International Journal of Agriculture and Biosciences 2023 12(4): 292-298.
https://doi.org/10.47278/journal.ijab/2023.080

SHORT COMMUNICATION
eISSN: 2306-3599; pISSN: 2305-6622

Effect of the Combination of Freezing and Packaging in an Acid Solution on the Stability of Arbutus Unedo L. Fruits

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ABSTRACT

The exploitation of *Arbutus unedo* L., a wild fruit, remains relatively limited due to its sensitivity and the dearth of information regarding its conservation methods. This study undertook a comprehensive examination of two preservation techniques: acid packaging utilizing a concentration of 3/4 acetic acid and freezing at -2°C over a span of 180 days. The research revealed that the 3/4 acetic acid concentration in packaging notably contributed to a significant reduction in pH and an elevation in titratable acidity when compared to packaging with a concentration of 1/2 acetic acid. However, it was observed that the increase in total aerobic mesophilic flora remained relatively insignificant in contrast to the control group. Simultaneously, an enhancement in fruit acidity was noted following the packaging process. Moreover, the preservation utilizing 1/2 acetic acid in packaging demonstrated favorable outcomes in terms of physico-chemical and organoleptic parameters of the *Arbutus unedo* L. fruit. Consequently, the amalgamation of packaging containing 1/2 acetic acid along with freezing at -2°C emerges as a pragmatic and cost-effective approach for preserving Arbutus unedo L. fruits.

Keywords: *Arbutus unedo* L. Storage, Packaging, Freezing, Acetic acid, Physicochemical.

Article History
Article # 23-519
Received: 20-Oct-2023
Revised: 22-Nov-2023
Accepted: 25-Oct-2023

INTRODUCTION

Situated within the Mediterranean basin, Morocco stands as one of the world's regions boasting rich ecological biodiversity. This biological wealth manifests through a diverse range of ecosystems and an extensive array of plant and animal species (EL Alami et al., 2016). Among these botanical species is the strawberry tree (Arbutus unedo L), known locally in Morocco as 'sasnou.' Belonging to the Ericaceae family, this fruit-bearing tree is indigenous to regions encompassing Greece, Lebanon, Ireland, Southern Europe, and Anatolia. Its presence extends across Western, Central, and Southern Europe, Northeast Africa, the Canary Islands, and Western Asia (Karadeniz and Şişman, 2003). Additionally, the strawberry tree is distributed throughout North American countries, along the Atlantic coast, in the Mediterranean regions of Portugal, Spain, France, Italy, Morocco, Albania, Croatia, Bosnia, Montenegro, Greece, Turkey and Mediterranean islands (Kim, 2012; Torres et al., 2002).

Thriving typically at elevations ranging between 700m to 1000m, the strawberry tree exhibits growth in both alkaline and relatively acidic rocky terrains (Celikel et al., 2008). The leaves of this plant hold significance in traditional therapy, recognized for their medicinal use in treating conditions such as hypertension, diabetes, and inflammatory disorders owing to their antioxidant, astringent, diuretic, and anti-diarrheal properties (Bnouham et al., 2007).

The fruit of the strawberry tree is a roughly spherical berry, approximately 2 cm in diameter, exhibiting a dark red hue and reaching its full flavor upon ripening in autumn (Alarcão-E-leitão et al., 2001; Pallauf et al., 2008). These fruits are rich in sugars and proteins (Elmejhed et al., 2022), with a mineral composition notably high in potassium and calcium. Furthermore, the presence of phenolic compounds such as gallic acid has been identified (Males et al., 2006). In Moroccan culinary practices, Arbutus unedo L. fruits find limited seasonal use, primarily employed in the production of alcoholic beverages, jams, jellies, and marmalades (Pawlowska et al., 2006; Simonetti et al., 2008).

Despite their nutritional and antioxidant richness, fresh consumption of strawberry fruits remains uncommon, with only local communities residing near forests partaking due to the fruit's sensitivity and the absence of effective preservation techniques. Vinegar, known for its attributes as a protein solvent, dietary acidity regulator, and antimicrobial food preservative, offers potential in fruit preservation (Mestiri et al., 2006).

Hence, ensuring sustainable preservation and utilization of these fruits remains imperative. This study aims to evaluate the efficacy of combining two traditional conservation methods over the shelf life while assessing the physico-chemical and organoleptic parameters of the strawberry tree fruit. This involves cold storage in a freezer subsequent to packing the fruit in a vinegar solution.

Cite this Article as: Mejhed BE, Kzaiber F and Terouzi W, 2023. Effect of the combination of freezing and packaging in an acid solution on the stability of Arbutus unedo L. fruits. International Journal of Agriculture and Biosciences 12(4): 292-298. https://doi.org/10.47278/journal.ijab/2023.080

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MATERIALS & METHODS

Our study utilized plant material comprising two samples of Arbutus unedo L. fruit. These samples were procured in November 2021 from the Oum Errabia forest in Khenifra, situated 124 kilometers away from Beni Mellal, at a latitude of 33°01' and a longitude of 5°29'. The region, part of the Beni Mellal Khenifra area, is positioned at an elevation of 1613 meters above sea level within the Middle Atlas range (Rahima et al., 2019).

Characterized by a sub-humid climate, this region experiences four distinct seasons-winter, spring, summer, and fall. The annual temperature averages 13.2°C, while the annual precipitation reaches up to 702 mm (Rahima et al., 2019).

To maintain the fruit's texture and prevent spoilage, the strawberries were used on the day of their harvest. Table (1) represents the geographical and ecological characteristics of the sampling area.

Method

The Arbutus unedo L. fruit was transported to the Laboratory of Engineering and Applied Technologies at the Beni Mellal College of Technology on the day of its harvest for preservation testing. A manual sorting process involved the segregation of 500 grams of fruit, removing waste and deformed fruits, followed by washing and categorization based on their ripeness.

The selected fruits underwent a random selection process, with each trial comprising 30 fruits of varying sizes and ripening stages. These fruits were then subjected to packaging in acetic acid solutions (vinegar) for a duration of 1 minute, using varying concentrations of 1/2 and 3/4 acetic acid. Subsequently, the prepared fruits were allowed to dry at room temperature and stored in a freezer set at -2°C under plastic wrap (Guo et al., 2008). Fig. (1) represents diagram of the fruit preparation courses.

A series of physico-chemical and organoleptic assessments were conducted on the strawberry fruits, following which they were stored in the freezer for a period of 180 days at -2°C subsequent to the conditioning process.

Physicochemical Analysis

Determination of pH

Ten grams of the fruit were weighed, sliced into small pieces, and combined with 100 ml of distilled water. The mixture was blended for a duration of 5 minutes to extract the juice. The pH of the resulting solution was measured using a pH meter electrode (AOAC, 2000).

Titratable Acidity

The determination of titratable acidity involved neutralizing the acid within a specified quantity of the sample using a base solution (NaOH).

Acidity assessment entailed the neutralization of the total free acidity found in 25 ml of juice using a solution of NaOH (0.1 N), alongside the utilization of phenolphthalein as a colored indicator. The resulting titratable acidity measurement is expressed in relation to the malic acid content, in accordance with the procedures outlined in (AOAC 2002).

Moisture Content

The water content corresponds to the weight loss incurred during desiccation.

Weigh separately 2 g of the fruit and chopped into small chunks in clean capsules. Place these capsules in the oven at 102°C until the weight remains steady. Cool the capsules prior to weighing (AOAC, 2000).

Organoleptic Analysis

The evaluation occurred both at the commencement and conclusion of the preservation process in room C6 of the Graduate School of Technology, illuminated by natural daylight. The tasting area was enhanced by the presence of white-painted walls, ensuring a visually conducive environment.

The panel comprised 26 tasters, spanning an age range from 18 to 30, inclusive of individuals of all genders. Participants consisted of first, second, and third-year students, along with members of the loading laboratory team, in accordance with the methodology described by (Saliba-Colombani et al. 2001).

Total Aerobic Mesophilic Flora

The stockpiling process involved utilizing 1 ml of the selected dilution (10^{-3}, 10^{-4}, 10^{-5}) in a Petri dish containing the nutrient agar medium. This mixture was allowed to cool, with a total of five Petri dishes prepared for each dilution. The subsequent step included incubating the dishes at 37°C for a duration of 72 hours.

The emergence of the total aerobic mesophilic flora was observed in the form of colonies displaying varied sizes and shapes. For the purpose of enumeration, containers with colony counts falling within the range of at least 30 to a maximum of 300 colonies were retained (NF V08-011, 1998).

Variance Analysis

The investigation focused on assessing the impact of two conservation techniques—acetic acid packaging and freezing at -2°C on multiple parameters: pH, titratable acidity, moisture content, total aerobic mesophilic flora, and sensory attributes. Statistical analysis was conducted via an analysis of variance (ANOVA) using JMP PRO 14 software, classifying the data accordingly. Comparisons among the averages of distinct factors were made. Any observed differences with a p-value below 0.05 were recognized as statistically significant (Monica and Gaddis, 1998).

RESULTS AND DISCUSSION

The results given in the table (2) below represent all of the major factor effects so their interaction of the levels studied based on the variance analysis (ANOVA). In this case, the primary effect of the regression is significant as the probability of the significance of therisk p-value is below 0.05.

pH value

Possessing a critical role in regulating microbial growth rates and enzymatic activity, pH serves as a fundamental parameter for ensuring the stability of food products (Brissonnet et al., 1994). The ANOVA analysis unveiled a significant variance by time in pH levels across 50 tests (F ratio=17.27; Prob F=<, 0001*), notably influenced by the concentration of acetic acid (F ratio=23.415; Prob F=<, 0001*) and the fruit's maturation stage (F ratio=35.61; Prob F=<, 0001*). These parameters distinctly impact the pH fluctuation in Arbutus unedo L.

Exploring potential interactions, Fig. (2) and Table (2) display a profound difference among the studied acetic acid concentrations. Notably, substantial variation was evident between the natural state and the inclusion of 3/4 acetic acid, followed by the presence of 1/2 acetic acid. A minimal pH change was observed when shifting from 1/2 to 3/4 acetic acid concentration, resulting in a less significant effect (p-Value = 0.0282*).

Variance analysis indicates a significant contrast between T0 (initial state) and TF (final state), and between T0 and T1, elucidating considerable variation between the fruit's natural state and the post-packaging status.

Table 1: The geographical and ecological characteristics of the sampling area

Havertz zone	Oum Errabia
Province	Khenifra
Administrative Region	Beni Mellal khenifra
Geographic Region	Middle Atlas
Latitude (N)	33°01’
Longitude (W)	5°29’
Altitude (m)	1613 m
Bioclimatic Zone	sub-humid
Annual Temperature (°C)	13,2°C
Annual Precipitation (mm)	702 mm

Table 2: The ANOVA table has three ways considering three factors with the interaction term of the levels studied. (0): without packaging; (1/2): 1/2 acetic acid packaging; (3/4): 3/4 acetic acid packaging; (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (I): intermediate; (R): ripe.

		Analysis of variance						*Means comparisons*		
Answers	Factors	*Source*	*DF*	*Sum ofsquares*	*Meansquare*	*F ratio*	*Prob >F*	*Level*	*-level*	*p-Value*
pH	Acetic acid	Acid	2	0,3528	0,1764	23,415	<,0001*	0	3/4	<,0001*
		Error	47	0,3541	0,0075			0	1/2	<,0001*
		C. total	49	0,7069				1/2	3/4	0,0282*
		Acid	2	0,2995	0,1497	17,27	<,0001*	T0	TF	<,0001*
	Time	Error	47	0,4073	0,0086			T0	T1	<,0001*
		C. total	49	0,7069				T1	TF	0,9994
		Acid	1	0,301	0,301	35,61	<,0001*	R	1,000	<,0001*
	Maturation	Error	48	0,4058	0,0084					
		C. total	49	0,7069				I	<,0001*	1,000
Titratable acidity	Acetic acid	Acid	2	0,247	0,1235	21,92	<,0001*	3/4	0	<,0001*
		Error	47	0,2647	0,0056			3/4	1/2	<,0001*
		C. total	49	0,5117				1/2	0	0,0494*
		Acid	2	0,126	0,063	7,677	0,0013*	T1	T0	0,0022*
	Time	Error	47	0,3857	0,008			TF	T0	0,0024*
		C. total	49	0,5117				T1	TF	0,9993
		Acid	1	0,2563	0,2563	48,17	<,0001*	R	1,000	<,0001*
	Maturation	Error	48	0,2553	0,0053					
		C. total	49	0,5117				I	<,0001*	1,000
Moisture content	Acetic acid	Acid	2	288,09	144,04	27,086	<,0001*	0	3/4	<,0001*
		Error	47	249,95	5,318			0	1/2	<,0001*
		C. total	49	538,04				1/2	3/4	0,0287*
		Acid	2	251,91	125,95	20,689	<,0001*	T0	T1	<,0001*
	Time	Error	47	286,13	6,088			T0	TF	<,0001*
		C. total	49	538,04				TF	T1	0,8924
		Acid	1	217,56	217,57	32,586	<,0001*	R	1,000	<,0001*
	Maturation	Error	48	320,48	6,677					
		C. total	49	538,04				I	<,0001*	1,000
TAMF	Acetic acid	Acid	2	129266	64633	0,2324	0,7935	0	3/4	0,807
		Error	47	13069	278064			1/2	3/4	0,87
		C. total	49	131982				0	1/2	0,975
		Acid	2	77323	38662	0,1385	0,871	T0	T1	0,86
	Time	Error	47	131209	279169			T0	TF	0,9473
		C. total	49	131982				TF	T1	0,641
		Acid	1	129428	129428	2432,2	<,0001*	R	1,000	<,0001*
	Maturation	Error	48	25542	5321,2					
		C. total	49	131982				I	<,0001*	1,000

Furthermore, the analysis shows no notable difference between T1 and TF (p-Value=0.9994), suggesting minimal pH alteration during storage at 2°C. This emphasizes the significant influence of fruit ripeness on pH variation.

In conclusion, the acid conditioning significantly influences fruit pH regardless of its ripening stage. Additionally, the efficacy of freezing at -2°C emerged as a contributor to preserving pH during storage.

Variation in Titratable Acidity

Titratable acidity denotes the total acid concentration in an agricultural food product, and it correlates proportionally with the food's pH, especially in terms of its shelf life (Al-Farsi et al., 2005). ANOVA analysis highlighted a significant disparity in titratable acidity values across the studied factors. Notably, the pH duration exhibited the most significant impact throughout the experiment (F ratio=7.677; Prob F=0.0013*), followed by the concentration of acetic acid (F ratio=21.92; Prob F=<.0001*) and the fruit's ripening rate (F ratio=48.17; Prob F=<.0001*). Consequently, all three factors significantly influence the evolution of titratable acidity in strawberry fruits. Fig. (3) provides a comparison of the various factor levels.

As depicted in Fig. (3) and detailed in Table (2), considering the pH variation outcomes, a substantial negative effect was observed between the 3/4 acetic acid concentration and the raw state, followed by the 1/2 and 3/4 acetic acid concentrations. A lesser effect was noticed between the raw state and packaging with a 1/2 acetic acid concentration (p-Value= 0.0494*).

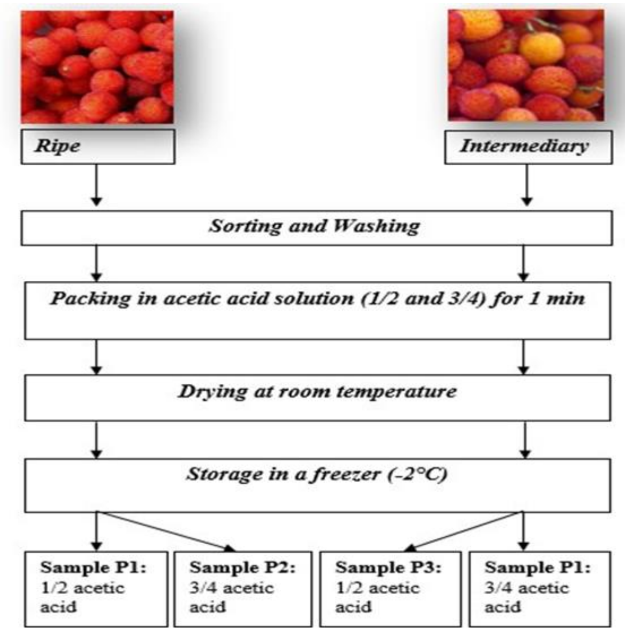

Fig. 1: Diagram of the fruit preparation stages of Arbutus unedo L. a conservation

Additionally, notable negative effects were identified between T0 and T1, and between T0 and TF, indicating a negative variation in titratable acidity concerning the pH alteration between the natural state of the fruit and its conditioned state, as well as between the initial state and the fruit's end-of-storage state. However, an insignificant difference was observed between T1 and TF, suggesting that titratable acidity variations are negligible during freezing.

Thus, the conditioning in an acidic environment significantly impacts titratable acidity, while freezing contributes to maintaining acidity levels during storage.

Variation in Moisture Content

Moisture content stands as a critical parameter in determining shelf life, where higher moisture levels in food elevate susceptibility to microbial interactions (Gray and Ward, 1981). Variance analysis exhibited a substantial difference primarily influenced by the fruit's ripening rate (F ratio=32.586; Prob F=<.0001*), followed by the concentration of acetic acid (F ratio=27.086; Prob F=<.0001*), and the duration of the experiment (F ratio=20.689; Prob F=<.0001*). It is evident that these three factors significantly impact moisture content variation.

The ANOVA findings revealed a substantial difference between the raw fruit state and the state following packaging with 3/4 acetic acid, as well as between the raw state and the packaging with 1/2 acetic acid. This indicates the conditioning effect on moisture content. As depicted in Fig. (4), there is a decrease in moisture content during the transition from the raw state to the end of packaging, followed by a minor reduction from 1/2 to 3/4 acetic acid concentration.

Moreover, ANOVA analysis showed a significant difference between T0 (raw state) and T1 (end of packaging), as well as between T0 and TF (end of freezing). This suggests that packaging affects changes in moisture content. However, minimal effects were observed between T1 and TF, implying slight variation in moisture content during freezing. Furthermore, the maturation rate of the fruit significantly influences moisture content.

These outcomes can be attributed to the migration of water from less concentrated to more concentrated environments.

Variation of Total Aerobic Mesophilic Flora (TAMF)

The total mesophilic aerobic flora encompasses all microorganisms, including bacteria, yeasts, and molds, directly impacting food consumption (Soro-Yao et al., 2014). ANOVA analysis confirmed a significant difference in the ripening rate (F ratio=2432.2; Prob F=<.0001*). However, there was no substantial difference observed in acetic acid concentration (F ratio=0.2324; Prob F=0.7935) or the duration of the experiment (F ratio=0.1385; Prob F=0.871). The comparative levels of these factors are presented in the Fig. (5).

Variance analysis showed no significant differences in the levels of the factors studied (experiment time and acetic acid concentration). These outcomes suggest that the comparison between the raw and end-of-packaging averages is minimal. Consequently, the variation in total aerobic mesophilic flora during the 180-day fruit storage is negligible.

Thus, the combined application of both preservation methods acid conditioning and freezing demonstrates effectiveness in managing the evolution of total aerobic mesophilic flora compared to the French regulation NFV45-065 (1996) with a load of 9.105 CFU/g."

Sensory Analysis

Organoleptic features encompass all sensory inputs gathered through sight (color), taste (flavor, residual taste, acidity, sweetness), and smell (Mariana et al., 2020). The accompanying Fig. (6) illustrates the impact of acid packaging and freezing on the organoleptic characteristics, graded on a scale of 1 to 5, in Arbutus unedo L. fruits.

Color, an essential parameter for assessing product freshness, exhibited noticeable differences in intensity between two ripening stages (intermediate and ripe). There was no significant difference in color between T0 (raw state) and T1 (after conditioning), suggesting no conditioning effect on fruit color. However, a substantial increase was observed between T0 and TF (after 180-day freezing at -2°C), compared to the control samples. This indicates that fruit preservation during freezing and with 3/4 acetic acid packaging significantly impacts fruit color intensity.

Regarding taste and residual taste, an average increase was observed between T0 and T1, indicating the influence of packaging on fruit taste. However, no significant difference was noticed between T1 and TF, suggesting that freezing doesn't affect taste and residual taste compared to the control samples.

In terms of smell, a slight increase was noted after acid packaging for both ripening stages, maintaining consistency over the 180-day freezer storage at -2°C.

The conditioning in 3/4 acetic acid notably influenced fruit acidity, showcasing a significant effect on the fruit's acidity. However, no significant difference was observed in sweetness across all experimental periods.

These techniques moderately impacted the overall acceptance of fruits during the experiment periods, resulting in a slight increase in overall acceptance.

Conclusion

The collective findings indicate that the combination of two preservation methods, acid conditioning, and freezing, indeed affects both the physico-chemical and organoleptic aspects of Arbutus unedo L. fruits.

The pH levels of the fruits noticeably increased post-packaging, likely due to the addition of acetic acid, and remained relatively stable during the subsequent 180-day freezing at -2°C. Similarly, the variations in titratable acidity and moisture content were unaffected by freezing, highlighting its pivotal role in maintaining the changes induced by acidic conditioning.

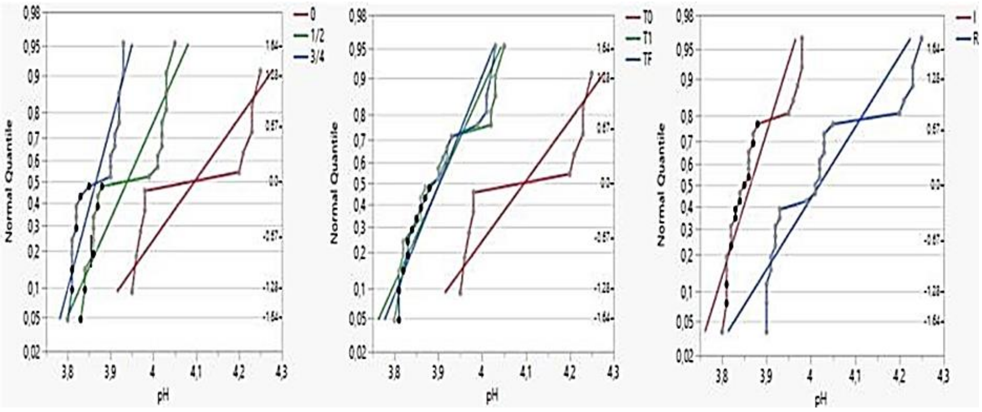

Fig. 2: Variation in fruit pH as a function of the levels of the factors studied. (0): without packaging; (1/2): 1/2 acetic acid packaging; (3/4): 3/4 acetic acid packaging; (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (I): intermediate; (R): ripe.

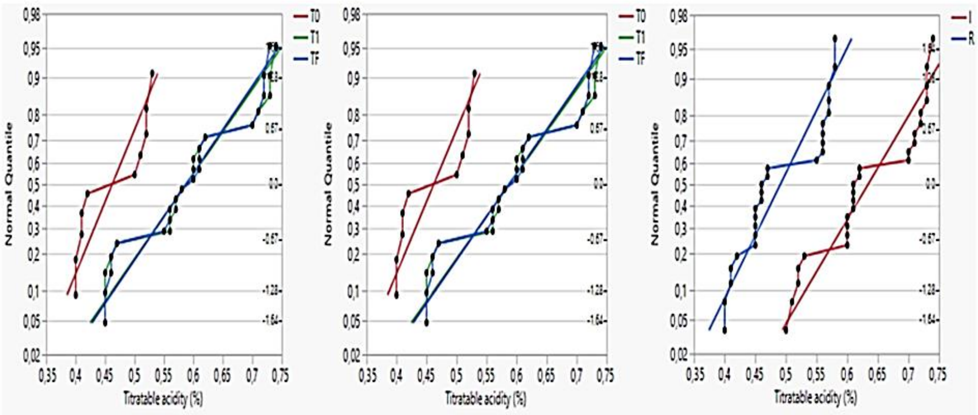

Fig. 3: Variation in titratable acidity of fruits as a function of the levels of the factors studied. (0): without packaging; (1/2): 1/2 acetic acid packaging; (3/4): 3/4 acetic acid packaging; (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (I): intermediate; (R): ripe.

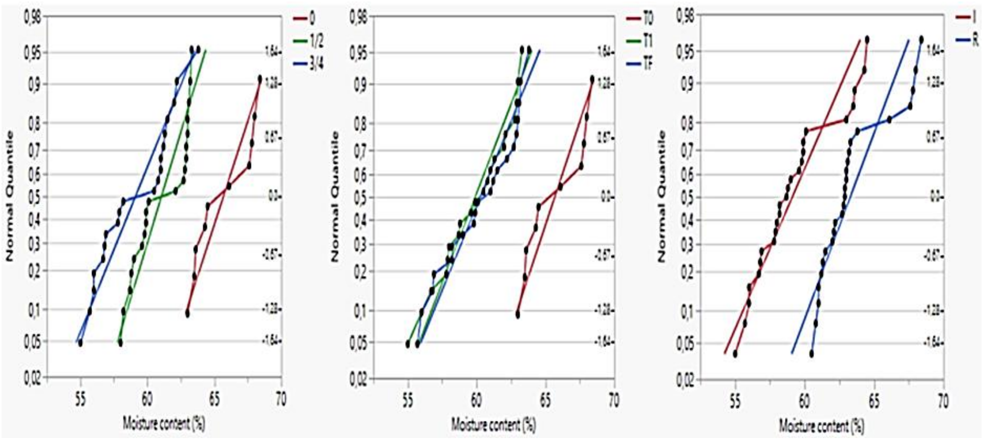

Fig. 4: Variation in fruit moisture content as a function of the levels of the factors studied. (0): without packaging; (1/2): 1/2 acetic acid packaging; (3/4): 3/4 acetic acid packaging; (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (I): intermediate; (R): ripe.

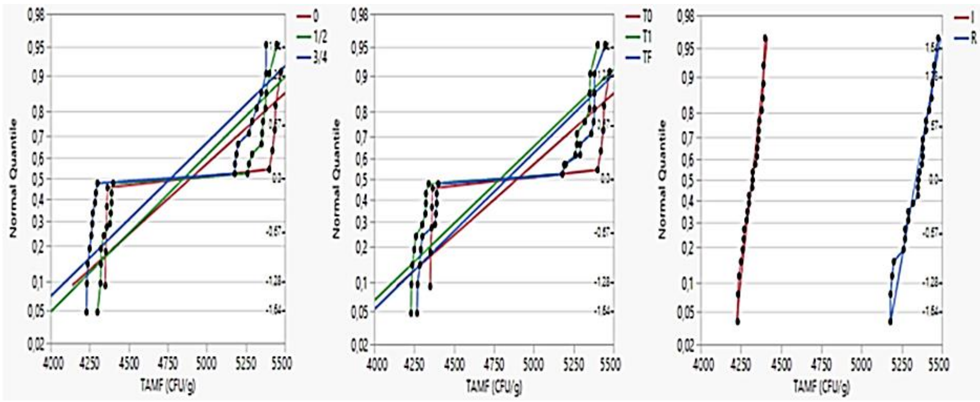

Fig. 5: Variation in total aerobic mesophilic fruit flora as a function of the levels of the factors studied. (0): without packaging; (1/2): 1/2 acetic acid packaging; (3/4): 3/4 acetic acid packaging; (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (I): intermediate; (R): ripe.

Neither freezing nor acetic acid conditioning significantly impacted the evolution of total aerobic mesophilic flora, evident in the minimal differences observed in the averages throughout the experiment. However, a slight increase in total aerobic mesophilic flora was noted across both ripening phases.

Moreover, both preservation techniques positively contributed to enhancing organoleptic attributes, particularly taste and overall acceptance.

Hence, it can be concluded that both ripening stages persist consistently throughout the entire 180-day preservation period, especially after conditioning at the optimal concentration of 1/2 acetic acid.

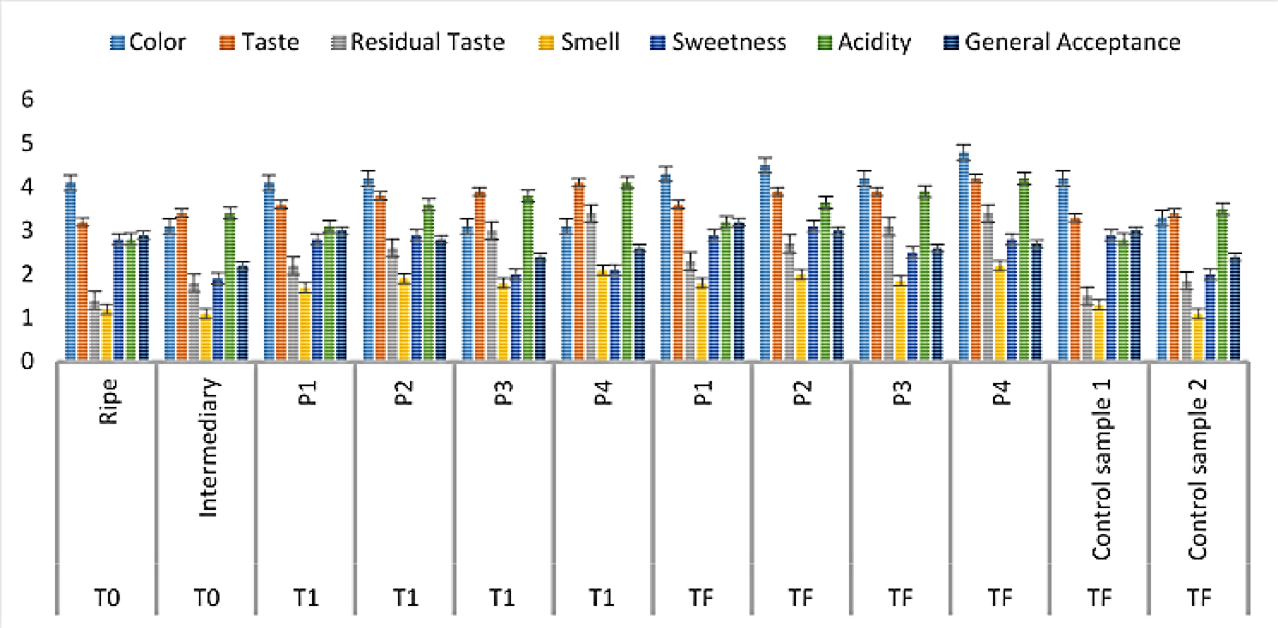

Fig. 6: Variation of organoleptic characteristics according to the time of the experiment. P1: (Ripe, 1/2 in acetic acid); P2: (Ripe, 3/4 in acetic acid); P3: (Intermediate, 1/2 in acetic acid); P4: (Intermediate, 3/4 in acetic acid); (T0): fruits in the raw state; (T1): after packaging; (TF): after 180 days of storage at -2°C; (control sample 1): ripe without packaging; (control sample 2): intermediary without packaging.

Acknowledgements

the authors would like to thank the students of the professional license Ip-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. Not to mention my high school English teacher for checking and correction.

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