Title

Impacts of Steam Pasteurization on Walnut Storage Quality and use of Low Oxygen to Mitigate Impacts

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Impacts of Steam Pasteurization on Walnut Storage Quality and use of Low Oxygen to Mitigate Impacts

By

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THESIS

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Overall Abstract

California walnut growers and processors sell product all across the globe, and like with any raw food, safety assurance is critically important. The walnut industry of California utilizes either a chemical method of pasteurization or heat pasteurization to provide an effective kill step on any surface bacteria that might be present on the walnuts. In this study I only focus on two heat pasteurization methods: continuous and batch. The impact of the heat pasteurization methods on walnut storage quality is unknown. My thesis research analyzed the impact each pasteurization method has on walnut quality during storage, and the effect low oxygen storage may have in mitigating potential negative impacts caused by each heat pasteurization method. Quality was analyzed in the lab by measuring lipid degradation compound accumulation (peroxide value, free-fatty acid content), kernel color change, volatile compound accumulation (hexanal), and vitamin E degradation (tocopherol). Additionally, a trained sensory panel created a lexicon of descriptors in order to analyze the walnuts for changes in aroma, taste, and texture. Results showed that continuous walnut pasteurization accelerated lipid degradation, degraded vitamin E content, and increased negative sensory characteristics such as rancidity. Low oxygen storage of continuous pasteurized walnuts significantly reduced the rate of lipid and tocopherol degradation, and resulted in a lower sensory scores for rancidity. However, low oxygen storage of continuous pasteurized walnuts had significantly lower nut quality in comparison to either unpasteurized or batch pasteurized walnuts. Batch pasteurized walnuts had reduced lipid degradation and an improved sensory profile compared to unpasteurized walnuts, while vitamin E content was unaffected. Low oxygen storage of batch pasteurized walnuts further reduced lipid degradation, and caused an even greater improvement on sensory characteristics especially compared to continuous pasteurized walnuts. The best method of pasteurization and storage was found to be batch pasteurization stored in low oxygen conditions.

Chapter 1: Pasteurization and Low Oxygen Storage of Walnuts

I. Walnuts Globally

The modern English walnut is a popular nut crop in the plant family *Juglandaceae* and genus *Juglans L*. The walnut fruit is considered a drupe because the edible seed is surrounded by a hard seed shell, which itself is contained within a soft exterior hull. The modern walnut is thought to be likely domesticated from a Southwest Asia walnut in the 8th century BCE due to their close genetic affinity (Aradhya et al., 2021). It was in the early 20th century that, through modern breeding, the popular English walnut varieties came into production (Bujdoso and Cseke, 2021). In 2020, 2.3 million tons of in-shell walnuts were produced globally. China is the largest producer of walnuts, with 85% of their crop used domestically. The United States is the second largest producer, making up a third of globally exported walnuts (Foreign Agricultural Service, 2021). In 2021, California produced 725,000 tons of walnuts on 390,000 bearing acres. In 2022, production increased to 400,000 bearing acres, but yield reduced by 1% to 720,000 tons, likely caused by frost damage and an ongoing drought throughout the state (*2022 California Walnut Objective Measurement Report*, 2022). Walnuts have been an important crop in California since they were first introduced commercially in the 1870s. During this time, a soft-shell variety was grown around the Santa Barbara area called Placentia. Walnut production slowly shifted from southern California to northern California throughout the mid-1900s due to competition with citrus production, and water quality problems (Kelley, 1928; David E. Ramos, 1997). In 1979, the high yielding and light kernel color variety Chandler was patented by Harold Forde at UC Davis. The Chandler variety helped to grow the California walnut industry into one of the leading global producers (University of California, 2010).

II. Processing

In California, walnuts are harvested in the fall, usually around October. Prior to harvesting, walnuts are analyzed for their maturity level. As walnuts fully mature the packing tissue between kernel halves turn brown (Bentley, 2004). Additionally, the fruit show signs of hull dehiscence or hull splitting. Ethephon is a common preharvest chemical treatment that can help initiate ethylene production, which in turn speeds up hull splitting and promotes fruit abscission (Atungulu, 2013). Once about 30% of the walnut fruit show signs of hull splitting, a mechanical shaker will be brought in to vibrate the tree trunks and cause fruit abscission. After the fruit are knocked to the orchard floor, a blower pushes all the fruit into windrows. Then, the fruit are collected from the windrows and brought to a processing facility (Amin et al., 2017; Sibbett et al., 1974). The first step of processing walnuts is to dehull and wash them. The washed, in-shell walnuts are dried to approximately 8% moisture content and placed into mass storage warehouses or silos (Christopoulos and Tsantili, 2012). In-shell walnuts can be sold as a whole in-shell product or processed into kernels after bulk storage on an as-needed basis. In-shell walnut processing starts with deshelling, which involves passing the walnuts through a small space next to a large metal spinning disk. This causes the outer shell to break apart with minimal damage to the walnut kernel inside. Kernels are then sorted based on color and size. Chandler variety walnuts were bred to be light in kernel color and highly uniform. However, color separation is still required to maintain high product quality. Figure 1.1 shows the four-point visual scale used by the industry today (USDA Walnut color chart). After kernels are separated by color, they are

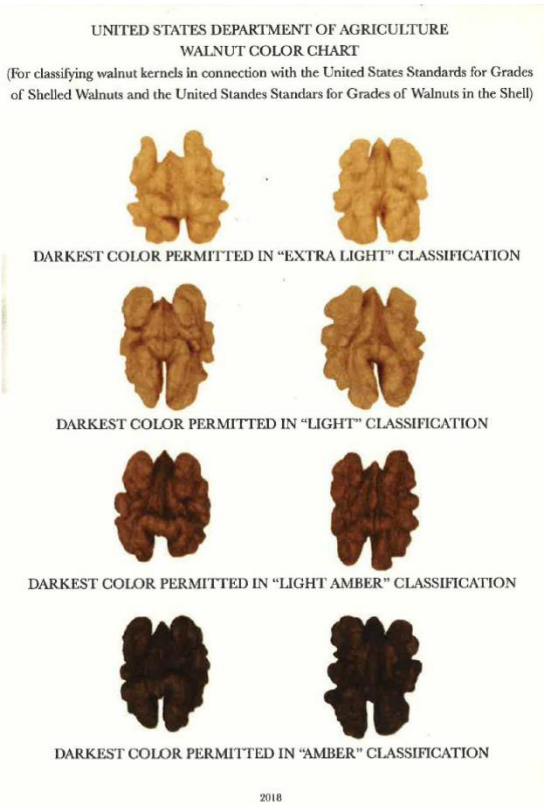

Figure 1.1. Visual aid used by the USDA and California walnut industry to determine kernel color grade. Source: USDA Specialty Crops Inspection Division

separated by size. The different kernel sizes range from small crumbs to entire half kernels. Once walnuts have been processed to kernels, they are packaged and sold within two weeks of processing (Shaw, 2021). A survey of California walnut processors found that walnuts are stored in-shell for up to a year in ambient air conditions. The temperature and relative humidity depend on the weather activity in the area (Adkison et al., 2021). Due to the large export market and the increased production of walnuts globally, there has been increased pressure on California walnut processors to keep in-shell product as long as possible so that walnuts can be sold year-round (D.E. Ramos, 1997). For many processors, year-round storage can be achieved, but the product can sometimes become rancid during the last few months before the next harvest (Österberg et al., 2001). Figure 1.2 highlights the order of each step involved from walnut harvest, to final processing.

III. Pasteurization

In the first decade of the 21st century, a growing concern for the general public centered around the possible microbial contamination of raw foods. Almost every year, there was a recall in the news for romaine lettuce due to contamination by food-borne pathogens, like Escherichia coli *(E. coli)*, Salmonella, or Listeria (Amanda Gardner, 2011; FDA, 2006; Food Chemical News, 2009). It was thought that microbial contamination was only a problem for the fresh, raw food industry because microbes could only survive and grow on high-water content products. However, this changed in 2008 when a case broke around the contamination of peanut butter with Salmonella that caused several people to become fatally ill (Bynum, 2014). This became a big concern for the walnut industry because during walnut harvest, the product is first knocked to the orchard floor before being taken to a sheller to be processed. The orchard floor contains a wide range of bacteria that can contaminate newly harvested walnuts (Blessington et al., 2014). It has been shown that *E. coli*, Salmonella, and Listeria can all survive on walnuts throughout the shelling process and remain on the product through long term storage (Blessington et al., 2013). Several recalls have occurred for walnuts due to contamination by all three microbes (RTT News, 2009; FDA, 2010; Tim Barker et al., 2014; Fisher, 2015).

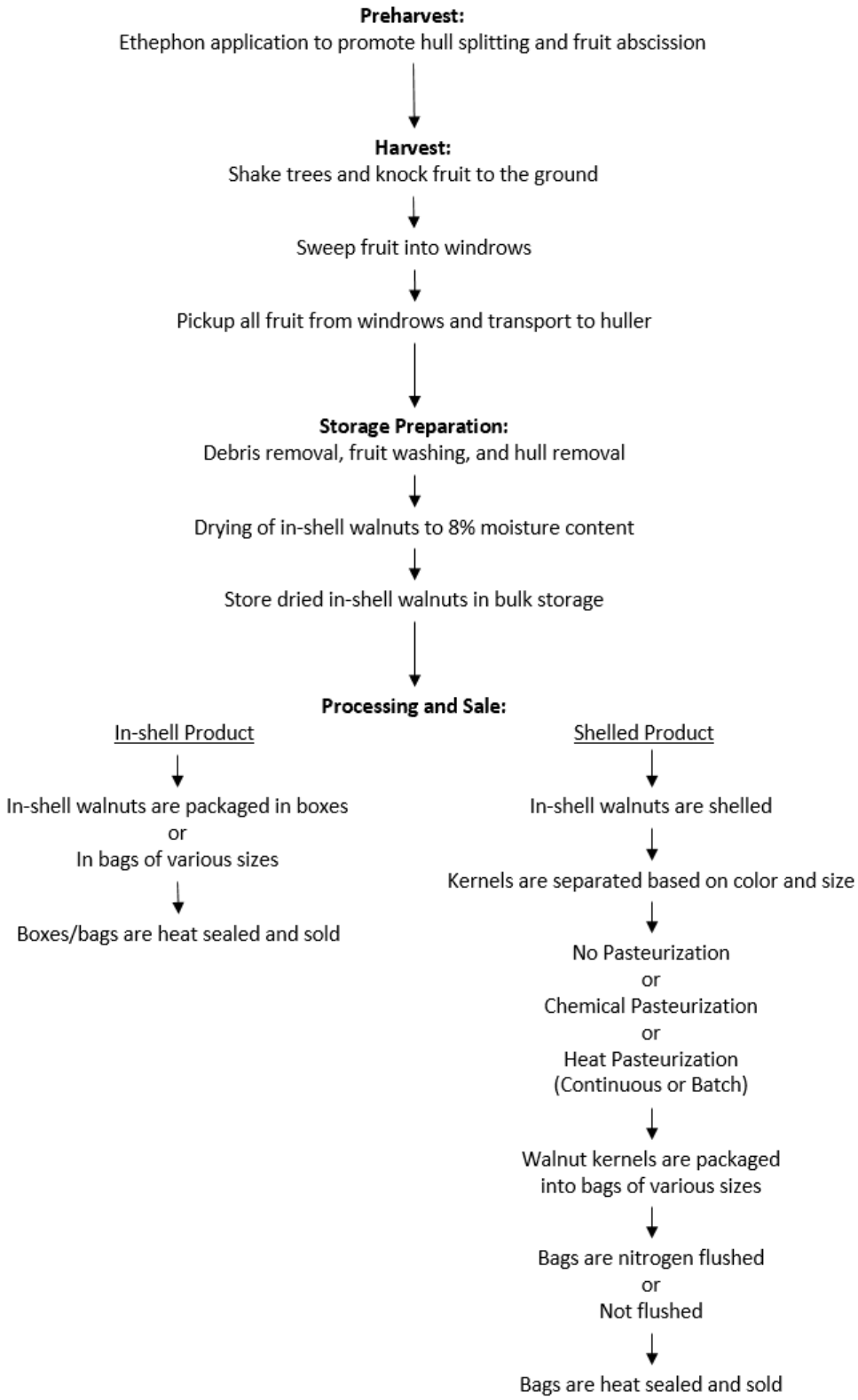

Figure 1.2. Walnut production process

Walnut pasteurization was developed to reduce the microbial load on shelled walnuts immediately before shipping to the customer (Poindexter, 2022). The idea was to ensure food safety by applying the process before packaging. Walnut pasteurization involves creating a log reduction of microbes on the surface of the product. To reach a log 4 reduction, 99.99% of all microbes must be eliminated from the walnut's surface. Many processors prefer to get the stricter log 5 reduction, which requires microbes to be reduced by 99.999%. Three different types of pasteurization were developed to achieve this reduction: a continuous heat treatment, a batch heat treatment, and a chemical treatment. In a continuous pasteurization system, walnuts are carried on a conveyer belt through a saturated steamer, a high-velocity oven dryer, then into a forced air-cooling system. In this process, walnuts are exposed to steam for a short time before quickly drying them to a low moisture content. In the batch pasteurization system, walnuts are preheated in large bins, then taken into a chamber where, using pressure manipulation, steam is injected and maintained at a temperature just above the boiling point of water. This allows the walnuts to be pasteurized without the possibility of water condensing on their surface. After the process is completed, the walnuts are quickly cooled in a forced air-cooling system to bring them back to ambient air conditions. For the chemical pasteurization treatment, the walnuts are exposed to propylene oxide (PPO) which causes irreversible damage to microbial DNA that is vital to the reproductive capabilities of the microbes (Ivarsson, 2013). This method is used by some segments of the walnut industry, but the chemical is not accepted as safe by some global markets, such as the EU (Jimenez et al., 2015). This thesis focuses on only heat treatment pasteurization processes.

IV. Health Benefits of Walnuts

Around the world, walnuts are sold and then consumed in various ways, as an ingredient, a topping, or individually as a snack. However, walnuts have been part of our diets for centuries, dating back to 7000 BCE in Persia. In the Mediterranean region, archeologists found evidence of walnuts being consumed in caves. The ancient Greeks were the first to cultivate walnuts on a large scale. They used walnuts as food, medicine, and dye for hair (Casas-Agustench et al., 2011). In 2000 BCE, on the other side of the world,

Native Americans consumed the Black walnut and used its sap for cooking. In ancient China, around 100 BCE, it was found that people consumed walnuts for their health benefits. The ancient Romans understood the high-energy properties of walnuts, and as a result many traditional foods from cultures around the Mediterranean Sea, such as in Italy, Israel, and Egypt, contain walnuts even today (Avanzato, 2010). Ancient people believed that walnuts were healthy, but not until recently did we find out exactly why they are good for us to consume.

A serving of walnuts (100g) contains high levels of both protein (14-24% of daily consumption) and fats (52-70% of daily consumption). They are a great energy source because of their high lipid content, which includes less unhealthy saturated and monounsaturated fatty acids and more healthy polyunsaturated fatty acids (PUFA). The leading PUFA in walnut is linoleic acid (omega-6) which makes up around 47 to 57% of all fats in walnut (Cannella and Dernini, 2005). The next most abundant fat in walnut is oleic acid (13-30%), followed by linolenic acid (omega-3, 11-14%). Linolenic acid metabolism has been shown to increase vasodilatory and anti-inflammatory oxylipins. It is also linked to neuroprotective effects and functions with phenols to improve brain health (Ros et al., 2018). These three compounds are considered essential dietary fatty acids because they are not synthesized in the body and must be consumed through food. Additionally, walnuts contain vitamin E and other antioxidant compounds (Beyhan et al., 2017). Vitamin E, or tocopherol, is primarily located in the skin of the walnut kernel, also known as the pellicle. The primary isomer of tocopherol found in walnuts is gamma-tocopherol which has positive health benefits when it comes to arterial health and anti-inflammatory impacts on the brain and heart (Li et al., 1999; Park et al., 2008).

V. Lipid Degradation

Like in any high fat containing food product, the downside to an abundant source of fatty acids is that they are prone to both oxidation and hydrolytic degradation. Oxidation of fatty acids occurs in two ways: autoxidation or catalytic oxidation. Autoxidation occurs primarily to PUFA, which contain more than one carbon-carbon double bond. These double bonds have allylic hydrogens that are C-H bonds adjacent to

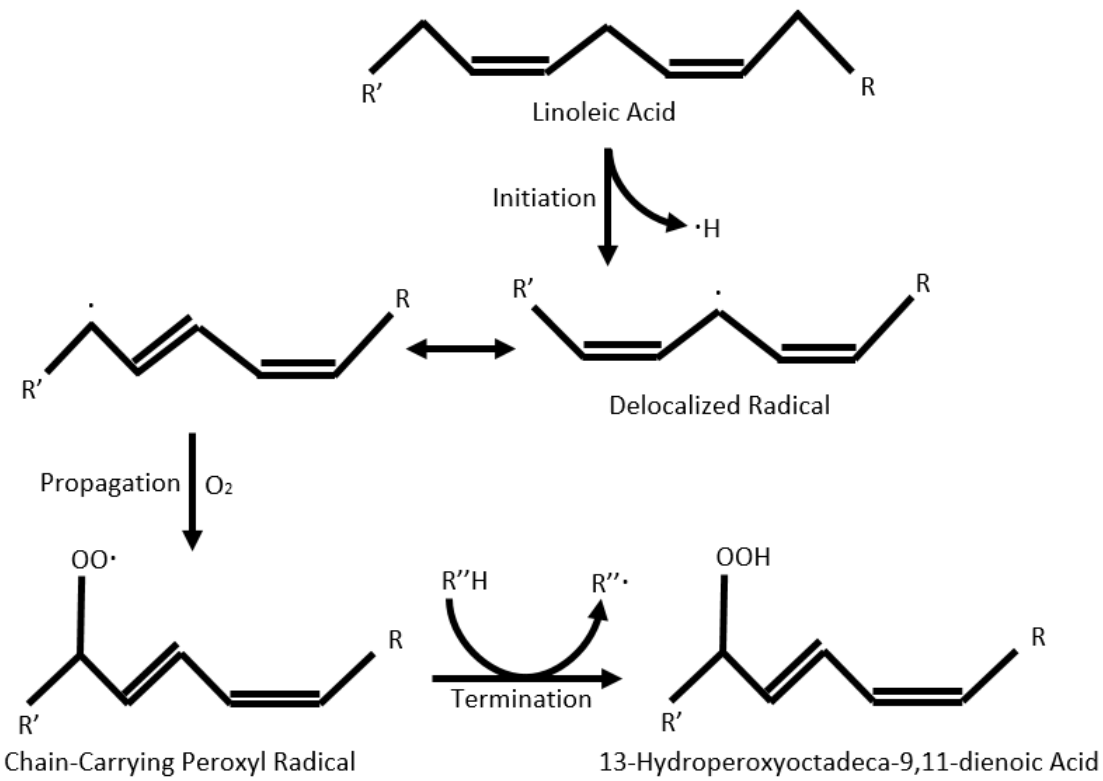

Figure 1.3. Autoxidation of linoleic acid and the formation of 13-hydroperoxyoctadeca-9,11-dienoic acid. Adapted from Frankel et al. 1984.

the double-bonded carbons. Allylic hydrogens are highly reactive and cause autoxidation to begin. The process occurs in three stages: initiation, propagation, and termination (Cosgrove et al., 1987). As shown in figure 1.3, starting in the initiation phase, the abstraction of the double allylic hydrogen atom (-RH) forms a carbon-centered delocalized radical (-R·). This cleavage process can be caused by an interior radical compound, such as hydroperoxides, or photochemically (Porter et al., 1995). A carbon-centered delocalized radical is highly reactive and causes the propagation phase to start. Oxygen in the atmosphere reacts with the radical creating a chain-carrying peroxyl radical (-ROO·). This peroxyl radical then reacts with another PUFA with an allylic hydrogen and creates a hydroperoxide (-ROOH) and another carbon-centered delocalized radical (-R·) (Dubrulle et al., 2017). In the last phase, termination, two peroxyl radicals can merge, creating a nonradical product and releasing oxygen back into the atmosphere. Catalytic oxidation is similar to autoxidation. Lipoxygenase enzyme facilitates the entire oxidation process. Lipoxygenase aids in the abstraction of the allylic hydrogen and catalyzes the attachment of oxygen to the exposed carbon-centered delocalized radical. Lastly, lipoxygenase neutralizes the chain-

carrying peroxyl by attaching a hydrogen atom to its end, creating a hydroperoxide (Gardner, 1991). Linoleic acid, the most abundant PUFA in walnuts, contains two double bonds on carbons 9 and 12. The next most abundant PUFA is linolenic acid which has three double bonds on carbons 9, 12, and 15. Linolenic acid autoxidizes at twice the rate of linoleic acid because it contains two doubly allylic hydrogen atoms instead of one (Cosgrove et al., 1987).

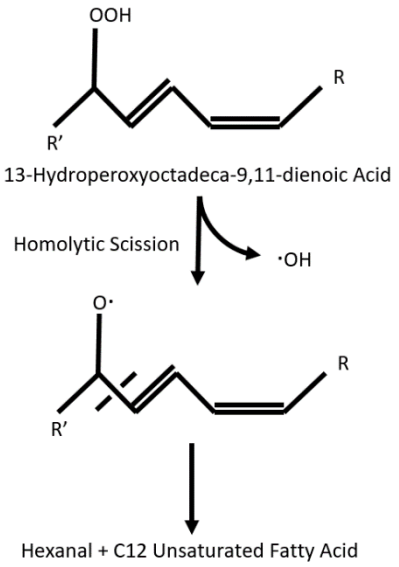

Figure 1.4. Autoxidation of 13-hydroperoxyoctadeca-9,11-dienoic acid and the formation of hexanal. Adapted from Frankel et al. 1984.

Another way walnut oil degrades is through hydrolytic degradation. Triglycerides make up the majority of fats in food products. They are composed of one glycerol molecule and three fatty acids. When the enzyme lipase comes into contact with triglyceride and water, it catalyzes the hydrolysis process creating individual fatty acid molecules (Murty et al., 2002). A common molecule analyzed in walnuts to determine the rate at which triglycerides degrade is oleic acid. This unsaturated fatty-acid does not rapidly oxidize because it contains only one carbon-carbon double bond (Porter et al., 1995). The presence of antioxidants like tocopherols decreases the process of rapid oxidation in walnuts. These molecules stabilize PUFA that have formed carbon-centered delocalized radicals, effectively stopping the oxidation process from proceeding to the propagation phase (Abdallah et al., 2015; Wagner and Elmadfa, 2000). Even with the presence of tocopherols, over time, hydroperoxides will eventually increase. As they accumulate, they further oxidize and break down into volatile organic compounds (VOC), such as aldehydes, alkyl furans, organic acids, and ketones (Grebenteuch et al., 2021). Hydroperoxides are both odorless and tasteless, but the VOC's they break down into are easily detectable organoleptically (Meijboom, 1964). Hexanal is the most common VOC created in walnuts through the autoxidation process or facilitated by the enzyme hydroperoxide lyase (Canoles et al., 2005; Grechkin et al., 2006). There are many proposed pathways that could lead to the creation of hexanal either via enzymes or autoxidation. In this paper, I explain two different chemical pathways, both of which start with a specific

hydroperoxide formed from linoleic acid, 13-hydroperoxyoctadeca-9,11-dienoic acid. Chemical pathways are adapted from Frankel et al., 1984 and Grechkin et al., 2006.

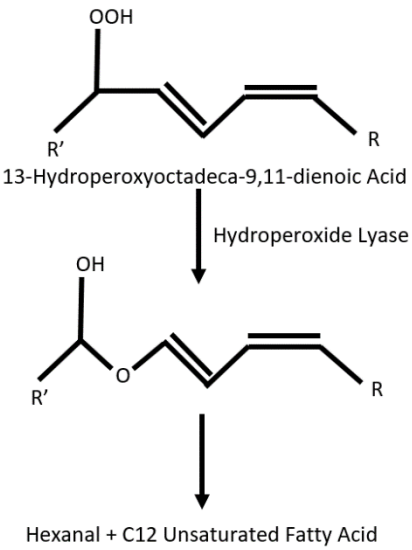

Figure 1.5. Catalytic oxidation of 13-Hydroperoxyoctadeca-9,11-dienoic acid by hydroperoxide lyase and the formation of hexanal. Adapted from Grechkin et al. 2006.

In the first pathway, as shown in figure 1.4, 13-hydroperoxyoctadeca-9,11-dienoic acid gets a hydroxide group removed through homolytic scission. Then the radical containing 13-hyroperoxide can be cleaved between carbon 13 and 14, forming hexanal and a 12-carbon unsaturated fatty acid. In this pathway, homolytic scission occurs, potentially caused by light exposure (Frankel, 1984; Shahidi and Pegg, 1994). In the second pathway, as shown in Figure 1.5, 13-hydroperoxyoctadeca-9,11-dienoic acid is rearranged to form a hemiacetal facilitated by the enzyme hydroperoxide lyase (Grechkin et al., 2006). The compound is then cleaved, creating hexanal and a 12-carbon unsaturated fatty acid again. Hexanal has been linked to off-flavor and off-odors in many other food products, causing them to become rancid. Other VOC's that are formed in walnuts are propanol and octanol, both of which are linked to increased rancidity (Abreu et al., 2017; Kaykhaii and Rahmani, 2007).

VI. Low Oxygen Storage

The chemical mechanism behind the formation of hydroperoxides from PUFAs requires the presence of atmospheric oxygen. In 1959, a test was conducted to demonstrate how low atmospheric oxygen could reduce the rate of development of rancid taste and odor in walnuts compared to walnuts stored in ambient air conditions (Wells and Barber, 1959). Different techniques for achieving a low oxygen atmosphere were developed and implemented into walnut packagings, such as nitrogen flushing and the addition of oxygen absorbing sachets (Jensen et al., 2003; Maté et al., 1996). In the absence of oxygen, the carbon-centered delocalized radical formed at the end of the initiation phase cannot proceed to the propagation

phase, and instead remains as a delocalized radical compound (Cosgrove et al., 1987; Porter et al., 1995). The reaction catalyzed by lipoxygenase is negated when oxygen is not present. The carbon-centered delocalized radical formed from the enzyme requires an oxidizing agent to move the reaction forward. Without oxygen, the radical remains until an oxidizing agent, such as oxygen, becomes available (Gardner, 1991).

VII. Impact of Pasteurization

The newly developed heat pasteurization processes can potentially impact walnut quality. The chemical mechanism of autoxidation is accelerated by heat (Ali et al., 2017; Iqbal and Bhanger, 2007). During the initiation phase, heat causes the abscission of the allylic hydrogen, pushing the reaction forward, changing the PUFA into a hydroperoxide (Cuesta et al., 1993). Slight heating can also cause enzymes, such as lipoxygenase and hydroperoxide lyase, to accelerate their reactions (Zhang et al., 2019). However, a high enough temperature application can denature many enzymes, reducing the formation of degraded lipid compounds over time. The specific impact of increased heat application on enzyme activity is dependent upon three parameters: specific temperature applied, duration of heat application, and method of heat application. For the continuous log 5 pasteurization process, walnuts enter the system at a minimum temperature of 16.6°C, then for 45 seconds, they pass through a saturated steamer set to 98.3°C. A saturated steam application with this large temperature difference will cause moisture to condense rapidly onto the walnut's surface. The gas to liquid phase change results in a large amount of heat being transferred into the walnut, and a significant increase in the walnut's moisture content. It has been shown that the rapid formation of water on the surface of nuts can also cause skin lifting. In almonds, steam pasteurization for 35 sec at 95°C caused skin detachment to occur (Chang et al., 2010). The almond's skin reattached after 10 min of cooling causing the skin to be wrinkled. Following the saturated steam application, the walnuts pass through an oven zone set to 165.5°C for 45 seconds. This dry heat application decreases the moisture content to around 8%. In the batch pasteurization system, pressure manipulation of the chamber allows for a lower temperature to be applied. Lower temperature prevents

moisture from condensing on the surface of the walnuts, but the application time needed for pasteurization is significantly longer than that for the continuous system.

The temperature applied during walnut pasteurization is significant and has the potential to denature the enzymes partly responsible for lipid degradation. A study in soy flour showed that lipoxygenase activity was reduced by 52% when the flour was heated to 82°C then immediately cooled. The enzyme's activity was reduced by 84% when the flour was heated to 100°C then cooled (Mustakas et al., 1969). In another study, tomato homogenates were heated to 65°C for 100 seconds, causing the lipoxygenase activity to be nearly reduced by 100%. In the same study hydroperoxide lyase was completely inhibited at 65°C in less than 50 seconds of application (Anthon and Barrett, 2003). The enzyme responsible for hydrolytic degradation seems to deactivate at a slower rate at high temperatures compared to the enzymes responsible for oxidative degradation. Lipase was shown to deactivate after 7 minutes at 90°C (Bendicho et al., 2002). In a different study, it took 40 minutes at 60°C to inactivate the lipase enzyme (Pimentel et al., 1997).

Based on the intensities of heat applied by both the continuous and batch pasteurization systems, most of the enzymes responsible for lipid degradation are likely significantly inactivated during pasteurization. This would only partially inhibit the accumulation of oxidation-degraded compounds over time because autoxidation would still take place. In the presence of atmospheric oxygen, PUFA can completely oxidize into hydroperoxides, which would cause the process to speed up as more oxidized compounds form. Significant heat application, as in the continuous pasteurization system, could cause PUFAs to rapidly oxidize and form an early abundance of hydroperoxides. This could then cause autoxidation to increase at a rapid pace immediately following pasteurization.

For antioxidants like tocopherols, it has been shown that high temperature applications impact their degradation. A study on tocopherols found that after applying 180°C heat for two hours, 50% of total tocopherols had degraded (Sabliov et al., 2009). The pasteurization of walnuts does not reach temperatures this high, but even lower temperatures being applied for a short period could cause some

tocopherols to be denatured, which in turn would impact the ability of tocopherols to prevent lipid oxidation. Another possible result of pasteurization is how it impacts the texture of walnut kernels. In a study on walnuts heated to 120°C, a sensory panel rated the product as more roasted and nuttier than the nontreated product (Liu et al., 2022). This has a mixed impact on consumers because some individuals prefer this textural change while others prefer fresh, unaltered walnuts.

VIII. Pasteurization and Low Oxygen

The process of walnut pasteurization has potentially positive or negative impacts on the product's immediate quality and its long-term shelf stability. One potential way to offset the negative effects brought on by pasteurization is to store the walnuts at very low oxygen atmospheres. With this combination of pasteurization and low oxygen storage, many factors would be impacted to slow the development of rancidity. Pasteurization would denature many enzymes responsible for catalytic oxidation (lipoxygenase and hydroperoxide lyase) and hydrolytic degradation (lipase). Then, with low oxygen storage, the propagation phase of autoxidation would be effectively blocked. This would prevent hydroperoxides from forming and, furthermore, slow the accumulation of VOCs such as hexanal. Any enzymes involved in catalytic oxidation that did not get denatured by the pasteurization process would likely be ineffective in a low oxygen environment. Without available oxygen, hydroperoxides do not have the opportunity to form even if enzymes are present to facilitate the reaction. As a result, many of the compounds responsible for rancid taste and odor would not have the ability to accumulate significantly. The upstream impact of slowing lipid degradation is that many PUFA's would be retained longer. Additionally, the hydrolytic degradation process would be significantly down regulated because the pasteurization process could denature the enzyme lipase, which is responsible for degrading triglycerides in walnut oil. Slowing this process will allow more fatty-acid compounds to be retained on the triglycerides. Pasteurization and low oxygen storage may have the ability to assure product safety while slowing lipid oxidation, slowing hydrolytic lipid degradation, extending overall shelf life, and retaining healthy PUFAs and triglycerides.

For both the walnut industry and the average walnut consumer, it is important to have an understanding of the impact heat pasteurization and low oxygen storage has on walnut quality. This thesis aims to understand the effect continuous and batch pasteurization methods have on walnut quality throughout long term storage. This includes both chemical effects, and organoleptic effects. Additionally, this paper will show how the various impacts on walnut quality by the heat pasteurization methods can be altered by long term low oxygen storage.

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Storage quality impacts of two heat pasteurization methods on walnut (*Juglans regia L.*) kernels

Abstract

The California walnut industry sells English walnuts (*Juglans regia* L.) all across the world, largely as a raw ingredient product. As a raw ingredient, implementation of pasteurization techniques before marketing is often utilized to provide assurance to customers of food safety; preventing illness due to possible contamination by human pathogens. Heat pasteurization has become popular in recent years; however, it is unclear how it may impact walnut quality. Two heat pasteurization methods (continuous and batch) were independently analyzed for their impact on walnut storage quality. The continuous pasteurization system (log 4 and log 5 samples) utilized a fast run time, high temperature saturated steam, and a high temperature drying step. The batch pasteurization system (log 5 samples) utilized a longer run time and a pressure/temperature manipulation chamber, but no drying step. Following pasteurization, kernel quality was assessed immediately and periodically during storage. Objective measures of walnut quality degradation were compared to a trained sensory panel's analysis of taste, texture, and aroma differences. Continuous pasteurization for both log 4 and log 5 intensities significantly increased oxidative rancidity accumulation over time, but reduced hydrolytic rancidity accumulation, compared to the unpasteurized product. The sensory panel perceived a higher degree of rancidity and off-flavor, but a crunchier, more brittle texture in continuous pasteurized walnuts stored for six months. Batch pasteurization for log 5 intensity significantly reduced both oxidative and hydrolytic rancidity accumulation over time compared to the unpasteurized product. The sensory panel found less rancidity and off-flavor, but a crispier texture for the batch pasteurized walnuts stored for 6 months compared to unpasteurized walnuts. Batch pasteurization of walnuts that had been in storage for different lengths of time prior to treatment did not have a significant impact on objective quality. Batch pasteurization seemed

to be highly effective in reducing the rate of quality degradation; further research is needed to explore how the system impacts the activity of enzymes responsible for lipid degradation during storage.

Introduction

In California, walnut (*Juglans regia L.*) production is estimated to grow steadily over the next few years as a result of increases in total bearing acreage.[1] Consumer demand for walnuts has also increased due to the nut's high content of healthy fats.[2] Additionally, walnuts are rich in bioactive nutrients that aid in cardiovascular and brain health.[3] Due to their positive health benefits, walnuts are a common ingredient in trail mixes and salads, and frequently eaten raw.[4] Because of the lack of a kill step, raw ingredients always pose a potential risk to food safety. In 2008, a salmonella outbreak in peanut butter prompted the walnut industry to consider that walnuts could one day cause a similar outbreak because of how they are harvested.[5,6]

Walnuts in California are harvested by shaking the trees and knocking the nuts to the orchard floor, which can be contaminated with bacteria such as *Escherichia coli*, Salmonella, and Listeria.[7] It has been shown that bacteria can survive on walnuts throughout processing.[8] In response to this potential health risk, the California walnut industry implemented two different techniques for pasteurizing shelled walnuts to ensure food safety. When walnut kernels are pasteurized they can be either heat or chemically treated to reduce potential bacterial populations.[9] Chemical treatment involves exposing walnut kernels to propylene oxide gas; however, this method is not fully accepted in all markets.[10] For heat treatment, the industry currently uses two different methods. The first is a continuous pasteurization technique that involves exposing the product to high temperature saturated and dry steam. The second is a batch pasteurization technique that consists in treating large bins of product with high temperature steam and manipulating both pressure and temperature. Both heat treatments provide an effective kill step assuring food safety. However, little is known about their impact on walnut quality and shelf-life after treatment.

Walnuts can be stored for up to 12 months or longer, initially as in-shell product, and further after shelling and packaging for distribution.[11] The main causes of quality degradation during storage of walnuts are darkening of the kernels, degradation of walnut oils, and development of fungal decay.[12,13] Chemical measurements are used to assess walnut oil degradation: peroxide value (PV) and free fatty acid content (FFA). PV is the measure of total hydroperoxides; the oxidized product formed from the two most abundant polyunsaturated fatty acids (PUFAs) in walnut: linoleic acid or omega-6 (47-57%) and linolenic acid or omega-3 (11-14%).[14] PUFAs degrade readily in the presence of atmospheric oxygen and have the potential to further break down into smaller compounds, such as aldehydes and ketones.[15] Hexanal is a volatile organic compound derived from the oxidative degradation of linoleic acid and has been positively correlated with negative sensory attributes such as bitter and rancid taste.[16] FFA is the total percent of free oleic acid, created through the hydrolytic degradation of triglycerides, facilitated by the enzyme lipase.[17] Oleic acid is a monounsaturated fatty acid (MUFA) and is the second most abundant fatty acid in walnuts (13-30%). The two pasteurization methods could have impacts on the rate of lipid degradation, and high temperature treatments have the potential to impact enzymatic activity and lipid autoxidation. Throughout the storage year, walnuts are shelled and pasteurized as needed, and continuously increase in rancidity as their PV and FFA values increase.[18,19] Pasteurization at different times during the storage year might impact the rate at which walnuts accumulate lipid degradation byproducts and become rancid.

High temperature heat application has several possible impacts on lipid degradation. Autoxidation is initiated via thermal or photochemical reactions, which could be altered by pasteurization.[20] Additionally, both oxidation and hydrolytic degradation can occur through enzymes, such as lipoxygenase (LOX), hydroperoxide lyase, and lipase.[17,21,22] All of these enzymes have the potential to be denatured at high temperatures.[23,24] The objectives of this study were to investigate the effects of continuous and batch pasteurization on the rate of walnut quality deterioration during storage, and to understand the effects of storage time prior to pasteurization on the kernel's response to pasteurization.

It is possible that the heat applied during the pasteurization treatment could denature enzymes that promote lipid oxidation, thereby reducing quality degradation during storage. However, it is also possible that the heat treatment could enhance the rate of walnut degradation, and the response could be different between the two methods of pasteurization.

Materials & Methods

Walnut sourcing

Large sized Chandler walnut kernels were sourced from walnut processing facilities in California over three seasons, including pasteurized and unpasteurized product from the same lot. The product was obtained from one facility using continuous-type pasteurization equipment (facility A), and two facilities using batch-type pasteurization equipment (facilities B and C). After sourcing, all walnuts were stored at 20°C with 60% RH in cardboard boxes with an interior plastic liner. Cardboard boxes provided physical protection and helped reduce the light exposure of the walnuts.

Season 1

Walnut kernels were obtained from facility A (continuous pasteurization), and facility B (batch pasteurization). All walnut kernels were received four months after harvest and were either pasteurized immediately before sourcing or unpasteurized (control) walnuts from the same lot within a facility. For facility A (continuous pasteurization), pasteurized products were treated using a system recipe designed to achieve either a log 4 or log 5 reduction in bacterial populations (Table 1). For facility B (batch pasteurization), the pasteurized product was treated using a system recipe designed to achieve a log 5 reduction in bacterial populations (Table 1). Walnuts were sampled and objective quality was evaluated every three months for a total of 12 months after sourcing. Sensory quality was assessed after 0, 6, and 12 months.

Season 2

Walnut kernels were obtained from facility A (continuous pasteurization) and packaged in 1.36kg plastic pouches. The pouches (*JenJil*, Turlock, CA, USA) were made of a tri-layer plastic with an oxygen transmission rate of 1.94cc cm^2/24hr.[25] All walnuts were received five months after harvest and were either pasteurized immediately before sourcing or unpasteurized (control) walnuts from the same lot within a facility. For location A (continuous pasteurization), pasteurized products were treated to obtain a log 4 or log 5 reduction in bacterial populations (Table 1). Walnuts were sampled and objective quality was evaluated every three months for a total of 12 months after sourcing. Sensory quality was evaluated after 0 and 6 months.

Season 3

Walnut kernels were obtained from facility C (batch pasteurization). Both pasteurized and unpasteurized (control) walnuts from the same lot were received immediately after pasteurization at three different time points after harvest: lot A (3 months after harvest), lot B (5 months after harvest), and lot C (8 months after harvest). Between harvest and pasteurization, walnuts were stored in-shell, in silos at ambient air conditions. The batch system pasteurized product was treated using a system recipe to achieve a log 5 reduction in bacterial populations (Table 1). Walnuts were sampled and evaluated for objective quality every three months for a total of 12 months after sourcing.

Kernel Quality Evaluation

At each evaluation time, walnuts from each treatment were divided for analysis. On a 10 x 10 cell counting tray (tray #1), 60 cells were filled with walnuts. Each cell had a collective of smaller walnut pieces that totaled in approximate mass to the kernels of one whole walnut. Color analysis (chromameter and color chart) was done nondestructively on the first 30 walnuts. Peroxide value (PV) and free-fatty acid content (FFA) analysis was conducted using all 60 walnuts. These walnuts were put into freezer safe 0.95L Ziplock bags (Ziplock®, *Johnson & Son, Inc.*) and stored at -80°C until oil extraction. On a

different 10 x 10 cell counting tray (tray #2), 30 cells were filled with walnuts using the same method as tray #1. These walnuts were used to test water activity (10 cells), moisture content (10 cells), and hexanal content (10 cells).

Kernel Color

Using a chromameter (Konica Minolta Sensing Americas, Inc, Ramsey, NJ, USA) CIELAB color space, the external color of the walnuts in the first 30 cells from tray #1 was analyzed. The chromameter was used to analyze the pellicle of 2 large walnut pieces from each cell. The L* value, which is a measure of white to black color, was used to measure kernel darkening.

A visual color score was given to the same 30 kernels from tray #1. The color score was based on the "Walnut Color Chart" which the United States Department of Agriculture created for use by the Dried Fruit and Tree Nut Association of California (USDA Equipment & Forms Depot, Fredericksburg, VA, USA). The chart has four color categories: extra light, light, light amber, and amber. These categories were assigned numerical scores of 1, 2, 3, and 4, respectively.

Moisture Content and Water Activity

The 30 walnuts from tray #2 were divided into three groups of 10. Ten walnuts were chopped and then sieved through a <1.7mm hole sieve (Advantech, New Berlin, WI). About 2 grams of sieved walnut pieces were placed in a water activity cup (Meter Group, Pullman, WA) and put into an AquaLab 4TE Duo Moisture Analyzer (Meter Group, Inc. USA) to test water activity. Approximately 5 grams of the sieved walnut pieces were weighed into an aluminum weigh boat (VWR, Radnor, PA). The weigh boats were placed in an oven at 105°C for 48 h before the samples were weighed a second time and the percent moisture content (MC, wet basis) was calculated using the following formula:[26]

% MC = (wet weight (g) – dry weight (g))/wet weight (g) *100

Peroxide Value and Free-Fatty Acid Content

The 60 walnuts from tray #1 were thawed and divided into six groups of 10, with two groups of ten for each replication. Ten walnuts were placed into the Stainless Steel test-cylinder-outfit of a bench-top hydraulic lab press (model #3925, Carver Inc., Wabash, IN). Oil was extracted and 14 mL was saved in a freezer safe tube. The tube was flushed with nitrogen before being stored in a -80°C freezer.

Frozen walnut oil was thawed, and 5 mL was analyzed for PV by titrating with sodium thiosulfate following the AOCS official method (Cd 8-53).[27] An additional aliquot of oil (5 mL) was used for free-fatty acid content following the AOCS official method (Cd 3d-63), which involved calculating the amount of potassium hydroxide needed to neutralize the oil.

Hexanal Content

Hexanal content was measured with a gas chromatograph (GC, *Agilent Technologies* Model *7890B*, Santa Clara, CA, USA) equipped with a splitless injector and a DB-wax UI column (30m, 0.25mm ID, 0.25µm film) (*Agilent Technologies*, Santa Clara, CA, USA). One extra gram of sieved walnut pieces was placed into a 20mL headspace screw top clear vial with 18mm magnetic headspace cap (*Agilent Technologies*, Santa Clara, CA). Samples were incubated for 10 min at 60°C, while a 50/30 µm DVB/CAR/PDMS gray SPME fiber (*Supelco/Sigma Aldrich/Millipore*, Bellefonte, Pennsylvania, USA) was conditioned for 7 min at 200°C. The fiber was then exposed to the headspace of the sample for 10 min, before the fiber was desorbed in the GC inlet for 1 min at 220°C. The initial oven temperature was 50°C, then increased at a rate of 10°C/min until 100°C was reached. A 1 min hold time proceeded, followed by a second ramp in temperature at 20°C/min until 200°C was reached. The hold time was 11 min. Then the temperature increased at a rate of 20°C/min until 240°C was reached, followed by a 3 min hold. A standard curve ranging from 0 to 5000 ppm was created and analyzed at the beginning, middle, and end of each batch of samples. A standard mix was created for each concentration along the standard curve using triacetin as a solvent to hexanal. For each standard mix, 10µL were placed into a 20mL vial and then closed with a septum cap. Each vial was prepared for a specific concentration along the standard curve.

Sensory Evaluation

A descriptive sensory analysis was used to evaluate the quality of the walnut samples. A group of 10-12 people was trained in sensory analysis of walnuts in order to calibrate their sensory perception. Panelists completed nine 1hr training sessions before the 0-month analysis of season 1. While in training, panelists were given a background on sensory analysis. The lexicon on walnut descriptors, and references for those descriptors, were decided upon by the panel. Panelists participated in a total of six different tastings over three years. Before each tasting, panelists completed a 30min refresher course to be re-familiarized with the tasting lexicon and methods.

Each tasting included three different sessions containing several walnut samples. Walnut samples (~16 g) were placed in 59 mL plastic cups with lids (Solo Cup Company, Lake Forest IL), which were labeled with randomly generated three-digit codes to identify the samples. Each session contained one sample from each treatment. Every evaluation was for walnuts stored for the same amount of time after pasteurization. The panelists scored walnut samples on eight aroma, four taste, and nine texture attributes (Table 2), using a 15 cm line anchored with less (0) and more (15). For some attributes, agreed upon references were used that were considered equivalent to the highest score for that specific attribute (Table 2). Tastings for season 1 were conducted in sensory booths (UC Davis) using computers running Compusense (Compusense Inc., Guelph, ON, Canada). Tastings for season 2 were conducted in panelist's homes due to pandemic restrictions using an online survey tool (Qualtrics^{XM}, Provo, UT, USA) to collect data. Results were analyzed using R studio.

During each session, the panelists analyzed the sensory quality of walnuts from one replication of all treatments at a time. Within each session, panelists evaluated one cup at a time, followed by a 1 min break. To begin, they first shook the cup with the sample inside to increase the volatile concentration. They then lifted the lid to smell the nuts and analyze their aroma attributes. Coffee grounds were used in between cups to clear their sinuses. Next, panelists pinched their noses and ate a few walnuts to assess the taste attributes. Pinching the nose helps to perceive only the taste of the nuts and remove the aroma

aspect. Lastly, for measuring texture, panelists were instructed to bite a couple of pieces with their front teeth and then bite a couple of pieces with their back teeth. They focused on how the walnut felt in their mouth, before and after swallowing. At the end of each session, the panelists paused for 5 min. During this time, they drank water and ate unsalted saltine crackers, and cut up cucumbers. These steps helped to reset the panelists and remove any lingering taste they still had in their mouths before they began the next session.

Results & Discussion

Continuous Pasteurization

Objective quality measures

For both harvest seasons and intensities of continuous pasteurization, the hydroperoxides (PV) accumulated at an accelerated rate compared to the unpasteurized samples. The initial increase in PV occurred immediately after pasteurization (0 months) (Figures 1A&B, Table 3). There was a significant difference in PV across seasons, between treatments, across months of storage and for treatment by storage month interactions (Table 3). During storage, walnuts treated at log 5 accumulated PV faster than walnuts treated at log 4 (Figure 1A&B, Table 3). The initial increase in PV for pasteurized walnuts was similarly observed in the heating of walnut paste (blended up walnut kernels) for 30 min at 60°C.[28] This result likely occurred because the walnut pellicle was significantly damaged, then the walnut meat was heated. The walnut pellicle acts as a natural oxygen diffusion barrier protecting the PUFA in walnut meat from autoxidizing rapidly.[29] In continuous walnut pasteurization, high temperature saturated steam could cause hot water to condense on the pellicle, resulting in both a significant amount of energy being transferred and for skin lifting to occur. Skin lifting was similarly observed in almond steam pasteurization.[30] Almonds heated by saturated steam at 95°C for less than 30 s resulted in skin lifting and a wrinkled appearance. After 10 min, the almond skin reattached to the almond meat. In walnuts, skin lifting could have allowed the walnut meat to be exposed to more oxygen due to a compromised pellicle.

Additionally, Perren et al.[31] showed that nuts roasted at high temperatures for short durations have higher cell porosity due to damaged cell tissues. The increased cell porosity may allow atmospheric oxygen to have significant penetration into lipid storage locations within the cells, resulting in a rapid accumulation of oxidative byproducts. After saturated steam application in continuously pasteurized walnuts, high temperature dry heat was applied. A combination of a compromised pellicle, a compromised cell structure and the application of high temperature heat could have resulted in rapid autoxidation and a sudden increase in PV.

Fluctuating weather conditions, differences in growing regions, and differences between processing facility equipment can all cause walnut quality to vary from season to season.[32] In Adkinson et al.[18] it was shown that walnut kernel quality steadily degraded over time in storage. Yet, continuous pasteurization caused PV to increase rapidly during storage, decreasing the walnut's shelf life significantly compared to unpasteurized product. Hydroperoxides are formed either through autoxidation or enzymatically. The enzyme lipoxygenase (LOX) can facilitate the oxidative degradation of PUFA, forming hydroperoxides. However, LOX is very susceptible to deactivation during high heat applications. Xu et al.[33] found that LOX was fully deactivated after a 4 min heating at 65°C. Due to the intensity of heat applied during continuous pasteurization, LOX was likely fully deactivated leading to a partial reduction in hydroperoxide creation. However, this reduction in hydroperoxide creation was likely insignificant compared to the increased impact of autoxidation. Autoxidation probably had a significant impact because of a combination of different factors. The observed sudden abundance of hydroperoxides would stimulate the production of exponentially more hydroperoxides via autoxidation. Hydroperoxides can facilitate the initiation step during autoxidation, leading to more hydroperoxide creation.[15,20,34] Additionally, after continuous pasteurization, the walnut's skin likely reattached to the walnut meat. However, it is likely that the process of detaching and reattaching did not allow for the pellicle to protect the walnut meat as effectively as before pasteurization.[29,35] Atmospheric oxygen might have significantly more access to the walnut meat and its PUFAs, stimulating the production of hydroperoxides. Lastly,

antioxidant levels can be impacted by heat treatments. It was shown that rapeseed oil slowly heated to 170°C for 6 h had significantly reduced total tocopherol content.[36] Like many seed oils, the most abundant antioxidants in walnut oils are tocopherols.[37] Tocopherols neutralize free-radicals that form during the autoxidation process of fatty-acids.[38] These antioxidants are present in the pellicle and, under significant heat stress, they can denature.[36] It is possible that due to the sudden condensation of water onto the continuously pasteurized walnut pellicle, which would cause a rapid amount of heat energy to be transferred at the point of condensation,[9] the tocopherols were significantly denatured. This would also contribute to the observed rapid accumulation of hydroperoxides in continuously pasteurized walnuts.

In season 1, continuously pasteurized walnuts contained a similar quantity of hexanal as the control samples immediately after pasteurization (month 0) (Figure 1C), but for season 2 at month 0, continuously pasteurized walnuts contained slightly less hexanal than the control samples (Figure 1D). The initial lack of secondary oxidative degradation byproducts was also observed by Damanik et al.[39] for palm oil. They found that it took about 12 h of heating palm oil at 120°C to cause a significant increase in compounds such as hexanal, propanol and octanol. In continuous pasteurization of walnuts, hexanal likely did not initially accumulate because hydroperoxides were not present in a significant enough quantity to translate into an increase in hexanal. Additionally, hexanal production might have been reduced initially due to the degradation of hydroperoxide lyase (HPL), a heat-labile enzyme found in walnuts that helps facilitate the degradation of hydroperoxides into hexanal,[21,40] by heat. Rodrigo et al.[41] found that HPL was nearly completely inactivated after 12 min in tomatoes heated to 55°C. In olive oil heated for 10 min at 60°C, HPL was almost fully deactivated.[42] For continuously pasteurized walnuts, the small but significant decrease in hexanal content in season 2 likely occurred because HPL activity was reduced due to the high temperature continuous pasteurization process.

For both seasons, hexanal accumulated at an accelerated rate during the first three months of storage of pasteurized walnuts compared to the control samples (Figure 1C&D). A rapid accumulation of hexanal was also observed in the heating of oats.[43] Hexanal is formed either through autoxidation or enzymatically

via HPL. Due to the high concentration of hydroperoxides present immediately after pasteurization, the likely decreased effectiveness of the pellicle to protect PUFA from oxygen, the likely disruption of cell structure and the likely decreased concentration of antioxidants in the pasteurized walnuts, hexanal was able to accumulate rapidly through the autoxidation process. The inactivation of HPL likely had a minor effect on the accumulation of hexanal in continuously pasteurized walnuts. This was also observed in walnut oil that was heated to 160°C for 20 min.[44] Even with a high enough temperature and long enough application time to deactivate HPL, primary and secondary oxidative byproducts still accumulated rapidly.

Walnut quality is also assessed by the hydrolytic degradation of triglycerides, resulting in an increased rate of oleic acid accumulation. In seasons 1 and 2, the accumulation of FFAs (% oleic acid) during walnut storage was significantly slowed in continuously pasteurized walnuts compared to the control samples, starting immediately after pasteurization (Figure 1E&F). These results were contrary to those of Ban et al.[45], who found that a saturated steam application on almonds and pistachios for 20 s at 100-200°C resulted in no change in FFAs after treatment. It is possible that a 20 s application was not long enough to significantly impact the activity of lipase, the enzyme responsible for facilitating hydrolytic degradation of triglycerides.[17] Like LOX and HPL, lipase is also susceptible to high temperature deactivation. Bendicho et al.[23] found that it took 7 min to fully deactivate lipase at 90°C. In continuous pasteurization of walnuts, 102°C heat for 35-45 s was applied, then 171°C heat for another 35-45 s. This temperature and time likely caused a significant decrease in walnut lipase activity, resulting in the initial decline in FFA immediately after pasteurization. FFAs accumulated at a slower rate throughout storage in continuously pasteurized walnuts compared to the control samples, likely because the treated samples contained less lipase activity.

After 12 months of storage, a significant difference in kernel color (L* value) was detected between pasteurized and control walnuts with pasteurized kernels being darker in color (Table 3). Kernel darkening is caused by the degradation of phenols found in the walnut pellicle. Phenols degrade after they

oxidize during storage.[29] Continuous pasteurization did not cause an immediate darkening of the walnut pellicle, but during long-term storage, more phenols were oxidized and more darkening occurred compared to unpasteurized kernels.

Sensory quality

After six months of storage in both seasons, the PCA biplots of sensory attributes showed that rancidity, bitter, woody, and astringent attributes were all associated with continuously pasteurized walnuts (Figure 2) and their mean values were higher in log 4 and log 5 pasteurized nuts compared with control (Table 4). The two lipid oxidation byproducts, PV and hexanal, were also associated with continuously pasteurized walnuts. Texturally, pasteurized walnuts were associated with attributes such as crispy, crunchy, and brittleness (Figure 2). The unpasteurized walnuts had positive aroma and taste attributes such as buttery and sweet; while texturally they were associated with the creamy attribute. Control walnuts were also associated with the hydrolytic degradation compound FFA (Figure 2).

The negative sensory attributes clustered near the continuously pasteurized treatment are associated with accumulating oxidative degradation byproducts such as hexanal. It has been shown that hexanal is directly linked to negative sensory quality,[16] and even though PV is odorless and tasteless, its accumulation is positively correlated to the accumulation of hexanal.[29,46] FFAs were clustered near the control treatment because pasteurization likely caused partial deactivation of lipase enzymes responsible for triglyceride hydrolytic degradation. These results were different from Mitcham et al.[47] who found that in stored unpasteurized walnut kernels, FFA accumulation was associated with oxidative degradation byproducts such as hexanal and PV along with negative sensory attributes. Even though FFAs accumulate during lipid degradation, they are not the compounds responsible for negative sensory attribute association.

The texture attributes associated with continuously pasteurized walnuts suggest that the mouthfeel is more brittle and has a harder texture than control walnuts. This was also found in walnuts roasted in air or in

oil.[48,49] The bending force required to break a roasted walnut kernel was significantly less than control walnuts, and their texture was described as "crunchier".[49] The positive sensory attributes associated with unpasteurized walnuts suggest they tasted better overall and were more palatable. They also seemed to have a softer texture due to a higher association with buttery and creamy texture attributes.

Batch Pasteurization

Objective quality measures

For walnuts from facilities B and C, PV and hexanal accumulated over time during storage, but at a slower rate for pasteurized compared to unpasteurized walnuts (Figures 3A,B,C&D, Table 5). The slower increase in PV and hexanal over time for pasteurized walnuts compared to unpasteurized product was likely due to the method and duration of heating in the batch system. The walnuts are preheated to 40-50°C prior to being sealed in the pasteurization chamber. The vaporization point within the chamber is held just above 88°C through pressure manipulation so that water does not condense on the walnut's surface.[9] This process likely allowed significant inactivation of enzymes such as LOX and HPL, while potentially preventing skin lifting.[24,30,42] Additionally, Perren et al.[31] showed that for hazelnuts and almonds, a lower temperature and longer time heat application allowed for cell structure to remain relatively undisturbed, resulting in decreased cell porosity and less accumulation of oxidative byproducts. Similarly, Buransompob et al.[50] showed that PV accumulated at a slower rate in walnuts heated at 60°C for 10 min compared to the control. Buransompob et al.[24] additionally found that after treating walnuts at 60°C for 10 min, LOX activity was reduced to 19%, and no skin lifting was reported. Padilla et al.[51] found that LOX was almost completely deactivated after treating olive oil at 50°C for 10 min. Olive oil treated at 60°C for 10 min resulted in about a 50% reduction in HPL activity. All of these studies show that longer treatment time and low temperature have a significant impact on decreasing both enzyme activity, and oxidation byproduct accumulation. The batch pasteurization of walnuts likely prevented water condensation while successfully deactivating lipid degradation enzymes, resulting in a significant reduction in both PV and hexanal accumulation over time.

FFAs accumulated over time during walnut storage, but the rate of accumulation was slower as a result of batch pasteurization (Figure 3E&F, Table 5). The extended heating time during batch pasteurization likely reduced the lipase activity, thereby reducing formation of FFAs. Lehtinen et al.[43] found that oats steam heated to a 100°C for 40 min had reduced lipase activity and a reduced rate of FFA accumulation.

The L* value for batch pasteurized walnuts was not significantly different from the control walnuts, and neither changed significantly with time in storage (Table 5). In both the batch pasteurized and control walnuts the overall oxidization was not large enough to cause a significant color change. Without phenol degradation, the color change was similar for both treatments.

Sensory quality

Batch pasteurized walnuts were more associated with sweet, buttery, and sweet aroma attributes, while control walnuts were on the other side of the biplot with the rancidity attribute and lipid degradation compounds PV, hexanal, and FFA (Figure 4). PC1 explained 82.5% of the variation in the data. The slower rate of accumulation of oxidized compounds in pasteurized walnuts likely caused this difference. The control walnuts not only had more oxidized compounds, but also more hydrolytic degradation compounds (FFAs).

Batch pasteurized walnuts were associated with the texture attributes crisp, brittle, and crunchy; while control walnuts were associated with creamy and chewy (Figure 4). The heat treatment caused the walnuts to become harder and more brittle in texture. Kita et al.[49] found that after dry oven roasting walnuts at 160°C for 15 min, the force required to break the nuts was significantly less than that of untreated walnuts. The decreased break force found in dry oven roasted walnuts was associated with a more brittle texture. For roasted pistachios, a sensory panel found that medium roasting provided the best overall flavor and texture compared to any other treatment, including no roasting.[52] Batch pasteurization of walnuts at 88°C for 6-9 min likely prevents moisture from condensing on the walnut's surface,

effectively deactivating enzymes involved in degradation. The batch pasteurized walnuts had improved sensory quality in all categories: taste, aroma, and texture.

Effect of Storage Prior to Pasteurization

Walnut kernels were batch pasteurized after three, five, and eight months in storage as in-shell product, to determine if stored nuts responded differently. It has been shown that in-shell walnuts accumulate PV at a significantly slower rate compared to shelled walnut kernels.[18] The exterior shell provides a gas barrier, decreasing the oxidation rate. There were no differences in the accumulation rate of PV or hexanal in walnuts pasteurized after different times in storage (Table 6). The initial PVs immediately after pasteurization were similar between walnuts stored for different times prior to pasteurization (Figures 5A,B,C). However, control walnuts removed from storage and shelled later during the storage year showed a higher rate of PV increase over time. This might have resulted from a decreased content of antioxidants in the older walnuts as shown by Ampofo et al.[53]. The lower content of antioxidants would allow for hydroperoxides to accumulate at an accelerated rate, therefore causing PV to increase more quickly. However, the walnuts from the batch pasteurized treatment did not show significant differences in the rate of increase in PV (Figure 5A,B,C) or hexanal (Figure 5D,E,F) after different months of storage. The impact of pasteurization heat on PV or hexanal accumulation exceeded any potential impact of differences in antioxidant content among walnuts stored for different lengths of time.

FFAs increased at similar rates regardless of storage time before sample collection for pasteurized and unpasteurized walnuts (Table 6, Figure 5G,H,I). As seen in earlier pasteurization data, all treatments resulted in a significant decrease in FFA accumulation over time compared to the control, regardless of the storage time prior to treatment. Because FFA accumulation is not caused oxidatively, but hydrolytically, any potential reduction in antioxidant levels resulting from time in storage would not impact FFA accumulation.

Conclusion

The batch pasteurization treatment reduced the rate of walnut oil oxidation and overall rancidity development compared to unpasteurized walnuts. However, in contrast, walnuts treated in the continuous pasteurization facility exhibited an increase in the rate of walnut oil oxidation and overall rancidity development compared to unpasteurized walnuts. We hypothesize that the difference between the two systems was likely due to the batch system having a longer heat application time, lower temperature applied, and lack of water condensation, which has been associated with skin lifting. This could be tested by running this experiment again, including a wide array of time/temperature combinations manipulated in the batch pasteurization system. However, to be commercially viable, any new combination would need to meet the specifications required for an effective log 5 kill step.

Both pasteurization systems reduced the rate of accumulation of FFA, but we did not find an association between FFA content and sensory rancidity in our study. There were no differences in the impact of pasteurization on walnut quality when the kernels were pasteurized after different lengths of in-shell storage. More research should be conducted to explore the amount of enzyme deactivation in batch pasteurized walnuts. This would provide the industry with a better understanding of what temperatures and durations of pasteurization work best to extend shelf life while also reducing the risk of microbial contamination. This study concludes that batch pasteurization had a positive effect on walnut shelf-life by retaining nut quality, and continuous pasteurization had a negative effect on walnut shelf-life by accelerating nut quality degradation.

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Table 1. Pasteurization recipes for facility A (continuous pasteurization) and facility B and C (batch pasteurization).

Facility	Treatment	Initial Nut Temp (°C)	Total Dwell Time (s)	Nut Bed Depth (cm)	Steam Zone Temp (°C)	Oven Zone Temp (°C)	Post Oven Zone MC (%)
A	Log 4	9	70[X]	2.8	102	171	8
A	Log 5	9	90[Y]	2.8	102	171	8

Facility	Treatment	Pre Heat Temp (°C)	Pre Heat Time (min)	Pasteurization Temp (°C)	Pasteurization Time (min)	Cooling Temp (°C)	Cooling Time (min)
B	Log 5	40-50	35-40	88-90	6-9	20-30	20
C	Log 5	40-50	35-40	88-90	6-9	20-30	20

[X]walnuts took 35s to go through the steam zone and 35s to go through the oven zone
[Y]walnuts took 45s to go through the steam zone and 45s to go through the oven zone

Table 2. Sensory attributes evaluated by panelists along with their descriptors and references.

Category	Attribute	Descriptor	Reference
Aroma	Intensity	Strength of initial aroma; Scale from bland to intense	No Reference
	Rancidity	Paint-like, grassy, oxidized, old	[V]Old walnut
	Buttery	Creamy aroma	[Z]Butter
	Sweet smell	Can you smell the sweetness?	[G]Marshmallow
	Nutty	Overall nuttiness	[Y]Mixed nuts
	Earthy	Soil-like, mushroom-like, musty	[U]Soil
	Woody	Woodiness	Pencil shavings
	Vegetal	Fresh plant smell, green smell	Cucumber peel
Taste	Bitter	Bitter taste on the tongue	No Reference
	Sweet	Sweet taste on the tongue	No Reference
	Bitter aftertaste	Does the bitterness linger at all?	No Reference
	Sweet aftertaste	Does the sweetness linger at all?	No Reference
Texture	Crisp	First initial bite is "snappy", audible sound or feel when biting into it	Honeycrisp apple
	Crunchy	Sample continues to be audible as you chew it	[R]Carrot
	Brittleness	Falls apart into many pieces upon biting or snapping	[L]Life cereal
	Astringent	Mouth puckering, drying. Imagine an unripe persimmon	No Reference
	Oily	Leaves an oily residue in mouth after chewing	[Q]Potato chips
	Moist	Makes mouth wet as you eat it	No Reference
	Creamy	Smooth mouthfeel	[W]Chocolate
	Chewy	Resistance to breaking into pieces	[K]Gummy bear
	Tooth-packing	Leaves residue in teeth after swallowing	[J]Goldfish

[Y]mixed nuts = chopped up mix of Planters® Deluxe salted mixed nuts. Contains: cashews, almonds, brazil nuts, pecans and pistachios
[Z]butter = slice of Challenge Butter® unsalted
[V]old walnut = walnuts that had been in 20C storage for over a year
[W]chocolate = Hershey's Kisses® milk chocolate
[Q]potato chip = Lay's® Classic
[R]carrot = Signature Farms® peeled baby-cut carrots washed and ready to eat
[U]soil = wet soil found outside of Wickson Hall
[L]life cereal = Quaker® life multigrain cereal
[K]gummy bear = Haribo® Gummy bear
[J]goldfish = Pepperidge Farm® Goldfish
[G]marshmallow = Jet-puffed mini marshmallows

Table 3. Main effect means and interaction for year, treatment and analysis month for continuous pasteurized walnuts for two seasons. Walnuts received from facility A (continuous pasteurization).

	PV[Z]	Hexanal	FFA[Z]	L*Value[Y]
Season				
1	9.89 b[X]	0.72 b	0.22 b	48.2 a
2	17.2 a	1.41 a	0.23 a	45.8 b
Treatment				
Control	1.23 c	0.45 b	0.32 a	47.5 a
Log 4	13.4 b	1.30 a	0.19 b	46.1 b
Log 5	26.0 a	1.44 a	0.17 c	47.4 a
Analysis Month				
0	1.72 e	0.07 e	0.11 e	46.5 b
3	4.60 d	0.22 d	0.18 d	48.0 a
6	12.4 c	0.77 c	0.22 c	47.6 ab
9	22.7 b	1.39 b	0.27 b	48.2 a
12	26.2 a	2.88 a	0.34 a	44.6 c
Treatment : Analysis Month				
Control:0	0.03 h	0.08 h	0.15 gh	46.6 abc
Control:3	0.18 gh	0.10 gh	0.24 def	48.6 a
Control:6	1.04 fg	0.32 f	0.34 bc	47.9 ab
Control:9	1.95 ef	0.31 f	0.39 ab	48.7 a
Control:12	2.95 ef	1.44 cd	0.46 a	45.6 bcd
Log4:0	1.63 f	0.06 h	0.11 ij	45.8 bc
Log4:3	4.43 e	0.33 f	0.15 ghi	46.9 abc
Log4:6	9.67 d	0.89 e	0.18 fg	47.1 abc
Log4:9	21.2 c	1.58 c	0.21 ef	47.6 ab
Log4:12	30.1 b	3.65 a	0.29 cd	43.4 d
Log5:0	3.51 ef	0.06 h	0.09 j	47.2 abc
Log5:3	9.19 d	0.24 fg	0.13 hij	48.6 a
Log5:6	26.6 bc	1.09 de	0.14 ghi	47.8 ab
Log5:9	44.9 a	2.29 b	0.21 ef	48.4 a
Log5:12	45.6 a	3.54 a	0.26 de	44.9 cd

[Z]PV = peroxide value; FFA = free fatty acid

[Y]L* value = darkness value (0 = black, 100 = white)

[X]means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 4. Main effect means and interactions for season, treatment, and analysis month for sensory attributes in continuous pasteurized walnuts over two seasons. Walnuts received from facility A (continuous pasteurization).

	Rancidity	Bitter	Astringent	Buttery	Sweet	Creamy	Crisp	Woody
Season								
1	3.8 a[X]	4.6 a	6.0 a	3.6 a	3.9 a	3.9 a	6.8 a	4.8 a
2	3.3 b	4.2 b	6.4 a	2.6 b	3.8 a	3.5 b	6.6 a	5.0 a
Treatmen								
Control	2.0 b	3.5 b	5.8 a	3.3 a	4.1 a	4.0 a	6.2 b	3.9 b
Log 4	4.3 a	4.8 a	6.6 a	3.2 a	3.8 a	3.4 b	7.0 a	5.2 a
Log 5	4.3 a	4.8 a	6.2 a	2.8 b	3.7 a	3.7 ab	6.9 a	5.5 a
Analysis Month								
0	1.4 b	3.8 b	5.5 b	3.7 a	4.4 a	4.2 a	6.7 a	4.0 b
6	5.7 a	5.0 a	6.9 a	2.5 b	3.3 b	3.2 b	6.7 a	5.8 a
Treatment: Analysis Month								
Control:0	1.4 c	3.4 b	5.5 b	3.6 ab	4.3 a	4.2 a	6.4	3.6 b
Control:6	2.5 b	3.6 b	6.1 b	3.0 bc	3.9 ab	3.8 ab	6.1 b	4.2 b
Log4:0	1.5 c	4.1 b	5.5 b	3.9 a	4.4 a	4.1 a	6.9 a	4.1 b
Log4:6	7.1 a	5.6 a	7.7 a	2.5 cd	3.2 bc	2.7 c	7.0 a	6.3 a
Log5:0	1.2 c	3.8 b	5.6 b	3.5 ab	4.5 a	4.3 bc	6.9	4.2 b
Log5:6	7.4 a	5.7 a	6.8 ab	2.1 d	2.8 c	3.1 bc	7.0 a	6.9 a

[X]means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 5. Main effect means and interaction for treatment and analysis month for batch pasteurized walnuts from two facilities and two seasons. Walnuts received from facility B in season 1 and facility C in season 3 (batch pasteurization).

	PV[Z]	Hexanal	FFA[Z]	L* Value[Y]
Treatment				
Control	3.47 a[X]	0.52 a	0.29 a	44.94 a
Log 5	1.31 b	0.23 b	0.14 b	44.96 a
Analysis Month				
0	0.22 d	0.02 d	0.07 c	46.30 a
6	1.92 c	0.28 c	0.23 b	45.39 a
9	3.07 b	0.50 b	0.24 b	43.20 b
12	4.44 a	0.72 a	0.33 a	44.92 a
Treatment : Analysis Month				
Control:0	0.28 d	0.02 e	0.06 f	46.28 a
Control:6	2.39 c	0.37 c	0.31 b	45.21 abc
Control:9	4.37 b	0.68 b	0.36 b	43.08 c
Control:12	6.83 a	1.02 a	0.44 a	45.20 abc
Log5:0	0.16 d	0.02 e	0.08 ef	46.32 a
Log5:6	1.25 c	0.18 d	0.14 d	45.58 ab
Log5:9	1.78 c	0.32 cd	0.12 de	43.31 bc
Log5:12	2.05 c	0.41 c	0.21 c	44.65 abc

[Z]PV = peroxide value; FFA = free fatty acid
[Y]L* value = darkness value (0 = black, 100 = white)
[X]means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 6. Main effect means and interaction for storage months prior to treatment, treatment and analysis month for walnuts pasteurized after 3, 5 and 8 months. Walnuts received from facility C in season 3 (batch pasteurization).

	PV[Z]	Hexanal	FFA[Z]	L* Value[Y]
Months prior				
3	1.48 b[X]	0.20 b	0.23 ab	42.27 b
5	1.33 b	0.15 c	0.21 b	47.64 a
8	2.95 a	0.48 a	0.23 a	48.03 a
Treatment				
Control	2.89 a	0.44 a	0.30 a	46.21 a
Log 5	0.95 b	0.11 b	0.15 b	45.75 a
Analysis Month				
0	0.34 d	0.02 d	0.09 c	48.53 a
6	1.51 c	0.11 c	0.23 b	45.51 b
9	2.23 b	0.31 b	0.23 b	44.44 c
12	3.60 a	0.67 a	0.35 a	45.44 bc
Months Prior : Treatment				
3:Control	1.88 b	0.27 b	0.31 a	42.27 b
5:Control	1.82 bc	0.21 bc	0.28 a	47.62 a
8:Control	4.99 a	0.84 a	0.32 a	48.74 a
3:Log5	1.08 cd	0.12 cd	0.15 b	42.28 b
5:Log5	0.84 d	0.09 d	0.14 b	47.65 a
8:Log5	0.92 d	0.13 cd	0.15 b	47.33 a

[Z]PV = peroxide value; FFA = free fatty acid
[Y]L* value = darkness value (0 = black, 100 = white)
[X]means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

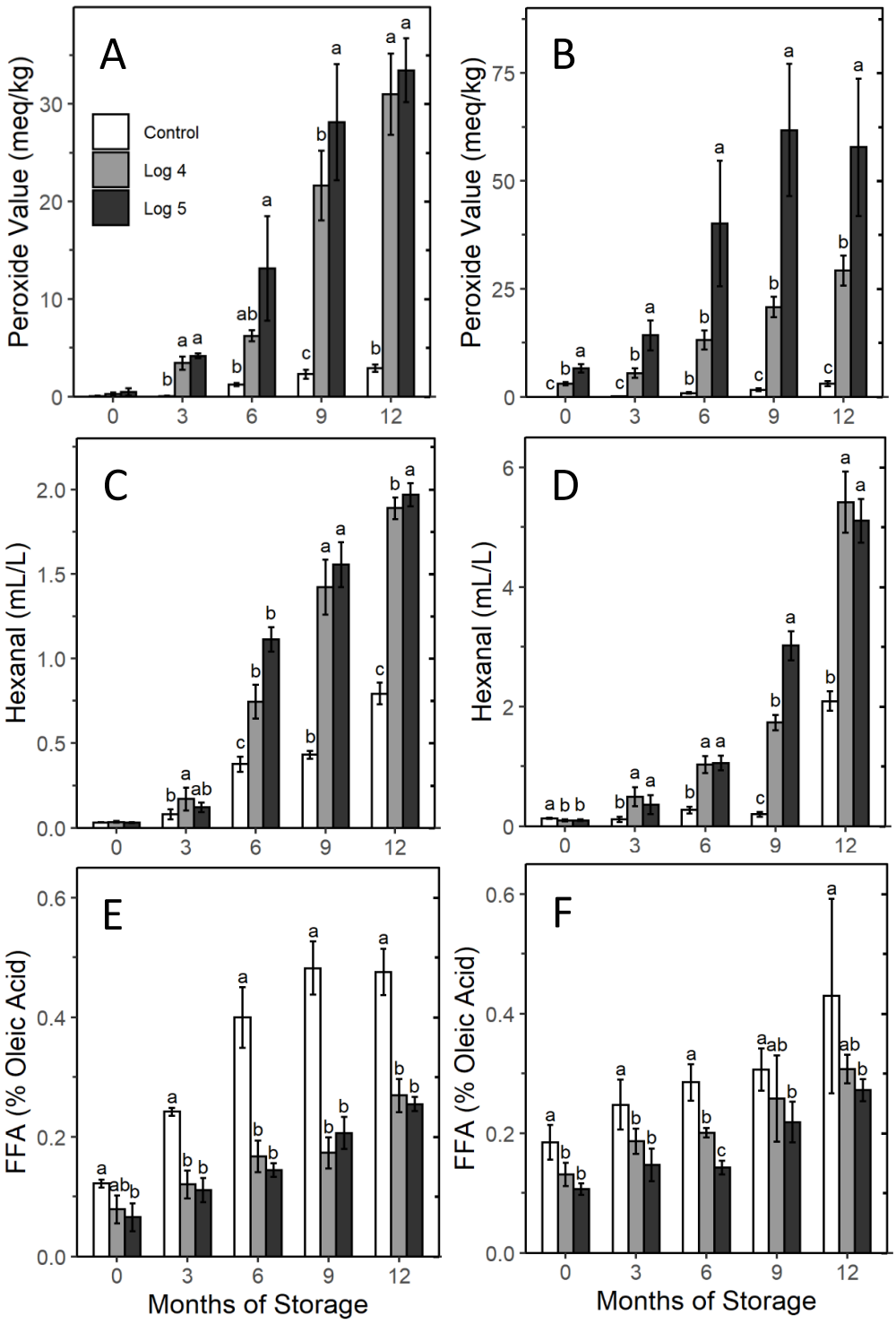

Fig. 1. The impact of continuous pasteurization on the content of peroxides, hexanal, and free fatty acids (FFA) across 12 months of storage at 20°C for walnuts received from facility A in season 1 (A,C,E) or season 2 (B, D, F). Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

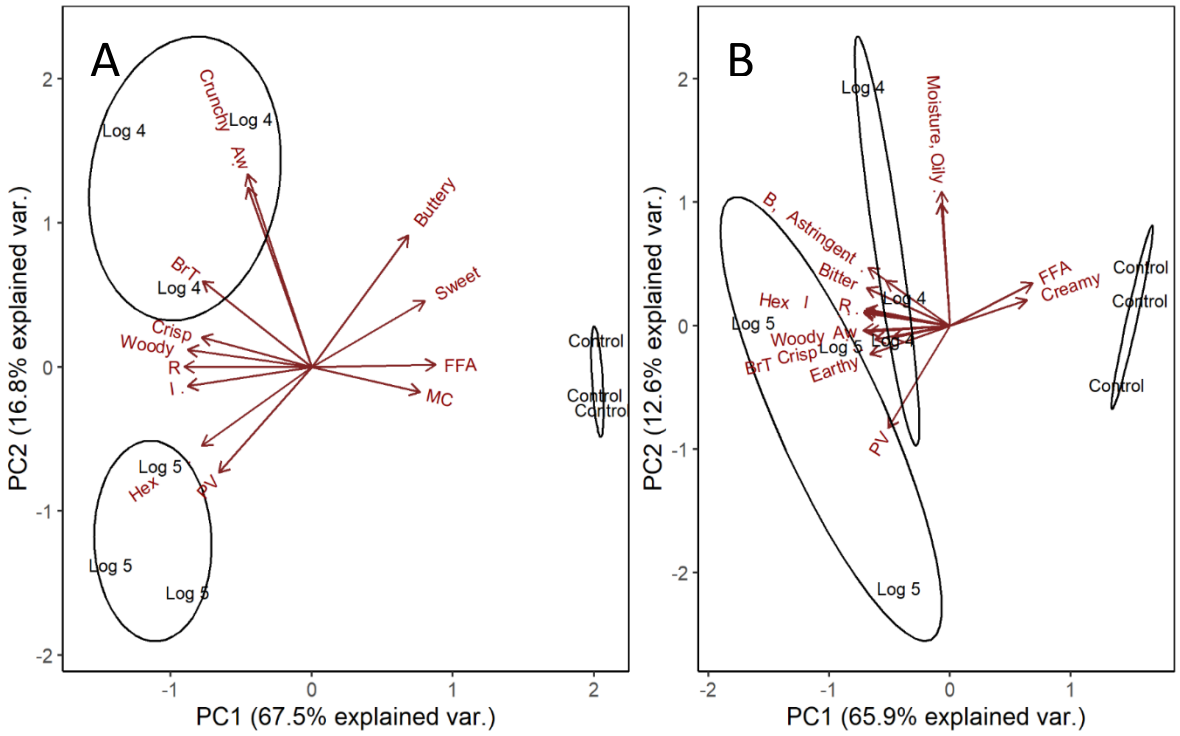

Fig. 2. Principle component analysis of both laboratory and sensory measurements of walnuts subjected to continuous pasteurization or unpasteurized after 6 months at 20°C of storage post-pasteurization in season 1 (A) and season 2 (B). Abbreviations used: I = intensity, R = rancidity, BrT = brittleness, B = bitter aftertaste, Aw = water activity, MC = moisture content, PV = peroxide value, FFA = free fatty acid, and Hex = hexanal.

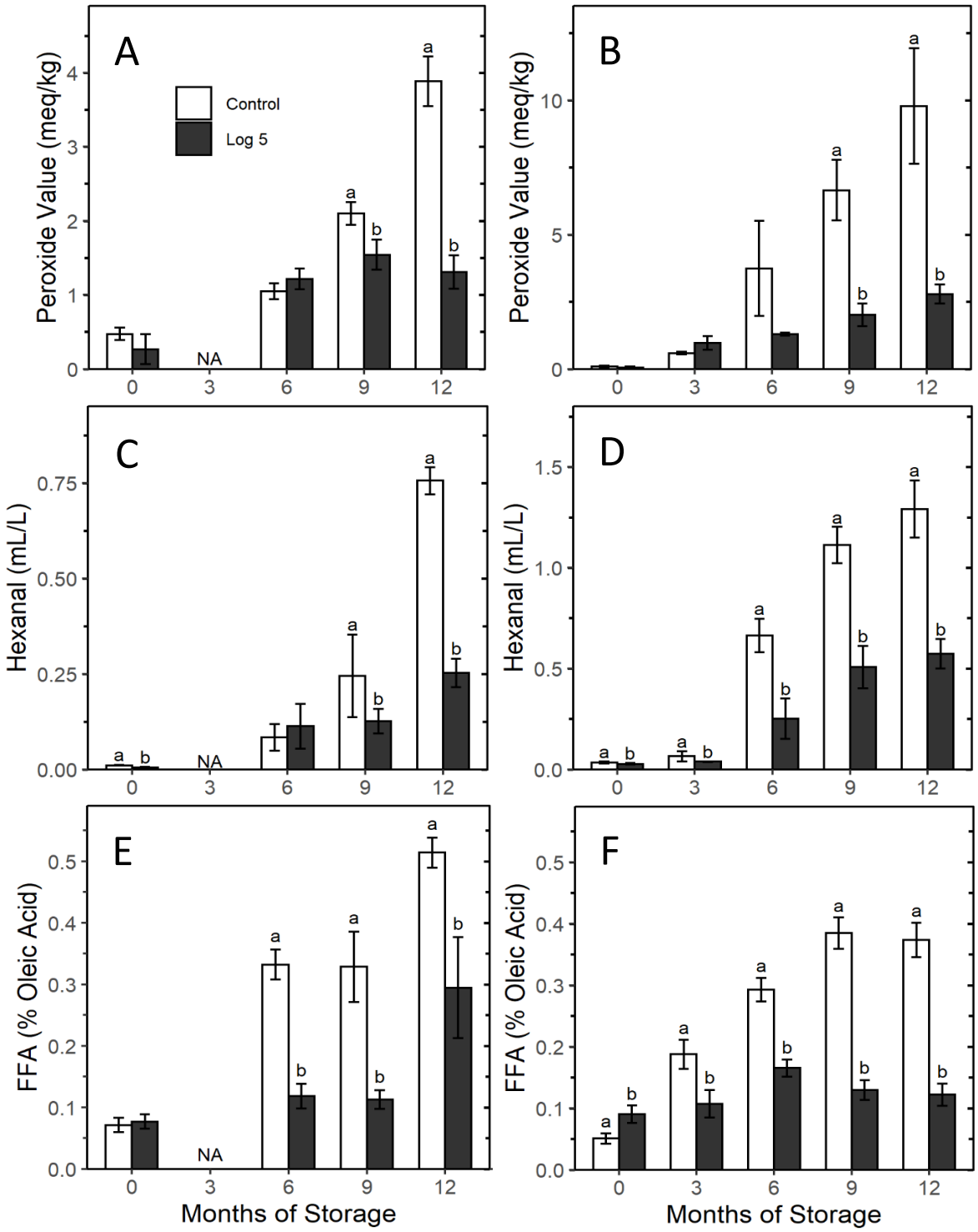

Fig. 3. The impact of batch pasteurization on the content of peroxides, hexanal, and free fatty acids (FFA) across 12 months of storage at 20°C for walnuts received from facility C in season 3 (A,C,E) or walnuts received from facility B in season 1 (B, D, F). Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

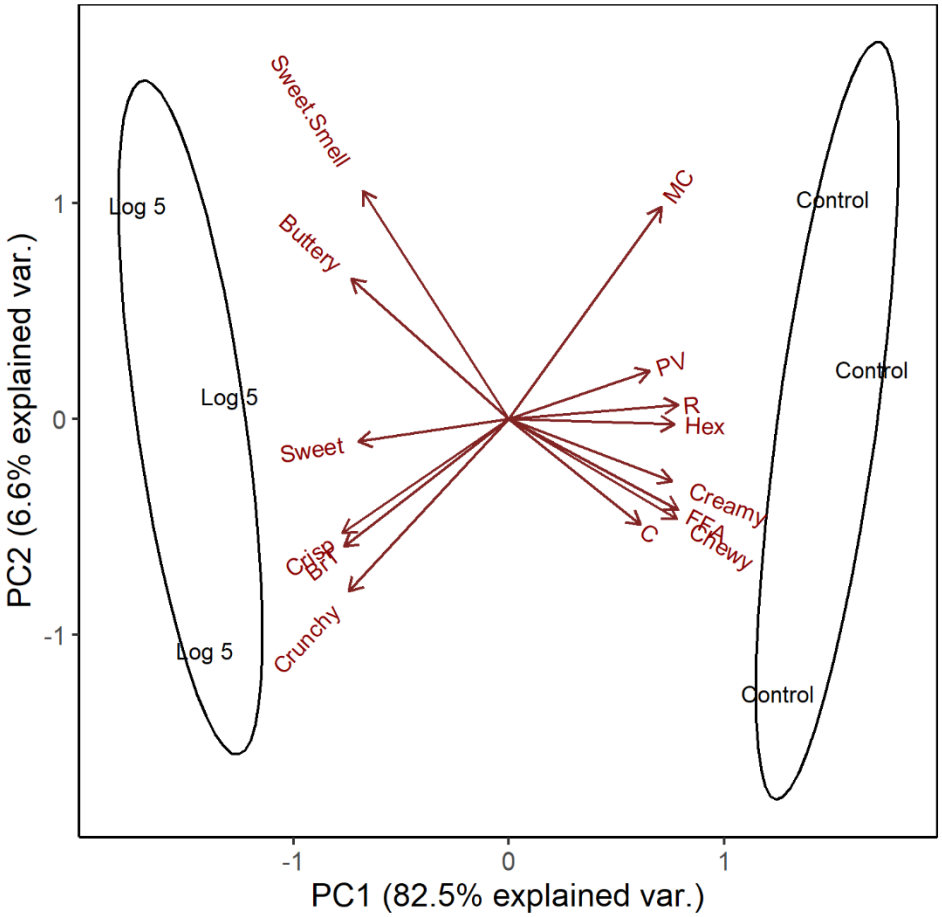

Fig. 4. Principle component analysis of both laboratory and sensory measurements of walnuts subjected to batch pasteurization or unpasteurized after 6 months at 20°C of storage post-pasteurization in season 1 from facility B. Abbreviations used: R = rancidity, BrT = brittleness, MC = moisture content, C = darker kernel color, PV = peroxide value, FFA = free fatty acid, and Hex = hexanal.

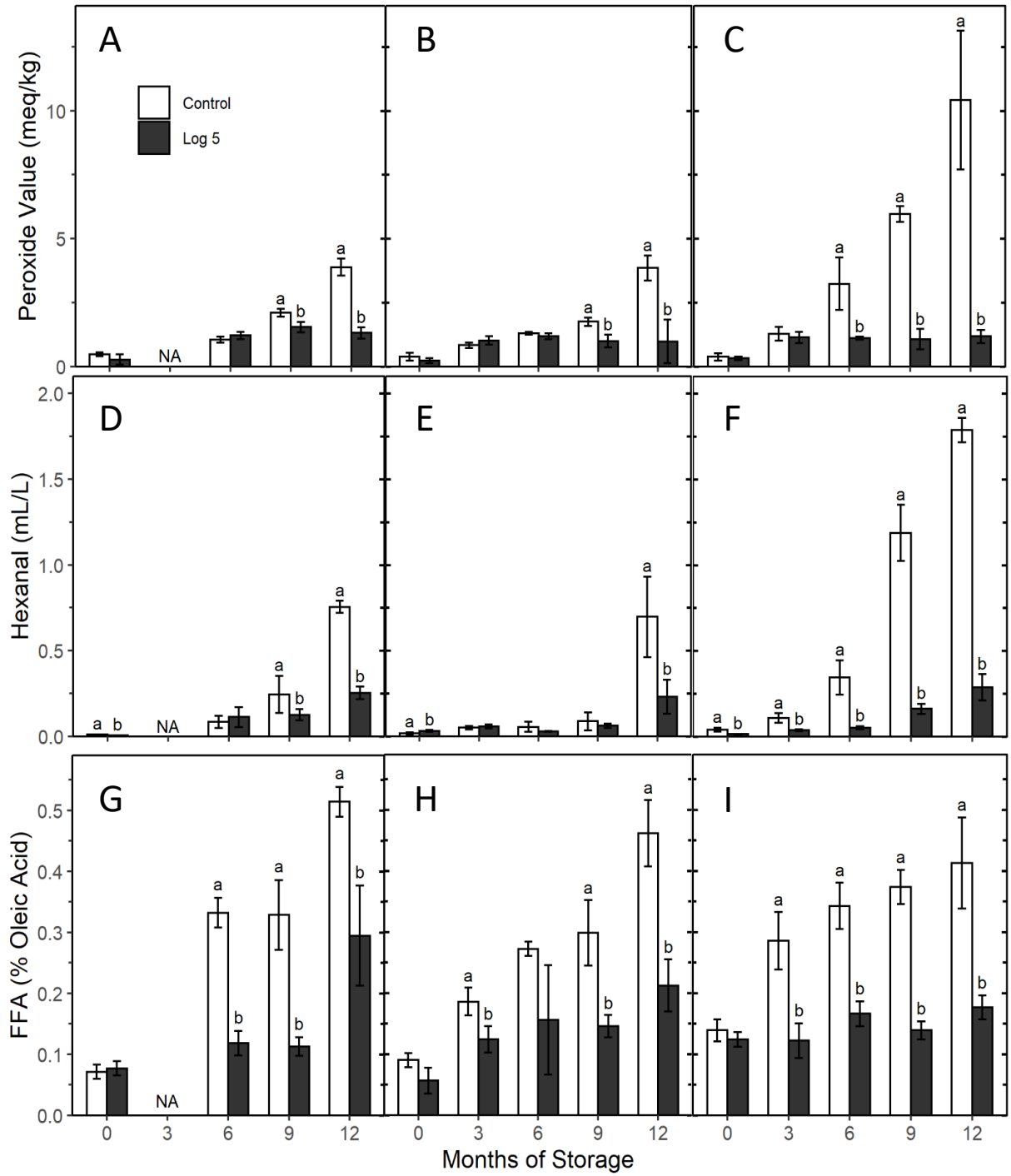

Fig. 5. The impact of batch pasteurization on the content of peroxides, hexanal, and free fatty acids (FFA) across 12 months of storage at 20°C for walnuts received from facility C, pasteurized 3 (A,D,G), 5 (B,E,H) and 8 (C,F,I) months after storage in season 3. Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

Impact of low oxygen storage on post-storage quality of pasteurized walnut (*Juglans regia L.*) kernels

Abstract

Walnut (*Juglans regia*) kernels were obtained from two facilities, each using a different method for pasteurization to achieve a log 4 and log 5 reduction in the bacterial load on the kernel surface. Continuous pasteurization utilized high temperature steam applied for a short period, and batch pasteurization utilized lower temperature steam applied in a pressurized chamber for a longer period. Walnut kernels from both types of log 4 and log 5 pasteurization treatments were stored in air or sealed in bags with a low oxygen transmission rate, and an oxygen absorbing sachet was inserted to reduce internal oxygen concentrations to <0.5%. After pasteurization and packaging, walnuts were stored and evaluated periodically for peroxide value, hexanal, free-fatty acids, and total tocopherols. These objective quality measures were compared to descriptive sensory analyses of walnut taste, texture, and aroma conducted by a trained sensory panel. Storage of walnuts in low oxygen atmospheres significantly mitigated some of the negative quality impacts that developed due to continuous pasteurization, which we suggest as a promising method to maintain walnut quality. However, continuous pasteurization resulted in an immediate decreased in total tocopherols prior to storage under low oxygen atmospheres; low oxygen reduced subsequent losses in tocopherols. Batch pasteurization did not cause negative quality impacts as compared to unpasteurized controls, but storage under low oxygen reduced the rate of quality degradation typically observed in stored walnuts. Storage under low oxygen reduced oil oxidation and slowed the degradation of total tocopherols.

1. Introduction

The third most lucrative nut crop grown in California in 2021 was walnuts (*Juglans regia L.*), only surpassed by almonds and pistachios (*California Agricultural Production Statistics*, 2022). California walnuts are world renowned for their great taste and high quality. People consume walnuts not only for

their flavor and aroma, but also for their high nutritional qualities. Walnuts are a great source of antioxidants such as vitamin E or tocopherols, (Kafkas et al., 2017), which have anti-inflammatory impacts on the brain and heart (Park et al., 2008). They also contain a substantial amount of healthy fatty acids linked to neuroprotective effects and help improve brain health (Ros et al., 2018). However, many fatty acids are also highly susceptible to oxidative lipid degradation during storage.

Walnuts are primarily composed of large lipid compounds called triglycerides. These triglycerides contain three fatty acid compounds, which can be polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), or saturated fatty acids (SFA) (Todorova et al., 2015). In walnut oil, the first and third most abundant fatty acids are PUFAs; linoleic acid (omega-6) (47-57%) and linolenic acid (omega-3) (11-14%), respectively (Beyhan et al., 2017). These compounds have two or more double bonds along their carbon chain tails, which makes the doubly allylic hydrogen atom between the double bonds susceptible to oxidation by atmospheric oxygen (Cosgrove et al., 1987). This chemical reaction can happen if PUFAs are exposed to oxygen in the atmosphere (Choe and Min, 2006). Removal of the allylic hydrogen by oxygen is considered the initiation step of autoxidation (Porter et al., 1995). Autoxidation eventually leads to the accumulation of hydroperoxides which can be measured using peroxide value (PV). PV is a common parameter used to determine walnut oil oxidative quality by the walnut industry. Hydroperoxides can further degrade in the presence of atmospheric oxygen through autoxidation into secondary byproducts such as aldehydes or ketones (hexanal, octanal, or propanol) (Frankel, 1984; Grebenteuch et al., 2021; Schieberle and Grosch, 1981; Shahidi and Pegg, 1994). These secondary byproducts are associated with off odors and rancid flavor (Jensen et al., 2001).

The presence of atmospheric oxygen primarily controls the process of autoxidation. Reducing oxygen concentrations during the storage of walnuts in order to reduce lipid oxidation and improve flavor was studied as early as 1959. Wells and Barber (1959) tested walnuts stored in sealed packages that were nitrogen flushed. The oxygen was reduced to less than 0.5%, and high quality flavor and aroma were better retained. Jensen et al. (2003) found that storing walnut kernels at 21°C in low oxygen transmission

rate (OTR) packaging with an oxygen absorbing sachet significantly reduced oxidative byproduct accumulation (hexanal), and also improved flavor and aroma throughout 12 months of storage. Until now, studies on the impact of low oxygen storage on walnut quality have largely focused on raw walnuts.

In 2008, a Salmonella outbreak in peanut butter (Bynum, 2014) prompted the walnut industry to consider methods to assure product safety for their consumers. Pasteurization was developed to provide an effective kill step for walnut kernels that were about to be packaged and sold (Black and Podolak, 2017). Currently, pasteurization of walnut kernels can be accomplished either by chemical or heat treatment. The chemical pasteurization method is commercially used, but the treatment may leave residues on the product, making it unacceptable in some markets (Jimenez et al., 2015). For heat pasteurization, there are two general methods to achieve an effective kill step: continuous and batch pasteurization.

Heat pasteurization can have a variety of impacts on walnut quality during storage after treatment, depending upon how the heat is applied (Perren and Escher, 2013). For continuous pasteurization, walnuts are carried along on a conveyor belt in a thin layer and exposed briefly to high heat saturated steam, then briefly to high heat dry steam, then rapidly cooled. This method applies high temperature heat for a short period. For batch pasteurized walnuts, the product is loaded into large metal bins, and preheated to an elevated temperature. The bins are then sealed and through pressure manipulation, saturated steam heats the walnuts to just below the condensation point. Later, the bins are depressurized and rapidly cooled. The batch pasteurization method utilizes a lower temperature heat for a longer period. Both methods can achieve an effective log 4 or log 5 kill step on the kernel's surface, 99.99% or 99.999% bacterial reduction, respectively. In either pasteurization method, after the walnuts are cooled to room temperature, they are packaged in market-ready bags with low OTR. Before the bags are sealed, a low oxygen treatment may be applied, either through nitrogen flushing or by adding an oxygen absorbing sachet.

Due to the high temperatures applied during heat pasteurization, many of the enzymatic pathways involved in the lipid degradation of walnut oil could be significantly slowed. The triglycerides that

makeup walnut oil can degrade hydrolytically through the enzyme lipase. Lipase utilizes water to remove fatty acids from triglycerides forming free-fatty acids (FFA) (Murty et al., 2002; Todorova et al., 2015). FFAs are measured through titration to determine walnut oil hydrolytic quality. They provide an accurate idea of the total percent of free oleic acid in a particular oil sample. Oleic acid is a MUFA and the second most abundant fatty acid in walnut oil (13-30%) (Beyhan et al., 2017). For PUFAs, enzymatic oxidation can occur via lipoxygenase (LOX), which utilizes atmospheric oxygen to degrade PUFAs into hydroperoxides (Buranasompob et al., 2007; Gardner, 1991). Lastly, the specific hydroperoxide derived from the most abundant fatty acid in walnuts, linoleic acid, is 13-hydroperoxyoctadeca-9,11-dienoic acid, which can be enzymatically degraded by hydroperoxide lyase (HPL) (Canoles et al., 2005). This enzyme utilizes atmospheric oxygen to degrade 13-hydroperoxyoctadeca-9,11-dienoic acid into hexanal (Grechkin et al., 2006), a volatile compound that can be measured on a gas chromatograph. All three enzymes are heat labile and have been shown to degrade after heat treatments. Lipase activity was almost entirely inhibited in tomatoes heated to 90°C for 7 min (Bendicho et al., 2002). LOX activity in wheat was also nearly completely inhibited after being heated to 70°C for 6 min (Sun et al., 2012). Lastly, HPL activity in tomatoes heated to 55°C for 12 min was significantly inhibited (Rodrigo et al., 2007). Due to the temperatures applied in these studies and the longer application time, both walnut pasteurization methods may have caused a significant reduction in enzyme activity.

The combination of heat pasteurization and low oxygen storage of walnuts has the potential to slow enzymatic lipid degradation due to the high temperatures during pasteurization and reduce the autoxidation of lipids due to the low oxygen conditions during storage. This study aimed to analyze the effect of low oxygen storage to mitigate the impacts of pasteurization on walnut quality degradation.

2. Methods

2.1 Walnut sourcing

Chandler walnut kernels were sourced two months after harvest in 2021 from two walnut processing facilities in California, including pasteurized and unpasteurized product. The walnuts were obtained from a facility using continuous-type pasteurization equipment (facility A) and a facility using batch-type pasteurization equipment (facility B). All walnuts were stored at the University of California Postharvest Pilot Plant at 20°C with 60% RH.

2.2 Initial packing

Walnut kernels obtained from facility A (continuous pasteurization) and facility B (batch pasteurization) were received in 9kg cardboard boxes with a plastic liner inside. All walnuts were either pasteurized immediately before sourcing or unpasteurized (control), both from the same lot. Pasteurized products from both facilities were treated to obtain a log 4 or log 5 reduction in bacterial populations, as described in table 1. Walnut boxes included 50% “large pieces” and 50% “halves.” Boxes were hand sorted to separate sizes into their respective groups. For each treatment (log 5, log 4, and control) and facility (A and B), walnuts were packaged in 19cm X 28.5cm plastic pouches (IMPAK Corporation, Los Angeles CA) that had a sealed zipper on one end. Eighteen pouches were filled with 0.35kg of “large pieces” sized walnuts for each treatment and facility for a total of 108 pouches. A 100cc oxygen absorbing sachet (IMPAK Corporation, Los Angeles CA) was placed in 9 of the 18 pouches for each treatment and facility. Pouches were sealed with a heat sealer (IMPAK Corporation, Los Angeles CA) to create an airtight package. Walnuts were sampled and evaluated upon receipt (0 months) and after 6, and 9 months of storage at the University of California. Sensory quality was assessed after 9 months.

2.3 Internal oxygen measurement

A port was created on each pouch’s surface to measure the oxygen concentration inside the pouches. A 2 cm circle of silicon (Grainger, Inc., Lake Forest, IL) was first placed on the pouch surface. A small rubber

septum (DAP Products Inc., Baltimore, MD) was pushed into the center of the unsolidified silicon circle. Finally, silicon was used to cover the top portion of the exposed septum. Pouches with septum newly attached were left to dry at 20°C for two days before being placed into cardboard boxes for the remainder of storage.

A bridge analyzer with a single sample analysis attachment (Bridge Analyzers, Inc., Bedford Heights, OH) was used to check the internal oxygen concentration using the attached septum 3, 6, and 9 months after initial packaging. The 3-month oxygen analysis revealed that all the sachet-containing pouches had air leaks. It was determined that the zipper closure was defective. After the pouch was opened and a new 100cc oxygen absorbing sachet was added, the zipper was cut off, and the pouch was heat sealed. A few pouches were analyzed three days after repacking to verify that low oxygen (< 0.5%) was accurately achieved. All oxygen sachet-containing pouches were repacked three months after initial packing. The pouch's internal oxygen concentrations at 6 and 9 months of storage are shown in table 2.

2.4 Kernel quality evaluation

At each evaluation time, three pouches (replications) from each treatment were opened for analysis. Walnuts from each replication were placed separately on a 10 x 10 cell counting tray (tray #1). Twenty cells were filled for each replication for a total of 60 cells. Each cell had a collective of smaller walnut pieces that totaled in the approximate mass of the kernels from one whole walnut. Color analysis (Minolta and color chart) was done nondestructively on the first 30 walnuts. Peroxide value (PV) and free-fatty acid content (FFA) analysis was conducted using all 60 cells, pressing each replication separately. The walnuts from each set of 20 cells per 3 replications were put into freezer safe 0.95L Ziplock bags (Ziplock®, *Johnson & Son, Inc.*) and stored at -80°C until oil extraction. On a different 10 x 10 cell counting tray (tray #2), 30 cells were filled with walnuts using the same method as tray #1. These walnuts were used to test water activity (10 cells), moisture content (10 cells) and hexanal content (10 cells).

2.5 Kernel color

The external color of walnuts in the first 30 cells of tray #1 were analyzed using a chromameter (Konica Minolta Sensing Americas, Inc, Ramsey, NJ, USA) CIELAB color space. The pellicle of two large walnut pieces from each cell were used for analysis. The measure of white to black color (L* value) was used to determine kernel darkening.

Additionally, a visual color score was determined for the same kernels from tray #1. The "Walnut Color Chart" created by the United States Department of Agriculture for use by the Dried Fruit and Tree Nut Association of California (USDA Equipment & Forms Depot, Fredericksburg, VA, USA) was used for color determination. The chart contains 4 color categories: extra light, light, light amber, and amber. Numerical scores of 1, 2, 3, and 4 were assigned to these categories respectively.

2.6 Moisture content and water activity

From tray #2, 30 walnuts were divided into 3 groups of 10. Each set of 10 walnuts were chopped, and sieved through a <1.7mm hole sieve (Advantech, New Berlin, WI). In a water activity cup (Meter Group, Pullman, WA) about 2 grams of sieved walnut pieces were placed, then the cup was placed into an AquaLab 4TE Duo Moisture Analyzer (Meter Group, Inc. USA) to test water activity.

In an aluminum weigh boat (VWR, Radnor, PA) about 5 grams of sieved walnut pieces were placed. The weigh boat containing sieved walnut pieces was placed in an oven set to 105°C for 48 hours. The sample and weigh boat was then weighed a second time and the percent moisture content (MC, wet basis) was calculated using the following formula:

% MC = (wet weight (g) – dry weight (g))/wet weight (g) *100

2.7 Peroxide value and free-fatty acid content

The walnuts from tray #1 that were previously frozen were thawed and separated. Each replication of 20 walnuts were split into 2 groups of 10. Each set of 10 walnuts were placed into a Stainless Steel test-cylinder-outfit of a bench-top hydraulic lab press (model #3925, Carver Inc., Wabash, IN). 14 mL of oil was extracted and saved in a freezer safe tube. After flushing the tube with nitrogen gas, the oil was stored at -80°C.

Once ready for analysis, frozen walnut oil was thawed, and 5 mL of oil was analyzed for PV by titration with sodium thiosulfate following the AOCS official method Dd 8-53 (American Oil Chemists' Society, 2017). An additional 5 mL of oil was used for free-fatty acid content following the AOCS official method Cd 3d-63, which involved neutralizing the oil with potassium hydroxide.

2.8 Hexanal content

A gas chromatograph (GC, *Agilent Technologies* Model *7890B,* Santa Clara, CA, USA) equipped with a splitless injector and a DB-wax UI column (30m, 0.25mm ID, 0.25µm film) (*Agilent Technologies*, Santa Clara, CA, USA) was used to measure hexanal content. An extra gram of sieved walnut pieces was placed in a 20mL headspace screw top clear vial with 18mm magnetic headspace cap (*Agilent Technologies,* Santa Clara, CA). For 10 min at 60°C samples were incubated while a 50/30 µm DVB/CAR/PDMS gray SPME fiber (*Supelco/Sigma Aldrich/Millipore*, Bellefonte, Pennsylvania, USA) was conditioned for 7 min at 200°C. For 10 min, the fiber was exposed to the headspace of the sample before desorption of the fiber in the GC inlet for 1 min at 220°C. The oven temperature was initially at 50°C, then increased to 100°C at a rate of 10°C /min. Followed by a 1 min hold time, proceeded by a 20°C /min second ramp in temperature to 200°C. A 11 min hold time followed. At a rate of 20°C/min, the temperature increased to 240°C, proceeded by a 3 min hold. The standard curve of hexanal (*Sigma-Aldrich*, St. Louis, Missouri, USA) was created with a range from 0 to 5000ppm. The standard curve was analyzed at the beginning, middle, and end of each set of samples. For each concentration along the standard curve, a standard mix

was created using triacetin as a solvent to hexanal. 10µL were placed into a 20mL vial and closed with a septum cap for each standard mix. For each specific concentration along the standard curve a vial was prepared.

2.9 Tocopherol analysis

Tocopherol content was measured using an Agilent 1290 Infinity II LC system with a diode-array detector (DAD) and an Agilent Eclipse XDB-C18 column (5µm, 4.6 x 150mm, *Agilent Technologies*, Santa Clara, CA, USA), according to the method of Simoes et al., (2020), with some modifications. Walnut oil was thawed and 40µL was vortexed with 160µL hexane. Then, 600µL methanol and 200µL internal standard solution was added. The internal standard consisted of α-tocopherol acetate in ethanol (300µg/mL). The sample was vortexed for 1 min and centrifuged for 5 min at 1789 x g (Sorvall Legend Micro 21R microcentrifuge, *Fisher Scientific*, Waltham, Massachusetts, USA). To allow for separation between the oil and organic phase, the samples were stored at -20°C for 10 min. The organic extract was filtered using a nylon filter (0.45µm, *Celltreat Scientific Products*, Pepperell, Massachusetts, USA) and then 20µL was analyzed by HPLC using methanol:water (96:4) as the mobile phase at a flow rate of 1.0 mL/min. The total run time was 12 min and a DAD signal was recorded at 292nm. A blank sample of ethanol followed by a standard mix was run every 15 samples. Specific tocopherols were determined based on their retention times in comparison with known standards. The standard mix consisted of delta-tocopherol, gamma-tocopherol, alpha-tocopherol, and alpha-tocopherol acetate (*Millipore Sigma*, Burlington, Massachusetts, USA) in ethanol at a concentration of 300µg/mL each.

2.10 Sensory evaluation

The evaluation of walnut quality was conducted using a descriptive sensory panel. A panel of 10-12 people were trained in walnut sensory analysis to calibrate their sensory perception. A total of 9, 1hr training sessions were completed by each panelist before the 6-month analysis of walnuts. Panelists were

given a background on sensory analysis during training. As a group, the panelists created a lexicon on walnut descriptors and references for those descriptors.

Included in each tasting, three different sessions containing several walnut samples were analyzed. About 16g of walnut samples that had been stored for 9 months were placed into 59 mL plastic cups with lids (Solo Cup Company, Lake Forest IL), all of which were labeled with randomly generated 3-digit codes for identification. Each tasting session had 1 replication from each treatment. Scoring of walnut samples was conducted on 8 aroma, 4 taste, and 9 texture attributes (Table 3) utilizing a 15 cm line anchored with less (0) and more (15). Several attributes had agreed upon references that were considered equivalent to a score of 15 (Table 3). The tasting was completed in panelist's homes using the online survey tool Qualtrics^{XM} (Provo, UT, USA) for data collection. Results were analyzed using R studio.

For each session, panelists analyzed sensory quality of walnuts from 1 replication of each treatment at one time. In each session, one sample was analyzed at a time proceeded by a 1 min break. First, panelists shook the cup containing the sample in order to increase volatile concentration. They then lifted the lid to measure nut aroma attributes. Between samples, coffee grounds were used to clear sinuses. After aroma, panelists pinched their noses and bite a few walnut pieces to measure the taste attributes. Nose pinching helps to only perceive the taste of the walnuts without the aroma aspect. Finally, panelists bite a couple pieces and chewed with their front teeth, then their back teeth to determine texture. Focus was placed on how the walnut felt in their mouth before and after swallowing. At the conclusion of each session, a 5 min paused was taken. During the pause, panelists drank water, ate unsalted saltine crackers, and ate cut up cucumbers in order to remove any lingering taste still on the pallet prior to starting the next session.

3. Results

3.1 Oxygen concentrations

The oxygen concentration within the low oxygen bags did not initially decrease to low enough oxygen levels due to a defect in the bag manufacturing resulting in leakiness. After three months, the oxygen

levels in the low oxygen bags with unpasteurized walnuts remained around 16%, while the oxygen level in bags with pasteurized walnuts was 7 to 8% for continuous pasteurization (Facility A) and 10 to 17% for batch pasteurization (Facility B). The oxygen level in bags with product pasteurized to log 5 had lower oxygen levels than those with the product pasteurized to log 4, regardless of the pasteurization facility. A similar trend was observed after 6 or 9 months in bags with the product from continuous pasteurization with or without the oxygen absorbing sachet (Table 2). However, the batch pasteurized walnuts did not impact the oxygen level in the packages as much as the continuous pasteurization. In some cases, the bags with the pasteurized product had higher oxygen concentrations than similar bags with unpasteurized product (Table 2).

3.2 Continuous pasteurization: Objective quality

Continuous pasteurization was conducted utilizing high temperature steam for a short period, as shown in table 1. Pasteurization treatment, storage atmosphere, and analysis month had significant effects on PV, with higher average values for log 4 and log 5 pasteurization intensities compared to the control. Walnuts stored in air had significantly higher PV than low oxygen stored walnuts. A significant quantity of PV was observed immediately after pasteurization (0 months) at both log 4 and log 5 intensities, while PV was very low in the control (0.01meq/kg) (Figure 1A, Table 4). Additionally, walnuts pasteurized at log 4 and log 5 and stored in air had the fastest accumulation of PV compared to all other treatments. Pasteurized walnuts stored in low oxygen exhibited a slower but significant accumulation of PV through six months of storage, followed by a decrease at nine months. Unpasteurized walnuts had much lower levels of PV accumulation, and storage in low oxygen further reduced PV accumulation.

For hexanal content, there was no significant difference in hexanal content between treatments, but there was significantly more hexanal in walnuts stored in air compared to a low oxygen atmosphere (Table 4, Figure 1B). All treatments had similar amounts of hexanal at 0 months. The pasteurization treatments were not significantly different in hexanal content compared to the control when walnuts were stored

under low oxygen. However, when walnuts were stored in air, the walnuts treated with log 4 and log 5 pasteurization intensities accumulated hexanal faster than the unpasteurized walnuts.

There was a significant effect of treatment and analysis month on FFA, a lipid hydrolytic byproduct (Table 4). Walnuts treated at both pasteurization intensities had significantly fewer FFAs than the control. There was no significant effect of storage atmosphere on FFA (Table 4, Figure 2A). Pasteurized walnuts accumulated FFAs much slower than unpasteurized walnuts (Figure 2A), with no difference between log 4 and log 5.

Treatment, storage atmosphere, and analysis month significantly affected total tocopherol content (Table 4). The control treatment had the highest tocopherol content overall, followed by the log 4 intensity, and then the log 5 intensity. Storage in low oxygen atmosphere maintained a higher content of tocopherols compared to air stored walnuts (Table 4, Figure 2B). There was an immediate effect of pasteurization treatment on tocopherol content, with lower levels in walnuts pasteurized at log 4 and log 5 at 0 months (Table 4, Figure 2B). The impact on tocopherol levels was greater from the log 5 treatment. After nine months of storage, the tocopherol content was similar in walnuts stored under low oxygen, regardless of the treatment (Figure 2B).

3.3 Continuous pasteurization: Sensory measures

Sensory analysis was conducted on all continuously pasteurized walnuts after nine months of storage using the attributes listed in table 3. There was no significant effect of treatment on the aroma attribute, rancidity, but there was a significant effect of storage atmosphere, with less rancidity in walnuts stored in low oxygen (Table 5). The lowest score for rancidity was observed for walnuts pasteurized and stored in low oxygen atmosphere (Table 5). Bitterness was greater in pasteurized walnuts but reduced by storage in low oxygen (Table 5). The average bitter score was highest for pasteurized walnuts stored in air. Unpasteurized walnuts stored in air had lower bitter scores than pasteurized walnuts stored in low oxygen (Table 5). For the aroma attribute, sweet smell, treatment, and atmosphere had a significant effect (Table

5). Both pasteurization intensities had slightly higher scores for sweet smell compared to the control, and storage under low oxygen atmosphere resulted in a slightly higher score for a sweet smell compared to walnuts stored in air.

Principal component analysis was used to observe associations between objective and sensory quality and treatments. PC1 explained 53.3% of the total variance in the x-axis, which showed that the sensory attributes intensity, rancidity, bitter, and woody were associated in the negative direction (Figure 3). Oxidative byproducts PV and hexanal were also associated in the negative direction of PC1. The treatment and storage atmosphere combinations most associated with the negative direction of PC1 were log 5 pasteurization stored in air and log 4 pasteurization stored in air. The treatment and storage atmosphere combination most related to the middle of PC1 was control in air. The sensory attributes in the positive direction of PC1 were sweet smell, and sweet aftertaste. Additionally, tocopherols were associated with the positive direction of PC1. All walnuts stored in low oxygen atmosphere, regardless of the treatment, were associated positively with PC1.

PC2 explained 23.4% of the total variance in the y-axis (Figure 3). The positive direction of PC2 was most associated with the hydrolytic degradation compound, FFA, and slightly with the antioxidant compounds, tocopherols. Regardless of the storage atmosphere, unpasteurized control walnuts were most associated with the positive direction of PC2. In the negative direction of PC2, sensory attributes, bitter and sweet smell, and laboratory measures of water activity and PV were most associated. Treatments at both pasteurization intensities, regardless of storage atmosphere, were associated in the negative direction of PC2.

3.4 Batch pasteurization: Objective measures

The batch pasteurization method utilized pressure manipulation within a chamber to steam treat walnuts in large bins (Table 1). There was a significant effect of treatment, atmosphere, and analysis month on PV and hexanal (Table 6). Both log 4 and log 5 pasteurization intensities had significantly lower overall PV

and hexanal compared to the control treatment; however, there was no difference between log 4 and log 5 intensities for PV, while hexanal was lower in log 5 compared to log 4. Low oxygen storage resulted in lower PV and hexanal overall compared to air storage atmosphere. PV was highest initially and throughout storage for the control walnuts stored in air (Table 6, Figure 4). Storage in low oxygen reduced PV and hexanal accumulation throughout storage, regardless of treatment.

There was a significant effect of treatment and analysis month on FFAs (Table 6). Both pasteurization intensities had lower average FFAs compared to the control walnuts; however, there was no difference in FFAs between storage atmospheres. Walnuts treated at both pasteurization intensities accumulated FFAs more slowly during storage compared to the control walnuts, regardless of storage atmosphere (Table 6, Figure 5A).

For total tocopherol content, there was a significant effect of atmosphere and analysis month. Storage under low oxygen maintained higher levels of tocopherols compared to air storage, regardless of treatment (Figure 5B). There was no significant effect of pasteurization treatment on total tocopherols.

3.5 Batch pasteurization: Sensory measures

There was a significant effect on rancidity, buttery, and creamy sensory attributes for treatment and storage atmosphere, but only the atmosphere affected the bitter attribute (Table 7). The average score for rancidity was lowest for log 5 pasteurization intensity, intermediate for log 4, and highest for control. Walnuts stored in air scored higher for rancidity overall compared to walnuts stored in low oxygen atmospheres, regardless of treatment (Table 7). Both pasteurization intensities had a similar overall score for buttery, which was higher than the score for control walnuts, while only log 5 pasteurization resulted in a decrease in the creamy score. Walnuts stored under low oxygen atmospheres had higher buttery and creamy scores than air stored walnuts.

In the PCA plot for batch pasteurization, PC1 explained 60.1% of the total variance in the x-axis (Figure 6). The attributes most associated with the negative direction of PC1 were intensity, rancidity, earthy,

woody, and bitter. The objective quality measurements most associated with the negative direction of PC1 are PV and hexanal. The treatment and atmosphere combination associated with the negative direction of PC1 are control stored in air and slightly with log 4 stored in air. The log 5 treatment stored in air is in the middle of the PC1 axis. In the positive direction of PC1 is the sensory attribute buttery and the antioxidant compounds tocopherols. Regardless of the treatment, all low oxygen stored walnuts were associated with the positive direction of PC1.

The PC2 explains 17.7% of total variance in the y-axis. In the positive direction of PC2, the texture attribute creamy, and the hydrolytic degradation byproduct FFA are highly associated. The control treatment, regardless of atmosphere, was highly associated with the positive direction of PC2. In the negative direction of PC2 is darker color and slightly the aroma attribute buttery and taste attribute bitter. All pasteurization treatments were associated in the negative direction of PC2, but air stored, pasteurized walnuts were more strongly associated with PC2 and the low oxygen stored, pasteurized walnuts were less strongly associated.

4. Discussion

4.1 Objective quality

Continuous pasteurization caused an immediate increase in PV levels. A similar increase in primary oxidative byproducts was observed for almonds and hazelnuts that were roasted at 160°C for 8 min (Ling et al., 2014). No initial increase in PV was observed in batch pasteurized walnuts. During storage, PV and hexanal did not accumulate as quickly for batch pasteurized walnuts compared to control and continuous pasteurized walnuts, indicating that batch pasteurization partially inhibited oxidation processes. The reduction in PV and hexanal accumulation caused by the batch treatment was obscured by the reduction in PV and hexanal accumulation caused by low oxygen storage. For continuous pasteurized walnuts PV and hexanal accumulated at a faster rate when stored in air, suggesting that continuous pasteurization accelerates oxidation processes. However, low oxygen storage of continuous pasteurized walnuts resulted

in the partial offset of PV accumulation and the total offset of hexanal accumulation normally caused by continuous pasteurization. For products stored in low oxygen conditions, Mexis and Kontominas (2010) observed a reduction in PV in almonds and Jensen et al., (2003) similarly observed a decrease in hexanal in shelled walnuts. The initial increase in PV from 0 to 6 months for continuous pasteurized walnuts stored in low oxygen atmospheres likely occurred due to the storage of walnuts in leaky packages for the first three months, which allowed PVs to form. Martín et al. (2001) stored hazelnuts at various concentrations of oxygen and found that the higher the oxygen concentration, the faster PV accumulated. Oxidation requires atmospheric oxygen to move the reaction forward. Without oxygen in the atmosphere, delocalized radicals on PUFAs can form, but they do not have the opportunity to further progress into hydroperoxides (Cosgrove et al., 1987). The slight decrease in PVs from 6 to 9 months in continuously pasteurized walnuts stored in low oxygen was likely caused by the slow degradation of hydroperoxides into smaller secondary byproducts, such as hexanal (Grebenteuch et al., 2021).

Hexanal is a volatile compound, and can shift from being dissolved within the walnut oil to a gaseous compound in the air (Canoles et al., 2005; Grechkin et al., 2006). This process readily occurs during storage and hexanal in a gaseous form directly contributes to the aroma of walnuts (Jensen et al., 2001). For continuous and batch pasteurized walnuts stored in low oxygen, even with the higher than desired oxygen concentrations during the first 3 months of storage, hexanal accumulated at a similar rate during storage of both pasteurized and control walnuts. Then, once low oxygen atmospheres were achieved within the bags, the hexanal content ceased to further accumulate, but instead primarily volatilized from the oil. As we observed, this would result in significantly low hexanal measurements at the 6- and 9-month analysis points. For batch pasteurization, the method has a small impact on decreasing oxidative byproduct accumulation, which, along with low oxygen storage, maintained a low concentration of hexanal.

Triglycerides primarily degrade through hydrolysis, and they have an extremely low rate of autoxidation (Cosgrove et al., 1987). Both continuous and batch pasteurizations effectively reduced the hydrolytic

degradation of triglycerides. FFA accumulation was significantly reduced in pasteurized walnuts regardless of the storage atmosphere. Buransompob et al. (2003) found that after heating walnuts and almonds at 60°C for 10 min, FFAs accumulated slower during storage than untreated nuts. Pimentel et al. (1997) found that heating lipase to 60°C for 40 min completely inactivated the enzyme. As with continuous and batch pasteurization, the heat treatments likely decreased the activity of the enzyme lipase resulting in reduced accumulation of FFAs. Low oxygen storage likely did not impact FFA accumulation because the process does not involve atmospheric oxygen.

Continuous pasteurization caused an immediate decrease in total tocopherols that did not occur in unpasteurized kernels. In contrast, Sabliov et al. (2009) found that slow heating of tocopherols required 180°C for 2 hours to achieve a substantial degradation of tocopherols. Additionally, Berasategi et al. (2012) found that heating olive oil for only 1 hour at 180°C significantly decrease total tocopherols. These authors may have seen these results because they applied heat slowly over a long period, which is different from continuous pasteurization of walnuts that suddenly applies high temperature heat in a short period. In continuous pasteurization, high temperature saturated steam may have caused water to condense on the nut's surface. This phase change from gas to liquid on the pellicle of the walnut would cause a significant amount of energy to transfer to the point of water contact (Ivarsson, 2013). This sudden energy transfer could have caused the immediate loss of tocopherols we observed in continuously pasteurized walnuts. In contrast, the total tocopherol content of batch pasteurized walnuts was statistically unaffected by treatment, and the rate of decrease in total tocopherol content between 0 and 6 months of storage was slower than in continuous pasteurized walnuts. An earlier study showed that tocopherols were significantly reduced after heating rapeseed oil for 6 hours at 170°C (Kmiecik et al., 2019). The lack of a significant impact on total tocopherols by batch pasteurization was likely because the heat being applied was too low in temperature and was used slowly over only a 6-8 min period.

The accumulation of PV in continuously pasteurized walnuts stored in air may have significantly contributed to the steady degradation of tocopherols during storage. Chun et al. (2005) found that roasted

peanuts had a faster accumulation of PV, which caused the degradation of tocopherols to occur faster than unroasted peanuts. Additionally, these authors observed that low oxygen storage of the roasted peanuts partially offset tocopherol loss, but not to the same degree as unroasted peanuts under low oxygen storage. The partial offset of tocopherol degradation was similarly observed for walnuts continuously pasteurized and stored in low oxygen in our study. Without oxygen in the atmosphere, the oxidation of PUFAs is significantly slowed, which reduces the need for tocopherols to quench the singlet oxygen that forms at the initiation of oxidation (Abdallah et al., 2015), and allows tocopherols to be maintained. In peanuts stored in vacuum packaging for 12 weeks, tocopherols were reduced by 50% while peanuts packaged in air had a 90% reduction (Shahidi and John, 2013). For batch pasteurized walnuts stored in low oxygen atmospheres, the absence of oxygen and the low PV levels likely contributed to the significant retention of tocopherols during storage.

4.2 Sensory quality

After nine months of storage, continuously pasteurized walnuts stored in air were highly associated with oxidative byproducts, PV and hexanal, and with negative sensory attributes, rancidity, and bitter. A higher quantity of hexanal in walnut kernels has been positively correlated with negative sensory attributes by Jensen et al. (2001). Batch pasteurized walnuts stored in air were slightly associated with oxidative byproducts PV and hexanal, along with negative sensory attributes, rancidity and bitter. However, compared to control walnuts stored in air, batch pasteurization had a positive impact on maintaining walnut quality during storage, but not to the same extent as storage in low oxygen atmospheres. Batch pasteurized low oxygen stored walnuts were not only positively associated with the buttery attribute but also negatively associated with the creamy attribute. Both batch and continuous pasteurizations have caused walnut texture to be harder and less creamy which is a potentially beneficial impact. For roasted hazelnuts, a crunchier texture was observed by tasters and was considered a desirable characteristic (Wani et al., 2020).

Continuously pasteurized walnuts stored in low oxygen were not associated with oxidative byproducts and were more associated with the positive sensory attributes of sweet smell and sweet aftertaste. Regardless of pasteurization treatment, storage of walnuts in low oxygen atmospheres resulted in walnuts that contained less hexanal and were perceived by panelists as having improved aroma and taste. This was similarly observed by Shayanfar et al. (2011), who stored pistachios in low oxygen packaging and found that they received higher overall sensory ratings as compared to air stored pistachios. When ground almonds were stored in low oxygen, oxidative volatiles accumulated significantly slower, and overall sensory quality was greatly improved during storage (Mexis et al., 2009). Roasting walnuts at 130°C for 10 min resulted in volatile changes and an overall improvement in flavor and aroma (Bi et al. 2022). The combination of batch pasteurization and low oxygen storage was the most successful treatment by atmosphere combination to maintain overall walnut quality. For continuous pasteurized walnuts, low oxygen storage avoided the production of off aroma and flavor.

5. Conclusion

Pasteurization of walnuts using a batch-type facility resulted in a reduced rate of oxidative byproduct accumulation, no impact on total tocopherols, and less association with negative sensory attributes compared to unpasteurized walnuts. This response was significantly different from what was observed for walnuts pasteurized in a continuous-type facility, which resulted in a faster rate of increase in oxidative byproducts, significant degradation in total tocopherols, and was overall attributed to negative sensory attributes as compared to unpasteurized product. Both pasteurization methods significantly reduced hydrolytic degradation byproducts throughout storage. Storage of pasteurized walnuts in low oxygen atmosphere significantly slowed the accumulation of PV and hexanal and the rate of degradation of tocopherols during storage and resulted in walnuts attributed to more positive sensory attributes than pasteurized walnuts stored in air. Storage of continuous pasteurized walnuts in low oxygen atmospheres could be a recommended treatment by storage method based on storage quality and overall sensory perception. However, continuous pasteurization caused a significant initial loss of tocopherols, reducing

the walnut's nutritional value. Batch pasteurizing walnuts and storing them in low oxygen atmospheres seems to provide the most successful treatment by storage method because it not only maintained high walnut quality and sensory characteristics but retained higher total tocopherol content, maintaining the walnut's nutritional value. Additionally, walnuts pasteurized in either method and stored in low oxygen atmosphere would benefit from low temperature storage compared to ambient air storage. This process would slow overall lipid degradation and aid in prolonging quality. In future research, it would be important to investigate the impact of various oxygen concentrations on walnut quality during storage. Based on this study it is likely that the lower oxygen atmospheres walnuts are stored under will allow for a longer shelf-life and prolonged retention of nutritional quality.

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Table 1. Pasteurization recipe for facility A (continuous pasteurization) and for facility B (batch pasteurization).

Facility	Treatment	Initial Nut Temp (°C)	Total Dwell Time (s)	Nut Bed Depth (cm)	Steam Zone Temp (°C)	Oven Zone Temp (°C)	Post Oven Zone MC[Z] (%)
A	Log 4	9	70[X]	2.8	102	171	8
A	Log 5	9	90[Y]	2.8	102	171	8

Facility	Treatment	Pre Heat Temp (°C)	Pre Heat Time (min)	Pasteurization Temp (°C)	Pasteurization Time (min)	Cooling Temp (°C)	Cooling Time (min)
B	Log 4	40-50	35-40	88-90	6-7	20-30	20
B	Log 5	40-50	35-40	88-90	8-9	20-30	20

[X]walnuts took 35s to go through the steam zone and 35s to go through the oven zone
[Y]walnuts took 45s to go through the steam zone and 45s to go through the oven zone
[Z]moisture content

Table 2. Average oxygen concentrations inside walnut pouches prior to opening at each evaluation time. Walnuts were previously pasteurized at facilities using either continuous (A) or batch (B) processes.

Storage Month	Facility	Modified Atmosphere Condition	Pasteurization	Average % Oxygen
6	A Continuous	Air	Control	18.71 a[X]
			Log 4	9.96 b
			Log 5	9.49 b
		Low Oxygen	Control	0.44 c
			Log 4	0.10 c
			Log 5	0.15 c
	B Batch	Air	Control	16.42 a
			Log 4	17.21 a
			Log 5	18.64 a
		Low Oxygen	Control	0.18 c
			Log 4	0.23 c
			Log 5	0.05 c
9	A Continuous	Air	Control	13.16 b
			Log 4	1.88 c
			Log 5	0.95 c
		Low Oxygen	Control	0.58 c
			Log 4	0.11 c
			Log 5	0.09 c
	B Batch	Air	Control	12.40 b
			Log 4	16.93 a
			Log 5	17.45 a
		Low Oxygen	Control	0.25 c
			Log 4	0.44 c
			Log 5	0.15 c

[X]Means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$). 6 month storage statistically analyzed separately from 9 month storage.

Table 3. Sensory attributes evaluated by panelists along with their descriptors and references.

Category	Attribute	Descriptor	Reference
Aroma	Intensity	Strength of initial aroma; Scale from bland to intense	No Reference
	Rancidity	Paint-like, grassy, oxidized, old	[V]Old walnut
	Buttery	Creamy aroma	[Z]Butter
	Sweet smell	Can you smell the sweetness?	[G]Marshmallow
	Nutty	Overall nuttiness	[Y]Mixed nuts
	Earthy	Soil-like, mushroom-like, musty	[U]Soil
	Woody	Woodiness	Pencil shavings
	Vegetal	Fresh plant smell, green smell	Cucumber peel
Taste	Bitter	Bitter taste on the tongue	No Reference
	Sweet	Sweet taste on the tongue	No Reference
	Bitter aftertaste	Does the bitterness linger at all?	No Reference
	Sweet aftertaste	Does the sweetness linger at all?	No Reference
Texture	Crisp	First initial bite is "snappy", audible sound or feel when biting into it	Honeycrisp apple
	Crunchy	Sample continues to be audible as you chew it	[R]Carrot
	Brittleness	Falls apart into many pieces upon biting or snapping	[L]Life cereal
	Astringent	Mouth puckering, drying. Imagine an unripe persimmon	No Reference
	Oily	Leaves an oily residue in mouth after chewing	[Q]Potato chips
	Moist	Makes mouth wet as you eat it	No Reference
	Creamy	Smooth mouthfeel	[W]Chocolate
	Chewy	Resistance to breaking into pieces	[K]Gummy bear
	Tooth-packing	Leaves residue in teeth after swallowing	[J]Goldfish

[Y]mixed nuts = chopped up mix of Planters® Deluxe salted mixed nuts. Contains: cashews, almonds, brazil nuts, pecans and pistachios
[Z]butter = slice of Challenge Butter® unsalted
[V]old walnut = walnuts that had been in 20C storage for over a year
[W]chocolate = Hershey's Kisses® milk chocolate
[Q]potato chip = Lay's® Classic
[R]carrot = Signature Farms® peeled baby-cut carrots washed and ready to eat
[U]soil = wet soil found outside of Wickson Hall
[L]life cereal = Quaker® life multigrain cereal
[K]gummy bear = Haribo® Gummy bear
[J]goldfish = Pepperidge Farm® Goldfish
[G]marshmallow = Jet-puffed mini marshmallows

Table 4. Main effect means and interaction for treatment, storage atmosphere, and analysis month for continuous pasteurized[Z] walnuts.

	PV[X]	Hexanal	FFA[X]	Toc[X]
Treatment				
Control	0.72 b[Y]	0.46 a	0.33 a	430.5 a
Log 4	16.1 a	0.54 a	0.11 b	357.8 b
Log 5	19.4 a	0.69 a	0.11 b	326.5 c
Storage Atmosphere				
Air	18.6 a	0.97 a	0.19 a	352.2 b
Low O_2	5.53 b	0.15 b	0.18 a	391.0 a
Analysis Month				
0	2.34 c	0.01 c	0.09 c	421.0 a
6	13.3 b	0.64 b	0.21 b	368.1 b
9	20.5 a	1.03 a	0.26 a	352.6 c
Treatment : Atmosphere : Analysis Month				
Control : Air : 0	0.01 g	0.02 d	0.12 bc	465.9 a
Control : Air : 6	0.64 efg	0.68 bc	0.38 a	445.2 a
Control : Air : 9	2.42 ef	1.50 ab	0.45 a	347.9 cde
Log4 : Air : 0	3.49 de	0.01 d	0.06 d	433.1 ab
Log4 : Air : 6	25.1 b	1.26 ab	0.12 bcd	314.2 def
Log4 : Air : 9	45.0 a	1.58 a	0.16 bc	265.8 fg
Log5 : Air : 0	3.57 de	0.01 d	0.09 cd	364.1 bcd
Log5 : Air : 6	30.6 b	1.28 ab	0.11 bcd	292.5 efg
Log5 : Air : 9	56.3 a	2.40 a	0.19 b	241.0 g
Control : Low O_2 : 0	0.01 g	0.02 d	0.12 bc	465.9 a
Control : Low O_2 : 6	0.49 fg	0.19 cd	0.41 a	456.6 a
Control : Low O_2 : 9	0.73 fg	0.32 c	0.51 a	401.3 abc
Log4 : Low O_2 : 0	3.49 de	0.01 d	0.06 d	433.1 ab
Log4 : Low O_2 : 6	10.5 c	0.18 cd	0.13 bc	356.5 cd
Log4 : Low O_2 : 9	8.75 cd	0.20 cd	0.13 bc	343.9 cde
Log5 : Low O_2 : 0	3.57 de	0.01 d	0.09 cd	364.1 bcd
Log5 : Low O_2 : 6	12.4 c	0.22 cd	0.09 cd	343.4 cde
Log5 : Low O_2 : 9	9.85 c	0.21 cd	0.12 bcd	353.7 cde

[Z]Walnuts received from facility A.
[X]PV = peroxide value; FFA = free fatty acid; Toc = total tocopherols
[Y]Means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 5. Main effect means and interaction for treatment and storage atmosphere for sensory attributes in continuous pasteurized[Z] walnuts.

	Rancidity	Bitter	Sweet Smell	Sweet Aftertaste
Treatment				
Control	3.7 a[X]	3.2 b	2.0 b	3.6 a
Log 4	4.1 a	4.6 a	2.8 a	3.4 a
Log 5	4.1 a	4.5 a	2.6 ab	3.6 a
Storage Atmosphere				
Air	5.8 a	4.8 a	2.1 b	3.2 a
Low O_2	2.1 b	3.5 b	2.8 a	3.8 a
Treatment : Atmosphere				
Control : Air	4.3 ab	3.3 c	1.8 b	3.8 ab
Log4 : Air	6.4 a	5.6 a	2.3 ab	3.4 ab
Log5 : Air	6.6 a	5.3 ab	2.1 ab	2.4 b
Control : Low O_2	3.0 abc	3.2 c	2.2 ab	3.3 ab
Log4 : Low O_2	1.8 bc	3.5 bc	3.2 a	3.4 ab
Log5 : Low O_2	1.6 c	3.7 bc	3.0 ab	4.7 a

[Z]Walnuts received from facility A.
[X]Means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 6. Main effect means and interaction for treatment, storage atmosphere, and analysis month for batch pasteurized[Z] walnuts.

	PV[X]	Hexanal	FFA[X]	Toc[X]
Treatment				
Control	2.43 a[Y]	1.09 a	0.36 a	422.5 a
Log 4	1.25 b	0.72 b	0.25 b	416.9 a
Log 5	1.18 b	0.29 c	0.16 b	419.8 a
Storage Atmosphere				
Air	2.85 a	1.26 a	0.27 a	406.2 b
Low O_2	0.39 b	0.14 b	0.25 a	433.3 a
Analysis Month				
0	0.09 c	0.01 c	0.22 b	436.4 a
6	1.62 b	0.66 b	0.25 ab	415.6 b
9	3.16 a	1.43 a	0.31 a	407.1 b
Treatment : Atmosphere : Analysis Month				
Control : Air : 0	0.24 ef	0.01 g	0.25 ab	440.5 ab
Control : Air : 6	3.66 bc	2.01 b	0.35 ab	424.3 ab
Control : Air : 9	9.15 a	4.05 a	0.52 a	362.2 c
Log4 : Air : 0	0.02 f	0.02 efg	0.31 ab	430.5 ab
Log4 : Air : 6	2.61 bc	1.12 c	0.22 ab	407.4 abc
Log4 : Air : 9	4.10 b	2.69 b	0.25 ab	365.4 c
Log5 : Air : 0	0.01 f	0.01 fg	0.11 b	438.0 ab
Log5 : Air : 6	1.88 cd	0.38 d	0.18 ab	397.5 abc
Log5 : Air : 9	3.99 bc	1.08 c	0.22 ab	389.6 bc
Control : Low O_2 :	0.24 ef	0.01 g	0.25 ab	440.5 ab
Control : Low O_2 :	0.54 e	0.19 d	0.39 ab	427.3 ab
Control : Low O_2 :	0.78 de	0.27 d	0.42 ab	439.9 ab
Log4 : Low O_2 : 0	0.02 f	0.02 efg	0.31 ab	430.5 ab
Log4 : Low O_2 : 6	0.38 ef	0.15 def	0.19 ab	418.9 ab
Log4 : Low O_2 : 9	0.36 ef	0.32 d	0.25 ab	448.7 a
Log5 : Low O_2 : 0	0.01 f	0.01 fg	0.11 b	438.0 ab
Log5 : Low O_2 : 6	0.65 de	0.12	0.15 ab	418.5 ab
Log5 : Low O_2 : 9	0.57 e	0.16 de	0.20 ab	437.1 ab

[Z]Walnuts received from facility B.
[X]PV = peroxide value ; FFA = free fatty acid ; Toc = total tocopherols
[Y]Means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

Table 7. Main effect means and interaction for treatment and storage atmosphere for sensory attributes in batch pasteurized[Z] walnuts.

	Rancidity	Bitter	Buttery	Creamy
Treatment				
Control	4.6 a[X]	3.6 a	2.1 b	3.4 a
Log 4	3.7 a	2.9 a	3.1 a	3.0 ab
Log 5	2.3 b	3.4 a	2.9 a	2.6 b
Storage Atmosphere				
Air	5.5 a	3.9 a	2.3 b	2.7 b
Low O_2	1.6 b	2.7 b	3.1 a	3.3 a
Treatment: Atmosphere				
Control : Air	7.5 a	4.3 a	1.5 b	2.9 ab
Log4 : Air	5.5 ab	3.6 ab	2.7 a	2.8 b
Log5 : Air	3.4 bc	3.9 ab	2.7 ab	2.4 b
Control : Low O_2	1.7 d	2.8 ab	2.6 ab	3.9 a
Log4 : Low O_2	1.9 cd	2.3 b	3.6 a	3.3 ab
Log5 : Low O_2	1.1 d	2.9 ab	3.1 a	2.8 b

[Z]Walnuts received from facility B.
[X]Means followed by different letters (Tukey HSD test) within main effects and columns were significantly different ($P < 0.05$).

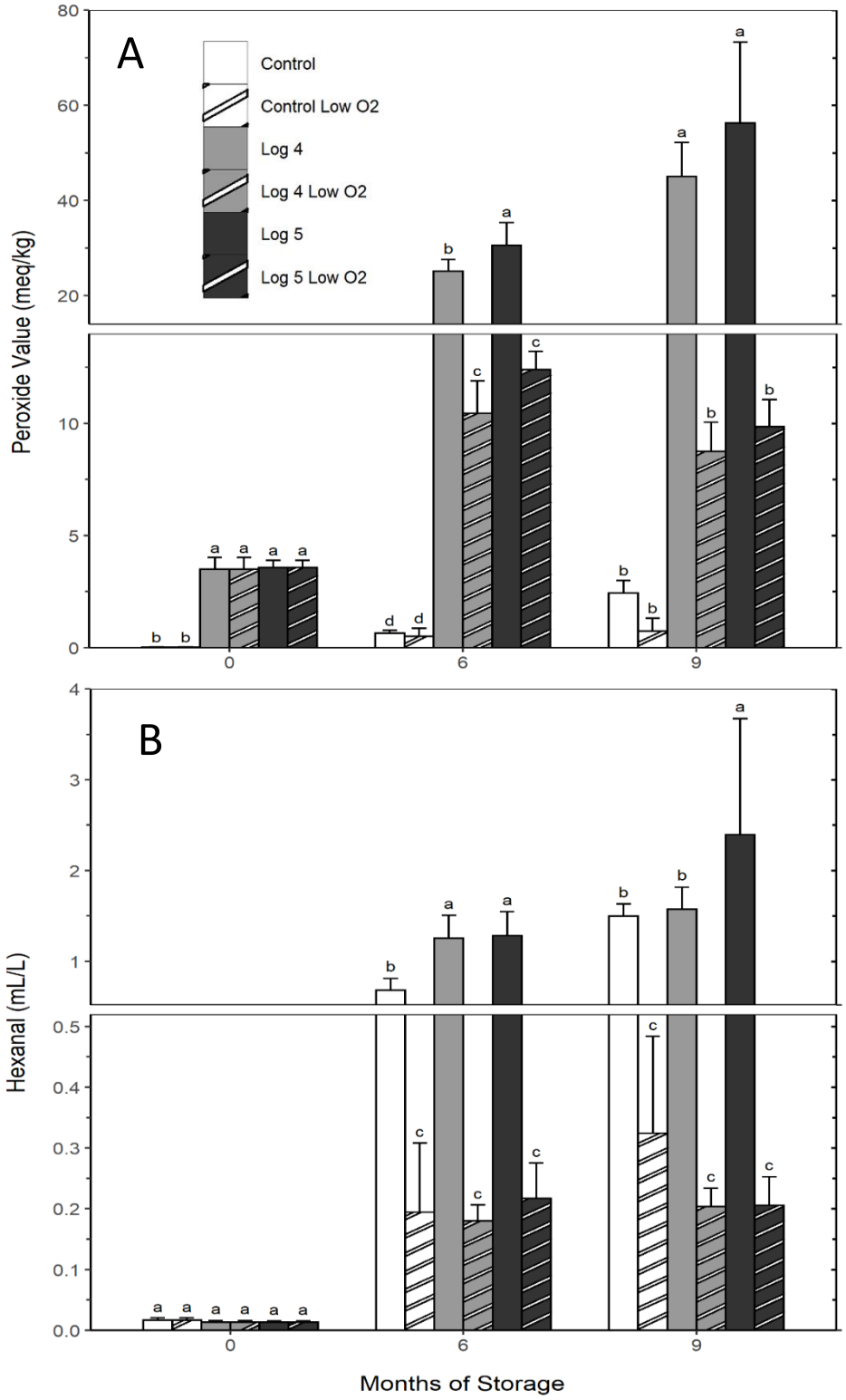

Fig. 1. The impact of continuous pasteurization and storage atmosphere on the content of peroxides (A) and hexanal (B) after 0, 6 and 9 months of storage at 20°C for walnuts received from facility A. Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

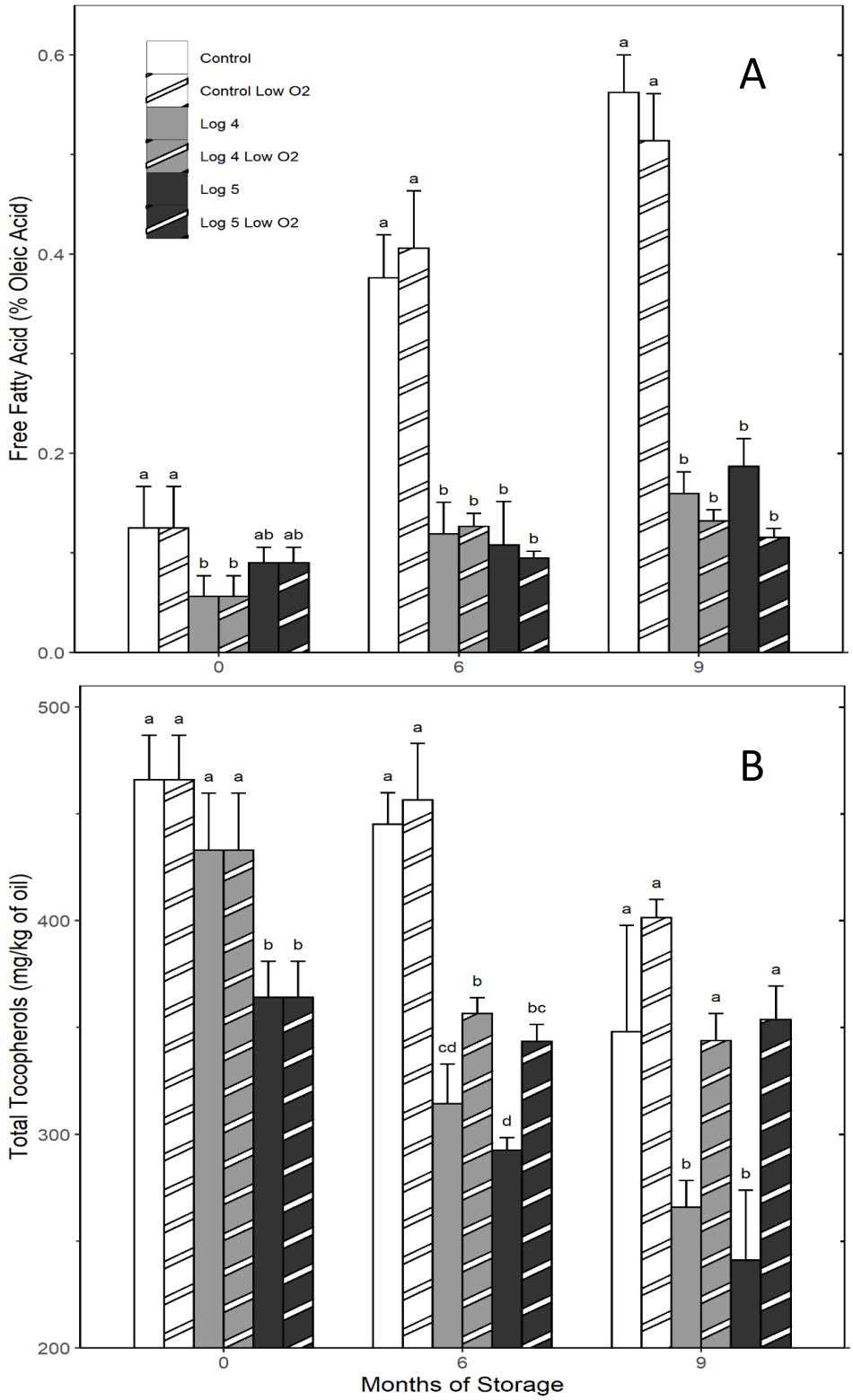

Fig. 2. The impact of continuous pasteurization and storage atmosphere on the content of free fatty acids (A) and total tocopherols (B) after 0, 6 and 9 months of storage at 20°C for walnuts received from facility A. Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

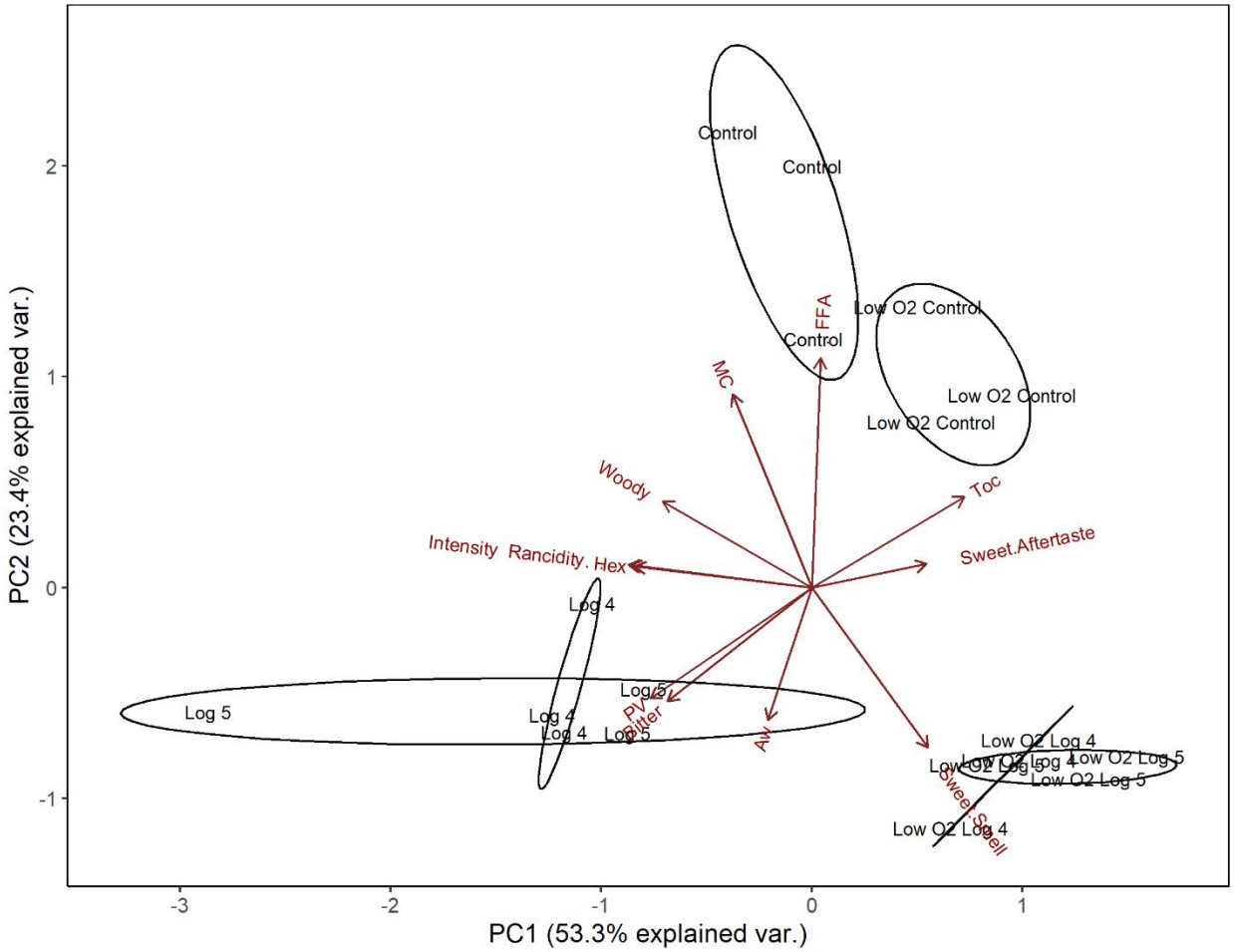

Fig. 3. Principle component analysis of both objective and sensory measurements of walnuts subjected to continuous pasteurization or unpasteurized control after 9 months of storage in air or low oxygen at 20°C post-pasteurization. Abbreviations used: Aw = water activity, MC = moisture content, PV = peroxide value, FFA = free fatty acid, Toc = total tocopherols and Hex = hexanal. Treatment abbreviation: Low O2 = Low oxygen stored.

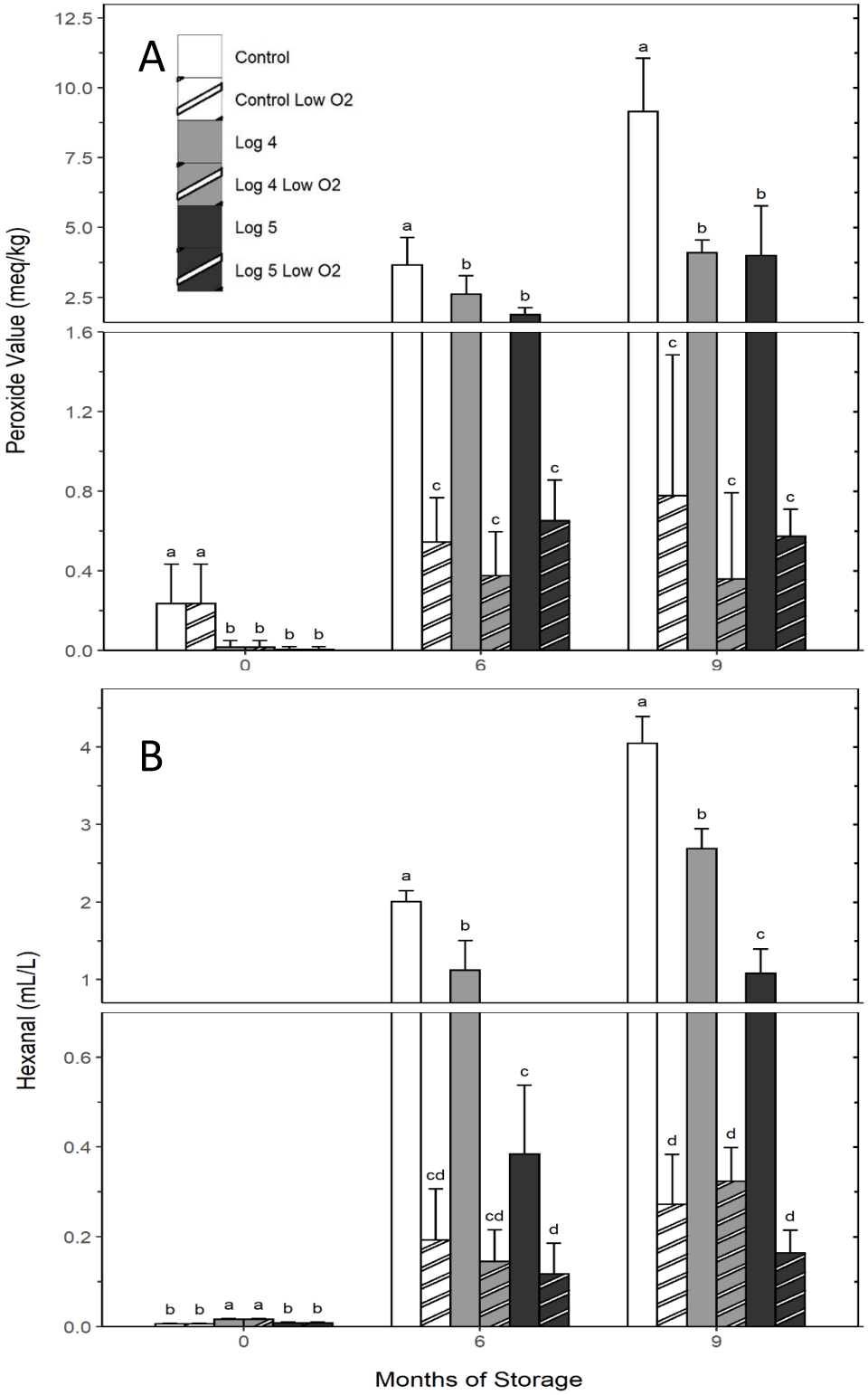

Fig. 4. The impact of batch pasteurization and storage atmosphere on the content of peroxides (A) and hexanal (B) after 0, 6 or 9 months of storage at 20°C for walnuts received from facility B. Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

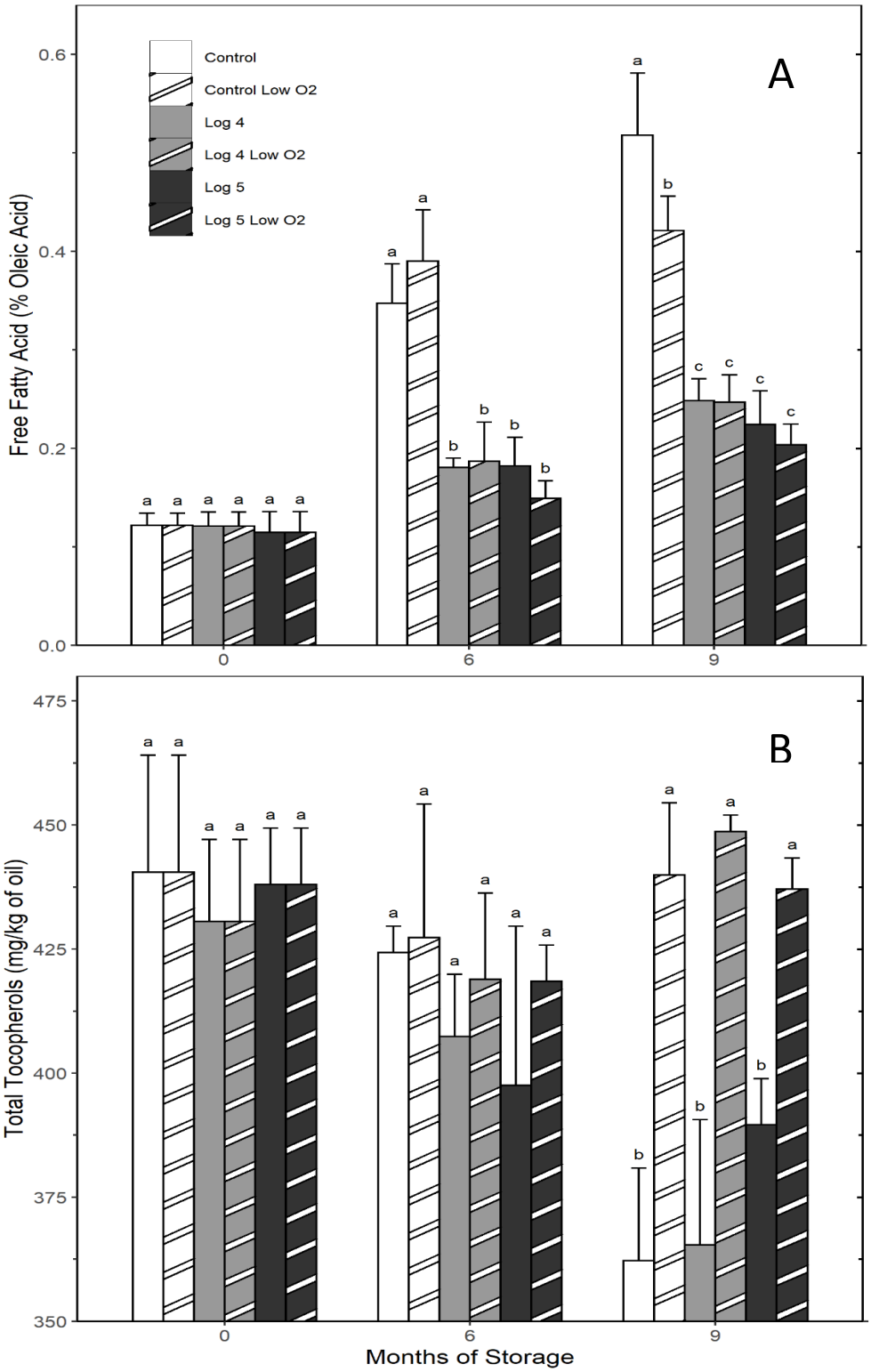

Fig. 5. The impact of batch pasteurization and storage atmosphere on the content of free fatty acids (A) and total tocopherols (B) after 0, 6 or 9 months of storage at 20°C for walnuts received from facility B. Bars with different letters (Tukey HSD test) represent a significant difference ($P < 0.05$) between treatments at specific months.

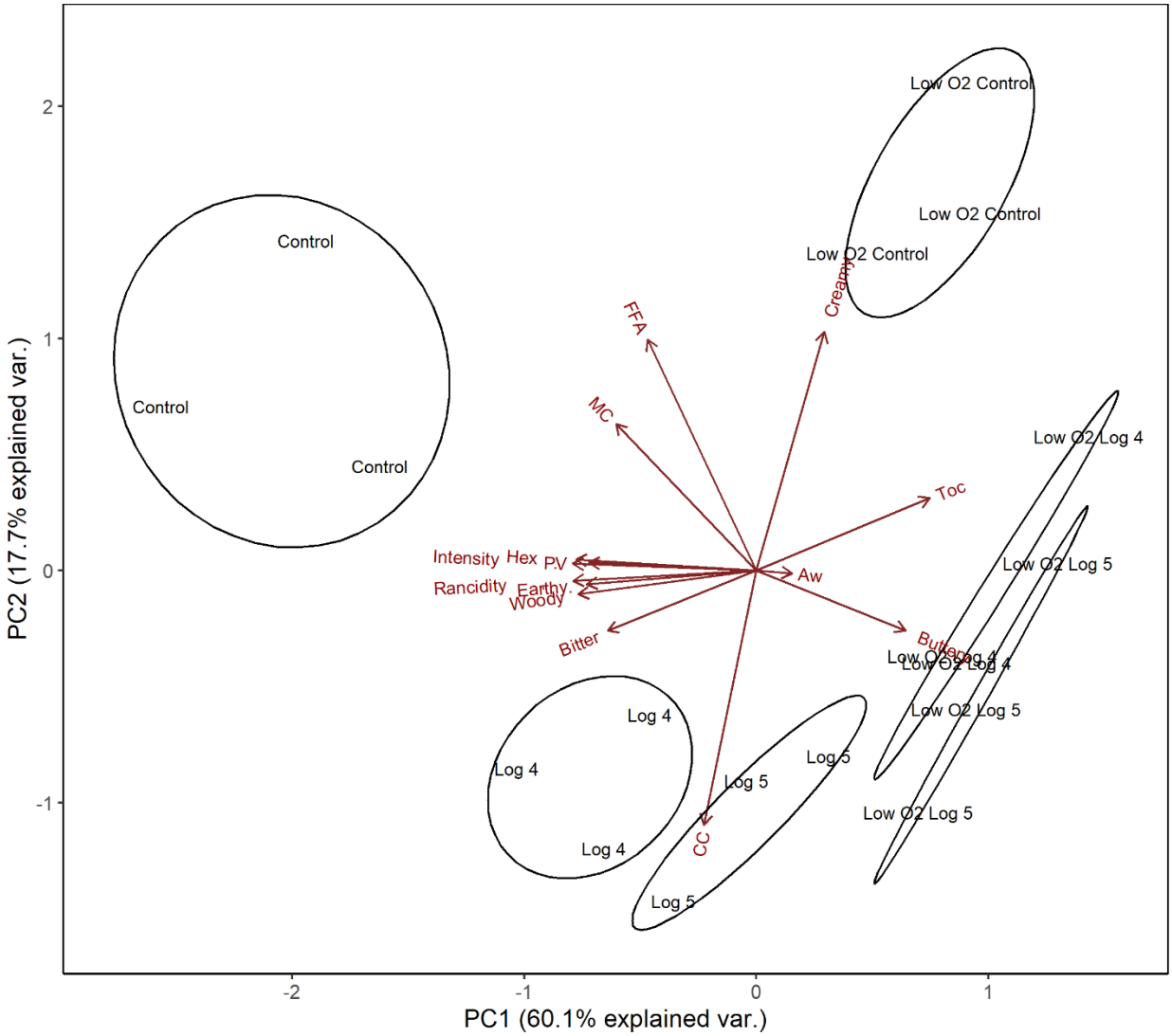

Fig. 6. Principle component analysis of both objective and sensory measurements of walnuts subjected to batch pasteurization or unpasteurized control after 9 months storage in air or low oxygen at 20°C post-pasteurization. Abbreviations used: Aw = water activity, MC = moisture content, CC = color chart PV = peroxide value, FFA = free fatty acid, Toc = total tocopherols and Hex = hexanal. Treatment abbreviation: Low O2 = Low oxygen stored.