

Food preservation methodologies for acerola (*Malpighia emarginata* DC) and wax apple (*Syzygium samarangense*) toward preserving their nutraceutical properties

Joyce GOVINDEN SOULANGE¹ (✉), Keershanee BALNAC¹, Nitisha HURKOO¹, Bibi Farhaanah AZUGUR HOSSEN¹, Joanna KORCZYK-SZABO²

¹ Faculty of Agriculture, University of Mauritius, Reduit, 80837, Moka, Mauritius

² Institute of Crop Production, Faculty of Agrobiological and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 01 Nitra, Slovak Republic

✉ Corresponding author: joyces@uom.ac.mu

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ABSTRACT

In this study, the effects of different preservation methods, namely salting, sun-drying, and starter culture-based fermentation, on the nutritional quality and bioactivity of two underutilised fruits, acerola and wax apple, were investigated. Phytochemical screening of the fruit extracts revealed the presence of bioactive compounds, including phenols, flavonoids, and tannins, in most samples. Vitamin A content was significantly ($P < 0.05$) higher in sun-dried wax apple and dry-salted acerola, with values of 0.225 ± 0.07 and 0.177 ± 0.01 mg beta-carotene/g, respectively. Vitamin C content was generally negatively affected by fermentation with starter cultures, yeast, and *Lactobacillus* species in both fruit types, except in dry-salted wax apple and sun-dried acerola samples. Antioxidant assays (FRAP and DPPH) further demonstrated that fermentation adversely affected antioxidant activity. The α -amylase inhibition assay indicated that only lactic acid-fermented acerola retained its antidiabetic potential after two weeks of preservation. Antibacterial activity, assessed using the microdilution method, showed that fermentation with starter cultures enhanced the bioactivity of wax apple, whereas dry-salting was the most effective method for preserving the antimicrobial properties of acerola. In contrast, sun-drying was found to be less effective overall. The effectiveness of preservation methods was shown to depend significantly on fruit type, suggesting that endogenous microflora may play a critical role in the preservation process. This study contributes to the development of sustainable food production systems and strategies for food waste reduction, thereby supporting local and global food security objectives.

Keywords: acerola, bioactivity, *Lactobacillus plantarum*, preservation methods, *Saccharomyces cerevisiae*, wax apples

INTRODUCTION

Globally, there is increasing interest in the nutraceutical elements of various fruits, as they exhibit many bioactive properties, including anticancer, anti-inflammatory, anti-diabetic, antioxidant, and antimicrobial activities. While many of these fruits are integral to traditional medicines in various cultures, most of them remain overlooked, likely due to the lack of knowledge on their food and nutritional value, non-availability of their complete botanical information, underestimation of their poten-

tial use, stigmatization as “poor man's food”, and inadequate research on their commercialization. Fruits are also categorised as nutraceuticals as they contain both nutritive elements and polyphenolic compounds such as flavonoids and anthocyanins, which are reputed for their antioxidant, anti-inflammatory, anticancer and immune-modulating attributes. Mauritius harbours a high level of fruit diversity that is underutilised and may represent a rich, untapped pool of nutraceuticals.

However, the main issue with these overlooked fruits is the fact that they are seasonal. Acerola, also known as Barbados cherry or West Indian cherry, is a tropical shrub that produces fruit year-round. The main attribute of the acerola for which it is greatly treasured involves its exceptionally high vitamin C content (Laurindo et al., 2024), which is better absorbed by the human body than synthetic ascorbic acid. Wax apple, commonly known as Jamalac in Mauritius, is also called Java apple, Semarang rose-apple and wax jambu. It grows in the coastal rainforests of the indigenous Archipelago, the Andaman and the Nicobar Islands. Its peak months are from March through April and October through December (Khandaker et al., 2012). Traditionally used in dishes and in medicines due to its seeds and juice being rich in a biochemical substance known as 'jamboline' and volatile oils. Jamboline has been found to inhibit the pathological conversion of starch into sugars when glucose is in excess (Saroj et al., 2023).

To preserve the nutritional and antioxidant properties of foods during processing, the food industry has considered new alternatives. Common methods like cooking, chilling, freezing, sugaring, salting and canning methods are known to conserve common fruit species, but reports on the preservation of underutilised fruits are scanty. Drying and salting are the most common and oldest fermentation practices of food preservation, and also part of fermentation processes (Ajibola et al., 2023). Adding 6g/100 g salt could result in a high *Lactobacillus* abundance and a nice flavour (Tang et al., 2022) by improving the sensory, nutritional, and aroma or taste properties of salted fruits while hindering browning (Ahmed et al., 2016).

Lactic acid fermentation is one of the oldest methods of food processing and preservation, known for enhancing safety, nutritional, sensory and shelf-life properties of foods (Zapašnik et al., 2022; Praveen and Brogi, 2025). It enhances the organoleptic and nutritional quality of the fermented fruits while retaining the nutrients

and coloured pigments (Swain et al., 2014). Natural fermentation often yields undesirable products due to a high level of microflora in raw materials, while lactic acid bacteria (LAB) play a vital role in preventing the growth of yeasts or molds. As a result, starter cultures are generally added to guarantee the product consistency (Aguirre-Garcia et al., 2024). Lactic acid bacteria (LAB), a gram-positive bacterium that produces lactic acid, also generate beneficial substances such as bacteriocins, exopolysaccharides (EPS), aroma compounds, enzymes, B vitamins (mainly folate, riboflavin, cobalamin) or low-calorie polyols (mannitol, sorbitol) (Fessard et al., 2017). Studies have investigated the ability of lactic acid fermentation to improve the antioxidant properties of fruits and vegetables. Fermentation with *Lactobacillus plantarum*, *Pediococcus pentosaceus* or *Weissella cibaria* of smoothies and tomato juice resulted in improved preservation of ascorbic acid, glutathione, phenolic compounds and antioxidant activity. Additionally, using *L. plantarum*, *Leuconostoc mesenteroides*, *P. pentosaceus*, *Lb. delbrueckii* subsp. *lactis*, *Bifidobacterium breve* and *B. thermophilum* increase Vitamin C concentration, phenolic content and antioxidant activity of carrots, French beans, marrows, black beans, pomegranate juice, soy milk and cowpeas (Fessard et al., 2017; Septembre-Malaterre et al., 2018). Having the same properties as the naturally occurring LAB in fruits, starter cultures also contribute to maintaining the food's sensory qualities (Septembre-Malaterre et al., 2018). However, the lactic acid produced lowers the pH of plant foods. Moreover, *Saccharomyces cerevisiae* has played a central role in fermented food production for centuries and remains the most commonly used microorganism in the traditional preparation of fermented products, mainly alcoholic beverages and baked goods (Que et al., 2024). Therefore, this study targets two nutrient-rich fruit species with health-promoting attributes that are undervalued locally as potential candidates for processing into fermented fruits and used as functional food.

MATERIALS AND METHODS

Fruits collection

Mature fruits of *Malpighia emarginata* (acerola) and *Syzygium samarangense* (Jamalac) were collected at Red-uit, Farm of the University of Mauritius.

Dry-salting fermentation

Fresh mature fruits were cleaned, washed in sterile distilled water, drained, peeled, sliced and blanched at 70 °C for 2-3 minutes. They were layered (2.5 cm depth) in airtight sterile fermenting jars, alternating with salt. Usually, for 100 kg of fruits, approximately 3 kg of salt is required (2.5 – 3.0% salt). Layers were repeated until jars were full. Fermentation was carried out for 1 to 4 weeks until the airtight jars were free of liquid.

Sun-dried fermentation

The same steps were repeated as above, but fruits were sun-dried at ambient temperature for 2-3 days before being placed in jars. No salt was added. Fermentation was done at 2- 10 °C for 1-2 weeks.

Lactobacillus plantarum starter cultures

Around 200 g of the fruit sample was measured, cleaned, washed, drained and blanched at 70 °C for 2-3 minutes, then transferred to a sterile fermenting jar under laminar air flow. *L. plantarum* (ATCC 8014) culture was prepared in MRS broth medium with 25 µg/ml nystatin at 35 °C for 24 hours, then standardized to 8 log CFU and added to jars. Fermentation occurred at 25 °C – 30 °C for 2-4 weeks.

Saccharomyces cerevisiae starter cultures

Same steps as above, using *Saccharomyces cerevisiae* (ATCC9763) cultured in YE broth with 25 µg/ml chloramphenicol. The culture was incubated for 24 hours, standardized to 7 log CFU and lastly added to jars for fermentation.

Metabolite extraction

50 g of each fruit sample was macerated in 150 ml of methanol for 48 hours on an orbital shaker set at 120 rpm. This was followed by vacuum filtration and flash evaporation at a temperature set at 58 °C.

Qualitative and quantitative phytochemical screening

Preliminary phytochemical screening was carried out using standard qualitative methods (Harborne et al., 1975; Trease and Evans, 1989). Total phenolic content (TPC), flavonoid (TFC) and anthocyanin content (TAC) were assessed using the method described by Singleton and Rossi (1965), Lamaison and Carnet (1990) and Porter et al. (1985), respectively.

Nutritional profile (vitamin A & C)

The Vitamin C content was determined using a method adapted from Chowdhury (2016), while the Vitamin A content was assessed using a modified procedure based on Aremu and Nweze (2017).

Antioxidant assay

Free Radical Scavenging Assay-DPPH by Bouyahya et al. (2017) to measure scavenging activity, and Ferric Reducing Antioxidant Power (FRAP) was determined according to the protocol developed by Benzie and Strain (1996).

Antidiabetic screening (alpha-amylase inhibitory activity)

The protocol from Amylase Activity Assay Kit Sigma-Aldrich Co. LLC Catalogue Number MAK009 was used to assess the anti-diabetic screening.

Antimicrobial activity

The microdilution assay to determine the minimum inhibitory concentration (MIC) of the extracts was carried out as described by Eloff (1998) with a few modifications. Two bacterial strains were tested: *Bacillus cereus* (Gram-positive, ATCC no. 11778) and *Escherichia coli* (Gram-negative, ATCC no. 25922).

Statistical analysis

Statistical analysis was performed to determine statistical differences between the samples. For this purpose, one-way ANOVA analysis was performed. Samples were determined as significantly different if $P < 0.05$. The LSD method was performed for a multiple comparison test. The experiments were done in triplicate ($n=3$).

RESULTS AND DISCUSSION

Percentage yield of wax apple & acerola

The percentage yield of crude extracts varied across treatments, highlighting the influence of processing methods on extraction efficiency. For wax apples, yeast fermentation yielded the highest extraction (14.8%), while sun-drying resulted in the lowest (2.68%). Lactic fermented extracts (10.2%) and fresh samples (5.9%) had intermediate yields. Similarly, for acerola, dry-salted extract produced the highest yield (59%), followed by sun-dried samples (17.3%), whereas yeast-fermented extract had the lowest (7%). Percentage yield differenc-

es depend not only on the type of treatment or solvent used but also on the type of fruit, growing conditions, the surface area per unit mass of the fruit samples during maceration, and extraction time.

Phytochemical screening

A plant's phytochemical profile is affected by environmental and genetic variables (Figueiredo et al., 2008).

The phytochemical profiles of wax apple and acerola are distinct, as shown in Table 1. Fresh and preserved wax apples were rich in saponins, while acerola showed saponin degradation as a result of preservation. It can be deduced that the presence of saponins depends on the type of fruit. Anthraquinone was present at trace levels in sun-dried, lactic acid and yeast-fermented wax apples but absent in other samples. Flavonols and phenols were detected in all the fruit extracts. Banadka et al. (2022) reported the richness of phenolic compounds, flavonoids, flavanones, proanthocyanidins, and terpenoids in *Syzygium samarangense*.

Table 1. The detailed phytochemical constituents of both fruits

Sample Name	Saponins	Anthraquinones	Flavonols	Leucoanthocyanins	Coumarins	Tannins	Alkaloids	Phenols	Steroids
Wax apples	Fresh	++	-	++	++	++	++	++	-
	SD	++	+	++	++	++	+	++	-
	DS	++	-	++	++	++	+	++	-
	LF	++	+	++	++	+	++	++	+
	YF	++	-	+	-	+	++	+	-
Acerola	Fresh	-	-	+	-	-	++	++	+
	SD	-	-	+	-	-	++	++	+
	DS	-	-	+	-	-	++	+	++
	LF	+	-	+	+	+	++	+	++
	YF	+	+	+	++	+	++	-	++

Key: ++: Present -: Absent +: Traces present.

Abbreviations: SD- Sun-dried, DS- Dry salted, L- Lactic acid fermented, Y - Yeast fermented.

Hydrolysable tannins were also detected in all fruit extracts except for fresh, sun-dried and dried-salted acerola, which yielded condensed tannins. Vasavilbazo-Saucedo et al. (2018) found condensed tannins in acerola *Malpighia umbellata* Rose, while leucoanthocyanidin was absent in yeast-fermented wax apples and in all fresh, sun-dried and dry salted acerola samples, indicating degradation.

Quantification of Total Phenolic Content (TPC)

The total phenolic content was determined by using Gallic acid as a standard. Table 2 represents the calculated concentration of phenol in the fruit extracts expressed as Gallic acid equivalent per dried mass using the calibration curve.

Fermented starter cultured wax apple extract exhibited higher TPC than fresh ones (Table 2). Microbial fermentation is reported to preserve and improve the phenolic content through mobilization of bound phenolics to free forms (Adebo et al., 2020). No significant difference ($P < 0.05$) was noted between fresh and dry-salted wax apples, indicating that the salt addition effectively preserved phenolic content. Sun-dried wax apples had the lowest TPC value (2768.71 mg GAE/DW g), probably due to thermal degradation, cell disruption and interactions with enzymes, oxygen and proteins. Therefore, this

supports findings that phenolic compounds degrade in direct sunlight (Chumroenphat et al., 2021).

The total phenolic content of acerola samples ranged from 1069.39 to 3500.68 mg GAE/ DW g (Table 2). Dry-salted extract had the highest TPC, followed by the starter cultured-fermented, while the sun-dried extract still shows the lowest TPC in both cases. Significant differences were noted between the treatments ($P < 0.05$) except between lactic acid and yeast-fermented acerola (LSD = 82.6)

Dry salting likely enhances TPC by reducing the water content of the fruits and also enhances the activity of endogenous LAB (Institute of Medicine (US) Committee on Strategies to Reduce Sodium Intake, 2010), promoting fermentation. This aligns with findings by Wijayanti et al. (2017) on fig fruit juice. Similarly, both lactic acid and yeast-fermented acerola also showed higher TPC than fresh acerola, with no significant difference between them. The increase in total phenol content in lactic acid fermented mulberry juice compared to mulberry fresh fruit juice was attributed to the ability of the bacterium's hydrolytic enzymes to break down complex phytochemicals into simple ones (Wang et al., 2022). In contrast, the lowest TPC in sun-dried acerola (1069.39 mg GAE/ DW g) indicated thermal degradation, making it the least suitable preservation method.

Table 2. TPC, TFC and TPAC values of wax apple and acerola

Preservation Methods	Wax apple			Acerola		
	TPC (mg GAE/g)	TFC (mg quercetin/g)	TPAC (mg tannic acid/g)	TPC (mg GAE/g)	TFC (mg quercetin/g)	TPAC (mg tannic acid/g)
Fresh	3556.46 ± 50.38 ^b	177.49 ± 1.95 ^a	109.95 ± 1.89 ^a	2911.57 ± 2.35 ^a	125.63 ± 0.62 ^a	12.73 ± 0.34 ^b
Sun-dried	2768.71 ± 27.18 ^a	123.82 ± 1.53 ^b	17.60 ± 3.88 ^c	1069.39 ± 2.32 ^c	79.04 ± 0.37 ^b	13.48 ± 0.37 ^b
Dry-salted	3534.69 ± 79.46 ^b	197.83 ± 2.19 ^c	40.66 ± 2.22 ^b	3500.68 ± 2.00 ^a	194.79 ± 0.58 ^c	18.22 ± 0.43 ^a
Lactic acid Fermented	5240.82 ± 88.30 ^c	364.22 ± 1.42 ^d	39.25 ± 1.54 ^b	3262.59 ± 14.33 ^b	207.42 ± 0.88 ^d	23.86 ± 1.55 ^b
Yeast fermented	4650.54 ± 41.89 ^d	349.37 ± 2.19 ^e	39.30 ± 7.86 ^b	3198.64 ± 15.45 ^b	181.58 ± 1.13 ^e	9.20 ± 1.19 ^c

Abbreviations: TPC - Total Phenolic Content, TFC - Total Flavonoid Content, TPAC - Total Proanthocyanidins Content.

Values are presented as mean ± standard deviation of three replicates (n = 3).

a-e Means with different superscripts within each column are significantly different (LSD, $P < 0.05$).

Quantification of Total Flavonoid Content (TFC)

The TFC of wax apple follows the same trend as TPC except that there are significant differences ($P < 0.05$) between dry-salted and fresh samples (Table 2). The highest TFC was recorded with fermented fruits (Yeast = 364.22 mg quercetin/DW g, Lactic = 349.37 mg quercetin/DW g), followed by dry-salted (197.83 mg quercetin / DW g), then fresh wax apples (177.49 mg quercetin / DW g) and eventually sun-dried (123.82 mg quercetin / DW g). Enzymes from yeasts and *L. plantarum* fermentation, such as beta-glucosidase, decarboxylases, esterase, hydrolases and reductases, can hydrolyse complex phenolic compounds to release free phenolics such as flavonoids. Hence, fermentation with starter cultures increased the extractability of phenols, increasing TFC as recorded in this study (Adebo et al., 2020). The higher TFC in dry-salted samples compared to fresh ones is likely attributed to salt-induced natural fermentation which promotes LAB growth under low-oxygen conditions (Liu et al., 2025). While all preservation methods preserved flavonoids, sun-drying led to significant degradation, making it the least effective method. Unlike TPC, TFC values in acerola followed a different trend, with the highest content in yeast-fermented acerola (207.42 mg quercetin / DW g) while sun-dried acerola had the lowest TFC content (79.040 mg quercetin/ DW g). Preservation methods were generally effective in maintaining flavonoids in wax apples, except for sun-drying. However, dry-salted acerola had higher TFC than lactic fermented acerola, suggesting that endogenous LAB may be more efficient at hydrolysing flavonoid precursors than *L. plantarum* in starter culture.

Quantification of Total Proanthocyanidins Content (TPAC)

Fresh wax apples demonstrated the highest tannin content (109.95 mg tannic acid/DW g), indicating that the preservation techniques effectively reduce antinutrients. Furthermore, Taiwo (1998) reported that salting reduces trypsin inhibitor and eliminates tannin contents in beans, which is consistent with the findings of this

study. The lowest tannin level was observed in sun-dried wax apples (17.60 mg tannic acid/DW g) and this is justified by the fact that tannins are unstable under high temperatures, light and oxygen. Madrau et al. (2008) showed that drying apricots in oxygen accelerates phenolic breakdown by polyphenol oxidase (PPO), which oxidizes phenolic compounds to quinones that polymerized melanin, explaining the dark colour after drying (Ala Eddine and Barkat, 2019). In acerola, yeast-fermented (23.86 mg tannic acid/DW g) and dry-salted (18.23 mg tannic acid/DW g) exhibited a higher TPAC value as compared to sun-dried and fresh fruits, while lactic acid fermented acerola showed the lowest. This suggests that yeast and salt were less effective in reducing antinutrient effects in the fruit but rather increased the levels of antinutrients. The preservation period may have impacted the results. No significant difference was observed between sun-dried and fresh acerola indicating that sunlight exposure did not significantly affect tannin content. In contrast, lactic acid fermentation proved more effective, aligning with findings of Shang et al. (2019), who reported the removal of approximately 78% of tannin content in fresh Xuan Mugua fruits after LAB fermentation.

Nutritional profile

Wax apple vitamin A

Sun-dried wax apples, despite exhibiting the lowest TPC and TFC, were found to be the richest in vitamin A of 0.41 μg beta carotene/g DW (Table 3). Dry-salted wax apples (0.32 μg beta carotene/g DW) also contained more vitamin A than fresh ones (0.07 μg beta carotene/g DW) ($P < 0.05$). In this study, sun-drying and dry-salting enhanced the vitamin A content of wax apples, whereas fermentation showed no significant effect, indicating its suitability for preservation. Sun-drying may degrade phenols and flavonoids, but remains effective for preserving vitamin A. Ultra-violet (UV) rays can be effective in rapidly inducing carotenogenesis during exposure to sunlight (Llewellyn et al., 2020), thereby improving the level of beta carotene in the fruit sample. An enhanced level of vitamin A was also observed in dry-salted wax

apples as compared to the fresh ones. This may be attributed to the ability of drying to maintain the integrity of cell structures in the fruits, hence preventing the breakdown or oxidation of vitamin A (Rahman, 2007). No significant difference ($P < 0.05$) was observed in lactic acid fermented fruits as compared to fresh fruit, as confirmed by Walther and Schmid (2017), who revealed that *Lactobacillus* does not influence or consume vitamins during fermentation.

Wax apple vitamin C

Ascorbic acid, which was the positive control, was found to contain 2318.91 mg/100 ml of ascorbic acid, which is higher than the preserved and fresh wax apples, as shown in Table 3. The highest vitamin C content for wax apples was recorded with dry-salted (874.73 mg/100 ml ascorbic acid), followed by fresh wax apples (769.06 mg/100ml ascorbic acid) and then sun-dried samples (484.33 mg/100ml ascorbic acid), showing a decline. Fermented acerola with starter cultures had the lowest vitamin C content, likely due to its inherent instability and quick oxidative degradation, or to the metabolic microbial consumption during the fermentation (Saud et al., 2024). Dry-salting enhances the level of vitamin C by lowering the water activity, whereby minimizing degradation. In contrast, the decline in the sun-dried sample is due to thermal degradation as vitamin C is highly vulnerable to oxidative damage, which is exacerbated by

heat and light (Yin et al., 2022). According to Subramani et al. (2024), drying of fruit leads to a decrease in Vitamin C content compared to fresh fruit. Furthermore, Knez et al. (2023) mentioned that with fermentation, the level of vitamin C decreases.

Acerola vitamin A

Dry-salted acerola demonstrated the highest vitamin A content (3.63 μg beta carotene / g DW), followed by sun-dried (2.37 μg beta carotene / g DW) and fresh fruit (2.33 μg beta carotene / g DW), as shown in Table 3. Significant differences were observed between the treatments as indicated by $P < 0.05$. However, no significant difference was found between the salt-fermented extract and the fresh extract. Sangiya et al. (2021) reported an increase in β -carotene content in lactic-fermented African nightshade pickles, which was attributed to factors such as carbon source, pH, temperature and Reactive Oxygen Species (ROS). The absence of Vitamin A increase in this study may be due to limited carbon sources available for bacterial carotenoid synthesis. Carotenoid liberation and bio-accessibility are caused by fermentation, which may also promote oxidation or destruction of the carotenoid compounds. In this study, no significant difference was noted in the vitamin A content of sun-dried extract compared to fresh acerola extract. In contrast, Karabulut et al. (2007) reported a decrease in β -carotene after the sun-drying of apricots process as

Table 3. Nutritional profile of wax apple and acerola

Preservation Methods	Wax apple		Acerola	
	Vitamin A (mg beta carotene/g)	Vitamin C (mg/100 ml ascorbic acid)	Vitamin A (mg beta carotene/g)	Vitamin C (mg/100 ml ascorbic acid)
Fresh	0.041 \pm 0.01 ^a	769.00 \pm 45.19 ^a	2.330 \pm 0.36 ^b	607.61 \pm 15.25 ^a
Sun-dried	0.225 \pm 0.07 ^c	484.00 \pm 40.35 ^b	2.370 \pm 0.29 ^b	737.00 \pm 36.66 ^b
Dry-salted	0.177 \pm 0.01 ^d	875.00 \pm 44.32 ^c	3.620 \pm 0.90 ^b	1185.85 \pm 22.16 ^c
Lactic acid fermented	0.014 \pm 0.00 ^b	238.00 \pm 49.03 ^d	0.045 \pm 0.00 ^a	299.40 \pm 15.25 ^d
Yeast fermented	0.006 \pm 0.00 ^b	285.00 \pm 5.08 ^e	0.032 \pm 0.01 ^a	387.00 \pm 23.30 ^e

Values are means \pm standard deviation of three replicates (n = 3).

a-e Means with different superscripts within each column are significantly different (LSD, $P < 0.05$).

β -carotene tends to diminish as drying times increase due to oxidation. Hence, the discrepancy between the findings may be explained by differences in drying conditions. In the present study, acerola fruits were chopped prior to drying, thereby increasing the surface area exposed to sunlight, leading to less drying time. In contrast, the apricots in the above-referred study were dried whole, resulting in longer exposure and greater surface area for oxidation. Consequently, in this study, the shorter drying duration did not cause oxidation and retained β -carotene. The lowest levels of vitamin A were recorded in fermented acerola using starter cultures.

Acerola vitamin C

In acerola extracts, the highest vitamin C content was recorded with dry-salted (1185.88 mg/100 ml ascorbic acid), followed by sun-dried (736.77 mg/100 ml ascorbic acid) and then the fresh fruit (607.61 mg/100 ml ascorbic acid) (Table 3). Fermented acerola with starter cultures had the lowest vitamin C content. Both dry-salting and sun-drying enhanced the vitamin C content as compared to the fresh fruit sample. However, studies such as Minuye et al. (2021) reported a decrease in vitamin C content on open solar drying of yellow papaya. This could be due to bruising and mechanical damage in fresh fruits, which exposes ascorbic acid to oxidation, lowering its concentration (Mditshwa et al., 2017). In contrast, starter-cultured fermentation led to a decrease in vitamin C content.

Determination of DPPH scavenging capacity

As shown in Figure 1, the lowest concentration (IC_{50}) for wax apples was recorded with sun-dried (4.22 mg/ml), followed by the fermented fruits (Yeast = 4.85 mg/ml, Lactic = 4.51 mg/ml) and eventually the dry-salted wax apples (5.88 mg/ml). Fresh wax apples had the highest antioxidant activity with DPPH (IC_{50} = 4.22 mg/ml).

The IC_{50} values indicate that none of the preservation methods maintained the free radical scavenging capacity of the species under study. The lowest concentration of the fresh sample extract was needed to inhibit 50% of the free radicals. No significant difference was ob-

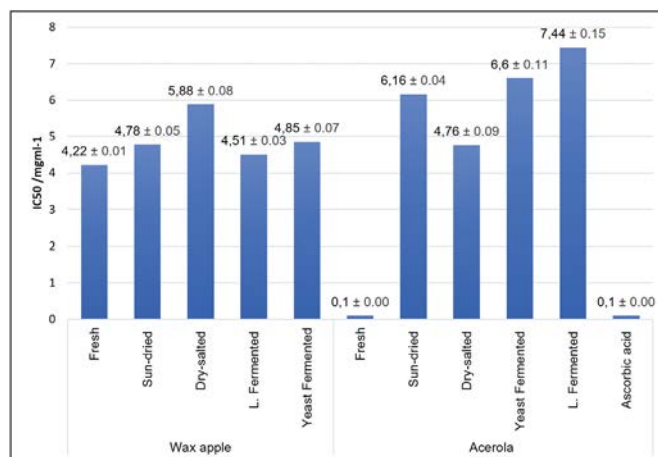


Figure 1. Scavenging capacity of wax apples and acerola

served between sun-dried and yeast-fermented. DPPH is sensitive to Lewis bases (Kedare et al., 2011). Plants have naturally-occurring Lewis bases such as alkaloids, terpenoids and phenols. Under acidic conditions, such as during fermentation, the Lewis bases are protonated and undergo structural changes, thereby reducing their reactivity with DPPH. Therefore, the sensitivity of the test decreases with fermentation. In addition, the DPPH assay is affected not only by pH but also by other factors, including oxygen and incubation time (Shahidi and Zhong, 2015), which may explain the inconsistency between DPPH results and Total Flavonoid Content (TFC). Despite having the lowest TFC, sun-dried and fresh wax apples exhibited the lowest IC_{50} , suggesting compounds other than flavonoids are probably acting as scavengers. For acerola, fresh extract showed the highest antioxidant activity against DPPH (IC_{50} = 0.09 mg/ml). Higher IC_{50} values were observed for dry-salted (4.76 mg/ml), followed by Lactic (7.435 mg/ml) (Figure 1). The lower DPPH activity observed in fermented samples is consistent with findings by Wijayanti et al. (2017), who reported a decreased antioxidant activity in lactic acid-fermented (using different lactic bacteria strains) fig juice. Similarly, a reduction in DPPH scavenging activity was observed in the sun-dried extract compared to the fresh extract. The result is in line with the investigation by Bey et al. (2016) on sun-drying of different fig varieties, who reported a decrease in antioxidant activity after sun-drying. Although dry-salted wax apple was the richest in vi-

tamin C, its antioxidant properties were found to be the lowest in this case.

Determination of FRAP

Figure 2 shows the FRAP activity of wax apples and acerola. The highest FRAP activity was obtained with the fermented wax apples (east = 1008.49 mM Fe²⁺/g DW, Lactic = 833.94 mM Fe²⁺/g DW), followed by dry-salted (443.73 mM Fe²⁺/g DW) and then fresh wax apples (303.71 mM Fe²⁺/g DW). Sun-dried wax apples had the lowest activity (299.83 mM Fe²⁺/g DW).

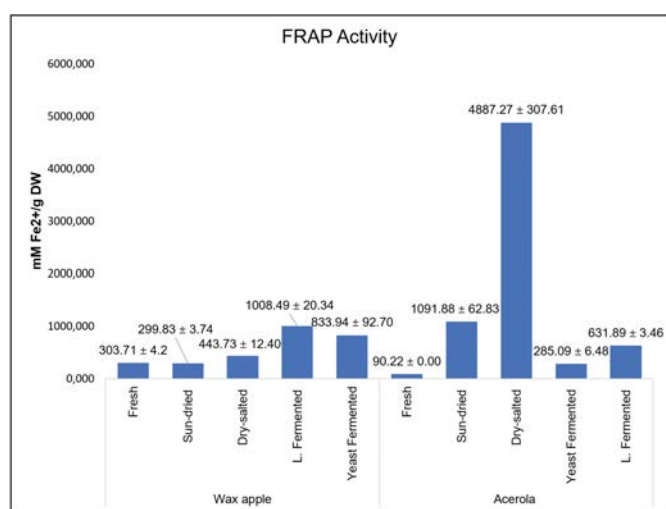


Figure 2. FRAP activity of wax apples and acerola

The trend in the results obtained mirrors the trend for the total flavonoid content in the fruits. Brahimi et al. (2022) found that there was an agreeable correlation between antioxidant activity and values of total phenolics and total flavonoids in Moroccan onions. Hence, the trend in the results obtained is probably due to the flavonoids present in the fruits. Fresh acerola exhibited the lowest activity (90.220 mM Fe²⁺/g DW) as compared to the preserved samples (Figure 2). This study shows that preservation enhances the bioactivity of acerola. Dry salted has the highest reducing power since it has the highest TPC and relatively high TFC. Despite having low TPC and TFC, sun-dried has the second highest reducing power, possibly due to the contribution of non-phenolics and non-flavonoids acting as antioxidants. Additionally, high levels of ascorbic acid in the sun-dried acerola

might have also contributed to its high FRAP value. The phenols and flavonoids detected in yeast-fermented and lactic acid fermented acerola may possess low reducing power or may act as slow-reacting antioxidants. The DPPH assay indicated that the preserved fruits possess lower antioxidant properties than fresh fruits. Borges et al. (2019) found that DPPH has higher efficiency in measuring antioxidant property in comparison with FRAP. However, since phenolic and flavonoid content aligns more closely with FRAP than with DPPH, it can be inferred that FRAP was the more effective assay in this study. Thus, the nutraceutical components in preserved acerola and wax apple exhibit greater reducing power but are lower in radical scavenging activity.

Determination of alpha-amylase inhibition

Figure 3 shows the range of the maximum inhibition of alpha-amylase recorded with the preserved and fresh wax apples, which varies from 13.02% to 86.79%. Metformin was used as a positive control, and the percentage inhibition was lower than the dry-salted and fermented fruits with starter cultures.

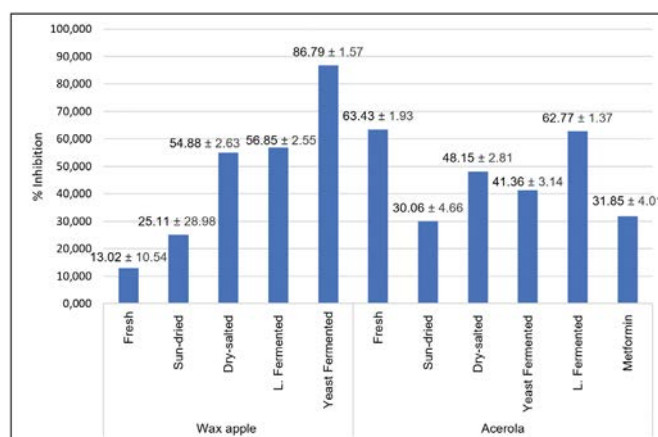


Figure 3. Alpha-amylase inhibition with wax apples and acerola

Fermented fruits with starter cultures and dry-salted wax apples have enhanced levels of phenolic compounds, especially flavonoids. Natural flavonoids may lower blood glucose by regulating gluconeogenesis in the liver. Kaempferol, a flavanol commonly found in medicinal plants, has been reported to improve hyperglycaemia by inhibiting hepatic gluconeogenesis and promoting

glucose utilization in skeletal muscle (Yi et al., 2023). This suggests that flavonoids could be valuable therapeutic candidates for managing type 2 diabetes mellitus (T2DM) by modulating intestinal hormones and enhancing glucose metabolism (Wen and Li, 2025). However, further study is needed to have a better understanding of the processes behind glucose-inhibiting activity with flavonoids. It has been noted from the study that fermentation and dry-salting enhance the antidiabetic properties of wax apples.

In acerola samples, the percentage inhibition with metformin was significantly ($P < 0.05$) lower than in fresh, fermented and dry-salted extracts alone (Figure 3). Only fermented lactic acerola has been able to preserve the glucose inhibitory properties of the fruit over a period of two weeks, while it decreases with the remaining preservation methods.

Antimicrobial properties using the microdilution assay

Table 4 shows the minimum concentration of fresh and preserved wax apples needed to inhibit the growth of *E. coli*, which ranges from 0.78 mg/ml to 1.56 mg/ml. The fresh sample was significantly ($P < 0.05$) better at inhibiting the bacteria as compared to the fermented and dry-salted wax apples.

Wax apples

Except for dry-salted, no significant differences were found between the preserved fruits and the fresh wax apples regarding the growth inhibition of *E. coli*, indicating that preservation techniques maintained antibacterial properties. However, a higher concentration of dry-salted extract was required for inhibition of the test organisms. Although flavonoid content was high, antibacterial activity also depends on the types and structures of the flavonoid. Flavonoid glycosides, including their prenylated, geranylated, methoxylated and hydroxylated derivatives, vary in structure and mode of antibacterial action (Górniak et al., 2019). These antibacterial compounds have huge structural diversity and wide biological activity arising from their frequent modifications. For instance, kaempferol exhibits antibacterial action against a variety of microorganisms, including *Escherichia coli*. However, it has a slower impact than more powerful flavonoids such as catechins (Periferakis et al., 2022). The minimum inhibitory concentration of fresh and preserved wax apples against *B. cereus* ranged from 0.391 mg/ml to 1.563 mg/ml (Table 4). For *B. cereus*, sun-dried extract exhibited the strongest inhibitory effect. This antibacterial activity correlated more closely with vitamin A content than with flavonoid content, which is consis-

Table 4. Antimicrobial effect of wax apple and acerola

Preservation Methods	Minimum inhibitory concentration value (mg/ml)			
	Wax apples		Acerola	
	<i>E. coli</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>B. cereus</i>
Fresh	0.78 ± 0.00	0.78 ± 0.00	12.50 ± 0.00	6.25 ± 0.00
Sun-dried	0.52 ± 0.20	0.39 ± 0.00	6.25 ± 0.00	3.13 ± 0.00
Dry-salted	1.56 ± 0.00	0.78 ± 0.00	8.33 ± 3.60	6.25 ± 0.00
Lactic acid fermented	0.91 ± 0.60	1.56 ± 0.00	12.50 ± 0.00	2.08 ± 0.90
Yeast fermented	1.30 ± 0.50	1.56 ± 0.00	6.25 ± 0.00	3.13 ± 0.00
Chloramphenicol (control)	0.39 ± 0.00	0.20 ± 0.00	0.39 ± 0.00	0.20 ± 0.0

Values are means ± standard deviation of three replicates (n = 3).

tent with Riol et al. (2018), who reported that vitamin A in milk inhibited Enteropathogenic *Bacillus cereus*. Dry salting, on the other hand, preserved the antibacterial property of wax apple against *B. cereus*, as no significant difference between the fresh fruit sample and the salted wax apples. *Bacillus* species can produce endospores in order to survive extreme environments (Ahmed et al., 2024) created by the phenolic compounds, which make them difficult to inhibit bacterial growth.

Acerola

Table 4 shows the minimum inhibitory concentration of fresh and preserved acerola needed to inhibit the growth of *B. cereus*, which ranges from 3.13 mg/ml to 6.250 mg/ml. Yeast-fermented and dry-salted acerola samples had better antibacterial properties against *E. coli* as compared to the fresh fruit samples, while lactic fermented samples preserved the antibacterial property of acerola. The sun-dried wax apple extract showed the strongest inhibition against *E. coli*, likely due to its elevated vitamin A and C content. Yeast and lactic fermented and sun-dried acerola showed the highest inhibitory effects at the lowest concentration (MIC: 3.125, 3.125, 2.084 mg/ml), respectively, with *B. cereus*, while dry-salted acerola preserved the antibacterial property of the fruit (MIC: 6.25 mg/ml). The acerola extract showed stronger antimicrobial activity against *B. cereus* than *E. coli*, likely due to the outer membrane of Gram-negative bacteria, which contains lipopolysaccharides that limit the penetration of antimicrobial compounds (Valle et al., 2015; Das and Goswami, 2019).

CONCLUSION

The selected preservation methods not only conserved but also distinctly positively affected the nutraceutical quality and bioactivity of wax apples and acerola. Sun-drying and dry-salting were the most effective techniques for preserving the nutritional quality of wax apples, while fermentation with starter cultures significantly enhanced their bioactivity. For acerola, dry-salting proved to be the most suitable preservation method.

The variation in results observed between acerola and wax apples points out the importance of factors such as fruit type, duration of preservation treatment, environmental conditions, and the inherent natural microflora in determining the effectiveness of preservation methods. Acerola, in particular, has recently attracted significant attention from researchers and the food industry; however, many of its potential health benefits remain to be fully explored. This study is the first report to examine the impact of preservation methods on the nutraceutical properties of both wax apple and acerola, thereby paving the way for more advanced investigations into their potential as functional foods.

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