JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF BARRIER TECHNOLOGIES ON THE CHEMICAL, NUTRITIONAL, SENSORY, AND MICROBIOLOGICAL QUALITY OF ARBUTUS UNEDO L. FRUITS DURING STORAGE

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https://doi.org/10.55251/jmbfs.10409

ARTICLE INFO

Received 28. 7. 2023
Revised 9. 5. 2024
Accepted 2. 7. 2024
Published 1. 10. 2024

Regular article

OPEN ACCESS

ABSTRACT

Arbutus unedo L. is a widespread tree known for its nutritional and antioxidant importance. However, the utilization of its berries remains limited due to their sensitivity and damage during storage. This study aims to evaluate the effect of barrier technologies with the addition of lemon juice (*Citrus limon*) and bitter orange juice (*Citrus aurantium*) as additives on the sensory, nutritional, and microbiological quality of fruits during storage. The combined fruits exhibited a significant decrease in pH (from 5.07±0.23 to 3.45±0.34 for fruits combined with bitter orange juice and from 5.07±0.23 to 3.64±0.12 for fruits combined with lemon juice), moisture content (from 62.48±2.32 to 45.47±3.54 for fruits combined with bitter orange juice and from 62.48±2.32 to 51.4±3.12 for fruits combined with lemon juice), and showed inactivation of aerobic mesophilic bacteria compared to non-treated fruits. Differential thermogravimetric analysis (DTG, DSC) demonstrated that fruits conditioned in the presence of bitter orange juice preserved their nutritional quality (vitamins B2, B6, B9, B12, and C) under light and oxygen exposure. The combination of acidity and sweetness improved the sensory quality of the combined fruits during storage.

Keywords: Barrier technology, *Arbutus unedo* L.; Conservation; DTG-DSC analyzes; vitamins

INTRODUCTION

Arbutus unedo L. is a fruit tree belonging to the Ericaceae family (**Celikel et al., 2008**), commonly known as "sasnou" in Morocco. It is an evergreen shrub ranging from 1.5 to 3 meters in height, native to the Mediterranean region including Morocco, Portugal, Spain, France, Italy, Albania, Croatia, Bosnia, Montenegro, Greece, and Turkey (**Molina et al., 2016; Torres et al., 2002**). It is also found in other regions characterized by hot summers and mild rainy winters, displaying great resistance to challenging environmental conditions such as drought, low temperatures, and poor or heavy soil conditions (**Gomes and Canhoto, 2009; Arnan et al., 2013**). Ecologically, the resistance of Arbutus unedo plays a central role in reforestation programs in southern European countries like Greece, Italy, Portugal, and Spain (**Schröter et al., 2005**). In Morocco, the arbutus tree is commonly found in the High Atlas, Middle Atlas, Pre-Rif, Central Rif, Western Rif, Northwest, and Central Plateau regions (**Faida et al., 2019**).
The appearance of fruits and flowers during the winter months: the flowers are clusters of small cream-colored lanterns (**Floris et al., 1992**). The fruits are spherical, orange, turning red when ripe, and rough, reaching up to 2 cm in diameter and a total mass of 4.7 g (**Elmejhed et al., 2022**). The fleshy edible fruits of *A. unedo* are part of the Mediterranean diet and are a source of health, containing vitamin C, dietary fiber, and bioactive compounds for dietary supplements or functional foods (**Molina et al., 2011; Ortuňo Moya, 2003**). The plant is traditionally known as the "bee plant," and strawberry honey is highly valued for its characteristic bitter taste and presumed biological properties (**Tubéroso et al., 2013**). The described medicinal properties are related to the presence of several pharmacotherapeutic compounds in different parts of *A. unedo* (**Kivregiscak and Mert, 2001; Spano et al., 2009; Verde et al., 2010**). The plant contains various gastrochemical compounds (carotenoids, organic acids, phenolics, tannins, vitamins E and C) and compounds with suitable antibacterial, anti-aggregant, antidiabetic, antihypertensive, anti-inflammatory, and antitumor effects (**Carcache-Blanco et al., 2006; Tavares et al., 2010**). The phytochemical constituents of different parts of the A. unedo tree (leaves, fruits, bark, wood/stem, and root) have been the subject of several studies (**Fiorentino et al. 2007; Kivçak et al. 2001; Soro and Paxton 1999**).
Despite its nutritional, health, and bioactive richness, *A. unedo* fruits remain underutilized and relatively unknown in several cities in Morocco due to their sensitivity and the lack of ideal conservation and transportation techniques. The term "barrier technology" (also known as combined method, combined processing, combined preservation, or combined technology) is often used to refer to food canning through the combination of multiple preservation factors. To ensure the stability, safety, and quality of food products, well-thought-out and judicious co-treatment appears as an extremely effective means of achieving microbial homeostasis responses while preserving desired nutritional and organoleptic properties (**Gould, 1995; Leitsner, 2000; Leitsner and Gould, 2002**).
Among the factors used is acidity (i.e., pH reduction). *Citrus limon* (lemon) belonging to the Rutaceae family is rich in various natural compounds such as ascorbic acid, citric acid, minerals, flavonoids, and essential oil (**Manabi et al., 2020**). Lemon juice contains citric acid, which is a crucial organic acid in the food preservation industry (**Sholeh et al., 2016**). Similarly, *Citrus aurantium* L. (sour orange) is well-known in Morocco but rarely used. It is a tree that reaches a height of 5 to 10 meters, and its leaves and flowers have numerous food applications, particularly in perfumery (**Carmen et al., 2018; El-Akhal et al., 2014**).
In this context, this article presents the application of barrier technology with the addition of two different juices (lemon and sour orange) to improve or preserve the nutritional and sensory quality of the studied food during storage. For this purpose, differential scanning calorimetry and thermogravimetric analysis (DTG-DSC) was used to monitor the degradation of essential vitamins B2, B6, B9, B12, and C, in addition to physicochemical analyses such as pH, acidity index, and moisture content. Subsequently, microbiological and sensory analyses were performed, followed by microscopic analyses of plant cells during storage.

MATERIALS AND METHODS

Sampling

A. unedo fruits used in this study were harvested on October 25, 2022, from an area of 100 m^2 with an average of 10 fruits per tree. The collection took place in the region of Béni-Mellal Mellal-khénifra, specifically in the Moudge forest near the city of Béni-Mellal Mellal. This region is located in the geographical zone of the Middle Atlas, ranging in altitude from 500 to 1170 meters, and covering an area of 7075 m^2. The population of the region is 869,749 inhabitants, with 483,244 residing in rural areas and 386,505 in urban areas. The region is characterized by a semi-humid climate with annual precipitation ranging from 400 to 700 mm, and an average annual temperature of 19°C (**Laamrani El Idrissi et al., 1999**).
The harvested fruits were carefully transported to the laboratory on the same day for the application of barrier technologies and the preparation of storage conditions.

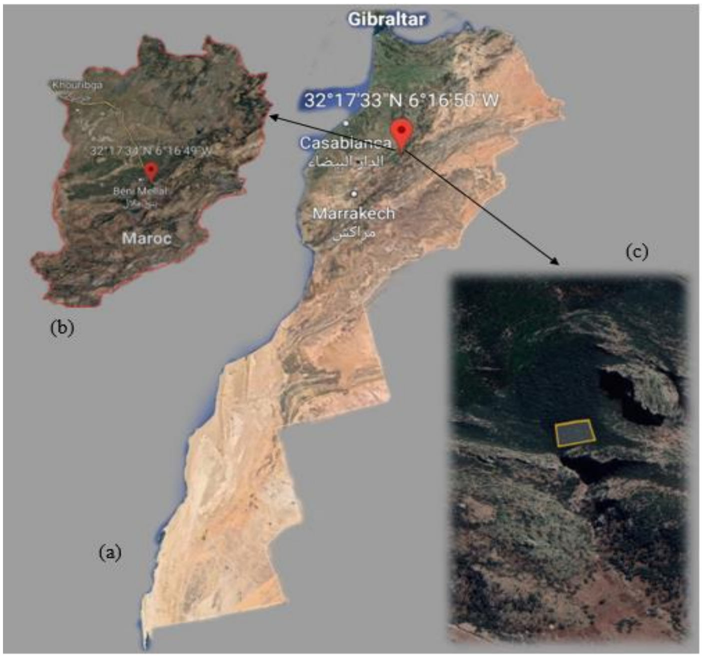

Figure 1 (a) Location on map of Morocco. (b) Location on map of Beni Mellal-khenifra region. (c): harvesting area.

Conservation method

In both industrialized and developing countries, barrier technology is increasingly employed to develop food products. Depending on the specific requirements, various methods can be applied (**Alzamora et al., 1993**). The method used in this study is based on two pathways: for high-moisture fruit products or intermediate-moisture fruits (**Jayaraman, 1995; López-Malo et al., 1995**). In the case of *A. unedo* fruits, they can be classified as high-moisture fruits, with a moisture content of up to 68.18%.

The studied fruits underwent preliminary operations, which involved selection and washing. After rinsing, they were blanched for 1 to 5 minutes in saturated steam and cooled with tap water. Then, the moisture content was reduced (during which the fruits lost water and gained sugar), followed by packaging in a solution with a sugar concentration of 3/4 (**El Mejhed et al., 2023**). At the same time, a pH reducer was added, consisting of two types of additives: lemon juice and bitter orange juice (4%). The fruits were balanced in glass jars for 5 to 10 days until reaching a pH between 3 and 4 and a decrease in moisture content.

Following the equilibration process, the prepared fruits were subjected to drying and packaging. Subsequently, they were stored at room temperature, and every 4 days, they underwent physicochemical and microbiological analysis. Both sensory and microscopic analyses were conducted before applying combined technologies and at the conclusion of the preservation period. Additionally, upon completing the storage duration, the samples were dried, ground, and subjected to thermal analysis using DTG-DSC.

Chemical analysis

The chemical properties of the fruits were analyzed according to the methods described in (**AOAC, 1984**). The pH measurement was performed by directly immersing the electrode into a solution containing 10 g of chopped fruit and 100 ml of distilled water using a pH meter. The titratable acidity was measured by neutralizing the total free acidity in 25 ml of the obtained juice with a 0.1 N NaOH solution until reaching a pH of 8.1 in the presence of phenolphthalein as a color indicator. The titratable acidity is expressed relative to the malic acid content (**Demir and Özcan, 2001**). Finally, the moisture content was determined by weighing 2 g of the chopped fruit into small pieces and drying it in an oven at 103°C + 2 until a constant weight was achieved (**AOAC, 2002**).

Microbiological analysis

The enumeration of the total aerobic mesophilic flora (TAMF) serves as a reliable measure to assess the overall quality and stability of food products. To conduct this analysis, a 1 g sample of the fruit was blended with 10 ml of distilled water, creating a uniform solution. Next, 15 ml of plate count agar was solidified, and petri dishes were inoculated with 0.1 ml of the diluted solution on their surface. Subsequently, these dishes were incubated for 72 hours at a temperature of 30°C. Finally, the number of colonies present on plates containing 20 to 300 colonies was quantified as per the method described by (**Mady et al. 2009**).

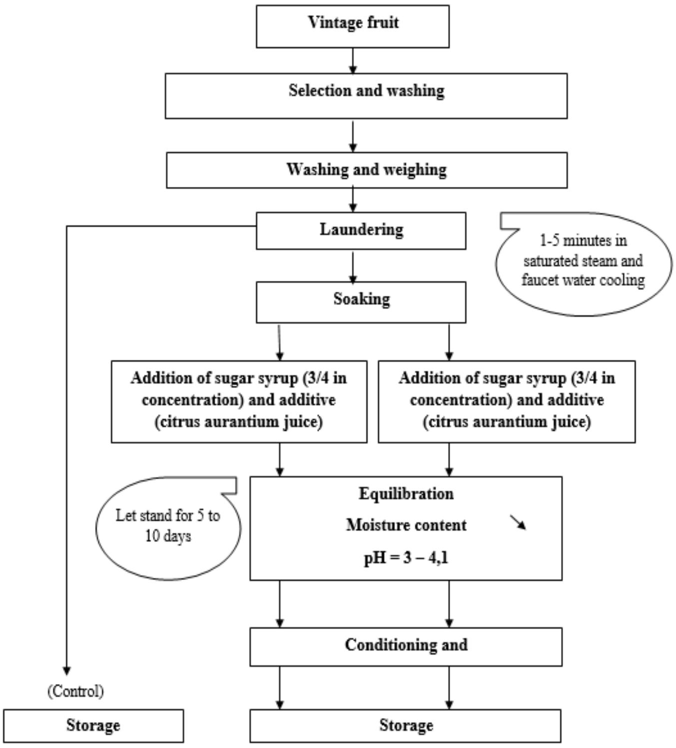

Figure 2 Barrier technology application diagram

Sensory analysis

The sensory characteristics of the fruits were evaluated in their raw state and after the application of barrier technology. The samples were assessed using a 1 to 5 point organoleptic test for taste, residual taste, acidity, sweetness, aroma, and overall acceptance (**Marc et al., 2001**). The sensory analysis was conducted with the participation of 50 students and doctoral researchers from the Laboratory of Engineering and Applied Technologies at the Higher School of Technology in Beni Mellal, Morocco.

Microscopic analysis

Preparation mounting was performed between a glass slide and cover slip, using a finely cut fragment of the fruit with a drop of distilled water (**Chafic et al., 2022**). The preparations were then observed under an optical microscope (Optika B-290, Italy) with direct light, at magnifications of *10 and *40. Images were captured for each magnification.

Thermal analysis

Thermogravimetric analysis was carried out using a DTA-DSC +1600 (LabSys evo) instrument, with a sample size ranging from 35 to 37.5 mg in platinum crucibles. The samples were heated from room temperature to 800°C at a heating rate of 1°C/min. The recorded curves represented temperature (°C), weight loss (TG in %), and derivative weight loss (DTG in %/min), which indicates the decomposition rate (**Mohit et al., 2020**).

Statistical analysis

The data were analyzed using statistical software (JMP Pro 14, for Windows) through one-way analysis of variance (ANOVA). The coefficients of variation (C.V) were calculated by dividing the relevant standard deviations by the means and multiplying by 100. Mean comparisons between different groups were performed at a significance level of p=0.05 (**Lotfi et al., 2010**).

RESULTS AND DISCUSSION

Chemical and microbiological analysis

The addition of an acidic solution is the most important factor in barrier technology. Table 1 presents the results of the one-way analysis of variance (ANOVA). ANOVA was conducted using a significance level of $p < 0.05$, corresponding to a confidence level of 95%.

Table 1 Results of the one-factor analysis of variance (ANOVA) for chemical and microbiological characteristics.

	Source	DF	Sum of square	Mean square	F Ratio	Prob>F	Level	-level	p-value
pH	Additives	2	59.80087	29.9004	2451.20	<0.0001*	Control	C.A Juice	<0.0001*
	Error	57	7.06599	0.124			Control	C.L Juice	<0.0001*
	C. total	59	66.86686				C.L Juice	C.A Juice	0.3384
Titratable acidity	Additives	2	0.694093	0.34704	221.32	<0.0001*	Control	C.A Juice	<0.0001*
	Error	57	0.08938	0.00156			Control	C.L Juice	<0.0001*
	C. total	59	0.783473				C.L Juice	C.A Juice	0.0004*
Moisture content	Additives	2	3989.63	1994.82	396.68	<0.0001*	Control	C.A Juice	<0.0001*
	Error	57	286.637	5.03			Control	C.L Juice	<0.0001*
	C. total	59	4276.26				C.L Juice	C.A Juice	<0.0001*
TAMF	Additives	2	1.289e+16	6.44e+15	16.249	<0.0001*	Control	C.A Juice	<0.0001*
	Error	57	2.262e+16	3.96e+14			Control	C.L Juice	<0.0001*
	C. total	59	3.552e+16				C.L Juice	C.A Juice	0.9948

Figure 3 Variation in chemical and microbiological parameters as a function of time and additive type: (a) pH; (b) titratable acidity; (c) moisture content and (d) total aerobic mesophilic flora.

Our findings clearly indicate that the use of additives has a significant impact on the chemical characteristics of fruits when applying combined technologies. Specifically, fruits conditioned with *C. limon* and citrus aurantium juice show a notable reduction in pH and moisture content compared to the control group. Furthermore, when comparing the pH evolution between *C. limon* and *C. aurantium*, no significant difference is observed ($p = 0.3384$). However, there is a significant decrease in moisture content for fruits conditioned with the solution containing citrus aurantium juice compared to those treated with *C. limon* juice. This observation is further supported by a significant coefficient between the two additives ($p < 0.0001$).

Regarding this decrease, a significant increase in acidity index of *A. unedo* fruits is observed after the application of barrier technologies with both types of additives. This increase is confirmed by a significant difference ($p < 0.0001$) compared to the control.

This effect is particularly notable in the development of total aerobic mesophilic flora (TAMF) when combined with technologies and the presence of lemon and orange juice, as confirmed by a significant effect ($p < 0.0001$).

However, the results depicted in Figure (3) demonstrate that the pH remains preserved throughout the conservation period for both types of additives: lemon and orange citrus juice. In contrast, there is a significant increase in pH between the eighth and twelfth days of conservation for the control group. Similarly, there is a considerable decrease in acidity index for the control group, whereas the acidity index remains almost constant for fruits combined with both types of additives. Moreover, the moisture percentage remains stable during the conservation period for fruits conditioned in the solution containing orange citrus juice, unlike the fruits conditioned in the solution containing lemon citrus juice and the control group, where an increase in moisture percentage is observed. Lastly, Figure (3, d) clearly demonstrates that the overall growth of mesophilic aerobic flora is negligible for fruits treated with both types of additives (lemon and orange citrus juice) compared to the control group, where a highly significant increase is

observed from the fourth day until the end of the conservation period. In fact, despite this increase, the fruits remain edible until the eighth day of conservation. However, beyond the twelfth day, the fruits deteriorate and become inedible according to the Quebec 2009 standard (**Norme Québec, 2009**), which requires a value of 107 CFU/g for total mesophilic aerobic germs.
The results obtained from this ANOVA study focusing on different types of additives used in the application of barrier technology and conservation of Arbutus unedo L. fruits highlight two crucial aspects. On the one hand, the addition of lemon and orange citrus juice presents a significant effect in the application of barrier technology, and on the other hand, citrus aurantium juice plays a role in preserving the chemical and microbiological charcateritics of the fruits during the conservation period.

Thermal analysis

To assess the resistance of vitamins B_2, B_6, B_9, B_{12}, and C during the conservation of *A. unedo* fruits, a thermal analysis (DTG, DSC) was conducted over a temperature range from room temperature to 800 °C. The onset of thermal degradation was identified by a decrease in the sample's weight. This loss of mass is attributable to the occurrence of endothermic and exothermic combustion reactions. The following graphs illustrate the obtained results.

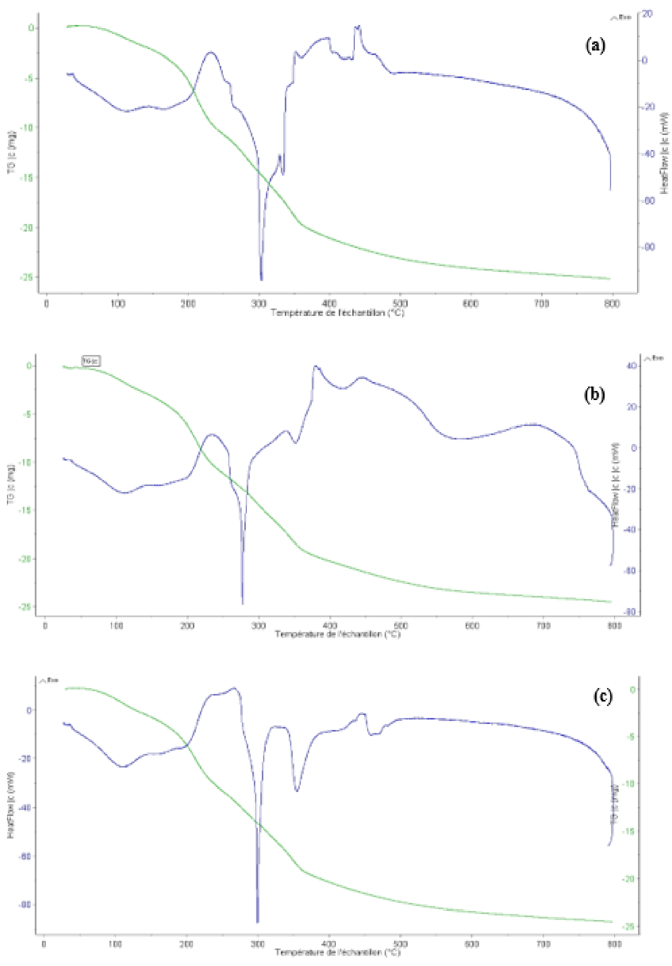

Figure 4 DTG-DSC curves of fruit at the end of the storage period. (a): control. (b): fruit combined in lemon juice. (c): fruit combined in sour orange juice.

To evaluate the stability of vitamins during the storage of fruits after the application of barrier technologies, an analysis was conducted using a thermal analyzer (DTG, DSC) at the end of the storage period.
Based on the results presented in Figure (4), three weight loss phases (10-150 °C, 150-500 °C, and 500-800 °C) can be observed for all three curves. The first phase is characterized by a slight weight loss, reaching 13.44%, 14.16%, and 10.95% for the control, fruits conditioned with lemon juice, and fruits conditioned with bitter orange juice, respectively. This weight loss is mainly due to water dehydration. The second phase, occurring between 150 and 500 °C, shows an increase in weight loss, ranging from 13.44% to 61.82%, 14.16% to 65.55%, and 10.95% to 63.1% for the control, fruits conditioned with lemon juice, and fruits conditioned with bitter orange juice, respectively. This increase can be attributed to vitamin degradation. Finally, the third phase (500-800 °C) is characterized by a weight loss of 68.09%, 70.05%, and 67.69%, corresponding to approximately 11.87 mg, 10.57 mg, and 11.86 mg of final residue in the form of black ashes at the end of the experiment, respectively, for the control, fruits conditioned with lemon juice, and fruits conditioned with bitter orange juice.
In the second stage of analysis, the DSC curves of all three samples display distinct characteristics. Firstly, an exothermic peak is observed between 150 and 250 °C in all cases, attributed to the decomposition of vitamin B6 (Borodi et al., 2009). Subsequently, a prominent intense peak appears between 280 and 300 °C, indicating the weight loss caused by the degradation of vitamin C (Juhász et al., 2012). Additionally, for curves (b) and (c), an identifiable peak emerges at a temperature range of 350-375 °C, corresponding to the degradation of vitamin B12 (Britto et al., 2016). This finding explains the absence of a peak in the DSC curve of the control sample, suggesting a negligible or low amount of vitamin B12 compared to the initial mass. Lastly, in curve (c), there is an endothermic peak observed around 450-500 °C, which is linked to the decomposition of vitamins B9 and B2 for fruits conditioned with bitter orange juice (Refat, 2010; Britto et al., 2016).
The thermal analyses demonstrate that the application of barrier technology with the use of bitter orange juice is more effective in preserving vitamins B2, B6, B9, B12, and C during the storage of fruits in the presence of light and oxygen. Conversely, for fruits conditioned with lemon juice, the absence of vitamins B9 and B2 is observed, while in the case of the control, the degradation of vitamins B2, B9, and B12 is noticeable during storage.

Sensory analysis

After conducting a session to train and select the panel of taste testers, we worked with three samples of *A. unedo* fruits: fruits conditioned with lemon juice, fruits conditioned with bitter orange juice, and non-conditioned fruits (control). The objective of this study was to evaluate the effect of barrier technologies on *A. unedo* fruits. The obtained results are presented in the following Figure (5).

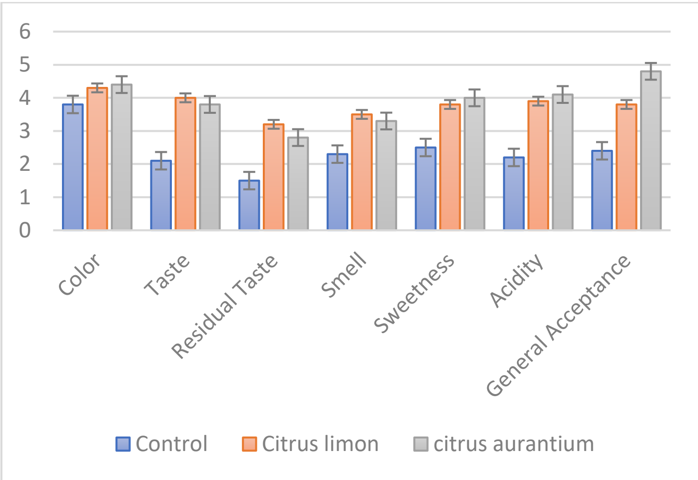

Figure 5 Organoleptic parameters as a function of additive type.

Figure (5) demonstrates that the application of the combined technology to *A. unedo* fruits leads to a noticeable increase in color intensity compared to the control. Additionally, there is a significant improvement in taste and residual taste when fruits are conditioned with lemon juice and bitter orange juice, indicating that the barrier technology has a positive impact on fruit taste. As for aroma, a moderate increase is observed when utilizing the combined technology with both lemon juice and bitter orange juice additives in comparison to the control.
Moreover, the application of barrier technologies has a significant impact on the acidity and sweetness of the fruits, resulting in a notable increase compared to the control. This increase can be attributed to the packaging in an environment that is both acidic and sweet.
Regarding the overall acceptance of the fruits, the panelists showed a strong preference for fruits conditioned with bitter orange juice, followed by moderate acceptance for fruits conditioned with lemon juice, while the non-conditioned fruits (control) were less preferred.

Microscopic analysis

Figure (6) presents the impact of barrier technology application on the plant cells of *A. unedo* fruits, as well as the color intensity. It is based on an image of a very thin cross-section of the fruit, magnified at a x40 magnification.

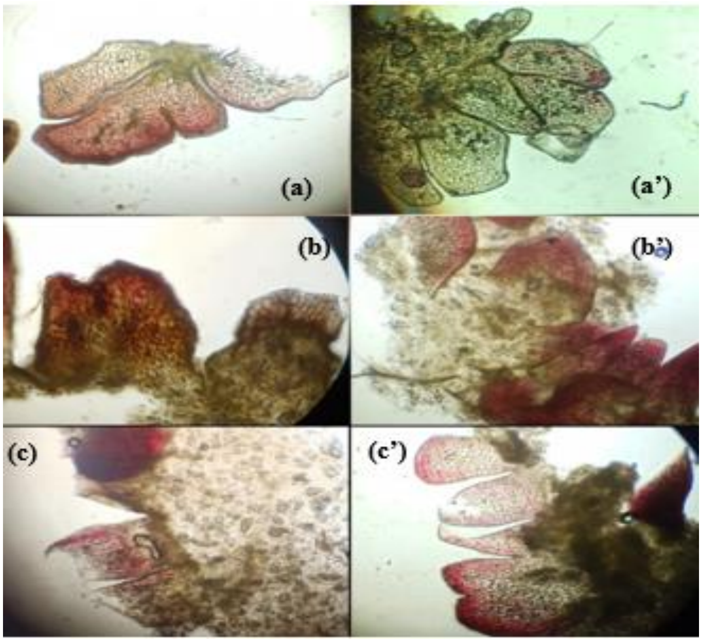

Figure 6 Image of plant cells after application of the combined technology (a, b and c), and after 12 days of storage (a', b' and c'). (a, a'): control. (b, b'): fruit combined in citrus limon juice. (c, c'): fruit combined in citrus aurantium juice.

The figure (6) presents the cells of *A. unedo* after the application of the combined technology (a, b, and c) and their state after 12 days of storage (a', b', and c'). The three conditions depicted are the control, fruits combined with lemon juice, and fruits combined with bitter orange juice.

According to our results, no changes in the shape of the cells were observed throughout the storage period. The cross-section, which includes multiple layers of cells, maintained its shape during the 12-day storage period.

A clear decrease in color intensity was observed during the storage period for the control (Figure 6) (a, a'). This decrease may have an impact on the acceptability of the fruits since color is the first encounter between the product and the consumer.

Our results highlight the impact of barrier technology application on color intensity. We clearly observe an increase in color intensity during the storage of fruits conditioned with both types of additives.

CONCLUSION

The results of this study have demonstrated a significant reduction in the chemical characteristics responsible for microbial growth, such as pH and moisture content, through the application of barrier technologies compared to the control group. Additionally, an inactivation of total aerobic mesophilic flora was observed. Thermal analysis (DTG-DSC) showed the beneficial influence of the addition of bitter orange juice on the preservation of vitamins B2, B6, B9, B12, and C. Furthermore, packaging in an acidic and sweet environment played a crucial role in improving the acceptance of the fruits and their color intensity in Arbutus unedo L.

The incorporation of bitter orange juice ensures the microbiological, chemical, and nutritional stability of the fruits while enhancing their sensory qualities during storage. Moreover, bitter orange fruits offer interesting prospects for potential use in industrial and biotechnological applications.

Acknowledgments: The authors would like to thank the students of the professional license LP-food industry, the students of the DUT food industry and the doctoral students of the Laboratory of Engineering and Applied Technologies, Higher School of Technology, University of Sultan Moulay Slimane; for their invaluable help when carrying out organoleptic testing.

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