

Research Article

Influence of Mid-Infrared Irradiation on Amla's (*Phyllanthus emblica*) Physicochemical Properties and Acceptability Index

Umakanthan^{1,*} , Madhu Mathi² , Umadevi³ , Sivaramakrishnan⁴

¹Veterinary Hospital, Gokulam Annadhanam Temple Complex, Theni, Tamil Nadu, India

²Veterinary Hospital, Vadakupudhu Palayam, Erode, Tamil Nadu, India

³Department of Botany, The Standard Fireworks Rajaratnam College for Women, Sivakasi, Tamil Nadu, India

⁴Veterinary Dispensary, Sivagangai, Tamil Nadu, India

Abstract

Amla (*Phyllanthus emblica*), commonly known as Indian gooseberry, is highly esteemed for its nutritional and medicinal properties. It possesses a rich abundance of bioactive compounds and exhibits a wide range of health benefits, including anti-hyperlipidemic, antidiabetic, anticancerous, anti-inflammatory, hepatoprotective, and neuroprotective effects. Amla lends itself to the production of diverse value-added goods such as powder, candy, juice, soup, and oil, offering a convenient and nutritious means of incorporating this fruit into one's daily regimen. Earlier studies suggest that irradiation can have both positive and negative effects on amla, depending on the specific parameters and doses used. Therefore, the primary aim of the present study was to positively enhance the inherent characteristics of amla by employing the safe and effective 2-6 μm mid-infrared rays, thereby further augmenting its value. Our research utilized a water-based atomizer known as the 2-6 μm mid-infrared radiation generating atomizer (MIRGA), which was recently developed by us. A panel of sensory experts conducted a thorough assessment of amla both before and after the application of mid-IR radiation. In addition, various analytical techniques, including FTIR, PXRD, TEM, and H1NMR, were employed to further characterize the irradiated amla. The results demonstrated that the application of mid-IR radiation positively influenced the sensory attributes of amla, enhancing its palatability. Furthermore, the findings revealed significant transformations at the atomic, bond, and compound levels. Hence, it can be inferred that the utilization of mid-IR radiation through an economical, easily accessible, and safe technology holds immense potential for elevating the quality of amla.

Keywords

2-6 μm Mid-IR, Amla, Sensory Attribute, Acceptability, Enhancement, Safe, Economy

*Corresponding author: rkbuma@gmail.com (Umakanthan)

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1. Introduction

Amla (*Phyllanthus emblica*) has multiple health benefits. Besides cooking it is also widely used in the treatment of colds, fevers, dyspepsia, diabetes mellitus, diuresis, and purgation cancers, etc. [1]. Several research studies have proven Amla to have hypoglycemic, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, and antioxidant properties in animal and human studies [2-4]. Amla's health benefits could be related to its high nutritional content. High levels of vitamin C, tannins, polyphenols, fibers, minerals, proteins, and amino acids are naturally found in fresh amla fruit [5].

Even though amla has many health benefits, the fruit has low acceptability for direct consumption. The reason is low palatability that stems from its high acidity and astringent taste. Thus, Amla is mainly consumed after processing, and it is easy to find Amla extracts being used in various food and beverage preparations [6]. However, fruit processing results in alterations in the bioactive molecules that could be responsible for some of the studied health benefits. It was found a loss of total phenolic contents and free radical scavenging activity after spray drying Amla fruit with maltodextrin [7]. It has also been found losses in ascorbic acid during Amla cooking [8]. Therefore, minimal processing of the Amla fruit would be preferable since it could benefit the acceptability of keeping its high nutritional content.

Infrared has been gaining popularity in recent years because of its thermal efficiency when compared to traditional technologies. Infrared transfers energy in the form of electromagnetic waves that are absorbed by several molecules and macromolecules present in food. However, there is still a lack of knowledge on the effects of mid-IR on fruit. Specifically, there is no work reporting the effects of mid-IR on fresh and powdered fruit sensorial aspects of the fruit. Thus, the objective of our work was to increase the sensorial acceptability of the fresh and powdered Amla fruit using our MIRGA technology.

2. Materials and Methods

2.1. Preparation of Samples

Hand-picked amla was bought at a local market. The fruits were cleaned and sanitized using an aqueous solution of sodium hypochlorite with a concentration of 200mg L⁻¹ of free chloride for 15 minutes and then rinsed with running water.

2.2. MIRGA Apparatus

MIRGA (patent no. 401387) – details in Supplementary file) is a 20 ml capacity polypropylene plastic atomizer containing an inorganic (molar mass 118.44 gm/mole) water-based solution (having approximately two sextillion cations and three sextillion anions). Dimensions of the unit are

86 x 55 x 11 mm, orifice diameter 0.375 mm, ejection volume 0.062 ± 0.005 ml, ejection time 0.2 sec, and average pressure 3900 pascal and cone liquid back pressure 2000 N/m². During spraying, approximately 1 µg weight of water as the mist is lost and the non-volatile material in the sprayed liquid is 153 mg/ml. Every time spraying emits 0.06ml which contains approximately seven quintillion cations and eleven quintillion anions. Depending on the pressure (varies with the user) applied to the plunger, every spraying is designed to generate 2-6 µm which was estimated by FTIR (retro-reflector) interferometer instrument (Detector type D* [cm HZ1/2 - 1] MCT [2-TE cooled]) at Lightwind, Petaluma, California. [9-11]

The spraying was carried out at a distance of 0.25 - 0.5 m toward packaged amla fruit, close spraying does not generate mid-IR.

2.3. Mid-IR Spraying of Fresh Amla

Amla fruit (200 g) was weighed and packed in polyethylene bags of different thicknesses (51-70 microns). Such fifty bags were prepared, and vacuum sealed (Trial). Additionally, ten packets were kept as controls. Each trial bag was sprayed from a distance of 0.25–0.50 meters. Twenty trial bags received one spraying, and another 20 trial bags received two times MIRGA sprayings. A subset of control and trial packets were kept at room temperature (approximately 32 °C; 10 one sprayed and 10 two sprayed + 5 control 25 bags). And another subset was kept in a refrigerator (4° C) (10 one sprayed and 10 two sprayed + 5 control = 25 bags). After each sensory trial, the samples were kept at room temperature or returned to the refrigerator.

The trial packets at room temperature were subjected to sensory expert panel (n=6) tests daily and those in refrigeration on alternative days. Expert panel members used a 9-point nominal structure as the hedonic scale: 1 - Dislike extremely, 2 - Dislike very much, 3 - Dislike moderately, 4 - Dislike slightly, 5 - Neither like nor dislike, 6 - Like slightly, 7 - Like moderately, 8 - Like very much, 9 - Like extremely [9-11]. For accuracy, the trials were repeated six times.

2.4. Mid-IR Spraying of Powdered Amla

Samples of commercial Amla fruit powders packed in polythene (70 microns) were taken, and sensory tests were performed (non-sprayed control). Then, MIRGA sprayings were externally applied. After every spraying, Amla powder was taken and subjected to sensory evaluation. The spraying continued until the powder became unpalatable. The reason is that input of extra energy (mid-IR) should denature the inherency of amla. The trials were repeated six times for accuracy.

2.5. Microbiological Assessment of the Fresh Amla Fruit

The microbiological assessment was performed to evaluate the presence/absence of total coliforms (35 °C) (method 2000.15), fecal coliforms (45 °C) (method 983.25), and *Salmonella* sp. (method 967.6).

2.6. Sensorial Analysis

The sensorial analysis was applied with 6 judges who were trained and potential consumers of the product. The judges were aware of the research goals, according to the Freely Given and Informed Consent Terms. The acceptance test was carried out using the 9-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely) for the sourness and bitterness. The Amla fruit samples were coded with random three-digit numbers.

2.7. Instrumentation analysis

2.7.1. High-Performance Liquid Chromatography

Instrument utilized: Agilent 1200 series; Reversed Phase-HPLC (RP-HPLC) with C₁₈ column. Standard used: 0.2 to 1 mg/ml of 100% pure Ascorbic acid. Protocol followed: 1 g Amla Powder sample in 25 ml of acidified water (pH=2). After dissolving powder, the solution was centrifuged and filtered through a 0.22-micron filter before HPLC analysis. Method: 90% 30mM O-phosphoric acid and 10% ACN with a flow rate of 0.5 ml/min and detection at 260 nm.

2.7.2. Gas Chromatography-Mass Spectrometry

Agilent 7890A with MS detector. Method: NERL method for FAME detection. Oven temperature: 100 °C for 1 min, 25 °C/min up to 200 °C and hold for 1 min, 5 °C/min up to 250 °C and hold for 7 min (23 min total). 1-μL injection at 10:1 split ratio, inlet temperature of 250 °C. Constant flow: 1 mL/min helium. FID: 280 °C, 450 mL/min zero air, 40 mL/min H₂, 30 mL/min helium. Processing of Samples: 100 mg sample dissolved in 5ml of HPLC. Method: Added 1 ml of BF₃-METHANOL. Heated at 80-90 °C for 2 min. Cooled it and added 5 ml of n-Hexane (HPLC grade) and water. Taken an n-hexane layer and washed with water two times. The n-hexane layer and filtered with a 0.22-micron filter and analyzed in a GC-MS instrument after suitable dilution.

2.7.3. Fourier Transform Infra-Red

FTIR analyses were carried out in the range 4000 - 600 cm⁻¹ with a resolution of 4000–400 cm⁻¹ at 4 cm⁻¹ and 32 scans in transmittance mode using an infrared spectrophotometer (Thermo Avtar 370 FTIR spectrometer).

A small quantity of the sample is added to KBr in the ratio of 1:100 approximately. The matrix is grind for 3-4 minutes

using mortar and pestle. The fine powder is transferred into a 13 mm diameter die and made into a pellet using a hydraulic press by applying a pressure of 7 tonnes. The fine pellet is subjected to FTIR analysis using a universal pellet holder (a single drop of oil is poured on the KBr pellet in the case of liquid samples). Infrared spectral data were collected.

2.7.4. Powdered X-ray Diffraction

The sample is smeared over a low background sample holder (amorphous silica holder) and fixed on the sample stage in a goniometer. The instrument is set with B-B geometry. The current and voltage is set to 40 mV and 35 mA and data has been collected. Instrument make: Bruker Model D8 Advance Goniometer: theta/2 theta.

2.7.5. Transmission Electron Microscopy

Transmission electron microscopy (TEM) observations were made with an FEI Technai Spirit G2, HT 120KV, Electron source LaB₆, Netherlands, with an accelerating voltage of 100kV. A droplet of a dilute suspension (0.02 wt%) of chitin nanowhiskers was deposited onto a carbon-coated grid and allowed to dry. No stain agent was used.

2.7.6. H¹ Nuclear Magnetic Resonance

The H¹NMR experiments were performed on a 600 MHz NMR spectrometer (ECZR Series, JEOL, JAPAN) using a 3.2mm CPMAS probe at 150MHz frequency. All the samples were run at 18 KHz spinning speed at Room Temp and with a delay of 5sec.

3. Result

3.1. Microbiological Assessment Revealed no Pathogens

Sensorial Analysis of Fresh and Powdered Amla

Table 1 presents the results of the sensorial analysis performed on fresh and powdered Amla fruit. The sensory attribute changes were perceived in 1-5 minutes after spraying. Considering the fresh Amla, an increase in both sourness and bitterness points was found after the first spraying irradiating mid-IR. Then, sourness rises to 8 points, while bitterness stays the same after the second irradiation. These increasing points indicate that Amla is less sour and less bitter. Thus, the result reveals that the fresh Amla fruits have an increase in palatability only after one irradiation. Considering the powdered samples, it is possible to observe from Table 1, that a similar increase in palatability was found after two irradiations. However, subsequent irradiation led to a decrease in both component points. A value of 3 points was found for sourness, while 2 points were found for bitterness after 6 irradiations. This result indicates that samples irradiated 6 times had a pungent taste and loss of palatability. Comparing the fresh and

powdered samples, it is observable that samples have similar moisture contents. susceptibility to mid-IR even though they have different

Table 1. Sensory profiling of fresh amla and amla powder samples.

| Number of sprayings | Fresh Amla | | Powdered Amla | |
|---------------------|------------|------------|---------------|------------|
| | Sourness | Bitterness | Sourness | Bitterness |
| Control | 5 | 5 | 5 | 5 |
| 1 | 6 | 6 | 6 | 6 |
| 2 | 8 | 6 | 8 | 7 |
| 3 | - | - | 6 | 6 |
| 4 | - | - | 5 | 5 |
| 5 | - | - | 3 | 3 |
| 6 | - | - | 3 | 2 |

3.2. Instrumentation Results

3.2.1. High-Performance Liquid Chromatography Analysis

Table 2. Vitamin C analysis in amla powder samples from HPLC spectra.

| Sample | AUC | Ascorbic acid conc. (mg/ml) | Ascorbic acid (mg/100g of amla powder) |
|-----------|----------|-----------------------------|--|
| Control | 17799.32 | 0.24427665 | 610 |
| 2 sprayed | 35187.14 | 0.53245504 | 1330 |
| 6 sprayed | 39940.94 | 0.61124252 | 1527 |

Table 2 presents Vitamin C analysis in amla powder samples from HPLC spectra, control sample has 610 mg of ascorbic acid per 100 grams which lies well within range. In 2 sprayed samples, the ascorbic acid concentration was nearly

doubled reaching 1330 mg per gram of sample. in 6 sprayed samples, the ascorbic acid concentration was 1527 mg per 100 grams. The results show promising improvement in ascorbic acid concentration after MIRGA sprayings.

3.2.2. Gas Chromatography-Mass Spectrometry Analysis (Figure 1)

Table 3. GC-MS analysis of amla powder samples.

| RT (Min) | Name of compound | % area present in each sample | | | Remarks |
|----------|--|-------------------------------|-----------|-----------|-------------------------------|
| | | Control | 2 sprayed | 6 sprayed | |
| 23.726 | 2-tert-butyl-5-methylarsole | 0.0 | 10.64 | 10.99 | Peaks in only sprayed samples |
| 29.206 | 2,6 Dimethyl -9-1 (methyl)-1-cyclodecanone | 2.24 | 0.0 | 0.0 | Unique peak in control |
| 29.319 | N-Hexadecanoic acid | 2.70 | 7.51 | 5.60 | |

| RT (Min) | Name of compound | % area present in each sample | | | Remarks |
|----------|--|-------------------------------|-----------|-----------|--|
| | | Control | 2 sprayed | 6 sprayed | |
| 29.778 | Ethylene Brassylate | 5.28 | 2.94 | 1.78 | |
| 30.741 | Oleic acid | 0.0 | 14.01 | 12.25 | Peaks in only sprayed samples |
| 30.750 | Octadecanoic acid (Stearic acid) | 18.33 | 2.86 | 2.31 | |
| 31.859 | Palmitic acid | 0.0 | 2.03 | 0.0 | Unique peak in 2 sprayed samples |
| 31.861 | Octadecanal-2-bromo | 0.0 | 0.0 | 1.25 | Unique peak in 6 sprayed sample |
| 33.291 | 2-Myristrynoyl-glycinamide | 5.59 | 0.0 | 0.0 | Unique peak in control |
| 33.343 | Olein, 2-Mono | 0.0 | 8.19 | 0.0 | Unique peak in 2 sprayed samples |
| 33.354 | Cis- Cis- Cis- 7,10,13, Hexadecatriene | 0.0 | 0.0 | 6.86 | Unique peak in 6 sprayed sample |
| 33.508 | 13-Docosenoic acid, methyl esters (2) | 2.95 | 0.0 | 0.0 | Unique peak in control |
| 39.45 | Nonacosane | 62.89 | 51.80 | 58.93 | The common and most abundant peak in all the samples |

Table 3 represents GC-MS analysis of amla powder samples, control sample contains Nonacosane, Stearic acid, 2-Myristrynoyl-glycinamide, etc. However, several of the peaks from the control sample disappeared after MIRGA spraying. 2 sprayed sample has unique peaks of Palmitic acid, Olein, 2-Mono, Oleic acid, and 2-tert-butyl-5-methylarisole. There was a sharp decrease in the peak of Nonacosane and an

increase in oleic acid. Thus 2 sprayed amla powder samples were found to be very soft and dissolved in the mouth quickly. 6 sprayed samples showed all unique peaks such as Octadecanal-2-bromo, Cis- Cis- Cis- 7,10,13, Hexadecatriene which is responsible for pungent taste, with reduction in sourness and bitterness.

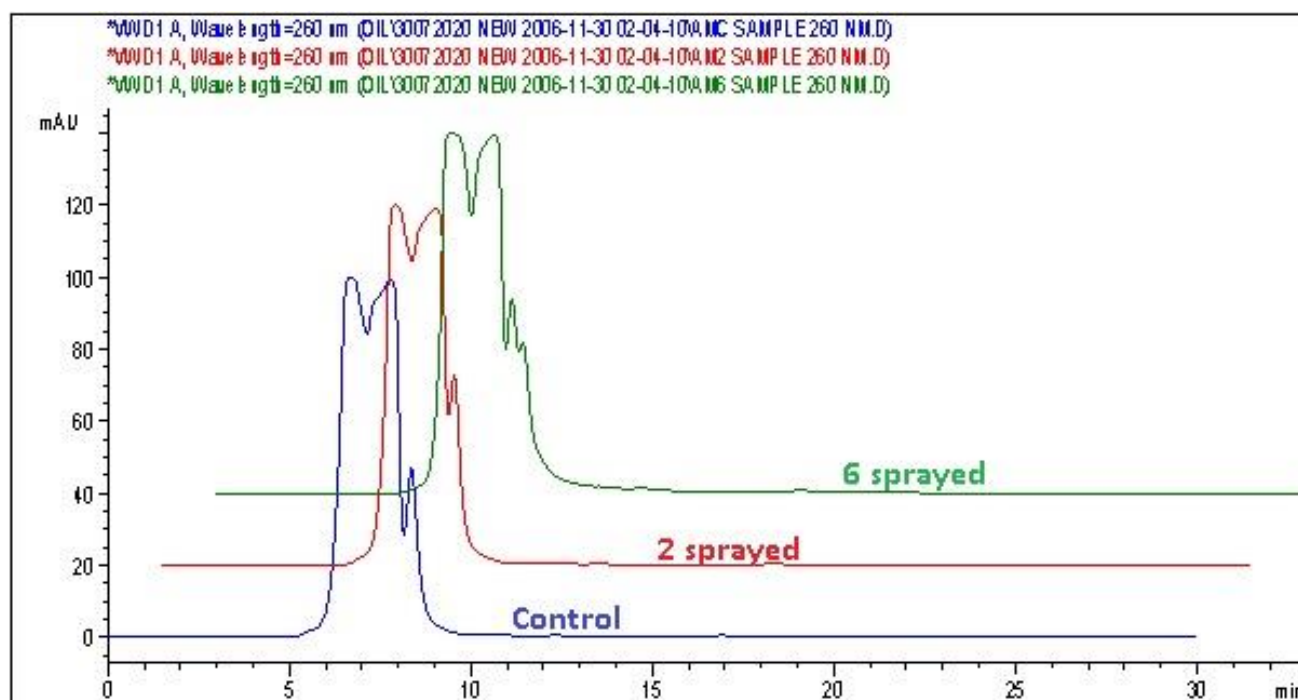


Figure 1. GC-MS of Amla powder samples.

3.2.3. Fourier Transform Infra-Red Spectroscopy Analysis (Figure 2)

All transmission peaks are the same between the sprayed samples. However, differences in intensity can be found when samples are compared. The transmission band around 3400cm^{-1} is attributed to the hydroxyl groups (-OH) present in cellulose [12]. The increase in intensity could be related to higher moisture content after two irradiations. After 6 irradiations, the (OH-) transmittance is the same, indicating that both samples have similar hydroxyl group content.

The transmittance around 1730cm^{-1} is attributed to pectins

with ester, the 1631cm^{-1} to free carboxyl groups, the 1230cm^{-1} to proteins, and 1015cm^{-1} to polysaccharides, sugars, and pectins [13].

2 sprayed sample: Bigger absorbance than control. Peaks 1631cm^{-1} and 618cm^{-1} are lower caused by protein reduction. The effect of applied mid-IR caused an increase in temperature enough to precipitate protein (denaturation) leading to reduction.

6 sprayed sample: A little bigger absorbance than 2 sprayed, peaks 1631cm^{-1} and 618cm^{-1} are lower might have been caused by protein reduction. The concentration of carbohydrate and carboxylic acid increased.

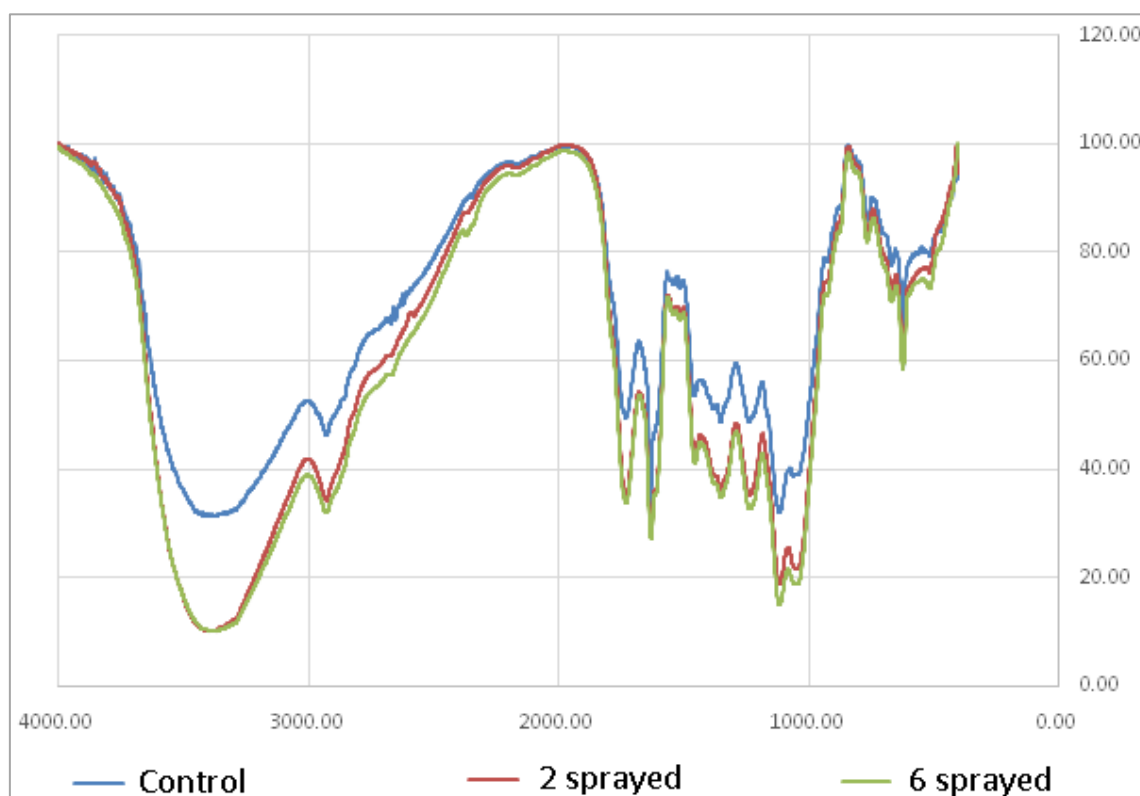


Figure 2. FTIR of Amla powder sample.

3.2.4. X-ray Crystallography Analysis

Figure 3 presents the diffractogram of the three powdered Amla samples. A broad peak between $\theta = 10^\circ$ and 30° centered on 21.5° is observed for the control sample. Two narrow and intense peaks are observed at $2\theta = 19.7^\circ$ and 30.9° , suggesting the presence of crystalline phases within Amla. Smaller peaks are discerned at $2\theta = 14.9^\circ$, 26.9° , and 37.8° . Samples sprayed 2 times presented less intensity at peak 21.5° ; but a higher intensity at 26.9° . The narrow, intense peak at 30.9° almost disappeared after the first two irradiations. However, this peak alteration did not result in a broader peak or an increase in the amorphous region of the sample.

Samples sprayed 6 times presented a much broader region

around the peak 21.5° ; indicating that the sample presents a more amorphous character. The crystalline peak around 14.9° has disappeared, but crystalline peaks at 19.7° , 26.9° , and 30.9° are present.

Comparing the effect of spraying repetition on the crystallographic nature of the samples, it is observed that all three samples show one intense, narrow peak followed by a broad peak between 2θ values 10° and 30° . The scattering in crystallographic planes corresponding to 21.5° (broad peak) and 19.6° (narrow peak) shows that all three samples maintain slightly similar crystalline structures. The broad peak can be attributed to the presence of semi-crystalline polysaccharides such as cellulose [14], while the crystalline peaks are attributed to specific crystalline molecules present in the pow-

der. These specific molecules have become crystallized after irradiation.

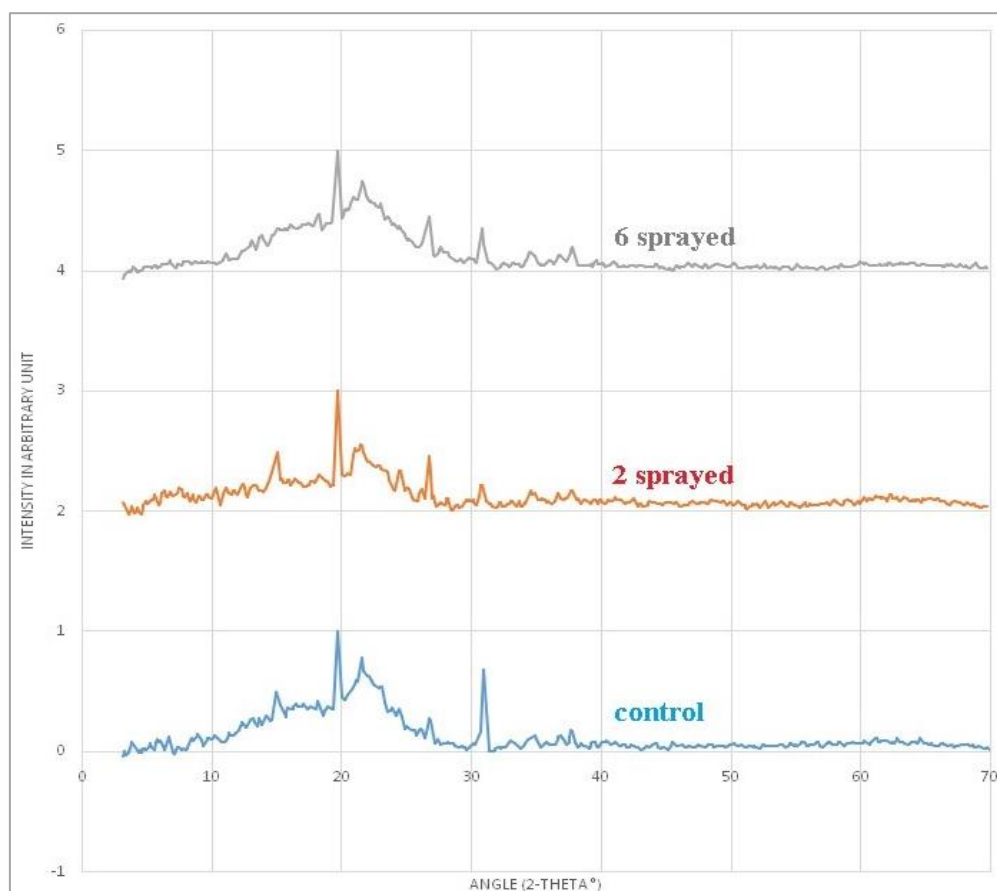


Figure 3. PXRD of the amla powder sample.

The reduction of overlapping peaks and width in the XRD of 2 sprayed samples allows more peaks to be seen at higher 2θ values. This result indicates that samples sprayed 2 times are more crystalline, due to some transition from an amorphous into a crystalline structure due to increased water activity.

Samples sprayed 6 times maintained a similar peak width to the control samples but presented more peaks at higher 2θ values. The peak at 14.9° increased in intensity from control to 2 sprayed but is absent in 6 sprayed showing the formation of new minority phases with the increase in spraying.

3.2.5. Transmission Electron Microscopy Analysis

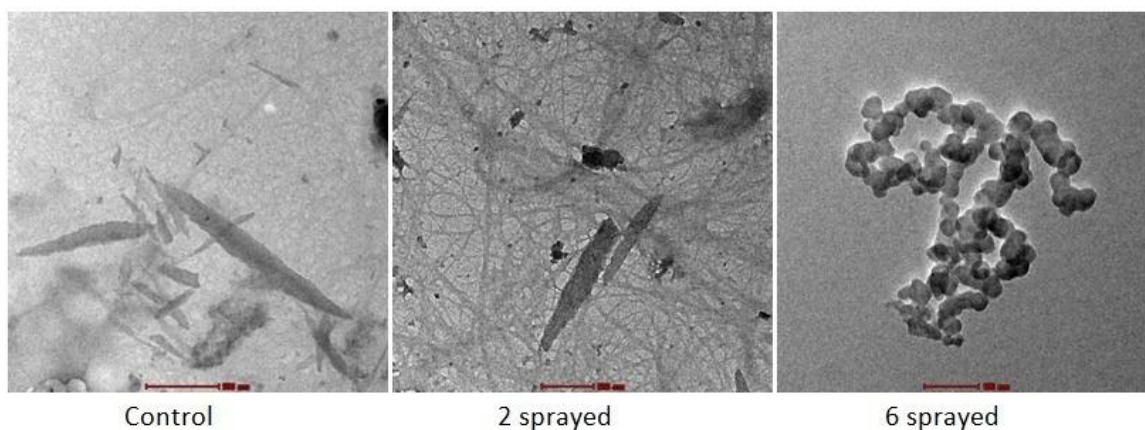


Figure 4. TEM of Amla powder.

Figure 4 presents the TEM micrographs of the irradiated samples. It is possible to observe that samples have become different after irradiation. The TEM of control samples presented elongated whisker structures (700 x100 microns) while the samples irradiated 2 times also presented whisker structures (600 x100 microns) but smaller. In both cases, the structures mentioned are independent and have no specific organization and orientation. The morphology of the samples became completely altered after six irradiations and presented much smaller spherical structures (diameter of about 25 mi-

crons) and an agglomerated arrangement.

3.2.6. ¹H-NMR

Figure 5 presents the magnetic resonance of Amla fruit samples. The 2 sprayings lead to a reduction of sourness and bitterness caused by a diminution of the concentration of ascorbic acid and polyphenolic compounds. In the spectrum, it is difficult to evaluate the variations in the concentration of ascorbic acid due to the overlap with other intense signals.

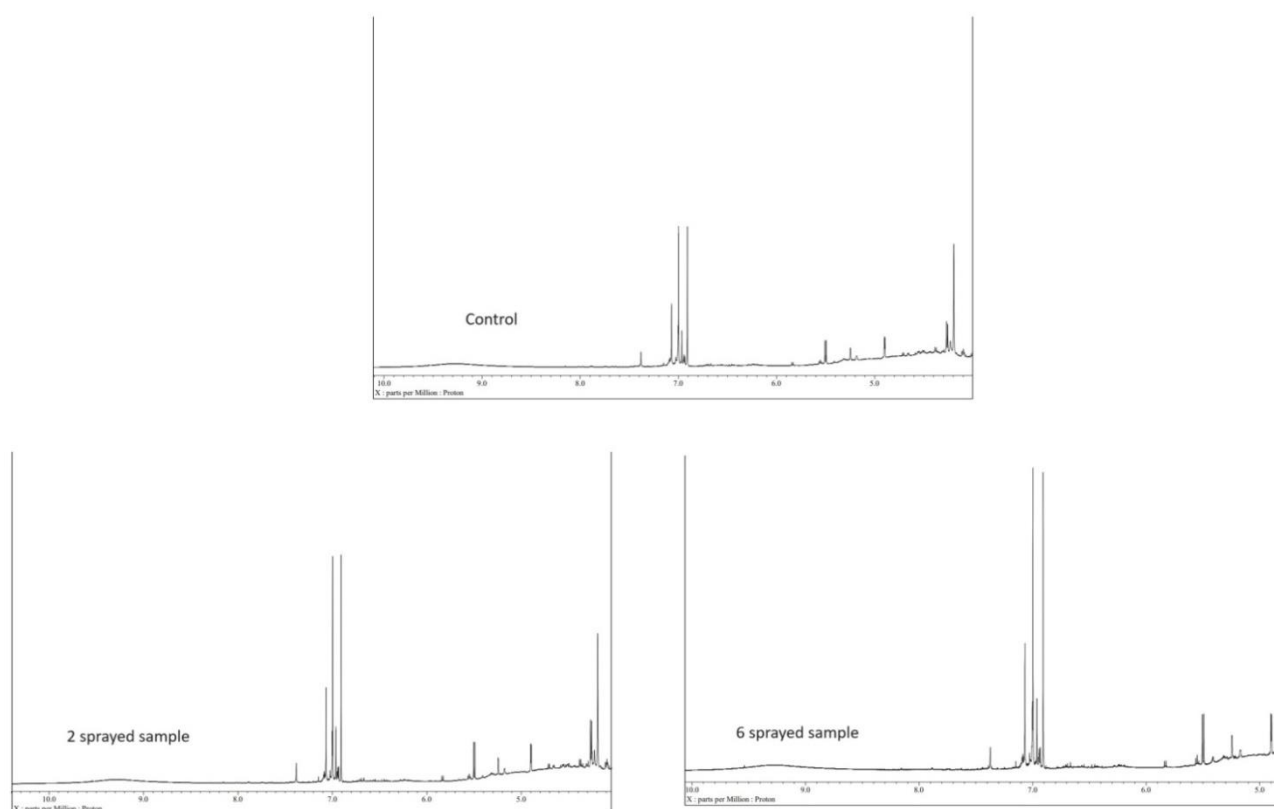


Figure 5. ¹H-NMR of Amla powder samples.

6 spraying leads to a similar effect on the sample, but the loss of spraying and bitterness is more pronounced. The interpretation of these observations not only considers the total concentration of the compounds involved in sourness and bitterness, but also looks at the total balance between substances contributing to bitterness, sourness, and sweetness (since the last ones could counteract the effect of the first ones).

The above trials and instrumentations validated the alterations (compared to non-sprayed control) in viz., chemical bond, chemical compounds, configuration, and sensory attributes in Amla caused by MIRGA spraying.

4. Discussion

The action of MIRGA emitted 2-6 μ m mid-IR on amla

While spraying MIRGA, most of the mid-IR energy scatters through the air and gets absorbed by receptors (Amla) molecules. Virtually all organic compounds absorb mid-IR radiation which causes a change in the molecule's vibrational state to move from the lower ground state to the excited higher energy state [15]. This leads to changes in chemical bonds [16] and these bond parameter changes lead to consequent changes in the receptor's physical and chemical characters, configuration, compound transformation depending on the dose of energy applied [17-20]. Nanostructured water layers can be triggered upon application of mid-IR radiation since water molecules absorb in this region [21, 22].

The 2-6 μ m mid-IR is non-ionizing, biologically safe, harmless, and capable of penetrating obscurants [23, 24], coincides with most of the biomolecules causing photostim-

ulation and photobiomodulation [25, 26], leading to chemical bond changes [16] and physicochemical alterations [17, 20]. This stereochemical configuration alteration influenced the amla's enhanced sensory attributes [27].

Though amla is nutritious, it is less palatable due to strong sourness and astringency [28] besides the short shelf-life [29] which can be extended by drying [30] nutritional value decrease and limited availability of sunlight in some countries. Other methods of amla processing are blanching, and pre-treatment with chemical addition. These methods have certain advantages but the occurrence of color and nutrition leaching and quality loss is inevitable [31, 32]. 1% NaCl pre-treated and 8 hrs 55°C drying of grated amla nearly overcame the said disadvantages [33]. This method does not apply to fresh amla fruit or marketed amla powder (with or without pretreatment). However, MIRGA technology is more advantageous, economical, rapid in action, and needs no skilled person, hence used at all levels.

The inorganic substances that are utilized to create MIR offer a potential avenue for biomedical uses [34, 35]. Additionally, it is a novel synthesis technique for producing functional material (2-6 μm mid-IR) [36, 37]. It is well known that the combination of different compounds with excellent electronic properties results in new composite materials, which have sparked significant technological interest in recent years [38, 39].

One of the advantages of this technology is depending on the number of MIRGA spraying a receptor's chemical bond configurations and subsequent physical and chemical characters can be altered to our desire [9-11].

5. Conclusion

The effects of mid-IR irradiation on Amla fruit were successfully studied. Amla fruit, both in the fresh and powdered form, presented increased acceptability when irradiated with mid-IR. The increase in acceptability results from several physicochemical alterations on the fruit. Therefore, it is concluded that MIRGA technology can be useful for the minimal processing of fruits.

Abbreviations

MIRGA: Mid-IR Generating Atomizer
GCMS: Gas Chromatography-Mass Spectrometry
FTIR: Fourier Transform Infra-Red
PXRD: Powdered X-Ray Diffraction
TEM: Transmission Electron Microscopy
NMR: Nuclear Magnetic Resonance

Supplementary Material

https://docs.google.com/document/d/1ugHu8azhlUyWrW_6knHElxqc0QFw75e0/edit?usp=sharing&oid=111101387151809704391&rtpof=true&sd=true

Author Contributions

Umakanthan: Conceptualization, Methodology, Project administration,

Madhu Mathi: Data curation, Investigation, Visualization, Writing - Original draft preparation.

Umadevi and Sivaramakrishnan: Resources, Supervision, Validation, Writing- Reviewing and Editing.

Data Availability Statement

All data is available in the manuscript and supplementary materials.

Conflicts of Interest

The authors declare no conflicts of interest.

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