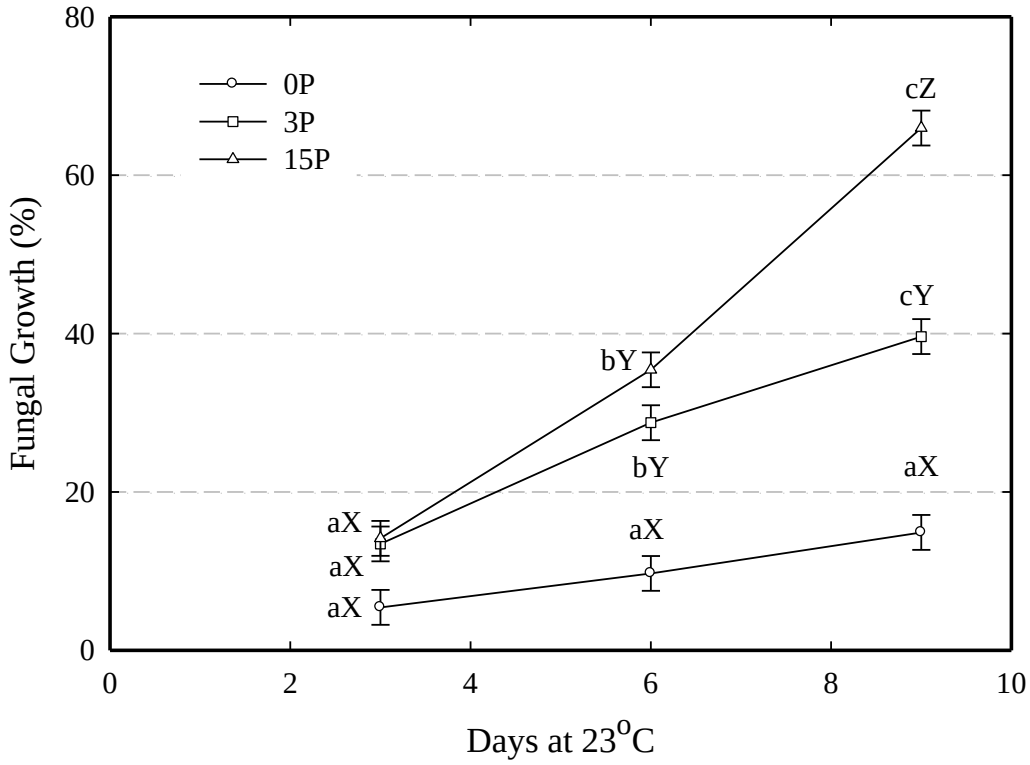


Figure 4-12b: The interaction effect of perforations, and days on fungal growth of blueberries stored at 23°C during 9 days. (a, b, c) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.4 pH

4.4.1 pH at 3°C

The evolution of pH of blueberries packaged in six types of packages ranged from 2.99 to 3.10 (Figure 4-13a). These values were comparable with the pH of the same cultivar ('Bluecrop') kept at 5°C, which was 3.10 (Saftner, Polashock, Ehlenfeldt & Vinyard, 2008). Blueberries packaged in PLA experience a decrease in pH, and blueberries in PET packages experience an increase in pH, in order of 0, 3, and 15 perforations. PLA with 15 perforations was statistically different than PET packages of

the same perforation (Figure 4-13b). The interaction effect of perforations, and days was also found significant particularly at the end of storage time (Figure not shown).

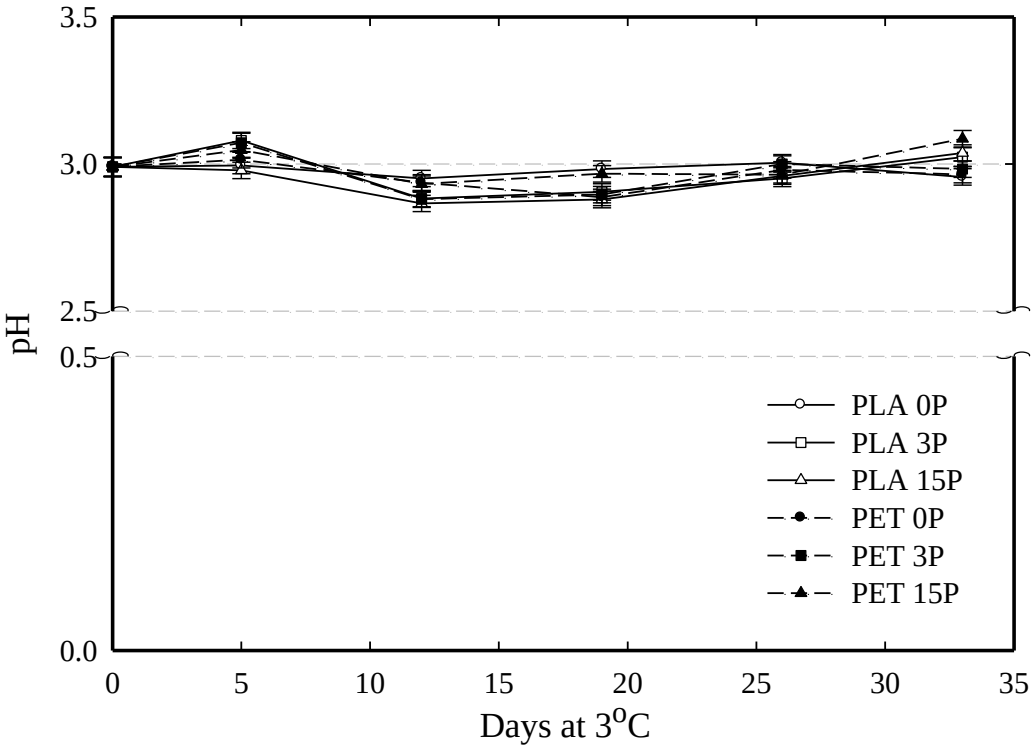


Figure 4-13a: The evolution of pH when stored at 3°C during 33 days of storage.

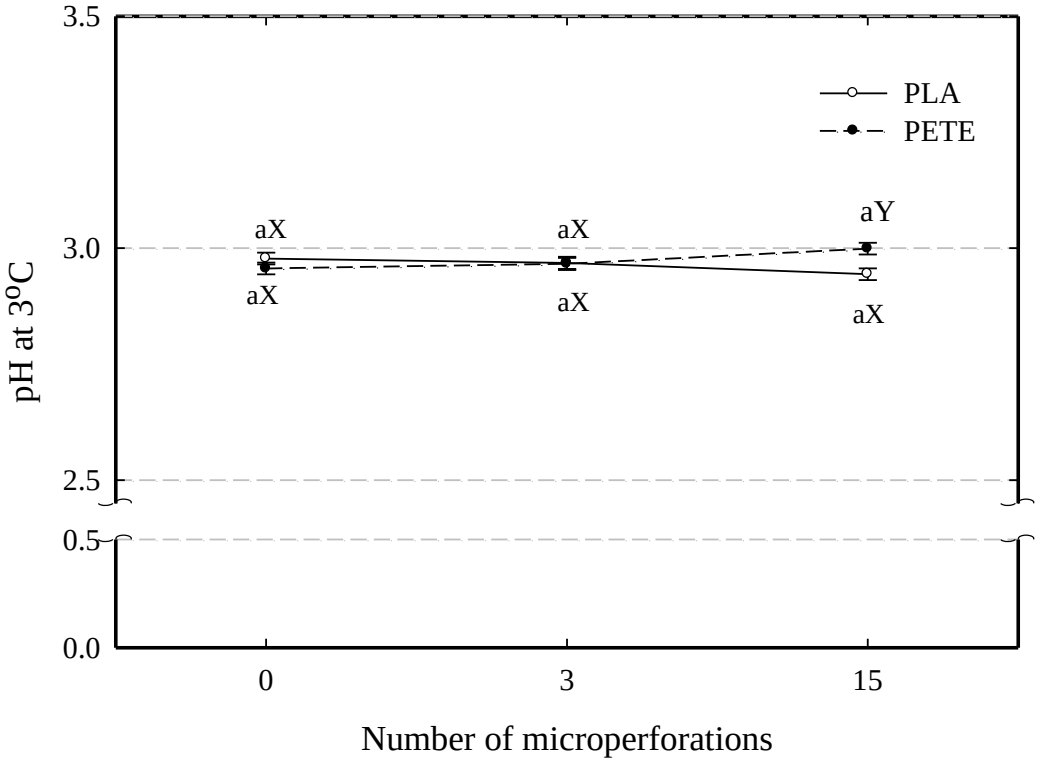


Figure 4-13b: The interaction effect of materials, and perforations on pH of blueberries stored at 3°C during 33 days. (a, b) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

4.4.2 pH at 10°C

The evolution of pH was seen fluctuating within the range of 3.18 to 3.26 at 10°C (Figure 4-14a). The interaction effect between materials, and perforations is shown in Figure 4-14b. Blueberries packed in PLA showed increasing value of pH with increasing number of perforations, while pH of blueberries packed in PET packages declined in the same order. Differences between 0, and 15 perforations of PLA on pH values were significant. The effect of materials with 15 perforations on pH was significant, similar to

the results obtained at 3°C (Figure 4-14b). Materials, and days effect on pH of blueberries was found significant. Both materials showed similar fluctuation pattern during 20 days (Figure not shown).

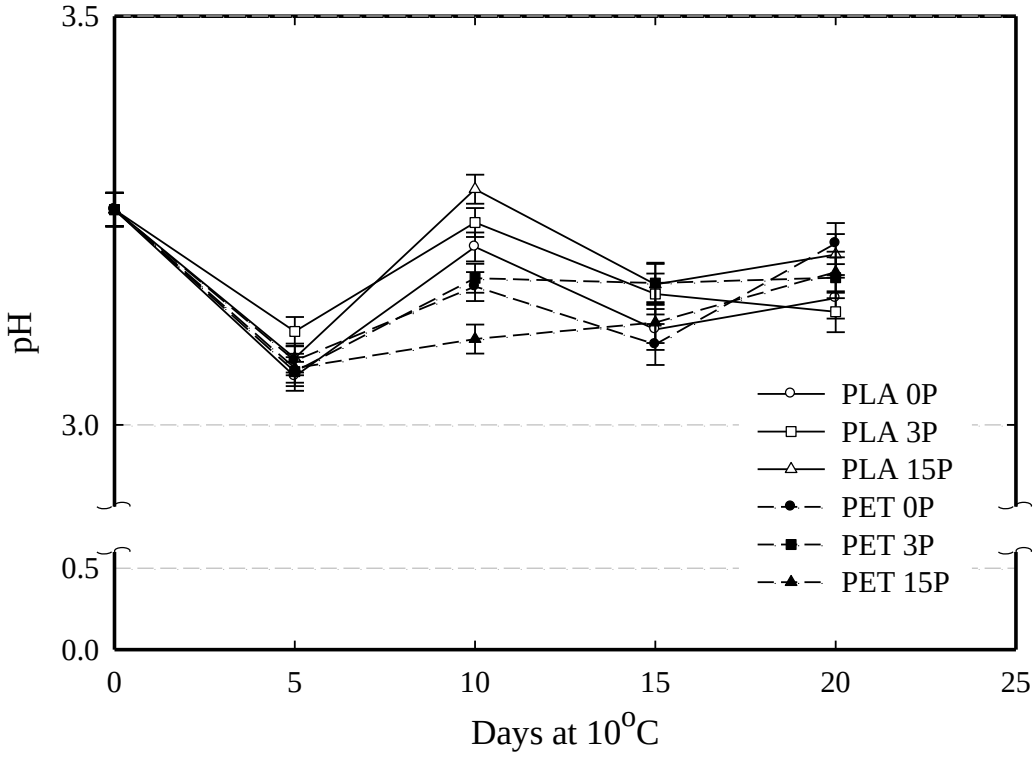


Figure 4-14a: The evolution of pH when stored at 10°C during 20 days of storage.

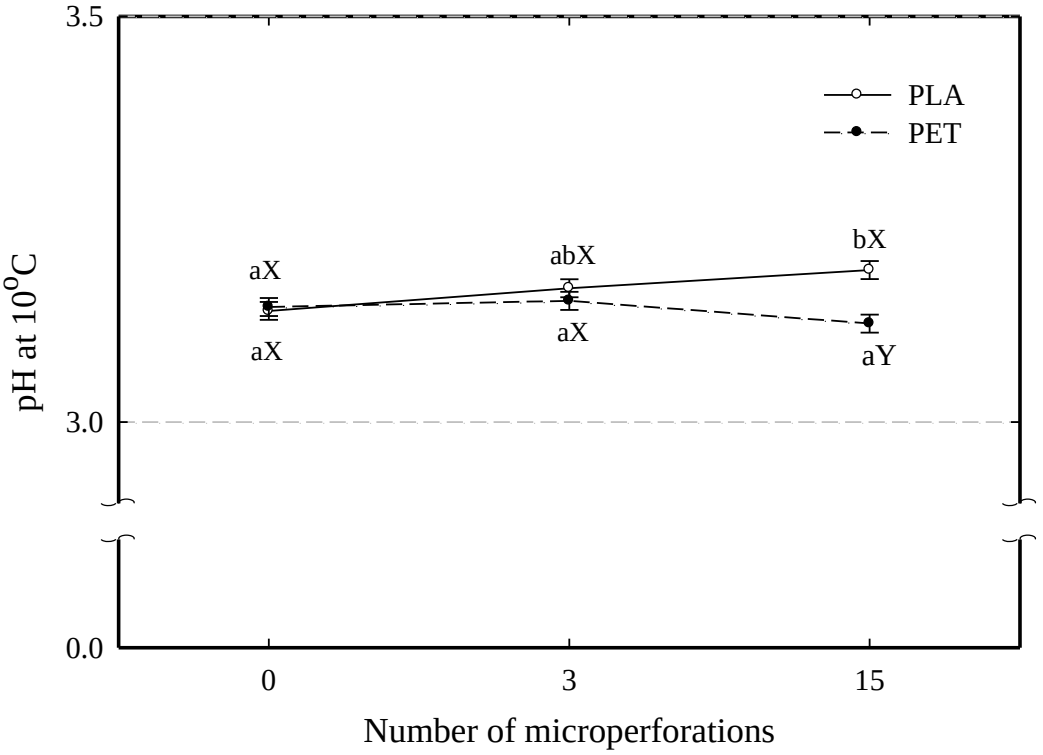


Figure 4-14b: The interaction effect of materials, and perforations on pH of blueberries stored at 10°C during 20 days. (a, b) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

4.4.3 pH at 23°C

Figure 4-15 showed the evolution of pH of blueberries in non-perforated, and perforated PLA, and PET packages at 23°C. The pH values remained constant within 3.2 to 3.27 for 9 days, which was resemblance to the results obtained at 3, and 10°C. Materials were found to have a highly significant effect on blueberries pH. All in all, the evolution of pH was consistent throughout storage time regardless of temperatures.

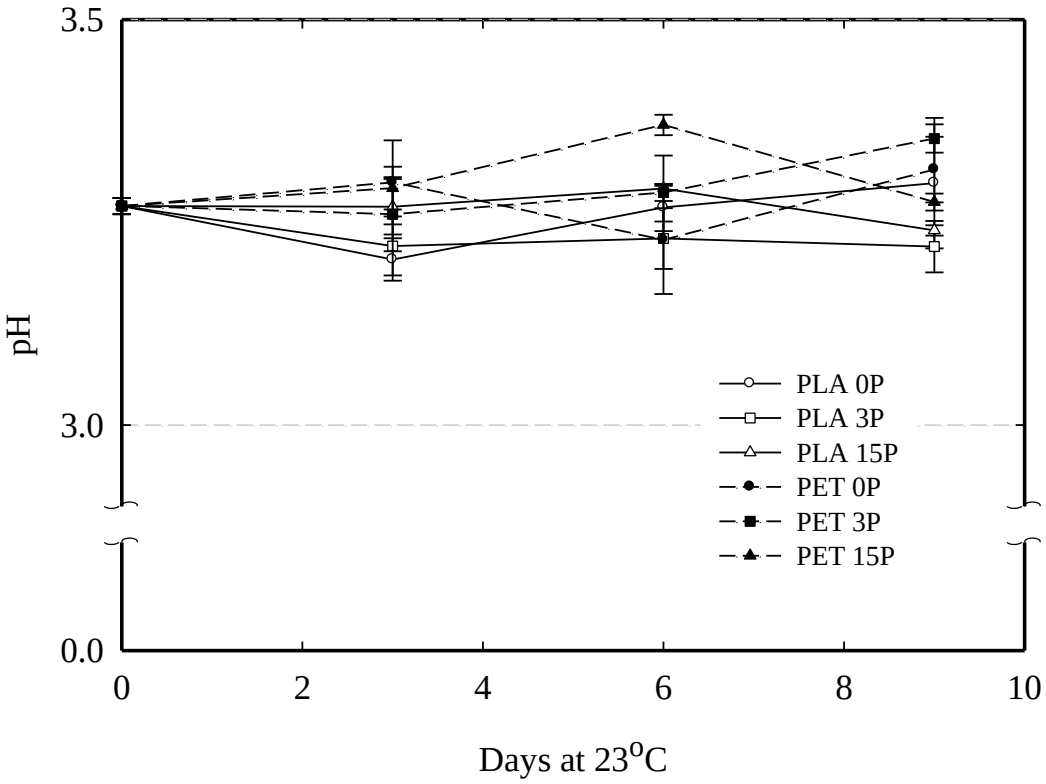


Figure 4-15: The evolution of pH when stored at 23°C during 9 days of storage.

4.5 Soluble solid contents (SSC)

4.5.1 SSC at 3°C

As can be seen in Figure 4-16a, soluble solid contents of blueberries in all packages at 3°C declined slightly through the end of storage. This decrease could be associated to the consumption of soluble solids of the fruits due to respiratory activity. Blueberries respire, and continue to ripen over time. This continuous metabolic process required energy which is generated by oxidative breakdown of complex substrates such as starch, sugar, and organic acids. The decrease in SSC value was also reported for 'Burlington' blueberries (Forney, Jordan & Nicholas, 2003). There was no significant different in SSC value between perforations for PLA. In terms of PET, the value of SSC

obtained for 0 perforation was not significantly different with 3 perforation, but 15 perforations was. The difference in SSC between materials within perforations was noticeable for 15 perforations (Figure 4-16b). The value of SSC obtained for all packages at day 33 shows significant reduction than the other analyzed days.

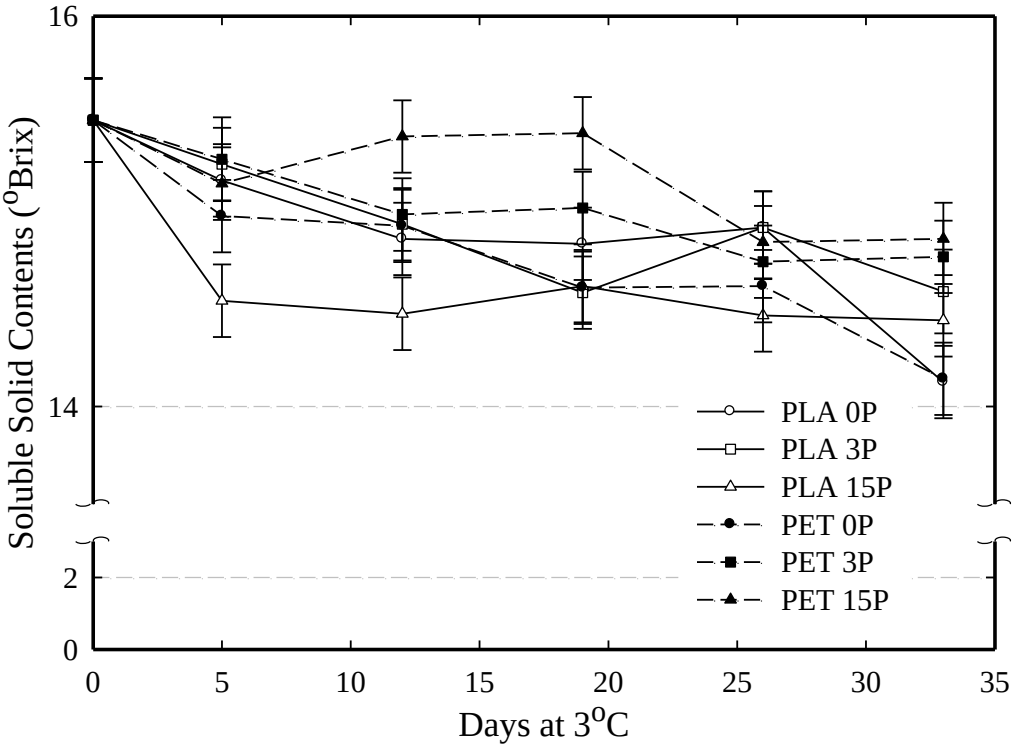


Figure 4-16a: The evolution of SSC when stored at 3°C during 33 days of storage.

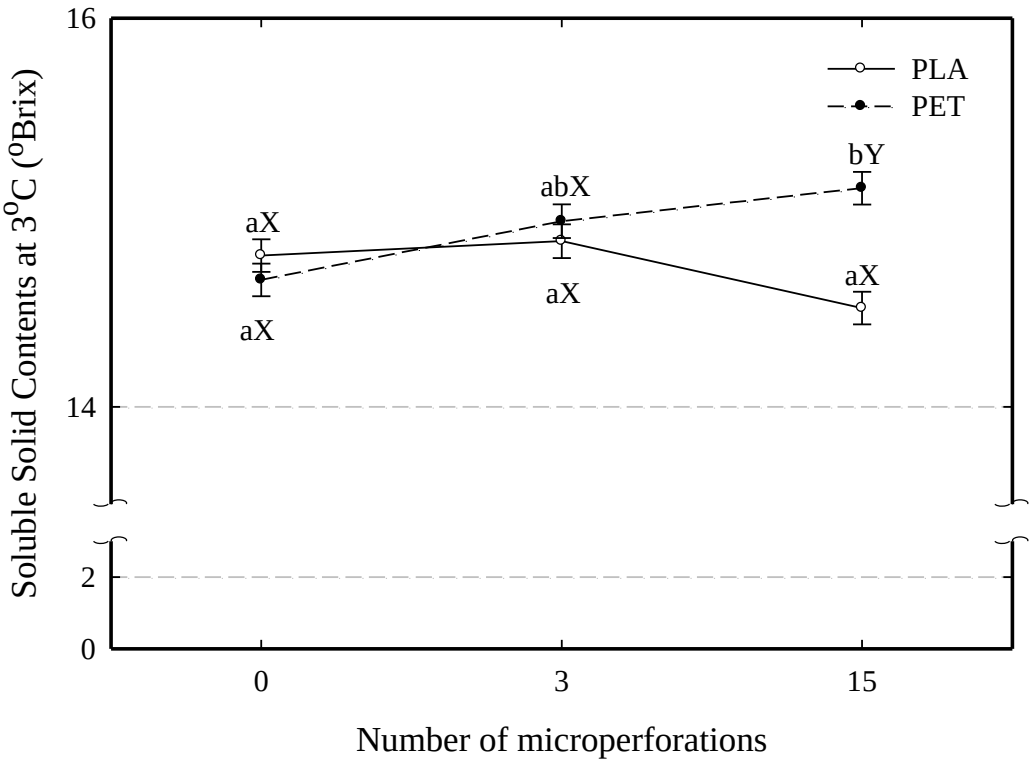


Figure 4-16b: The interaction effect of materials, and perforations on SSC of blueberries stored at 3°C during 33 days. (a, b, c) Differences between perforations within materials ($p<0.05$); (X, Y) Differences between materials within perforations ($p<0.05$).

4.5.2 SSC at 10°C

SSC of blueberries packed in all packages at 10°C was considerably consistent throughout storage (Figure 4-17a). However, PET without perforation had significantly less SSC than PET with 3, and 15 perforations. No differences were observed in SSC of blueberries between perforations of PLA (Figure 4-17b). 'Elliot' blueberries packed in PLA containers were reported to have a constant evolution of SSC during storage at similar temperature (Almenar et al., 2008b).

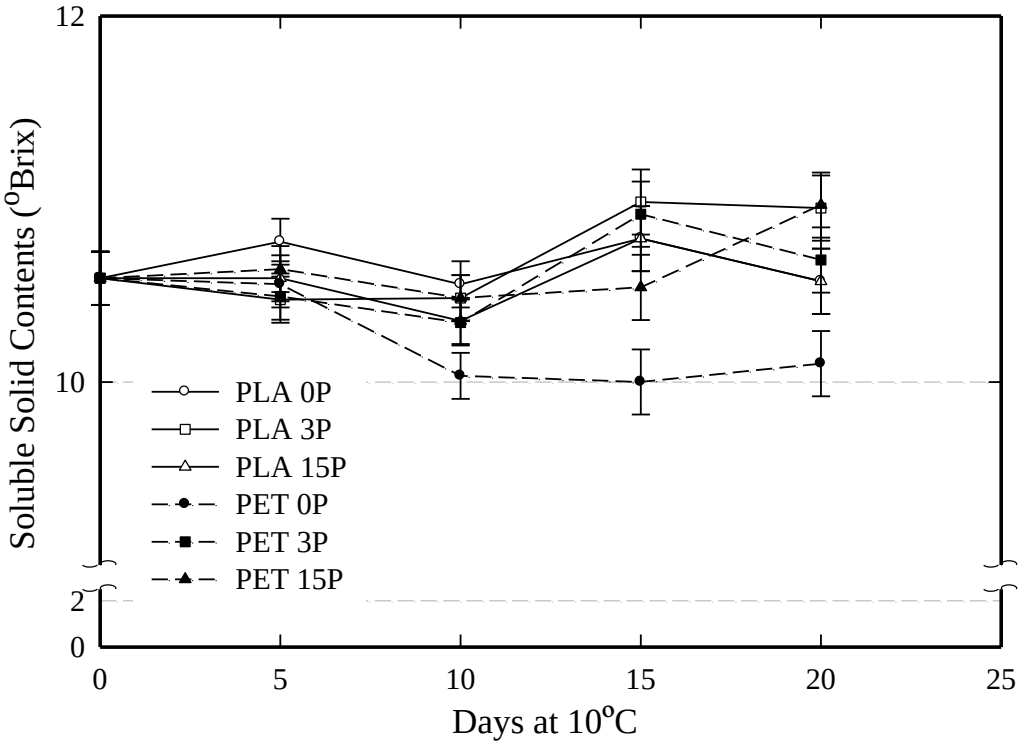


Figure 4-17a: The evolution of SSC when stored at 10°C during 20 days of storage.

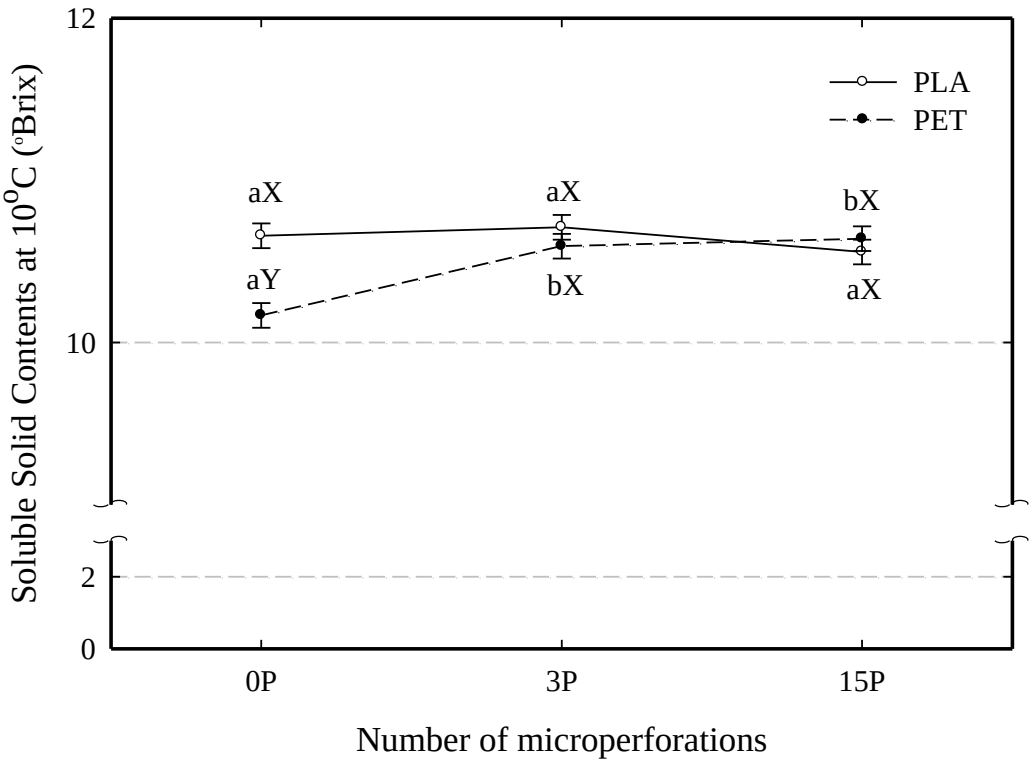


Figure 4-17b: The interaction effect of materials, and perforations on SSC of blueberries stored at 10°C during 20 days. (a, b) Differences between perforations within materials ($p<0.05$);(X, Y) Differences between materials within perforations ($p<0.05$).

4.5.3 SSC at 23°C

Figure 4-18a shows the evolution of SSC of blueberries packed in all packages at 23°C. The evolution of SSC at this temperature was similar to SSC obtained at 3°C. SSC of blueberries in all packages decreased towards the end of storage time. Almenar et al. (2008b) reported that SSC of blueberries packed in PLA container showed a decrease of 5% at the same storage temperature (Almenar et al., 2008b). The evolution of SSC was significantly different between PLA, and PET ($P<0.05$). Packages with 0 perforation had greater reduction in SSC than packages with 3, and 15 perforations during 9 days of

storage (Figure 4-18b). This could be related to the fast rate of oxidation breakdown because of higher storage temperature. The level of gases developed in the package headspace could also be the reason since high concentration of the gases induces stress in fruit in which affecting metabolic activity of fruits. Evidently, the amount of CO_2 in non-perforated packages was found greater than perforated packages.

SSC values obtained at 3, 10, and 23°C varied from 14.1-15.5 °Brix, 10-11 °Brix, and 13-14.5 °Brix, respectively. Saftner et al.(2008) reported SSC value of 11.5 for 'Bluecrop' blueberries. Kader (1999) proposed a value of 10 as a minimum SSC that is acceptable for blueberries flavor. This value was assured based on the majority of consumers. Therefore, it could be expected that the flavor of blueberries packaged in PLA, and PET 0, 3 and 15 perforations would still be acceptable for an extended time based on the SSC results.

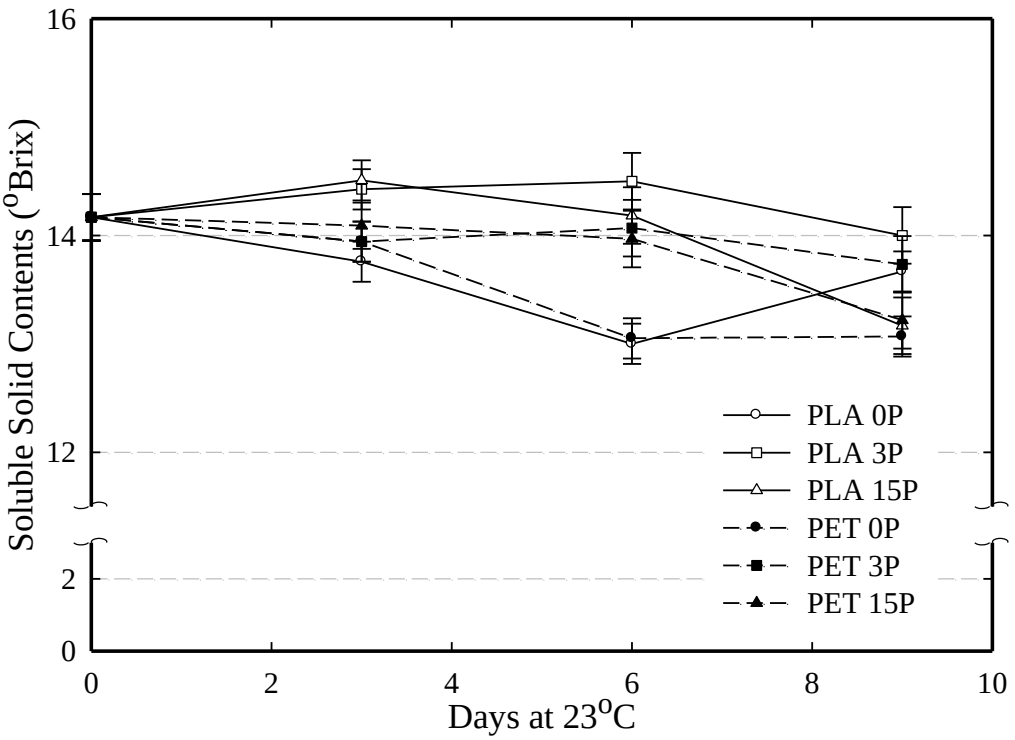


Figure 4-18a: The evolution of SSC when stored at 23°C during 9 days of storage.

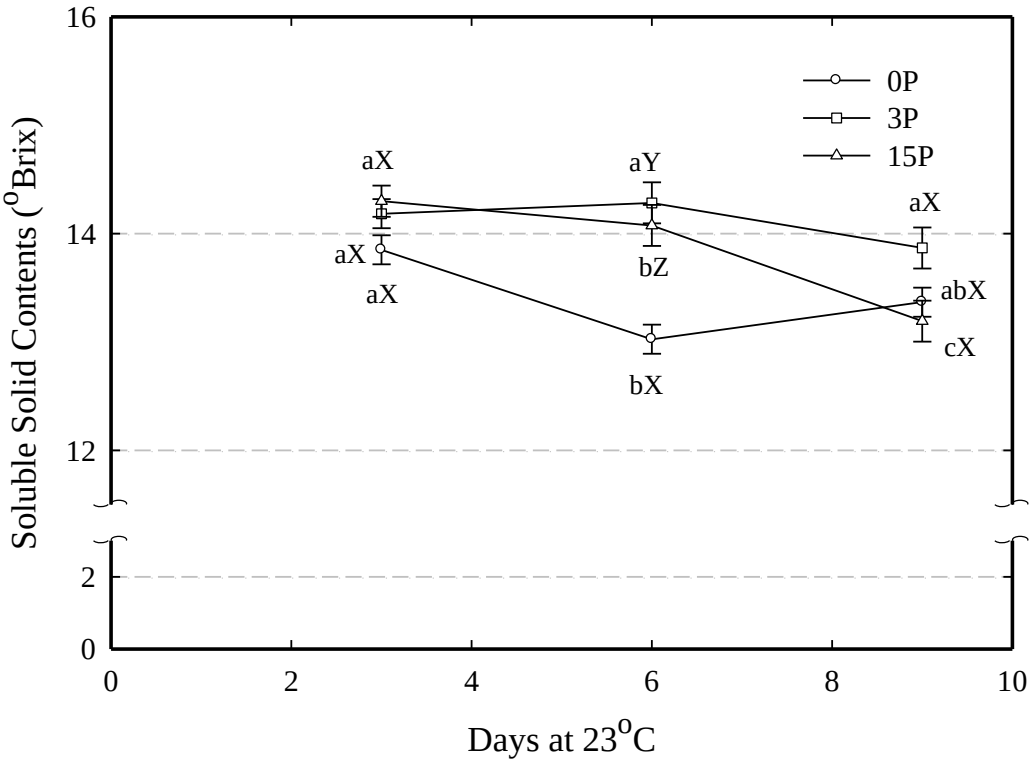


Figure 4-18b: The interaction effect of perforations, and days on SSC of blueberries stored at 3°C during 33 days. (a, b) Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.6 Off-flavors and aroma

Blueberries' taste and odor are the result of a complex multi-component relationship of many aromatic constituents (Almenar et al., 2008b). These compounds could be esters, alcohols, aldehydes and/or ketones. There are a lots of aroma compounds that being identified as the vital contributor to the aroma profile of blueberries such as 2(E)-Hexenal, linalool, nonanal, benzaldehyde, 2-ethyl-1-hexanol, to name a few. The presence and generation of these compounds however, often being regarded as cultivar, and atmosphere and storage condition dependent, respectively. These constituents when presence at optimum level provides desirable sensorial properties for consumers.

However, there are some constituents for instance, ethanol, ethyl acetate, and acetaldehydes that when their concentration is accumulated beyond the threshold level, they would result in the formation of fermentative metabolites (off-flavor).

In this study, six typical blueberries aroma compounds: ethanol, ethyl acetate, hexanal, 2(E)-Hexenal, 1-Hexanol, and linalool were analyzed. Figure 4-19 shows the chromatogram of these six compounds.

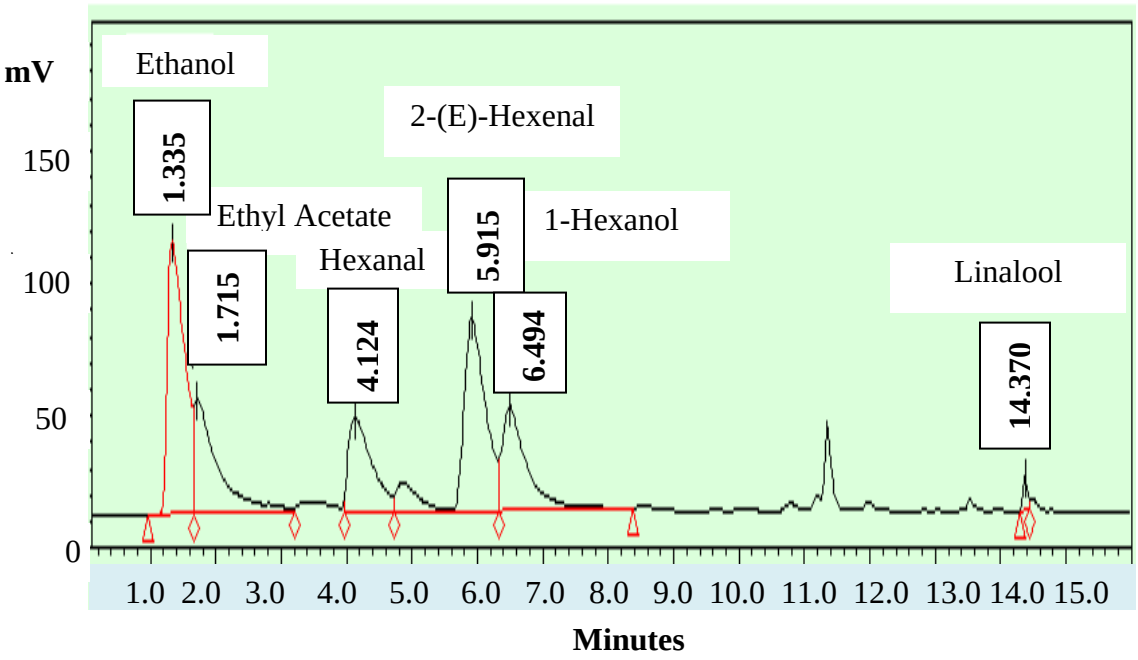


Figure 4-19: Gas chromatography of selected aroma compounds of blueberries.

4.6.1 Off-flavors (fermentative metabolites)

4.6.1.1 Ethanol at 3°C

Ethanol is an immediate product of acetaldehyde via the reaction of enzyme alcohol dehydrogenase (ADH). This compound exists naturally in fruits mainly due to the ripening process and its level could be increased as a results of prolong storage or an

exposure to anaerobic environment (low level of O_2 and/or high level of CO_2). Ethanol is also precursors of natural aroma compounds (Pesis, 2005). Figure 4-20a shows the accumulation of ethanol throughout storage at 3°C during 33 days expressed in chromatographic area (c.a). Non-perforated PLA and PET packages had the highest development of ethanol compared to PLA and PET packages with 3 and 15 perforations (4.3×10^5 to 7.5×10^6 c.a). This result was expected since the non-perforated packages had the higher evolution of CO_2 level up to 60% during storage. The riched-CO_2 atmosphere has been associated with the induction of ethanol generation (Almenar, Hernández-Muñoz, J.M. Lagarón & Gavara, 2006). The evolution of ethanol in perforated PLA and PET packages were constant from day 0 to the last day of the storage within the range of 4.3×10^5 to 7.2×10^5 c.a. The perforation and days interaction was found significant. The packages without perforation were statistically different than packages with 3 and 15 perforations regardless of materials (Figure 4-20b).

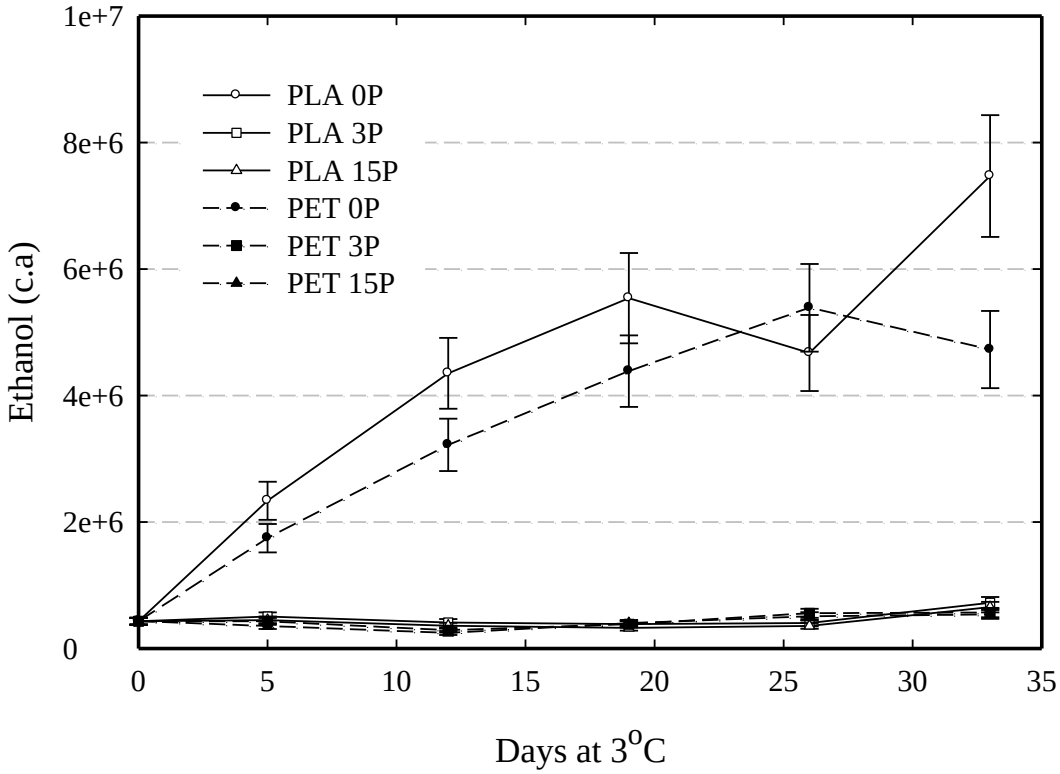


Figure 4-20a: The evolution of ethanol in the blueberries in different packages stored at 3°C during 33 days of storage.

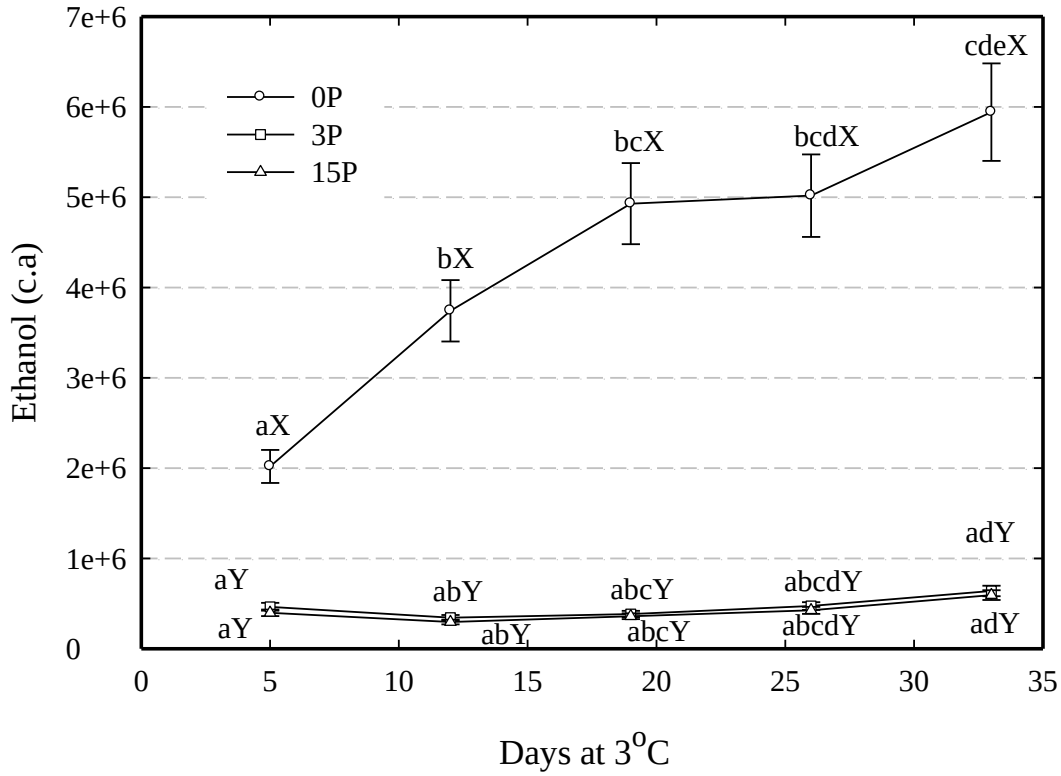


Figure 4-20b: The interaction effect of perforations and days on ethanol of blueberries stored at 3°C during 33 days.(a, b,c,d,e) Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.6.1.2 Ethanol at 10°C

Figure 4-21a shows the development of ethanol in PLA and PET packages without perforation and with 3 and 15 perforations during 20 days of storage at 10°C. The result at this temperature shows similar trend as observed at 3°C. Non-perforated PLA and PET packages accumulated higher amount of ethanol than that of packages with 3 and 15 perforations (Figure 4-21b). These packages also had the highest level of CO_2 (98-100%) in comparison with packages of 3 and 15 perforations (6-13% CO_2).

However, there were no noticeable differences that can be found in the ethanol evolution during storage between these two temperatures (3 versus 10°C). This result was not expected since in general, the higher the temperature, the higher the volatile production would be. In addition, the level of ethanol of blueberries packaged in non-perforated PLA packages was found higher (3.0×10^6 c.a) than blueberries packaged in rigid PLA containers ($3.0 – 4.0 \times 10^5$ c.a) at the same temperature at day 15 by Almenar et al. (2008b). This huge difference could be related to the difference in cultivar ('Bluecrop' versus 'Elliot') and also could be due to the difference between the packages used (packages sealed with film versus snap-fit container).

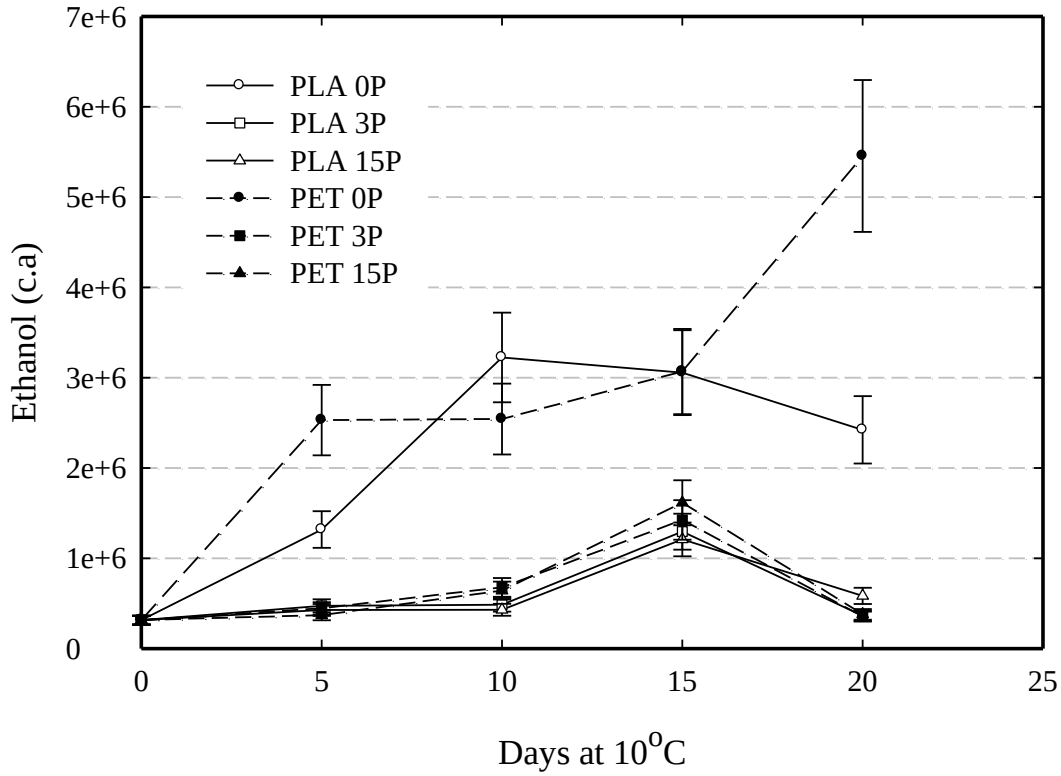


Figure 4-21a: The evolution of ethanol in the blueberries in different packages stored at $10^{\circ}C$ during 20 days of storage.

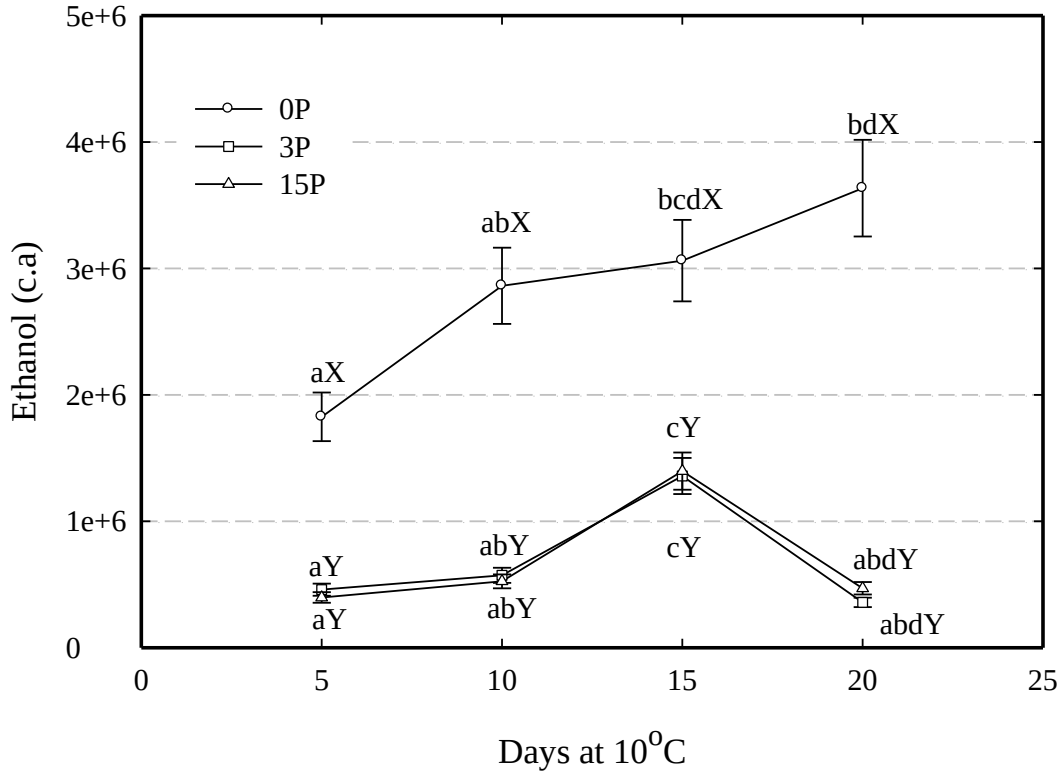


Figure 4-21 b: The interaction effect of perforations, and days on ethanol of blueberries stored at 10°C during 20 days. (a, b,c,d). Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.6.1.3 Ethanol at 23°C

The evolution of ethanol at 23°C for blueberries packaged in PLA and PET with 0, 3, and 15 perforations during 9 days of storage is shown in Figure 4-22. The main effect of days and perforations were statistically significant. The evolution of ethanol was higher for non-perforated packages, followed by packages with 3 perforations and 15 perforations. These results were found consistent with decreasing level of CO_2 obtained for those packages in the same order (0P up to 100% CO_2; 3P up to 71% CO_2; 15P up to

12% CO_2). The evolution of ethanol was also found significantly higher with days of storage. The effect of high temperature was seen to increase the accumulation of ethanol to greater extent in comparison to the results obtained at refrigerated temperatures (3 and 10°C).

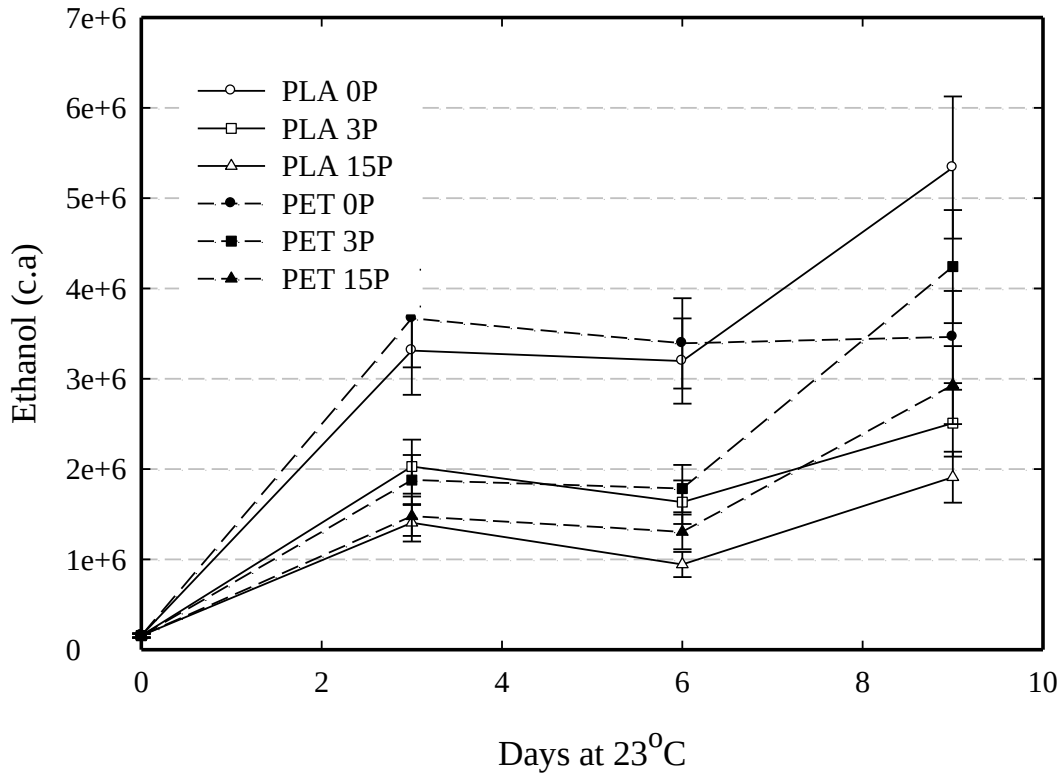


Figure 4-22: The evolution of ethanol in the blueberries in different packages stored at 23°C during 9 days of storage.

4.6.2.1 Ethyl acetate at 3°C

Ethyl acetate is formed by utilizing ethanol and acetyl coenzyme A (CoA) as substrates via an esterification reaction catalyzed by the enzyme alcohol acetyltransferase (AAT). The concentration of this compound depends on both AAT activity and ethanol

concentration (Ke, Zhou & Kader, 1994). Ethyl acetate is one of the compounds that contribute towards the development of off-flavor in blueberries. It was observed that PLA and PET packages without perforations showed higher evolution of ethyl acetate compared to the packages with 3 and 15 perforations at 3°C during 33 days of storage (Figure 4-23a). This result was expected since the production of ethanol was also higher for the same packages at the same temperature. According to Ke, Zhou and Kader (1994), the presence of higher amount of ethanol contributes to an increase in the production of ethyl acetate. In the presence of high levels of ethanol, the synthesis of ethyl esters is more preferable as opposed to other esters due to the activity of the AAT that apparently depends on substrate availability rather than on substrate specificity (Pelayo, Ebeler & Kader, 2003). The evolution of ethyl acetate of blueberries packaged in PLA and PET with 3 and 15 perforations started at relatively low amount at day 5 and had been constant throughout 33 days of storage. Material and days interaction was found significant for ethyl acetate evolution particularly among days within the same materials (Figure 4-23b).

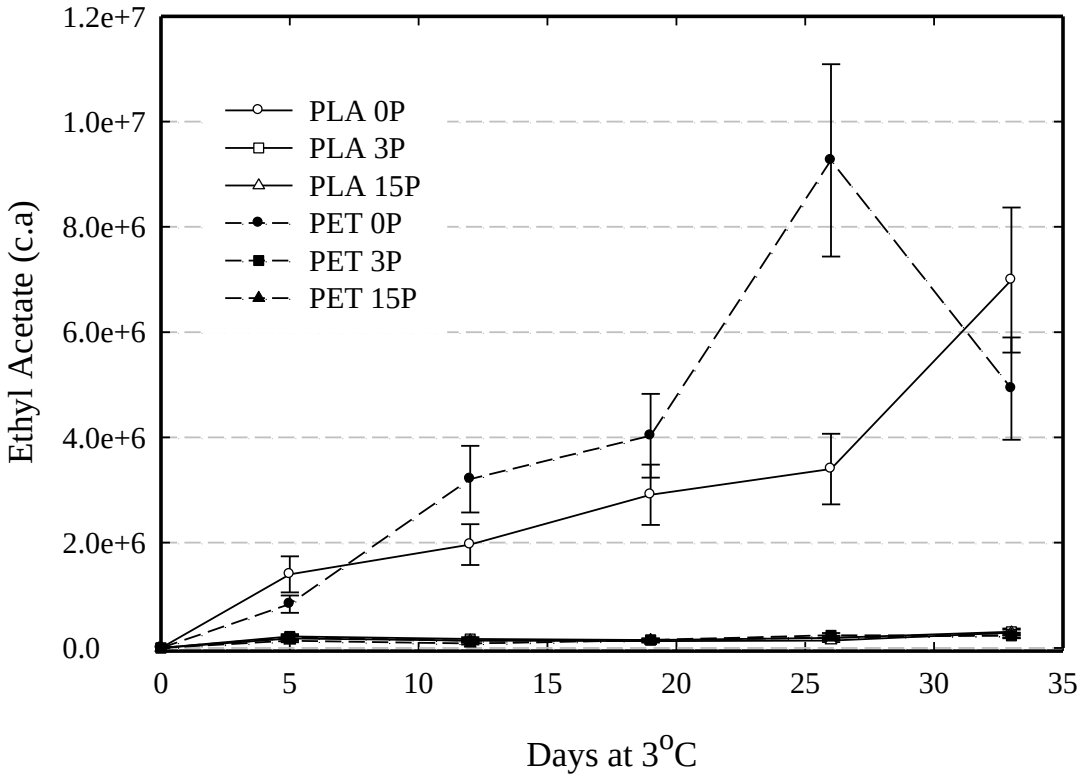


Figure 4-23a: The evolution of ethyl acetate in the blueberries in different packages stored at 3°C during 33 days of storage.

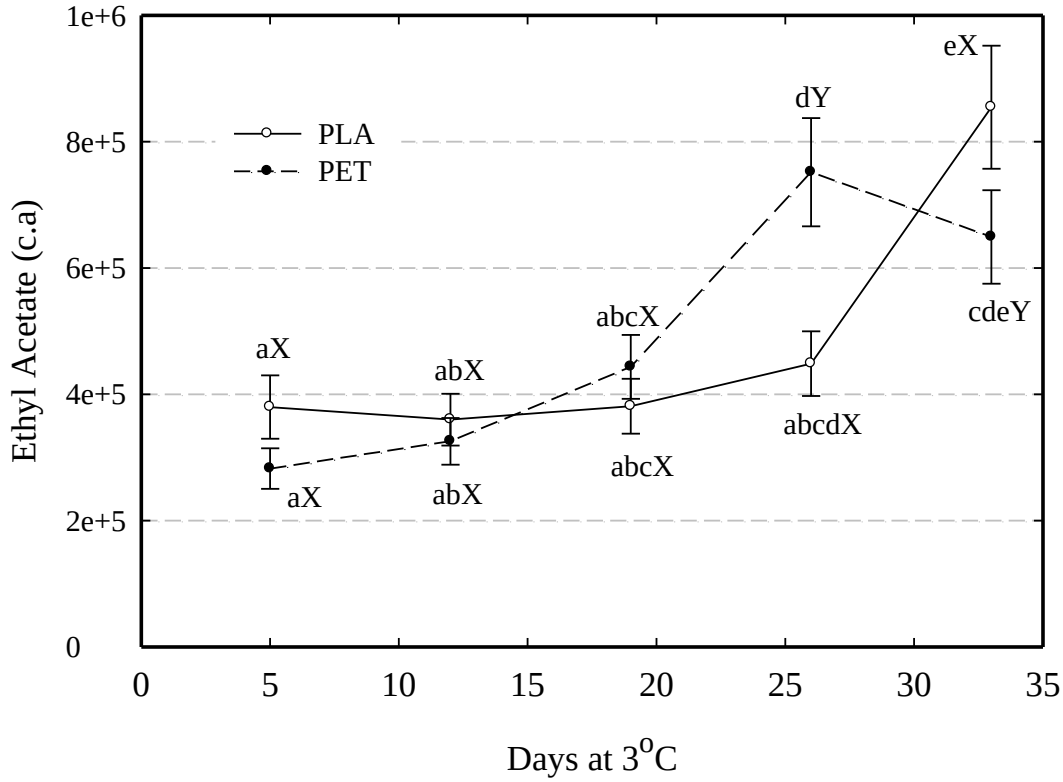


Figure 4-23b: The interaction effect of materials, and days on ethyl acetate of blueberries stored at 3°C during 33 days.(a,b,c,d,e). Differences between days within materials ($p<0.05$); (X, Y) Differences between materials within days ($p<0.05$).

4.6.2.2 Ethyl acetate at 10°C

The evolution of ethyl acetate at 10°C during 20 days of storage is presented in Table 4-1. This result indicated that the evolution of ethyl acetate was slow since this compound was not detected until the last day of storage. PLA package with 3 perforations and PET package with 15 perforations did not show any evolution of ethyl acetate during 20 days of storage. The levels of ethyl acetate of blueberries in PLA and PET packages with 15 and 3 perforations, respectively, started at a relatively lower value compared to non-perforated packages at day 20. However, the levels of ethyl acetate was

conclusive with the levels of CO_2 at 10°C, in which the higher the level of CO_2, the greater the level of this compound was.

Table 4-1: The evolution of ethyl acetate in the blueberries in different packages stored at 10°C during 20 days of storage.

PACKAGES	**Days at 10°C**				
	0	**5**	**10**	**15**	**20**
PLA 0P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1213840.6722 ± 121019a
PLA 3P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PLA 15P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	241107.9409 ± 24038b
PET 0P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2073399.316 ± 206716a
PET 3P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	115162.5629 ± 11482bc
PET 15P	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Note: Values expressed are in chromatographic area (c.a) unit.

4.6.2.3 Ethyl acetate at 23°C

Figure 4-24a shows the evolution of ethyl acetate of blueberries packaged in PLA and PET packages with 0, 3, and 15 perforations at 23°C during 9 days of storage. The production of ethyl acetate was observed to start at day 6 for all types of packages. The perforations and days interaction was found significant ($p<0.05$). At day 6 the packages

of the same perforation was significantly different, however at day 9, there was no differences observed between packages with 3 and 15 perforations but there was for non-perforated packages. The packages without perforation had the highest levels of ethyl acetate over the other two temperatures (3 and 10°C). This was because the higher the temperature, the more the accumulation of CO_2 levels would be, which in turns, increase the production of volatile compounds.

The evolution of fermentative metabolites in fruits is not desirable since it changes the flavor profile, thus reducing the organoleptic properties. Three main aroma compounds that often denoted to the off-flavor development are acetaldehyde, ethanol, and ethyl acetate. In fruits, acetaldehyde is produced from pyruvate by the pyruvate decarboxylase enzyme (PDC). Through the action of enzyme alcohol dehydrogenase (ADH) and enzyme aldehyde dehydrogenase (ALDH), ethanol and acetyl coenzyme A (CoA) are formed, respectively. As mentioned earlier, these two latter compounds are responsible in the formation of ethyl acetate via the action of enzyme alcohol acetyltransferase (AAT) in the esterification process (Pesis, 2005). In addition, the development of off-flavor is strongly influenced by ethanol, followed by ethyl acetate and acetaldehyde accumulation (Almenar et al., 2007a; Ke, Goldstein, O'Mahony & Kader, 1991).

In this study, there were only two fermentative metabolites observed (ethanol and ethyl acetate). It was found that the greater the storage temperature, the higher the level of these compounds obtained with an exceptional to 10°C. As supported by literature, the

accumulation of ethanol and ethyl acetate was favored by elevated CO_2 level throughout storage for blueberries at all temperatures.

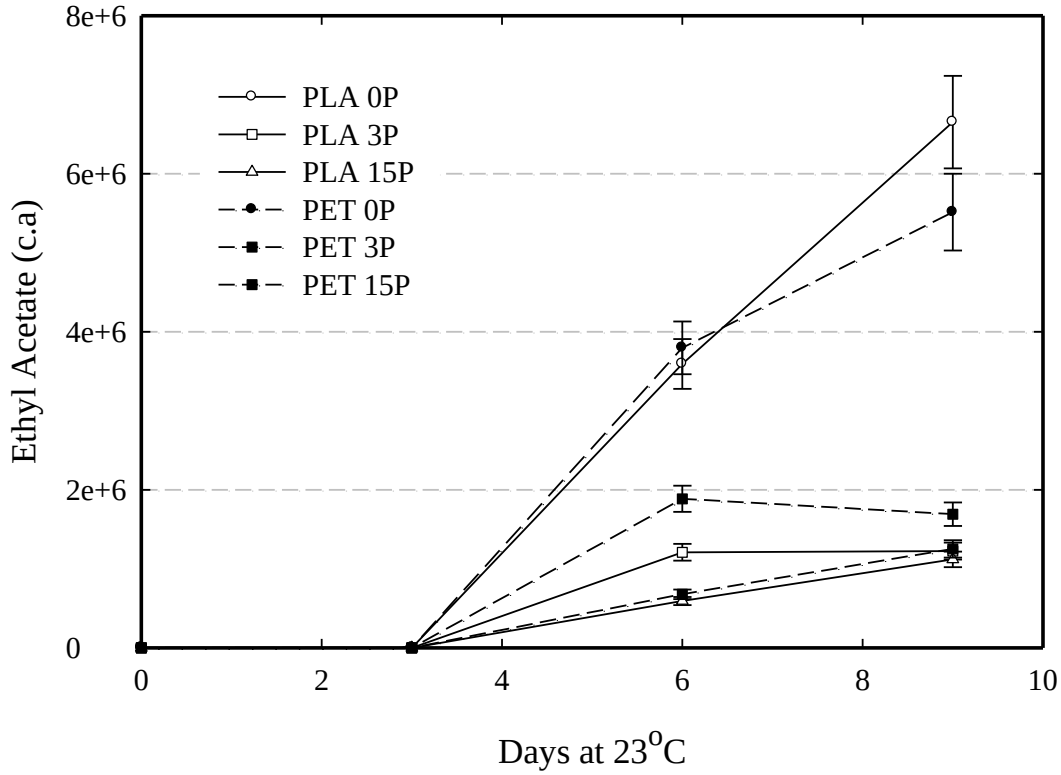


Figure 4-24a: The evolution of ethyl acetate in the blueberries in different packages stored at 23°C during 9 days of storage.

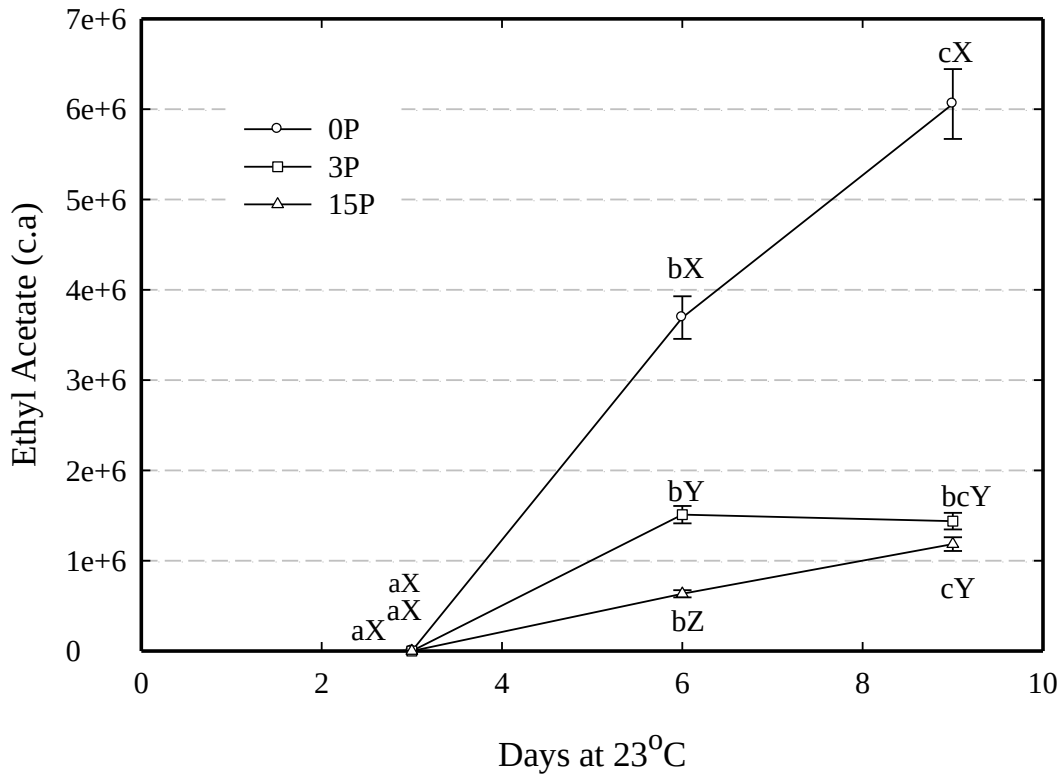


Figure 4-24b: The interaction effect of perforations, and days on ethyl acetate of blueberries stored at 23°C during 9 days.(a, b,c) Differences between days within perforations ($p<0.05$); (X, Y,Z) Differences between perforations within days ($p<0.05$).

4.6.2 Aroma compounds

4.6.2.1.1 Hexanal at 3°C

Hexanal is always associated with rancidity flavor. However, this compound is one of the typical aroma compounds found in blueberries. It is produced through the lipoxygenase-lyase oxidation of linoleic and linolenic acids (Almenar, Hernandez-Munoz & Gavara, 2009). Figure 4-25 shows the evolution of hexanal during 33 days of storage at 3°C. The main effect of perforation was found significant, of which the packages without perforation had lower evolution of hexanal in comparison with perforated

packages. The level of hexanal was also observed remained within the same range during the storage time.

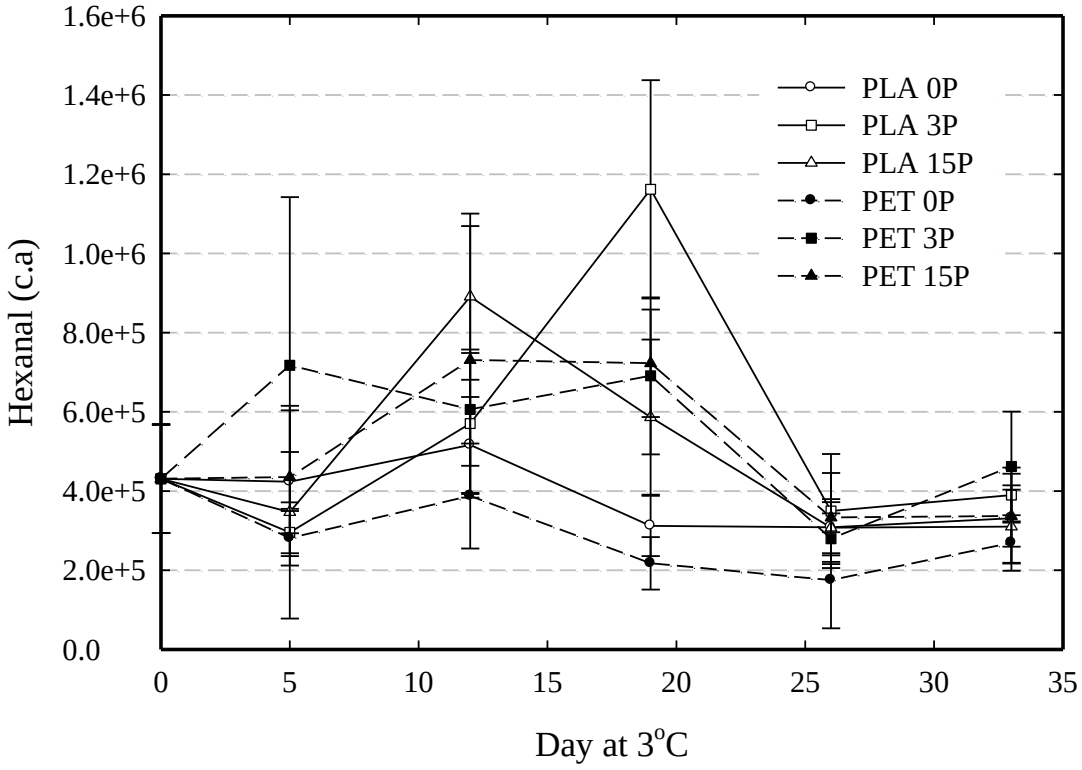


Figure 4-25: The evolution of hexanal in the blueberries in different packages stored at 3°C during 33 days of storage.

4.6.2.1.2 Hexanal at 10°C

Elevation of hexanal level during 20 days of storage at 10°C can be observed from Figure 4-26a. The accumulation of hexanal of blueberries in all packages was seen constant for the first 15 days (4.0×10^5 to 5.5×10^5 c.a) before increased to 2.0×10^6 c.a. Figure 4-26b indicated that the level of hexanal increased with increasing storage time within the same perforation. There was also no effect of perforation found on the

evolution of hexanal at this temperature. Interestingly, the evolution of hexanal at this temperature was slightly greater than that of 3°C, as opposed to the evolution of fermentative metabolites of 3 and 10°C. Moreover, Almenar et al. (2008b) reported that the level of hexanal of blueberries packaged in rigid PLA containers was approximately 2.0×10^6 c.a at 10°C at day 15 (Almenar et al., 2008b). This result was comparatively higher than the ones obtained in this study, of which the hexanal level of blueberries in non-perforated PLA packages was 7.5×10^5 c.a. This may be due to differences between varieties.

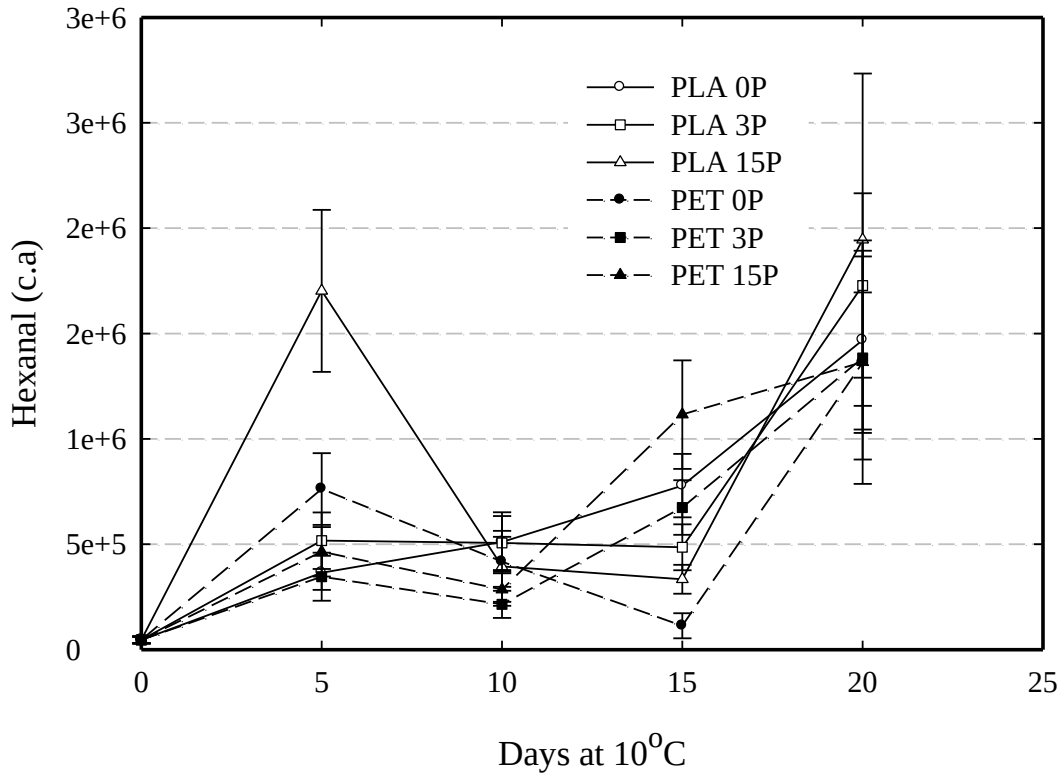


Figure 4-26a: The evolution of hexanal in the blueberries in different packages stored at 10°C during 20 days of storage.

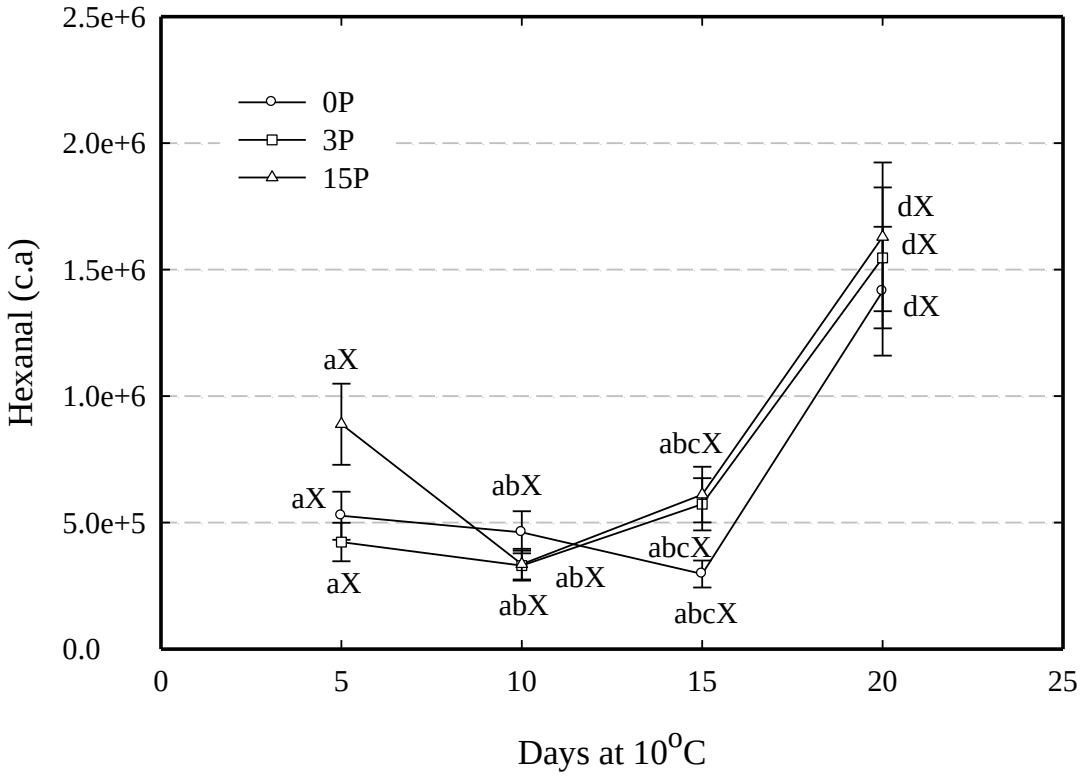


Figure 4-26b: The interaction effect of perforations, and days on hexanal of blueberries stored at 10°C during 20 days.(a, b,c,d) Differences between days within perforations ($p<0.05$); (X, Y) Differences between perforations within days ($p<0.05$).

4.6.2.1.3 Hexanal at 23°C

Figure 4-27 shows the evolution of hexanal of blueberries packaged in PLA and PET packages with 0, 3, and 15 perforations during 9 days of storage at 23°C. As can be seen, there was no noticeable differences in the level of hexanal during storage as it remained constant for all types of packages. It can also be concluded that the effect of temperature on the evolution of hexanal of blueberries in all packages was not pronounced since the results indicated that at day 5 of 3 and 10°C and day 6 of 23°C, the level of hexanal was found closely related to each other.

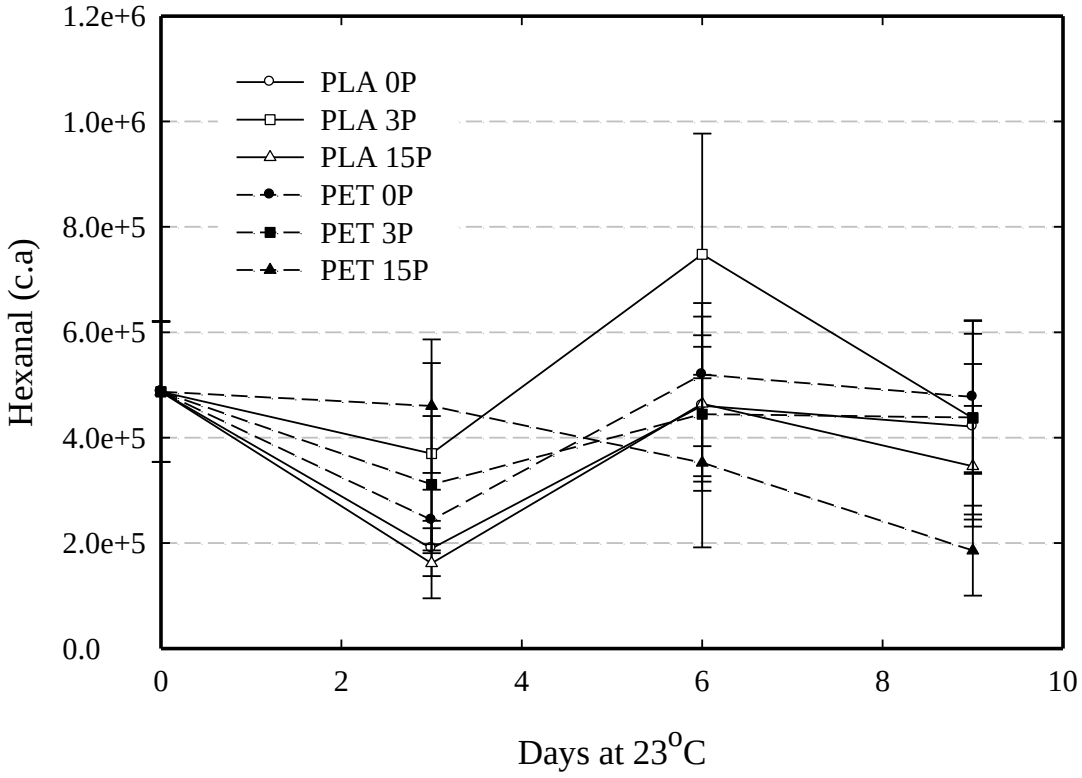


Figure 4-27: The evolution of hexanal in the blueberries in different packages stored at 23°C during 9 days of storage.

4.6.2.2.1 2(E)-Hexenal at 3°C

2(E)-Hexenal is one of the naturally-occurring important aroma compounds that contribute to pleasant blueberries flavor. Figure 4-28a shows the evolution of 2(E)-Hexenal compounds of blueberries packaged in non-perforated and perforated (3 and 15 perforations) PLA and PET packages at 3°C during 33 days of storage. The level of this compound was found higher for blueberries packaged in non-perforated PLA and PET packages. On the other hand, the evolution of this volatile was consistent from day 0 to day 33 for blueberries packaged in perforated packages. The effects of perforations

within days on the evolution of this compound can be seen clearly in Figure 4-28b. The level of 2(E)-Hexenal was initially similar at day 5 for all packages.

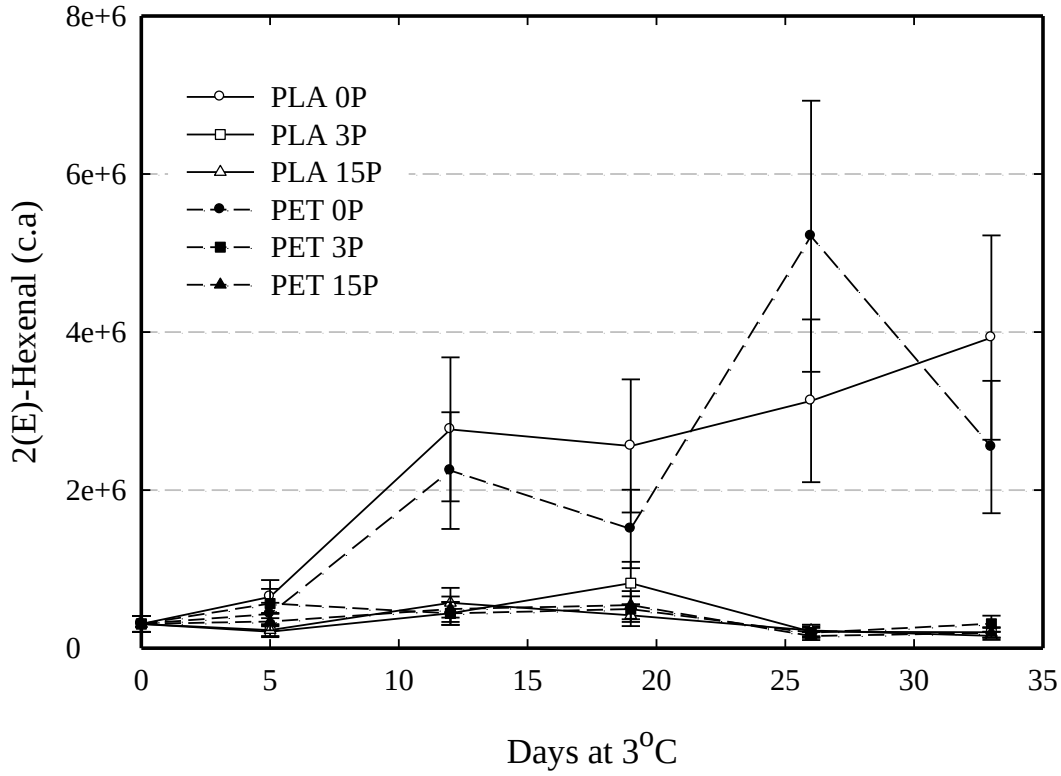


Figure 4-28a: The evolution of 2(E)-Hexenal in the blueberries in different packages stored at 3°C during 33 days of storage.

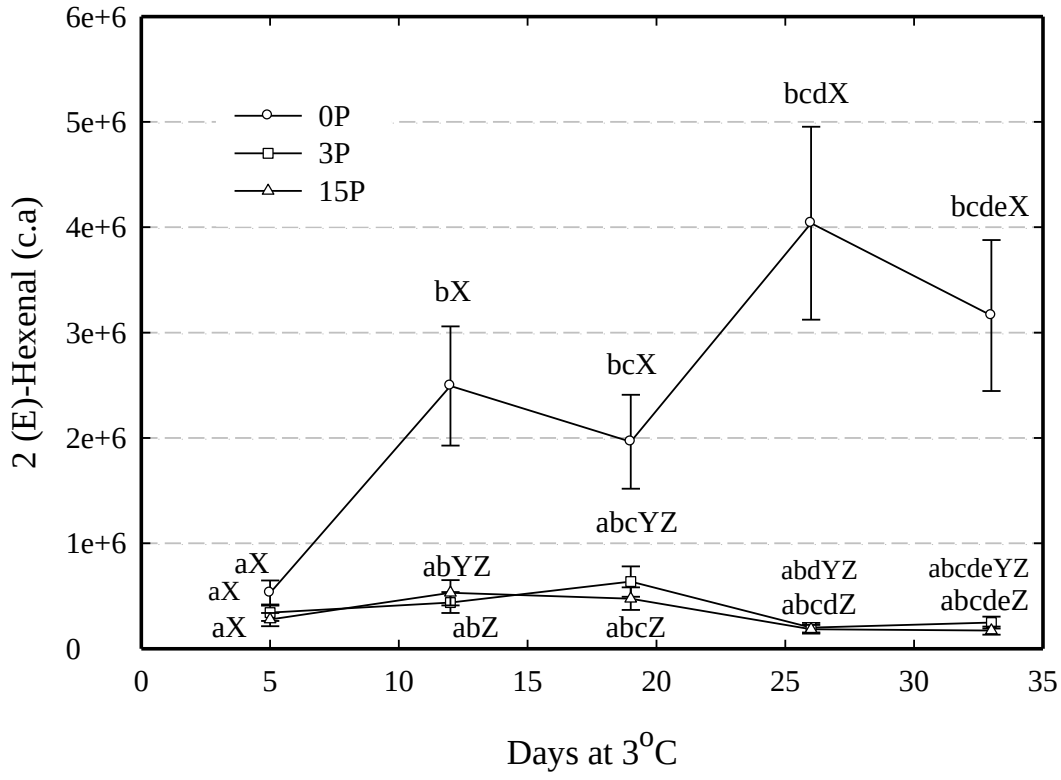


Figure 4-28b: The interaction effect of perforations, and days on 2(E)-Hexenal of blueberries stored at 3°C during 33 days. (a, b, c, d, e) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.6.2.2.2 2 (E)-Hexenal at 10°C

The evolution of 2(E)-Hexenal of blueberries packaged in PLA and PET packages with 0, 3, and 15 perforations at 10°C during 20 days of storage is shown in Figure 4-29a. The level of this compound was approximately similar for blueberries in all perforated packages for the whole storage time. While for PLA and PET packages without perforation, after day 15, an increase in 2(E)-Hexenal level was observed. The interaction effect of perforation and days is also shown in Figure 4-29b, in which at day 20, packages without perforation had significantly higher level of 2(E)-Hexenal

compared to the other perforations. In addition, it can be seen that the level of 2(E)-Hexenal was greater at 10°C (day 20) than it was at 3°C (day 19). These differences were particularly obvious for blueberries packaged in non-perforated packages.

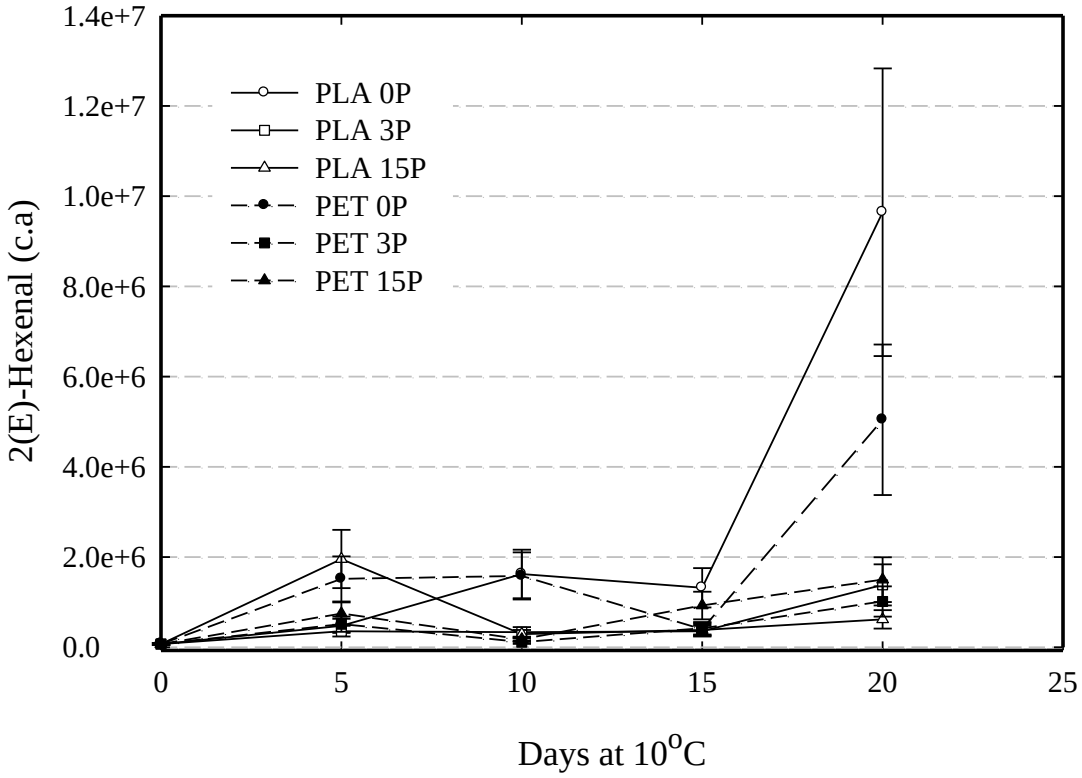


Figure 4-29a: The evolution of 2(E)-Hexenal in the blueberries in different packages stored at 10°C during 20 days of storage.

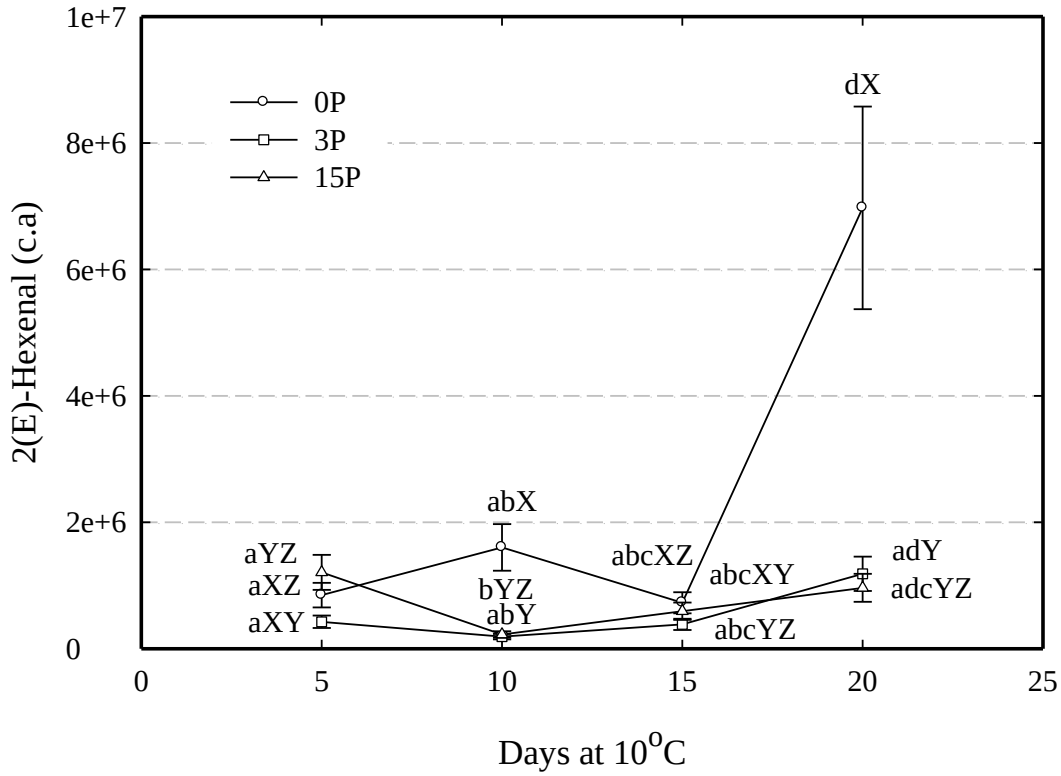


Figure 4-29b: The interaction effect of perforations, and days on 2(E)-Hexenal of blueberries stored at 10°C during 20 days.(a, b, c, d) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.6.2.2.3 2(E)-Hexenal at 23°C

The evolution of 2(E)-Hexenal of blueberries in all packages at 23°C during 9 days of storage was markedly different than the results obtained at 3 and 10°C (Figure 4-30a). The trend of the evolution towards different perforation was noticeable at this particular temperature as it was expected. As the temperature increases, the accumulation of aroma compounds is expected to increase as well. The level of 2(E)-Hexenal of blueberries in the perforated packages was observed secluded from each other at day 6, however this compound later reached at roughly the same level at day 9. On the other

hand, the level of 2(E)-Hexenal of blueberries in the non-perforated packages was seen elevated to a greater extent from day 0 to day 9 (Figure 4-30b). It can also be observed that the higher the temperature, the more effect it has on the development of 2(E)-Hexenal during storage time.

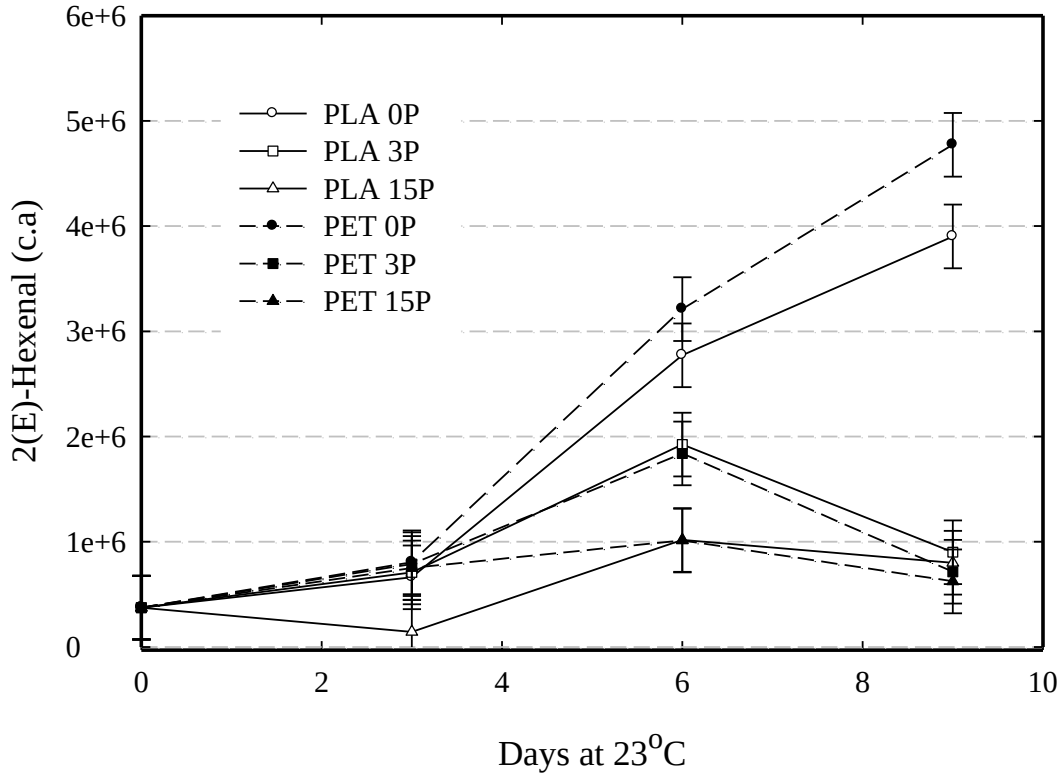


Figure 4-30a: The evolution of 2(E)-Hexenal in the blueberries in different packages stored at 23°C during 9 days of storage.

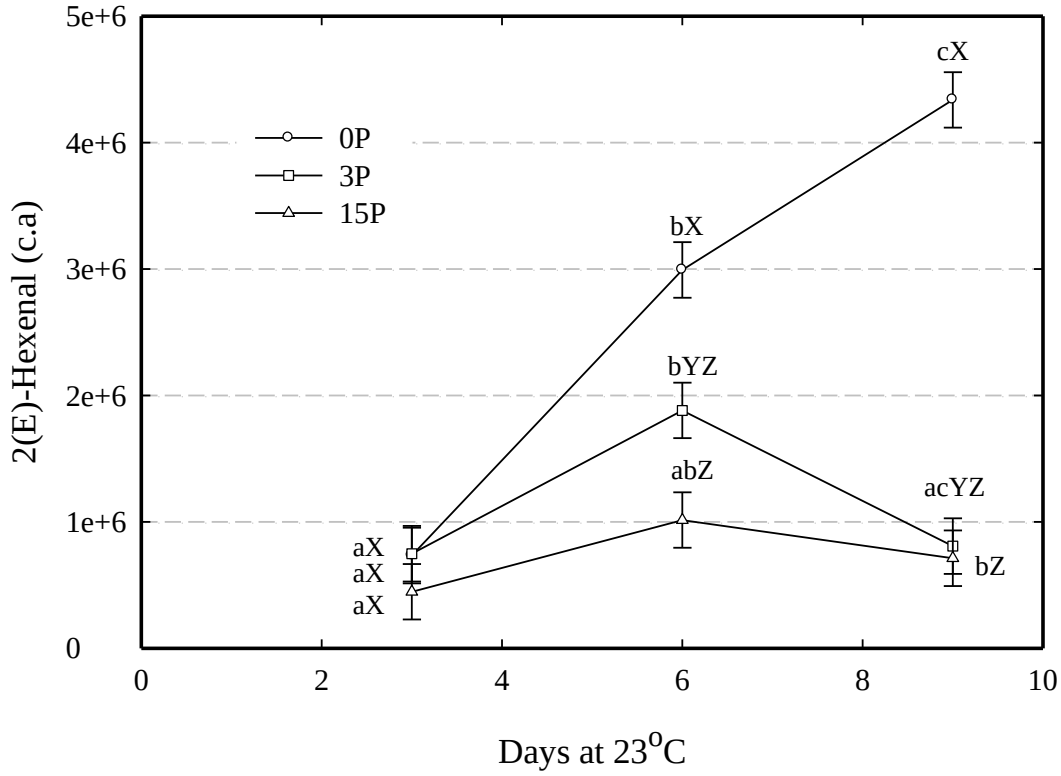


Figure 4-30b: The interaction effect of perforations, and days on 2(E)-Hexenal of blueberries stored at 23°C during 9 days. (a, b, c) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.6.2.3.1 1-Hexanol at 3°C

1-Hexanol is a six carbon alcohol that is produced from the lipoxygenase-hydroperoxide lyase metabolic pathway (Hamilton-Kemp, Archbold, Loughrin, Collins & Byers, 1996). This compound is commonly found in berry fruits such as strawberries, blueberries, raspberry and others. The evolution of 1-hexanol of blueberries in non-perforated and perforated PLA and PET packages at 3°C during 33 days of storage is presented in Figure 4-31a. The trend of this volatile was different than other volatiles measured in this study because its evolution decreased over storage time. The reduction

of 1-hexanol observed from non-perforated packages was highly significant than perforated ones, in which this volatile could not be detected from day 26 onwards (Figure 4-31b).

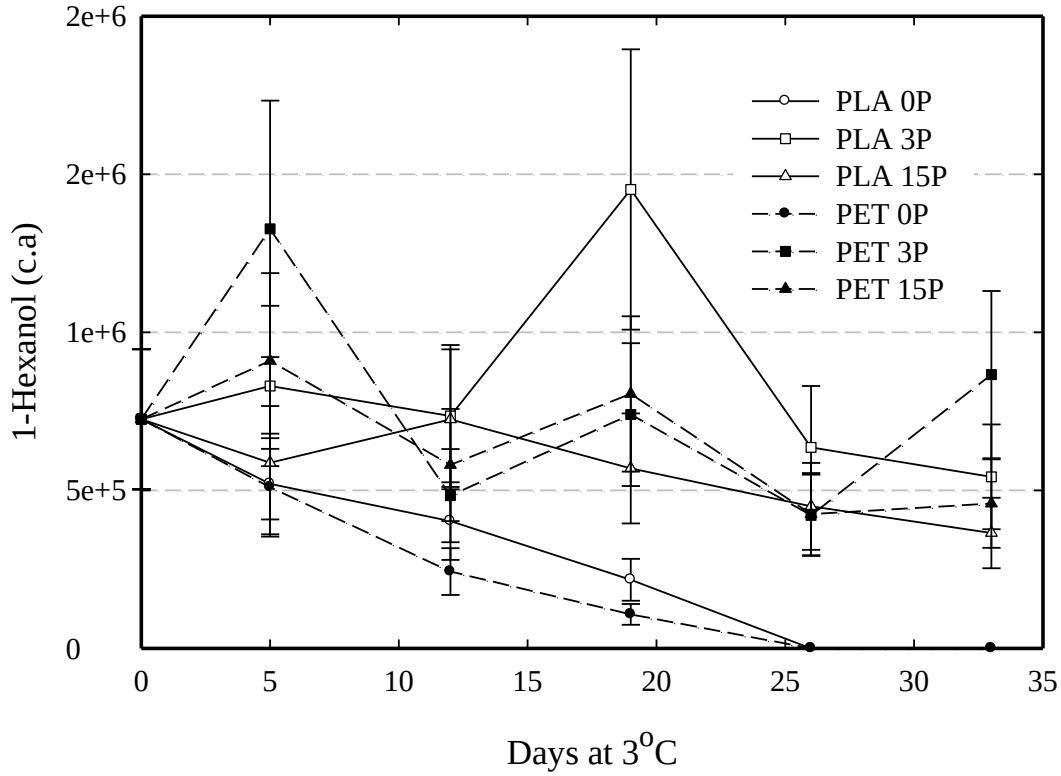


Figure 4-31a: The evolution of 1-Hexanol in the blueberries in different packages stored at 3°C during 33 days of storage.

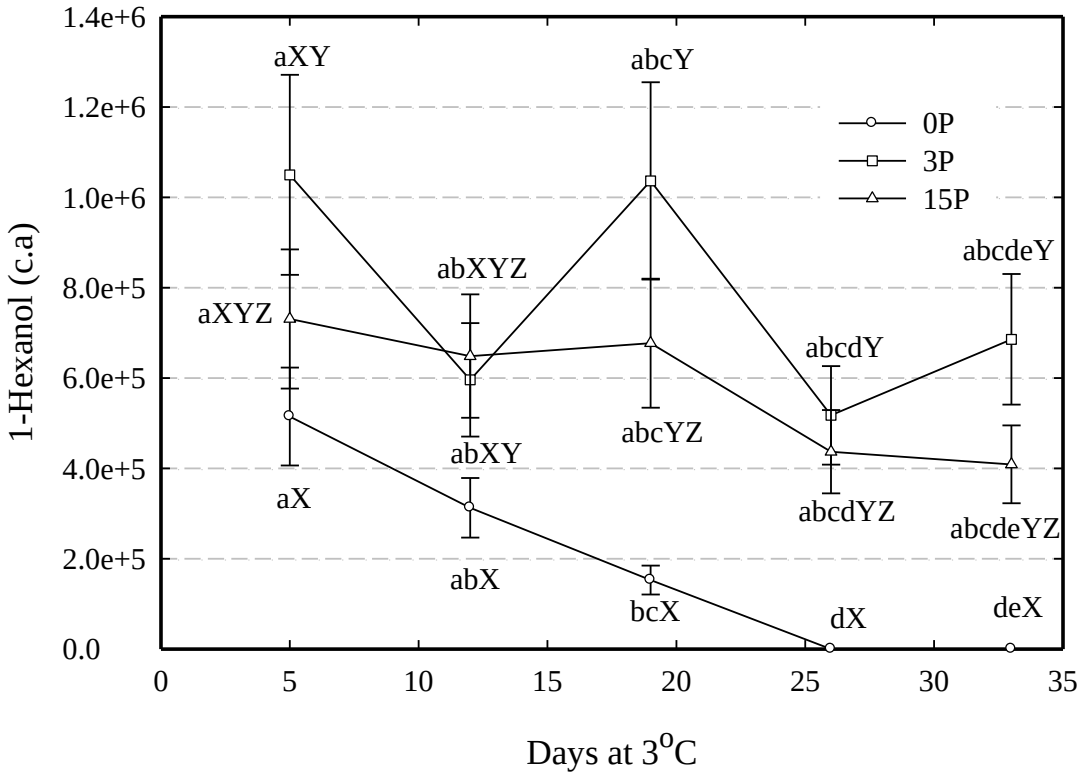


Figure 4-31b: The interaction effect of perforations, and days on 1-hexenol of blueberries stored at 3°C during 33 days.(a, b, c, d, e) Differences between days within perforations ($p<0.05$); (X, Y, Z) Differences between perforations within days ($p<0.05$).

4.6.2.3.2 1-Hexanol at 10°C

The evolution of 1-hexanol of blueberries in all packages at 10°C during 20 days of storage (Figure 4-32a) was slightly different than the ones observed at 3°C. The volatile development was not seen at day 0, and then an increased was observed during approximately half of the storage time, in which later the level of this compound started to decrease towards the end of storage time. No obvious pattern can be observed in terms of perforations effect on each day of evaluation. However, there was significant different

noticed for the effect of materials and days. For each material, the evolution of 1-hexanol decreased significantly from day 10 onwards (Figure 4-32b).

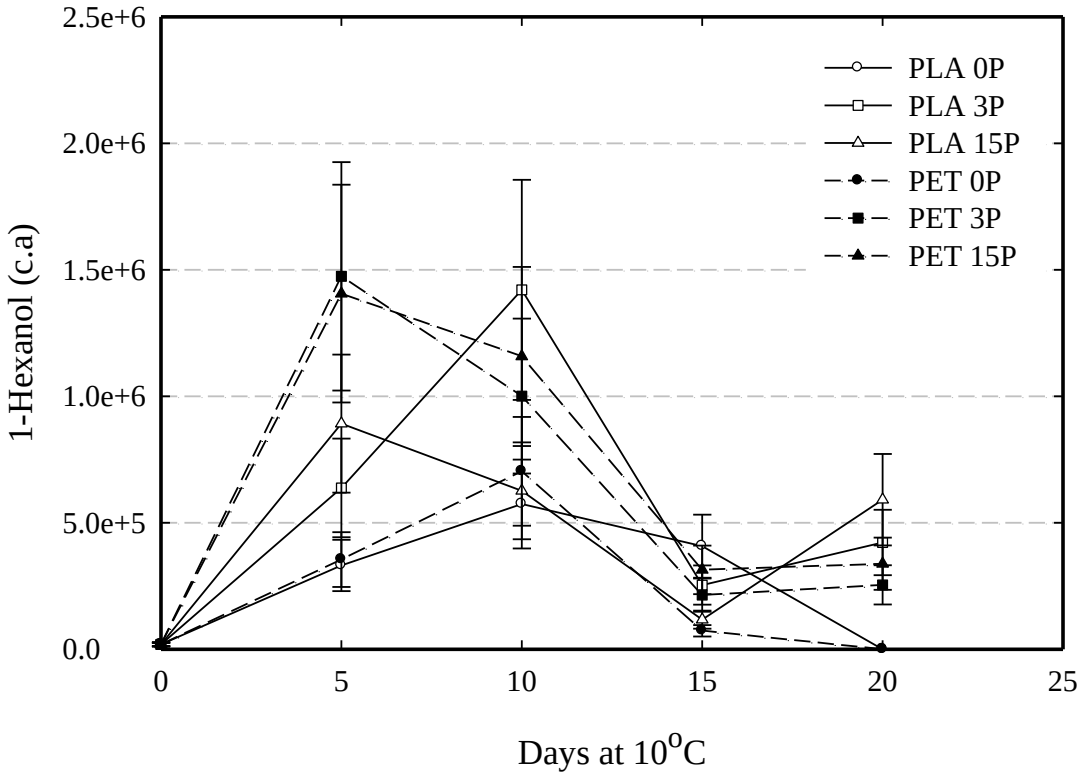


Figure 4-32a: The evolution of 1-Hexanol in the blueberries in different packages stored at 10°C during 20 days of storage.

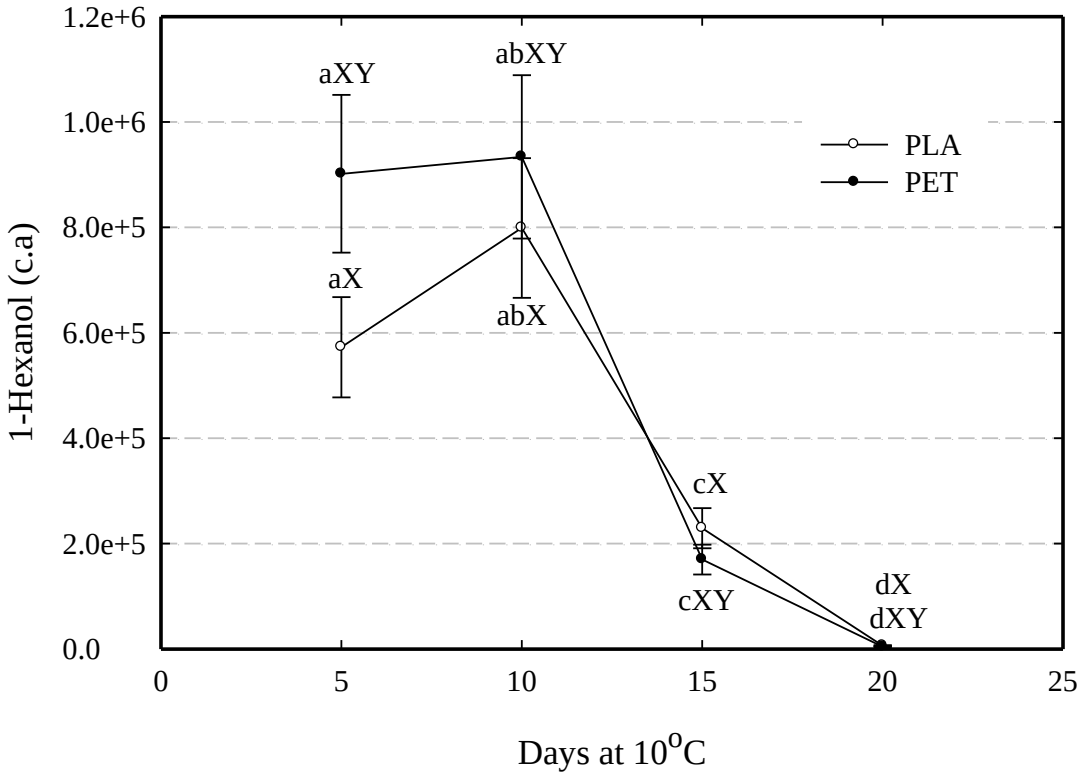


Figure 4-32b: The interaction effect of materials, and days on 1-hexenol of blueberries stored at 10°C during 20 days.(a, b, c, d, e) Differences between days within materials ($p<0.05$); (X, Y, Z) Differences between materials within days ($p<0.05$).

4.6.2.3.3 1-Hexanol at 23°C

Figure 4-33 shows the evolution of 1-hexanol of blueberries in all packages at higher temperature (23°C) during 9 days of storage. The evolution of this compound at 23°C indicated comparable pattern as it was observed at 3°C, in which the level of 1-hexanol reduced from day 0 to the last day of storage. In general, the evolution of 1-hexanol of blueberries in non-perforated packages was markedly different than perforated packages during the storage time, and the level of this compound decreased with increasing days of storage.

In this study, it is revealed that the temperature has an effect on the evolution of 1-hexanol of blueberries in all packages. For instance, at 3°C, there was no level of 1-hexanol observed in the non-perforated packages at day 26 onwards, however at 10°C, for the same situation it was much faster where no production of this volatile was observed as early as day 20. In addition, at 23°C, the evolution of this volatile reached at the same level even faster (day 9) than those at refrigerated temperatures. Therefore, the higher the temperature, the greater the reduction level of 1-hexanol was observed.

The reduction of 1-hexanol volatile of blueberries in all packages at all temperature was contradicted to the results obtained by Almenar et al. (2008b), in which the blueberries packaged in the rigid PLA containers showed slight increase in this compound level. However, according to Hamilton-Kemp et al. (1996), low levels of 1-hexanol and hexyl acetate was detected in the headspace of the jar containing strawberries when there was a presence of 2(E)-Hexenal. It was stated that this situation happened due to the capacity of strawberries to reduce a carbon-carbon double bond. The same situation could also be applied in this study, since it can be observed that the evolution of 2(E)-Hexenal was in fact greater in comparison to the evolution of 1-hexanol of blueberries in all packages at all temperatures. However, further work is needed.

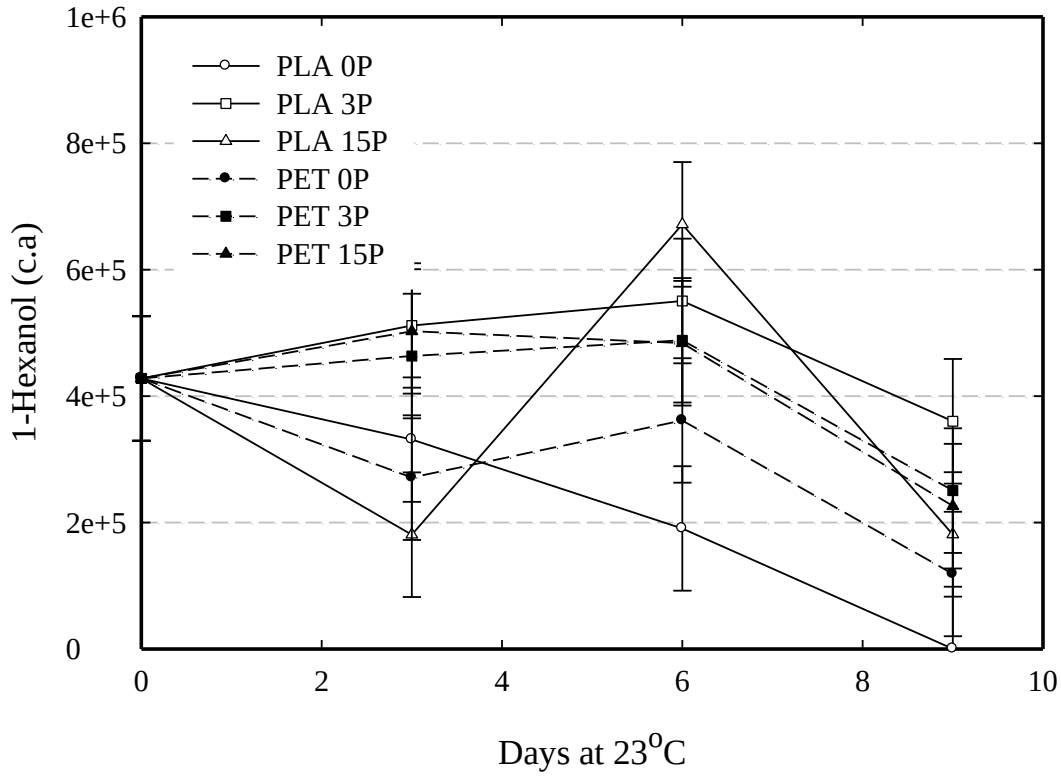


Figure 4-33: The evolution of 1-Hexanol in the blueberries in different packages stored at 23°C during 9 days of storage.

4.6.2.4.1 Linalool at 3°C

Figure 4-34 shows the evolution of linalool of blueberries in PLA and PET packages with 0, 3, and 15 perforations at 3°C during 33 days of storage. There was no significant different observed for this volatile evolution through the whole storage time. This aroma compound retained it level since day 0 to the end of day 33.

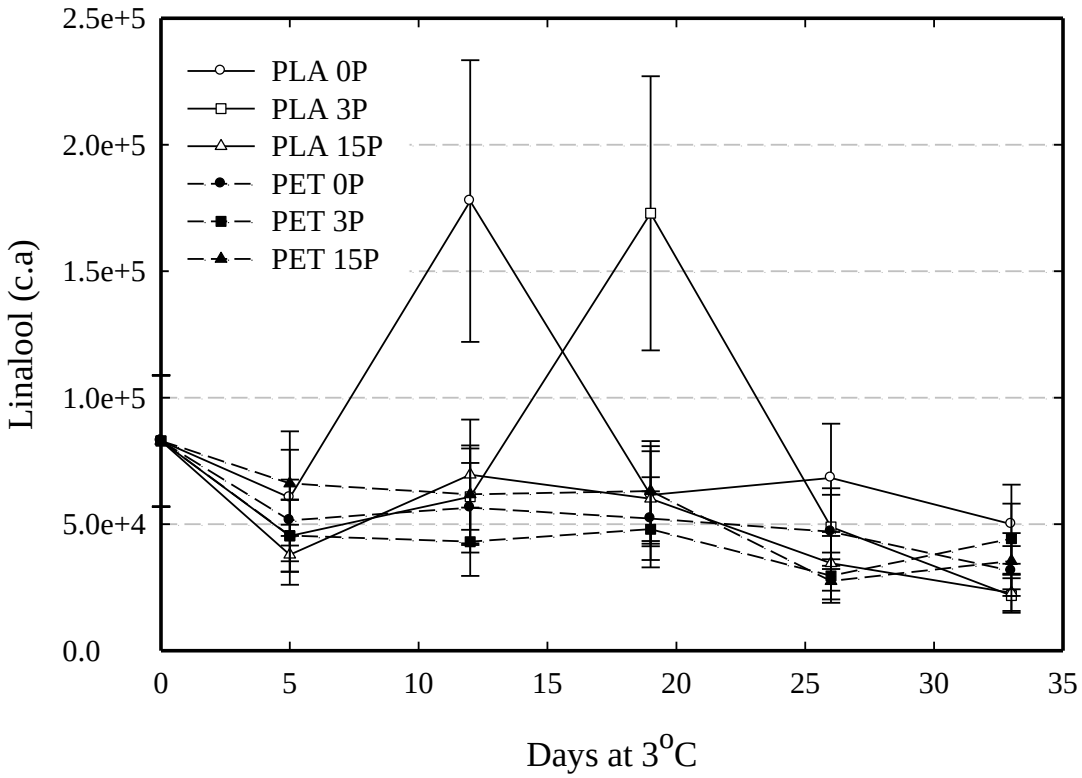


Figure 4-34: The evolution of linalool in the blueberries in different packages stored at 3°C during 33 days of storage.

4.6.2.4.2 Linalool at 10°C

The evolution of linalool of blueberries in all packages at 10°C during 20 days of storage is shown in Figure 4-35. There was fluctuation pattern observed in this compound evolution. However, there was no obvious trend in the evolution of linalool of blueberries in PLA 3P, PET 0P, and PET 15P, of which they were significant evolution compared to day 0 before they remained at approximately the same level during storage time.

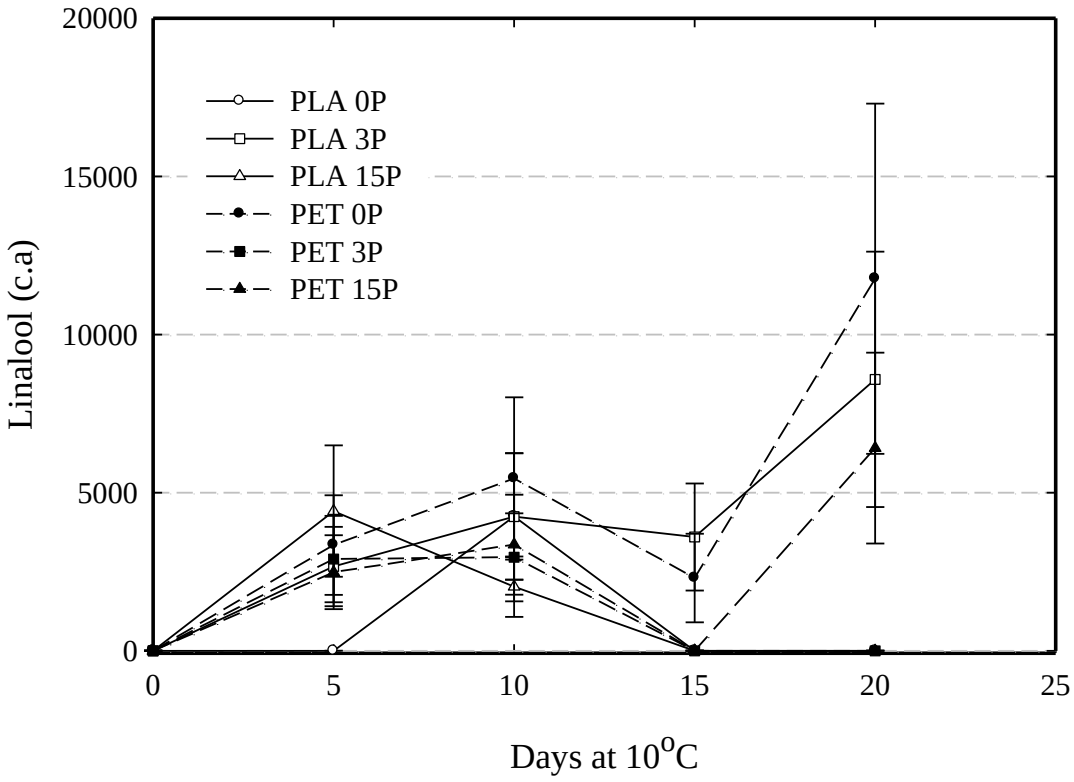


Figure 4-35: The evolution of linalool in the blueberries in different packages stored at 10°C during 20 days of storage.

4.6.2.4.3 Linalool at 23°C

Figure 4-36 shows the evolution of linalool of blueberries in non-perforated and perforated PLA and PET packages during 9 days of storage at 23°C. Although it was an increasing trend in this volatile evolution, there was no significant different observed for all of the packages during the storage time. The effect of the temperature was also not clear in the evolution of linalool of blueberries in all packages.

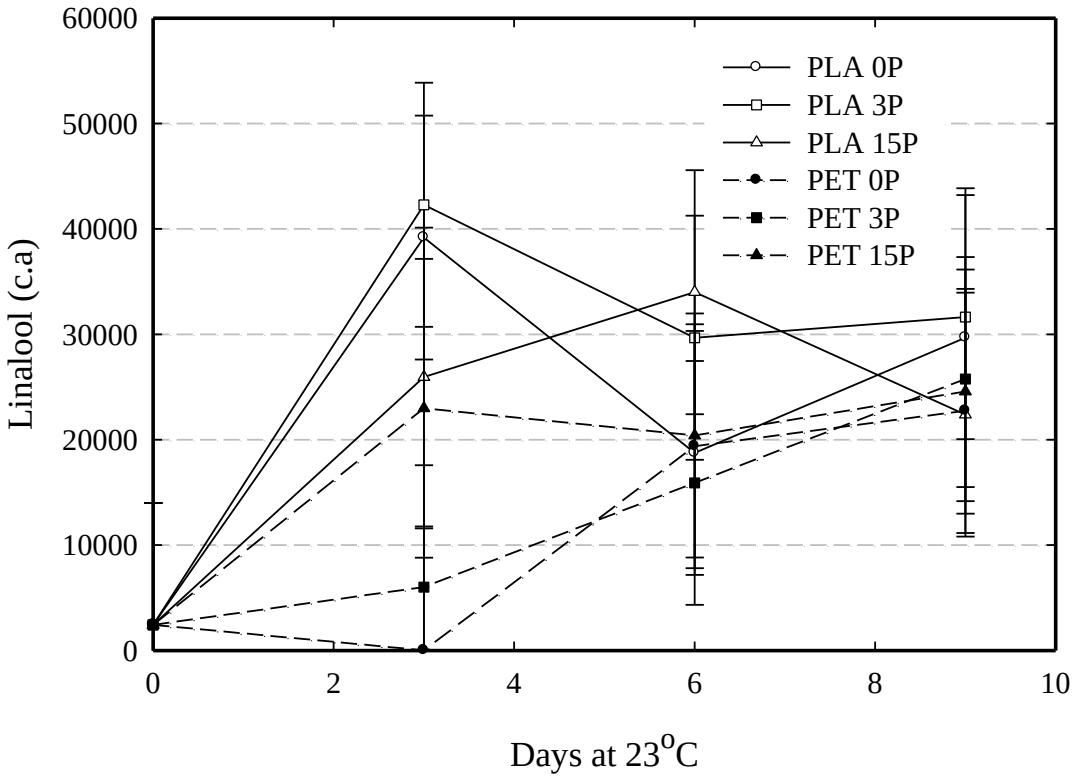


Figure 4-36: The evolution of linalool in the blueberries in different packages stored at 23°C during 9 days of storage.

4.7 Sensory analysis

Descriptive analysis is the most sophisticated and highly informative methodology of the sensory evaluations (Stone & Sidel, 2004). This analysis is commonly used due to its usefulness in specifying any sensory changes that may possibly occur in product, and or product development in terms of variation in ingredient, packaging, processing, etc. It is also used to provide prediction or explanation models of factors driving 'likes' and 'dislikes' of consumers (Lawless, 2001). In this study, descriptive analysis was used in order to evaluate the quality of blueberries packaged in different type of packages at the end of storage time at three different temperatures.

4.7.1 Sensory analysis at 3°C

From Figure 4-37, it can be seen that non-perforated packages were rated higher for off-flavor development by trained panelist. These results are consistent with instrumental results where CO_2 developed in non-perforated packages was higher than those found in perforated packages. Moreover, the evolution of ethanol and ethyl acetate of blueberries packaged in non-perforated packages measured by gas chromatography was also observed to be comparatively higher than those in perforated packages.

There were no significant differences found for appearance. This could be related to the low percentage of weight loss of blueberries in all packages at 3°C (maximum weight loss at day 33= 8%). Few of trained panelists commented that some of the fruits showed slight sign of shrivel.

In terms of texture of blueberries, the non-perforated packages had significantly lower rating than that of perforated packages. Forney et al. (2003) stated that elevated CO_2 in the package headspace retarded postharvest firming, thus causing fruit softening. This phenomenon however depends on CO_2 concentration, and exposure time. This would explain the possible cause of low texture scores given to blueberries packed in the non-perforated PLA and PET packages. The firmness of 'Bluecrop' blueberries was also reported decreased as CO_2 level increase to 15kPa (Fan, Patterson, Robbins, Fellman & Cavalieri, 1993). On the contrary, several blueberry cultivars were reported to increase in firmness during three weeks of cold storage (Mitcham, Biasi, Gaskell, Faber & Lobo, 2006).

The sweetness characteristic of blueberries was significant different at $p<0.05$. Blueberries packed in PET with 15 perforations had the highest absolute score for sweetness, even though it was not significant with the fruits in PLA with 0 perforation and 15 perforations. The value of empirical SSC was in agreement with the sensory result found for blueberries packed in PET with 15 perforations. However, the relationship of SSC with sweetness is unclear for blueberries in other packages. Kader (2008) stated that the measurement of SSC by refractometer considers sugar, organic acids, soluble pectins, anthocyanins and other phenolic compounds, and ascorbic acid, therefore reduces the correlation between soluble solids and sweetness. No significant differences were found of blueberries flavor with regards to tartness, and typical blueberry quality.

The overall quality of blueberries comprises of balance quality of all attributes tested. PET and PLA with 3 perforations scored the highest for overall blueberry quality. This is followed by PET and PLA with 15 perforations. Based on the overall description by trained panelists, it could be concluded that blueberries in non-perforated packages had the lowest score for this attributes mainly due to the high development of fermentative metabolites and softening of texture, and this was due to the high CO_2 levels achieved during storage.

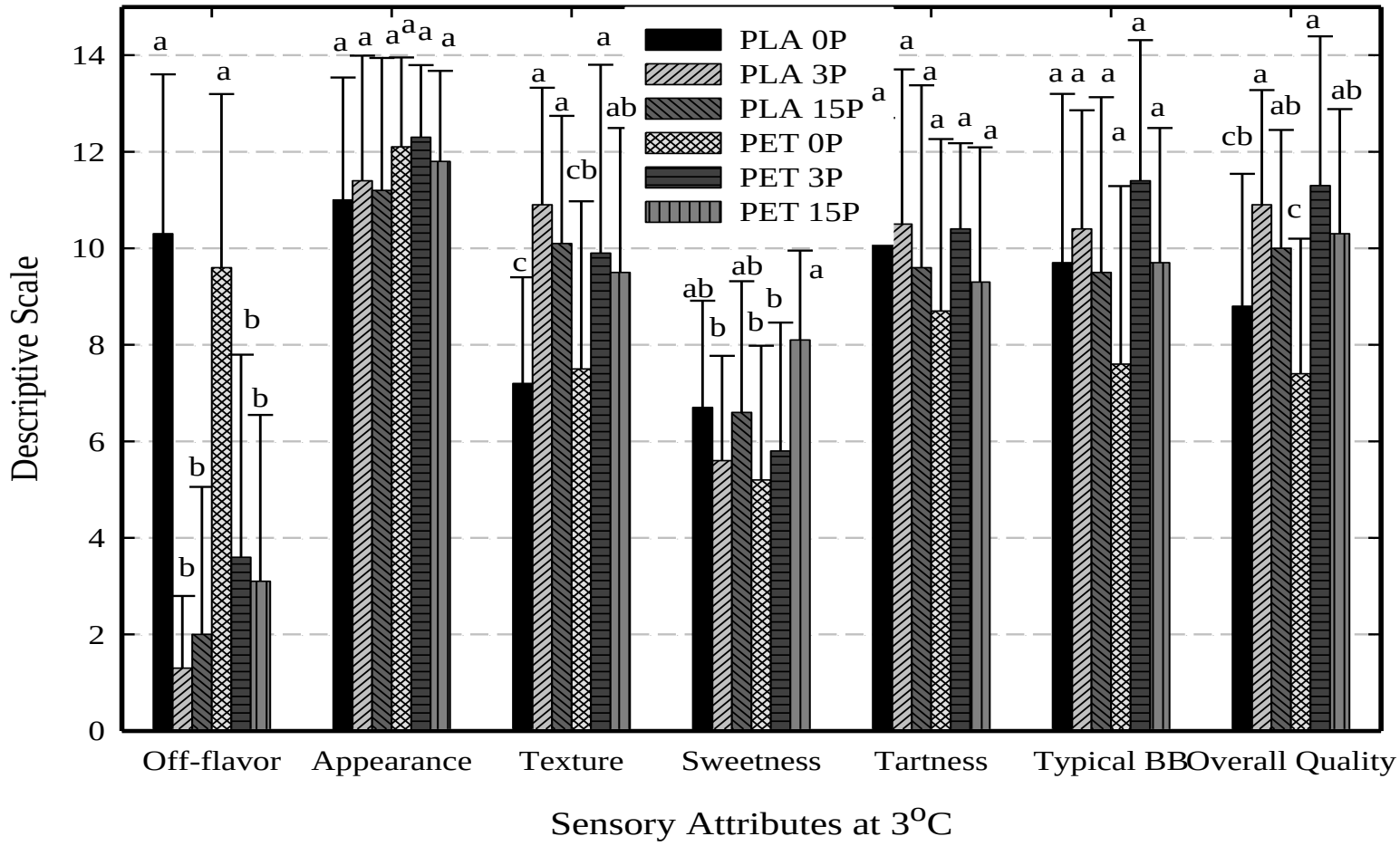


Figure 4-37: Sensory evaluation of blueberries packaged in non-perforated, and perforated PLA, and PET at the end of storage at 3°C and days 33 (n=10).

4.7.2 Sensory analysis at 10°C

Off-flavor development was detected the most in the non-perforated packages containing blueberries. The score given to non-perforated packages at this temperature was slightly lower (8-9) than at 3°C (9.5-10.5) possibly because their storage time was relatively shorter 2 weeks than at 3°C. This result was also in an agreement with the result from fermentative metabolites. The combination of ethanol and ethyl acetate of blueberries in non-perforated packages at 3°C (day 33) (ethanol: 5.0 – 7.5 × 10^6 c.a , ethyl acetate: 5.0 -7.0 × 10^6 c.a) was greatly higher than those found at 10°C (day 20) (ethanol: 2.5 – 6.0 × 10^6 c.a , ethyl acetate: 1.2 -2.1 × 10^6 c.a). No significant differences were observed between perforated packages except for PLA with 15 perforations which had the lowest rate for off-flavor, and it was difference than PET with 3 perforations (Figure 4-38). The presence of perforation on the package lid avoids excessive accumulation of CO_2 while balancing the level of O_2 in the headspace, thus preventing anaerobic respiration.

No significant differences were observed for appearance attribute. As previously discussed, this could be due to low weight loss of blueberries in those packages (maximum weight loss at day 20= 5%). The panelists commented that are some of the blueberries showed slight sign of shrivel.

The texture of blueberries packaged in non-perforated packages was lower in comparison to blueberries in perforated packages. These results were following the same

pattern of rating as observed at 3°C. However, it was only non-perforated PET found to be significantly difference than other packages. There were no differences in flavor in terms of sweetness. Nevertheless, for tartness, blueberries in non-perforated and perforated PLA packages had higher score than non-perforated and perforated PET packages. Identically, the results for typical blueberries flavor were also rated in the same order as tartness attribute.

The overall quality of blueberries in non-perforated PET packages was scored significantly lower than blueberries in other packages. Trained panelists described flavor, and texture, together with off-flavor as the contributing factors that reduced the quality of the fruits.

There were no significant different observed between PLA 0P, PLA 15P, PLA 3P, PET 15P, and PET 3P packages on the overall quality of blueberries (packages are presented in decreasing order of absolute score).

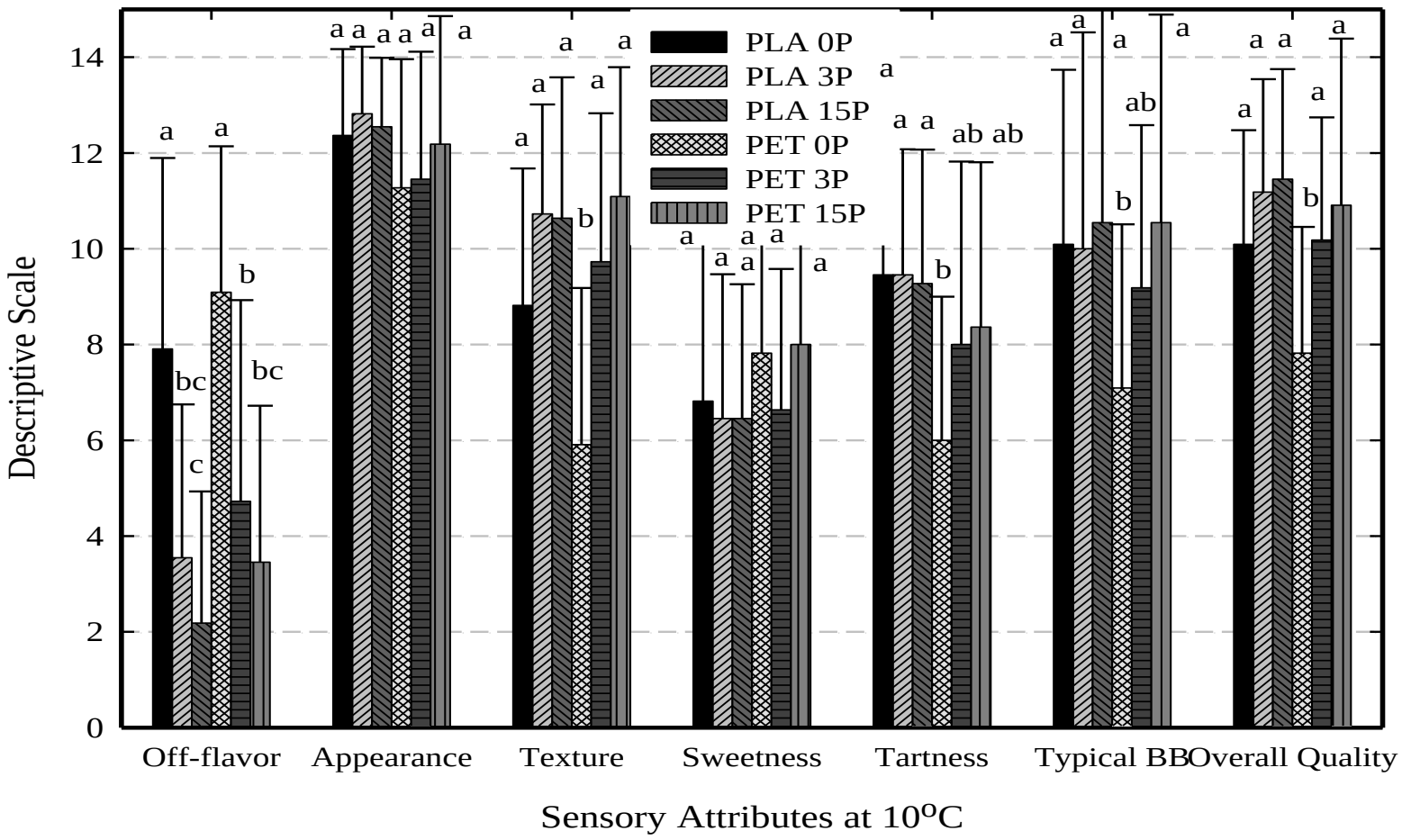


Figure 4-38: Sensory evaluation of blueberries packaged in non-perforated, and perforated PLA, and PET at the end of storage at 10°C and days 20 (n=11).

4.7.3 Sensory analysis at 23°C

Figure 4-39 shows the results obtained for all sensory attributes of blueberries in six different packages kept at 23°C for 7 days. Similar to off-flavor results at 3 and 10°C, blueberries in non-perforated packages were detected to develop strong off-flavor as a result of riched-CO_2 atmosphere in the headspace that led to fermentation. This result was also supported by the result of fermentative metabolites of which the level of ethanol and ethyl acetate was markedly higher for blueberries in non-perforated packages as opposed to perforated packages. In addition, trained panelists noticed the presence of slight off-flavor in perforated packages. This condition is possible due to the high storage temperature that induce respiration rate of the fruits, which in turn, accelerate senescence, and decay.

There were no differences observed for blueberries in all packages with regards to appearance. As had been discussed previously, this could be associated with low weight loss of blueberries (maximum weight loss at day 7=6%). The sign of slight shrivel was also described by panelists.

The texture of blueberries stored at this temperature was rated differently than at 3 and 10°C. Blueberries in non-perforated packages were comparably firm in texture to those fruits in perforated packages. This incidence could be related to storage temperature considering that previously blueberries were kept at refrigerated temperature. Forney et al. (2003) mentioned that increase in blueberries firmness was temperature dependent

since ‘Burlington’ blueberries were found firmer when they were kept at 7°C than at 0°C. In addition, fruit firmness was reported to

be related to corrugation and thickening of the epidermal and hypodermal cell walls (Allan-Wojtas, Forney, Carbyn & Nicholas, 2001).

Flavors with regards to its sweetness, tartness, and typical blueberry flavor were found not significantly different for blueberries in all packages. Overall quality was found higher for blueberries packed in PLA 3P, followed by PET 15P, PET 3P, PLA 0P, PLA 15P, and PET 0P. At this temperature, blueberries in PET 0P were noted for their strong off-flavor development, undesirable flavor and appearance.

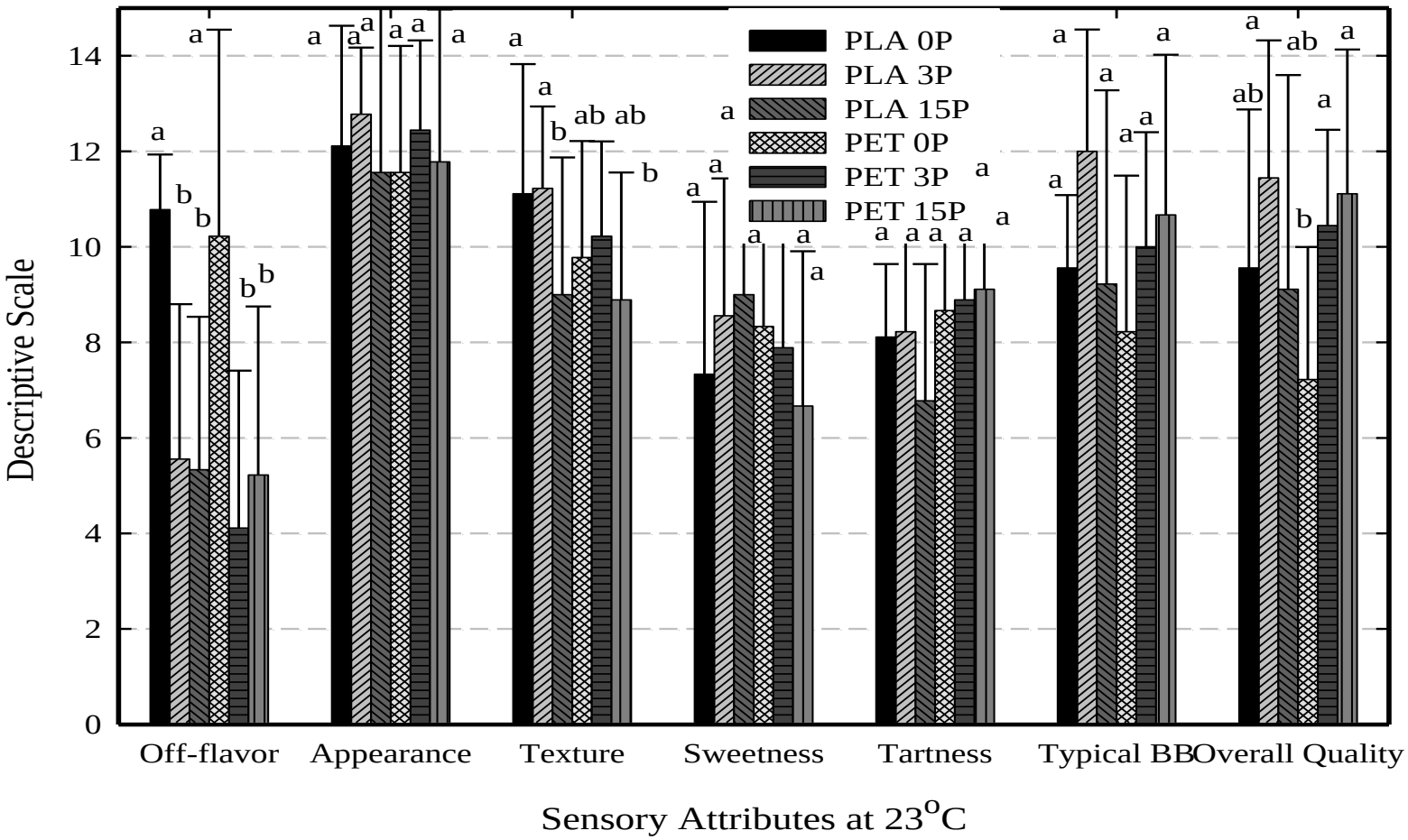


Figure 4-39: Sensory evaluation of blueberries packaged in non-perforated, and perforated PLA, and PET at the end of storage at 23°C and days 9 (n=9).

4.8 Characterization of perforation

The perforations diameters were obtained by measuring in two cross direction since the perforations were mostly in elliptical shape. Although the shape of perforations was varied from one to another, in general they had roughly the same diameter and position. This variation in shape was due to the use of mechanical microperforation technique instead of laser technique that is commonly used in the industry. Due to the irregularity of the perforation, the void area was calculated with the help of Adobe Photoshop CS3. In general the diameter of R_L was within the range of 15 to 24 μm and the diameter of R_S was within the range of 3 to 10 μm. Even though it seems the variation was relatively wide, the formation of perforation was actually balance in terms of its diameter. For instance, if one perforation had a wide length of R_L, its R_S would be relatively short, and vice versa. The calculated area was found varied from 28 to 40 μm^2 for the lidding material with 3 perforations. Meanwhile, the area was bigger (73 to 120 μm^2) for the lidding material with 15 perforations. Table 4-2 shows the calculated area for corresponding lidding materials. The differences of the area between the lidding material with 3 and 15 perforations could be related to the distance between the needles on the stainless steel device. The presence of more needles on this devices reduced the distance from one needle to another, caused a high tension on the lidding material, thus resulted into bigger perforation area. Figure 4-40 and 4-41 shows some of the perforation images.

Table 4-2: Calculated area for lidding materials

Number of microperforations	Material (μm^2)	
	PLA	**PET**
3	43.7 ± 11.5	41.9 ± 3.1
15	88.0 ± 40.2	70.4 ± 36.5

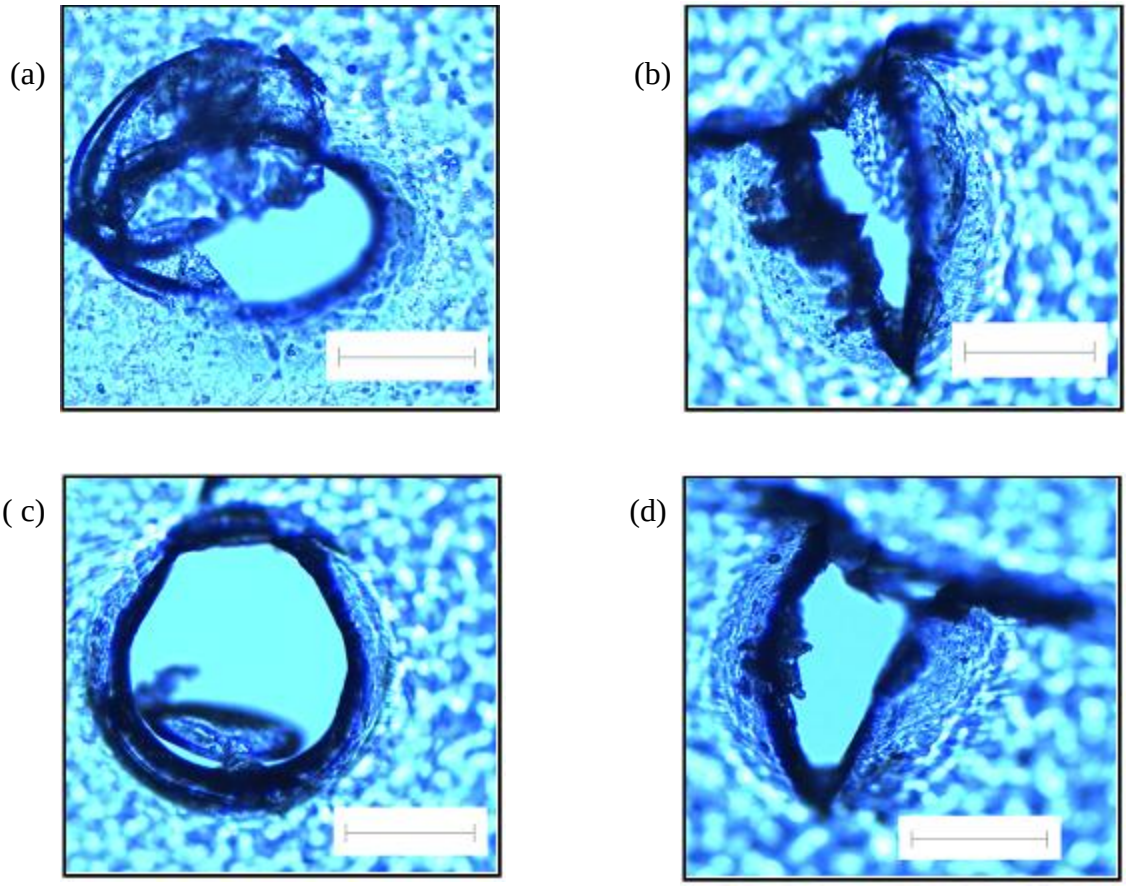


Figure 4-40: (a), (b) Microperforation of PLA lidding material obtained from 3 perforations; (c), (d) Microperforation of PLA lidding material obtained from 15 perforation. Bar represents 10 μm.

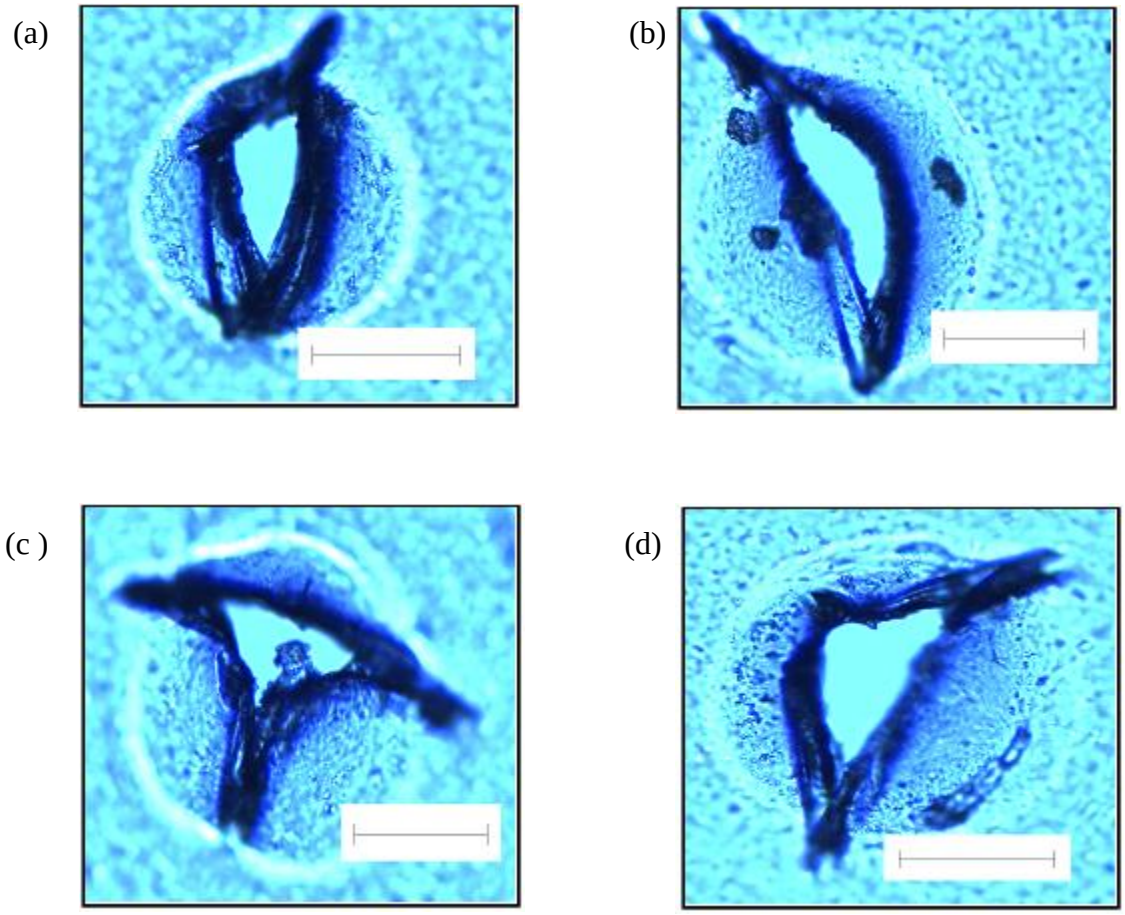


Figure 4-41: (a), (b) Microperforation of PET lidding material obtained from 3 perforations;(c), (d) Microperforation of PET lidding material obtained from 15 perforation. Bar represents 10µm.

4.9 Permeation analysis

Table 4-3: CO_2, O_2 permeation rate and water vapor permeance of PLA and PET packages with 0 perforation.

Package	$CO_2 \times 10^{-10}$ (kg/pkg-s)	$O_2 \times 10^{-11}$ (kg/pkg-s)	WATER $\times 10^{-12}$ (kg/pkg-s-Pa)
PLA 0P	9.79 ± 0.09 a	3.41 ± 0.20 a	*1.37 ± 0.01 a
PET 0P	1.24 ± 0.09 b	34.7 ± 0.02 b	**0.62 ± 0.02 b

Note: Value(s): lsmean ± standard error; *Test was done at 23 °C, 15% RH; ** Test was done at 23 °C, 100% RH.

4.9.1 Permeation rate of CO_2

The results obtained in the permeation rate of CO_2 shown in Table 4-2, indicated that non-perforated PLA had higher permeation rate in comparison to those non-perforated PET. This result in general was in agreement with other reported data. This result, however, was found inconclusive with the results of CO_2 evolution of blueberries, since it can be seen that the non-perforated PLA packages contained slightly higher level of CO_2 in the package headspace compared to the non-perforated PET packages. This could be due to the influence of external factor such as the presence/absence of fruits in the package, which in turn affect the continuous production of this gas.

4.9.2 Permeation rate of O_2

The data obtained for O_2 permeation rate showed that non-perforated PLA packages had higher value than non-perforated PET packages. This result was in

agreement with the result cited in the literature in term of its order. Since the permeation rate in this study was measured as the whole package, the result was not comparable to other finding because most of the research was done to determine the permeation rate of films/sheets. In addition, for both packages, the level of O_2 reduced fast due to the package low barrier that limits O_2 permeation into the headspace, and also due to the continuous consumption of this gas for the metabolic process of blueberries.

4.9.3 Water vapor permeance

The water vapor permeance of PLA and PET packages regardless of perforations was measured by using two separate conditions. The water vapor permeance of PLA packages were extremely high to be measured by the instrument at 100% RH. Therefore, lower RH (15%) was used for this measurement.

Based on the results obtained, it can be concluded that water vapor permeance of PLA packages was greater than that of PET packages. This noticeable trend was also observed in the result of weight loss of blueberries.

Chapter 5

5. Conclusions and Recommendations

5.1.1 General conclusions

The first bio-based microperforated packaging system was developed in this study to prolong the shelf life of respiring products such as blueberries. For CO_2 and O_2 evolution of blueberries, it was demonstrated that the absence and presence of perforation had the most pronounced effect for this criteria. Blueberries packaged in non-perforated packages showed greater development of CO_2 level and greater reduction of O_2 level in the packages' headspace. The effect of temperature was not obvious for the gas evolution. The results obtained for weight loss at all temperature was observed as material dependent. The weight loss was observed greater in PLA packages rather than PET packages regardless of perforations. Higher temperature was found to have significant effect on the weight loss. Fungal growth was observed increased with increasing in temperature. The correlation between fungal growth and level of CO_2 was noticeable in this study especially at lower temperature ($3^{\circ}C$). The combination of low temperature with high level of CO_2 was observed effective in inhibiting the growth of fungal. pH and SSC of blueberries packaged in PLA and PET packages with 0, 3, and 15 perforations showed no differences. Those values were constant from day 0 towards the end of storage time at all temperatures. As for flavor evolution of blueberries, the production of ethanol and ethyl acetate was found higher for blueberries in the non-perforated packages. This result was consistent with the result observed for CO_2 level.

Fermentative metabolites development was affected by the temperature. The evolution of hexanal of blueberries in all packages remained constant during the whole storage time. There was also no effect of temperature observed in this volatile generation. Higher level of 2(E)-hexenal was seen for blueberries in the non-perforated packages at all temperatures. The level of this compound was found increased as the temperature increases. Blueberries in the perforated packages revealed constant evolution of 2(E)-hexenal during storage. In contrast, the level of 1-hexanol of blueberries in all packages was decreased over time for all temperatures. The higher the temperature, the greater the reduction of 1-hexanol was observed. The evolution of linalool remained the same throughout storage time for blueberries in all packages. There was no clear effect of temperature noticed for this value. Overall, it was observed that the aroma profiles obtained for blueberries stored at 10°C was different than those blueberries kept at 3 and 23°C. This may be associated with the difference in early and late cultivar since the batch of blueberries used for analysis at 10°C was an early cultivar of 'Bluecrop'.

For sensory analysis, the development of off-flavors was noticeable in the non-perforated PLA and PET packages. There were also no significant differences observed for blueberries appearance in all packages at all temperatures. The texture of blueberries in non-perforated packages was evaluated as firmer at 23°C in comparison to those at 3 and 10°C. The results obtained for sweetness, tartness and typical blueberry flavor showed similar behavior at all temperatures. No clear correlations could be drawn between sensory and instrumental analysis for these attributes (sweetness, tartness,

typical blueberry flavor). In terms of overall quality, it was demonstrated that PLA and PET packages with 3 perforations had the highest scores for evaluation of blueberries at 3 and 23°C. No obvious differences were observed for blueberries in all packages at 10°C, except for blueberries in non-perforated PET, of which was rated as the worst quality.

Meanwhile, water permeance, CO_2 and O_2 permeation rates were higher for non-perforated PLA packages in comparison to non-perforated PET packages.

5.1.2 Final conclusions

Based on overall results, it was revealed that the presence of 3 perforations at 3°C had contributed to the close to optimal level of CO_2 and O_2 in the packages headspace in comparison to other packaging systems. PLA 3P had the level of CO_2 and O_2 of 5 and 16 %, respectively, while PET 3P had the level of CO_2 and O_2 of 6 and 18 %, respectively, between day 12 to 19 at 3°C. This packaging system had an acceptable amount of weight loss during storage. PLA 3P had a weight loss of approximately 4.9% at day 25, while PET 3P had a weight loss of 2.7% at day 33. These blueberries would still be marketable since their weight loss in both packages was lowered than that of 5% cut-off point as cited in the literature. The sign of fungal growth for these particular packaging systems at 3°C was only observed after 19 days of storage. pH values of blueberries packaged in these packaging systems were found consistent throughout storage. The SSC of blueberries was also within the range of acceptable and preferable for blueberries flavor (14.6-15.5 °Brix). The evolution of fermentative metabolites

(ethanol and ethyl acetate) of blueberries in PLA and PET 3P was found maintained throughout 33 days of storage at the same level obtained for day 0. No off-flavor development noticed for blueberries packaged in these packaging systems by trained panelist. The evolution of hexanal, 2(E)-hexenal, 1-hexanol, and linalool was observed constant throughout the storage time for blueberries packaged in PLA 3P and PET 3P packages. Trained panelists had rated higher absolute score for blueberries packaged in these packaging systems for overall quality at 3°C. Therefore, it could be concluded that PLA and PET packages with 3 perforations kept at 3°C have demonstrated the potential for maintaining the quality and prolonging the shelf life of blueberries for at least 19 days.

5.2 Future recommendations

In general, this study has covered most of the important criteria such as the development of bio-based microperforated packaging system, characterization of the packages and perforations, determination of physico-chemical properties of blueberries, microbiological and sensory evaluation. However, there are some limitations and missing scope that could be addressed in the future:

1. To improve microperforation technique in order to obtain uniform shape of perforation, thus the gas exchange process through perforation can be predicted by using mathematical model and compared with the instrumental data. The improvement could be done by adjusting the distance between needles on cylinder device or by modifying the length of the needles.

2. A similar study could also be conducted on other fresh produces with different mode of respiration rate such as high, medium and low respiration rate, thus validating this packaging system for wider application.
3. Another technology/ testing method could be used to validate the barrier properties data obtained in this study.

Appendices

Appendix A- Consent form

The School of Packaging, Michigan State University

TRAINED PANEL CONSENT FORM

Sensory Evaluation of Blueberries

Dear Participant:

Before you decide to sign this consent form and continue to participate in this study, please read this document carefully for the information related to the study, ingredients, packaging material and procedures used in the study. Potential risks and benefits from your study, assurance of your privacy and your rights as a human subject in our study are also listed.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator, either Dr. Rafael Auras by phone at 517-432-3254 or by email at aurasraf@msu.edu or Dr. Eva Almenar by phone at 517-432-1431 and email at ealmenar@msu.edu for any inquiry you might have related to your participation in the study. In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Ph.D., Director of Human Research Protections, by phone: (517) 355-2180, fax: (517) 432-4503, email: irb@msu.edu or regular mail: 202 Olds Hall, East Lansing, MI 48824-1047.

PLEASE NOTE THAT UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN THIS STUDY. YOUR

SIGNATURES INDICATE YOU HAVE READ ALL THE INFORMATION PROVIDED IN THIS CONSENT FORM AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS CAN BE PROVIDED UPON YOUR REQUEST.

I voluntarily agree to participate in the study.

SIGNED _____ DATE_____

Invitation to Participate: You are invited to participate in the study that assesses the effect of different packaging material on blueberries.

Purpose of the study: We are investigating the effect of different packaging materials on the blueberry fruits in terms of aroma, appearance, texture, flavor and overall acceptance.

Procedure of the study: Each panelist would be served blueberry packed in the cup and each cup would be coded with a random 3-digit code. We are asking that panelists participate in this study at which will last for 3-month period. Training will consist of approximately 3-4 sessions of 30-60 minutes. Instructions to the test would be provided on a given sheet. Participants will be asked to rate the samples based on Universal Scale in which consists of 15 points spectrum scale on five attributes (aroma, appearance, texture, flavor and overall acceptance).

Samples Preparation: Blueberry fruits were sorted and repacked before stored at different conditions.

Potential Risks: Since there is no treatment on the blueberry fruits used in this study, these samples pose no adverse health risk. Though none is anticipated, if you have a problem **upon consuming these samples**, please notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for

lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. Your response is confidential and we will protect your confidentiality to the full extent of the law.

Expected Benefits: This study will enable the researchers to establish the relationship between sensory evaluation and experimental data on physicochemical properties of blueberry fruits.

Assurance of confidentiality: Any information obtained in connection with this study that could be identified with you will be kept confidential by ensuring that all consent forms and response sheets are securely stored. All data collected and analyzed will be reported in an aggregate format that will not permit associating subjects with specific responses or findings. **Your privacy will be protected to the maximum extent allowable by law.**

Withdrawal from the study: Participation in this study is voluntary. You may refuse to perform the evaluation on these samples without penalty, and your decision to refuse participation or discontinue participation during this study will be honored promptly and unconditionally.

Appendix B-Questionnaire

SENSORY EVALUATION OF BLUEBERRIES

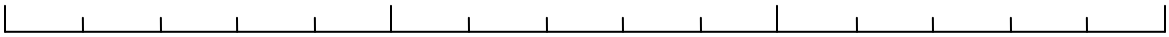
NAME _____ **DATE** _____

SAMPLE CODE _____

Fermentative metabolites:

Please open the lid and take a sniff of the aroma.

How do you perceive the aroma?



0 5 10 15

Weakest Strongest

Do you notice any odd odor? If yes, please comment(s).

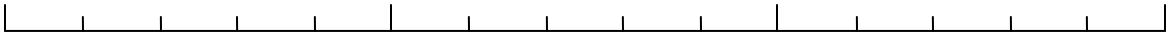
_____

_____

Appearance:

Please observe for any noticeable change on fruit appearance as compared to fresh blueberry fruits (disregard the color).

How do you perceive the appearance?



0 5 10 15

Least Pleasant Most Pleasant

Comment (s): (e.g shriveling)

_____

_____

Texture:

Please bite the sample using front teeth.

How do you perceive the texture?

0 5 10 15

Soft Firm

Comment (s): _____

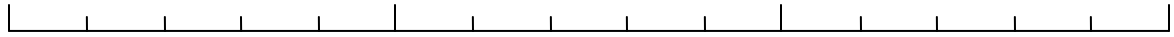
_____

Flavor:
Please taste the fruit(s).

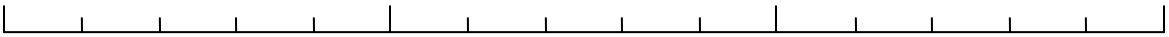
Sweetness:

Bland, not sweet slightly sweet moderately sweet sweet

Tartness:



Bland, tartless slightly tart moderately tart tart



0 5 10 15
Atypical blueberry flavor
Typical blueberry flavor

Comment (s):

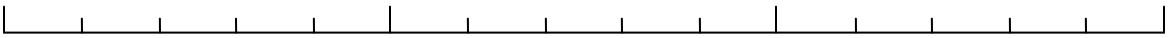
_____

Do you notice any off flavor? If yes, please explain (s).

_____

_____

Overall Quality:



0 5 10
15
Comment (s):
Do you think the sample quality is appropriate for consumer to eat and purchase this product?

_____

_____

Appendix C- Trained panelists performance

Trained panelists were evaluated two times during the whole training sessions for their performance in rating the provided samples. This evaluation helped researcher to identify the obstacles, to address the problems and to determine the trained panelists' readiness for the actual testing. A reference score was set up by the researcher for the same samples of each tested attributes. This reference score was later compared with the score rated by the trained panelist.

SESSION 1

During this session, a separate sample for fermentative metabolites attributes was prepared. Fresh blueberries were placed in an air-tight glass container and kept for 5 hours at room temperature prior to evaluation time to generate the development of fermentative metabolites. While for the other attributes, fruits were kept in a tray at 3°C for 5 hours before the testing.

Table C-1: The comparison between the score rated by the trained panelists and the reference score for fermentative metabolites attribute.

	FERMENTATIVE METABOLITES	
PANELISTS	REFERENCE	SCORE
1	14	14
2	14	15
3	14	15
4	14	15
5	14	15
6	14	15
7	14	15
8	14	15
9	14	15
	175	

Table C-2: The comparison between the score rated by the trained panelists and the reference score for appearance attribute.

	APPEARANCE	
PANELISTS	REFERENCE	SCORE
1	13	13
2	13	13
3	13	11
4	13	13
5	13	13
6	13	13
7	13	12
8	13	12
9	13	13

Table C-3: The comparison between the score rated by the trained panelists and the reference score for texture attribute.

	TEXTURE	
PANELISTS	REFERENCE	SCORE
1	12	13
2	12	12
3	12	12
4	12	12
5	12	9
6	12	12
7	12	11
8	12	12
9	12	13

Table C-4: The comparison between the score rated by the trained panelists and the reference score for sweetness attribute.

	FLAVOR: SWEETNESS	
PANELISTS	REFERENCE	SCORE
1	4	5
2	4	5
3	4	10
4	4	5
5	4	2
6	4	13
7	4	5
8	4	5
9	4	5

Table C-5: The comparison between the score rated by the trained panelists and the reference score for tartness attribute.

	FLAVOR: TARTNESS	
PANELISTS	REFERENCE	SCORE
1	10	10
2	10	10
3	10	10
4	10	10
5	10	10
6	10	10
7	10	4
8	10	5
9	10	9

Table C-6: The comparison between the score rated by the trained panelists and the reference score for typical blueberry flavor attribute.

	FLAVOR: TYPICAL BB FLAVOR	
PANELISTS	REFERENCE	SCORE
1	11	15
2	11	10
3	11	14
4	11	14
5	11	9
6	11	12
7	11	12
8	11	10
9	11	9

Table C-7: The comparison between the score rated by the trained panelists and the reference score for overall quality attribute.

	OVERALL QUALITY	
PANELISTS	REFERENCE	SCORE
1	12	13
2	12	11
3	12	13
4	12	14
5	12	12
6	12	12
7	12	15
8	12	13
9	12	10

SESSION 2

In this session, two separate samples were also provided for fermentative metabolites attribute. Fresh blueberries were packed in PLA (122) and PET (124) packages and kept at 23°C for 6 days prior to testing. For the other attributes, fruits were placed on a tray and stored at 10°C (937) and 23°C (275) for overnight before the evaluation.

Table C-8: The comparison between the score rated by the trained panelists and the reference score and between two samples for fermentative metabolites attribute.

	FERMENTATIVE METABOLITES			
PANELISTS	REFERENCE	122	REFERENCE	124
1	15	13	10	11
2	15	11	10	8
3	15	14	10	10
4	15	14	10	10
5	15	15	10	10
6	15	13	10	10
7	15	13	10	10
8	15	13	10	11
9	15	13	10	10
10	15	14	10	10

Table C-9: The comparison between the score rated by the trained panelists and the reference score and between two samples for appearance attribute.

	APPEARANCE			
PANELISTS	REFERENCE	275	REFERENCE	937
1	13	13	13	13
2	13	13	13	13
3	13	13	13	13
4	13	14	13	13
5	13	15	13	13
6	13	14	13	15
7	13	13	13	13
8	13	13	13	13
9	13	13	13	14
10	13	13	13	13

Table C-10: The comparison between the score rated by the trained panelists and the reference score and between two samples for texture attribute.

	TEXTURE			
PANELISTS	REFERENCE	275	REFERENCE	937
1	10	10	11	11
2	10	11	11	11
3	10	10	11	11
4	10	10	11	12
5	10	10	11	10
6	10	10	11	10
7	10	10	11	13
8	10	8	11	10
9	10	9	11	5
10	10	9	11	9

Table C-11: The comparison between the score rated by the trained panelists and the reference score and between two samples for sweetness attribute.

	FLAVOR: SWEETNESS			
PANELISTS	REFERENCE	275	REFERENCE	937
1	10	15	3	3
2	10	10	3	4
3	10	14	3	2
4	10	12	3	2
5	10	11	3	1
6	10	10	3	3
7	10	10	3	3
8	10	10	3	11
9	10	10	3	0
10	10	10	3	2

Table C-12: The comparison between the score rated by the trained panelists and the reference score and between two samples for tartness attribute.

	FLAVOR: TARTNESS			
PANELISTS	REFERENCE	275	REFERENCE	937
1	5	1	12	13
2	5	4	12	7
3	5	3	12	13
4	5	3	12	13
5	5	3	12	13
6	5	10	12	13
7	5	4	12	11
8	5	2	12	15
9	5	5	12	12
10	5	4	12	11

Table C-13: The comparison between the score rated by the trained panelists and the reference score and between two samples for typical blueberry flavor attribute.

	FLAVOR: TYPICAL BB FLAVOR			
PANELISTS	REFERENCE	275	REFERENCE	937
1	5	6	10	10
2	5	5	10	10
3	5	5	10	10
4	5	4	10	10
5	5	8	10	11
6	5	4	10	12
7	5	4	10	10
8	5	10	10	12
9	5	9	10	12
10	5	5	10	10

Table C-14: The comparison between the score rated by the trained panelists and the reference score and between two samples for overall quality attribute.

	OVERALL QUALITY			
PANELISTS	REFERENCE	275	REFERENCE	937
1	6	5	11	11
2	6	5	11	11
3	6	6	11	10
4	6	6	11	12
5	6	6	11	13
6	6	11	11	10
7	6	5	11	12
8	6	8	11	11
9	6	6	11	12
10	6	4	11	11

Appendix D- Sensory evaluation: appearance guideline

Figure D-1: The appearance guideline for sensory evaluation.

Note: Blueberry with 0 score – The least pleasant

Blueberry with 15 score-The most pleasant

Appendix E -IRB Certificate

Michigan State University
Office of Regulatory Affairs

This certifies that

HAYATI SAMSUDIN

has successfully completed the Training Tutorial on human subject research protections. This training is conducted under the terms of Michigan State University's Federal-Wide Assurance with the U.S. Department of Health and Human Services, Office for Human Research Protections, FWA #00004556, and Federal Regulations in 45 CFR 46.103.

Training Date: 8/14/2008 **Record ID:** 0026006

Appendix F-SAS code for PROC MIXED

```
data ph;
input Mat    Perf   temp   days   rep    ph group;
cards;
1      1      3      5      1      2.916666667 1
1      1      3      5      2      3.06 1
1      1      3      5      3      2.906666667 1
1      1      3      5      4      3.096666667 1
1      1      3      12     1      2.85 2
1      1      3      12     2      2.933333333 2
1      1      3      12     3      3.053333333 2
1      1      3      12     4      2.966666667 2
1      1      3      19     1      2.946666667 3
1      1      3      19     2      2.936666667 3
1      1      3      19     3      2.983333333 3
1      1      3      19     4      3.063333333 3
1      1      3      26     1      2.976666667 4
1      1      3      26     2      3.016666667 4
1      1      3      26     3      2.99 4
1      1      3      26     4      3.033333333 4
1      1      3      33     1      2.986666667 5
1      1      3      33     2      2.893333333 5
1      1      3      33     3      2.91 5
1      1      3      33     4      3.033333333 5
1      2      3      5      1      3.146666667 6
1      2      3      5      2      3.03 6
1      2      3      5      3      3.1 6
1      2      3      5      4      3.043333333 6
1      2      3      12     1      2.886666667 7
1      2      3      12     2      2.803333333 7
1      2      3      12     3      2.93 7
1      2      3      12     4      2.91 7
1      2      3      19     1      2.833333333 8
1      2      3      19     2      2.93 8
1      2      3      19     3      2.916666667 8
1      2      3      19     4      2.94 8
1      2      3      26     1      2.85 9
1      2      3      26     2      2.966666667 9
1      2      3      26     3      2.96 9
1      2      3      26     4      3.026666667 9
1      2      3      33     1      3.083333333 10
1      2      3      33     2      3.006666667 10
1      2      3      33     3      2.98 10
1      2      3      33     4      . 10
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1      3      3      5      2      2.96 11
1      3      3      5      3      2.95 11
1      3      3      5      4      2.956666667 11
1      3      3      12     1      2.766666667 12
1      3      3      12     2      2.903333333 12
1      3      3      12     3      2.9 12
1      3      3      12     4      2.893333333 12
1      3      3      19     1      2.863333333 13
1      3      3      19     2      2.886666667 13
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1	3	3	19	3	2.916666667 13
1	3	3	19	4	2.85 13
1	3	3	26	1	2.953333333 14
1	3	3	26	2	2.953333333 14
1	3	3	26	3	2.986666667 14
1	3	3	26	4	2.943333333 14
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1	3	3	33	3	3.016666667 15
1	3	3	33	4	3.113333333 15
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2	1	3	5	2	3.053333333 16
2	1	3	5	3	3 16
2	1	3	5	4	3.013333333 16
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2	1	3	12	3	2.91 17
2	1	3	12	4	2.946666667 17
2	1	3	19	1	2.78 18
2	1	3	19	2	2.91 18
2	1	3	19	3	2.936666667 18
2	1	3	19	4	2.92 18
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2	1	3	26	3	3.01 19
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2	2	3	5	4	. 21
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2	3	3	5	2	3.05 26
2	3	3	5	3	3.003333333 26
2	3	3	5	4	3.073333333 26
2	3	3	12	1	2.883333333 27

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2       3       3       12      2       2.9 27
2       3       3       12      3       2.983333333 27
2       3       3       12      4       2.96 27
2       3       3       19      1       2.83 28
2       3       3       19      2       2.953333333 28
2       3       3       19      3       3.006666667 28
2       3       3       19      4       3.076666667 28
2       3       3       26      1       2.94 29
2       3       3       26      2       2.963333333 29
2       3       3       26      3       2.97 29
2       3       3       26      4       2.98 29
2       3       3       33      1       3.156666667 30
2       3       3       33      2       3.05 30
2       3       3       33      3       3.076666667 30
2       3       3       33      4       3.06 30
;
run;

title 'ph at 3C';
proc mixed data= ph;
class mat perf days temp;
where temp=3;
model ph = mat perf mat*perf days days*mat days*perf
mat*perf*days/outp=predict;
lsmeans mat*perf mat*perf*days perf*days mat*days/ diffs;
run;
title 'residual plot for ph at 3C';
proc gplot data=predict;
plot resid*pred;
where temp=3;
run;
title 'one-way anova PH at 3C';
proc glm data= ph;
class mat perf days group;
model ph = group/solution ss1 ss3;
lsmeans group/adjust = bon pdiff;
output out=fitdata p=predict r=resid;
run;
title 'residual one-way anova ph  at 3C';
proc gplot;
plot resid*group;
run;
```

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