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Thin-Layer Chromatography Profiles of Non-Commercial Turmeric (*Curcuma longa* L.) Products Obtained via Partial Hydrothermal Hydrolysis

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Abstract This study verifies an alternative use of waste turmeric from extraction processes via partial-hydrothermal hydrolysis, which provides partial hydrolyzed-deflavored and depigmented turmeric (PHDDT) and turmeric hydrolysates (TH). Thin-layer chromatography was used to fingerprint and evaluate the chemical compositions of these products in terms of phenolics, antioxidants, volatiles and sugars, which verified the possibility of adding value to these waste products. Reaction temperature variations influenced the quality of the analyzed compounds. Based on a qualitative approach, PHDDT provided a considerable source of bioactive compounds, while TH samples were determined to be a source of low-chain sugars. The production of various sugars and bioactive compounds from non-commercial turmeric represents a promising alternative for future methodologies that can diminish the quantity of agro-waste and improve economic profitability.

Keywords Turmeric, Partial Hydrolysis, Thin-Layer Chromatography, NP, DPPH, Vanillin, Alpha-naphthol

1. Introduction

Turmeric (*Curcuma longa L.*) is an important medicinal plant and source of phenolic compounds, volatile oil, sugars, proteins and resins [1, 2]. The pigments in the colorant extracts obtained from turmeric are collectively known as curcuminoids, which are phenolic compounds. The major constituent is curcumin (60-80%) in addition to small amounts of demethoxycurcumin (15-30%) and bisdemethoxycurcumin (2-6%) [3].

Curcumin is widely used for foods and dyes, and possesses numerous biological benefits, including antioxidant, anti-inflammatory, antimicrobial, antiparasitic, antimutagenic, anticancer and antivirus properties [4].

Brazil possesses favorable turmeric cultivation conditions. The dry basis curcuminoid pigment levels in Brazilian turmeric range from 1.4 to 6.1 g/100 g, while the volatile oil fraction varies between 1.0 and 7.6 ml/100 g [5].

Several techniques have been used to qualitatively and quantitatively analyze turmeric species and byproducts. High-performance liquid chromatography (HPLC) was used to detect curcuminoid in extracts obtained via pressurized liquid extraction (PLE) [6]. The extracts were then used to standardize a beauty cream formulated with turmeric powder [7]. The curcumin contents of various *curcuma* varieties collected from different regions of India have been analyzed [2]. Random Amplified Polymorphic DNA was used to detect adulterants in the *Curcuma zeodoaria* and *Curcuma malabarica* species [1]. Thin Layer Chromatography (TLC) was used to identify curcumin in *curcuma* varieties [8], and turmeric extracts were obtained by supercritical fluid extraction (SFE), low-pressure solvent extraction (LPSE) and hydrodistillation [9].

TLC is one of the easiest and most versatile methods for identifying and separating compounds due to its low cost, simplicity, short development time, high sensitivity and good reproducibility [10].

TLC simultaneously separates substances in space. The R_F (retardation factor, ratio of fronts or retention index) value is the standard measure of retention. Given the general difficulty of controlling absolute R_F values, it is common to separate standards and samples in the same system for identification purposes [11].

SFE has been used to extract volatile oils [12], and turmeric curcuminoids have been obtained using pressurized liquid extraction (PLE) with ethanol [6]. This study uses partial-hydrothermal hydrolysis to obtain new products from deflavored and depigmented turmeric (DDT) using pressurized water; DDT is the residue of the SFE and PLE processes. A partial-hydrothermal hydrolysis was performed to generate two main products: the partial-hydrolyzed deflavored and depigmented turmeric (PHDDT), which is a mixed biopolymer, and turmeric hydrolysate (TH), which is the liquid fraction source of low-chain sugars.

Few studies exist regarding the potential use of turmeric

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rhizomes after the deodorization and depigmenting processes. In most of cases, the material is not reused, increasing the amount of environmental waste [13]. Processes are needed that can generate added-value products from food waste, which can be used for feed, cosmetics, energy and pharmaceuticals.

This study qualitatively determines the chemical composition of PHDDT and TH obtained via the partial-hydrothermal hydrolysis of DDT using TLC.

Standards were used to assist in the identification of compounds present in the extracts when known compounds were present in the mixture, including phenolics, antioxidants, volatile oil compounds and sugars. The theoretically known compounds presented in the samples were identified via R_F calculations of the separated zones. The resulting R_F values of the samples were compared to the R_F values of the standards.

2. Materials and Methods

2.1. Material

Deflavored and depigmented turmeric (DDT) was obtained from the crude raw material purchased from the Oficina de Ervas Farmácia de Manipulação Ltda (lot 065DM, Ribeirão Preto, Brazil), from which volatile oil was removed using supercritical CO_2 at 60°C and 250bar, while curcuminoids were removed using pressurized ethanol at 60°C and 100bar [6].

2.2. Experimental Methods

Partial hydrolysis procedures were performed in a home-made PLE apparatus, shown in Figure 1. Three temperature levels (40, 70 and 100°C) and seven pressure levels (10, 20, 30, 40, 50, 60 and 70bar) were used.

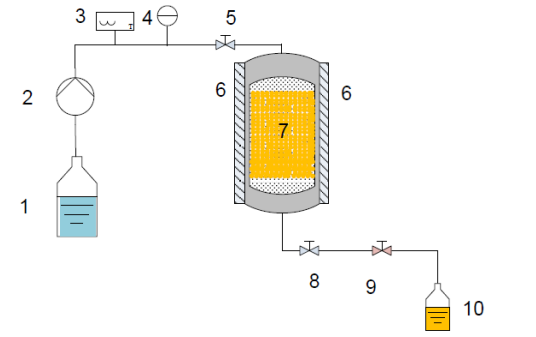

Figure 1. Home-made PLE system

Milli-Q water (EMD Millipore Corporation, Merck, Darmstadt, Germany) was used as a solvent, which was pumped into the extraction cell using an HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, Fremoni, USA). The extraction cell was placed in an electrical heating jacket at the desired temperature until the required pressure was obtained. Stainless steel tubes were used for all connections in the system.

Approximately 3.3 g of DDT was placed in a 5 ml extraction cell (MV-10 ASFE, Waters, Milford, USA) containing a sintered metal filter at the top and bottom.

The cell containing the sample was heated for 5 min to ensure that the extraction cell would remain at the desired temperature. Then, the first blocking valve was opened to pressurize the cell with solvent until the desired pressure was reached for 20 minutes (static time).

The second blocking valve and the back pressure valve were carefully opened, keeping the pressure at an appropriate level for the desired flow (1 ml/min). The extraction cell was rinsed with fresh extraction solvent for approximately 15 min (dynamic time, i.e., the period of time that the TH samples are collected).

After the experiments, the partial-hydrolyzed deflavored and depigmented turmeric (PHDDT) and turmeric hydrolysates (TH) were maintained in the dark at -18 °C until further analysis.

2.3. Thin Layer Chromatography

Silica gel plates with aluminum backs were used as the stationary phase, including ultraviolet light-sensitive (UV_{254}, Alugram®, Xtra SIL G, Macherey-Nagel, Germany) and non-UV-sensitive plates (Alugram®, Xtra SIL G, Macherey-Nagel, Germany) with 10 cm × 10 cm dimensions. The TLC plates were prepared by establishing a 1 cm distance from the origin, 8 cm solvent travel distance and 1 cm distance from the solvent front.

Approximately 10 µL of the samples were spotted on the TLC plates using capillary glass tubes with an approximately 1 cm distance from each band. The plates were then developed in glass chambers via mobile phase elution.

The bands of compounds generated by the constituents that could not be detected in the visible region were visualized using a UV (Multiband UV – 254-366 nm, UVGL-58, Mineralight® Lamp, Upland, CA, EUA) equipped with a cabinet (UVP-Chromato-VUE, CC-10, Upland, CA, EUA) for short wavelength (254 nm) and long wavelength (366 nm) analyses.

2.3.1. Sample Preparation and Identification

Ethanol was used as solvent to identify phenolics, antioxidants and volatiles because bioactive turmeric compounds are insoluble in water and soluble in organic solvents, such as ethanol, ethyl acetate and acetone. The standards used to identify these PHDDT and TH compounds included curcumin, demethoxycurcumin, bisdemethoxycurcumin and turmeric volatile oil, which were obtained from the SFE process (Table 1). These standards were diluted in ethanol until a concentration of 15 mg/ml was reached. All solvents and chemicals were of analytical grade.

PHDDT samples were diluted in ethanol until the adequate concentration (30 mg/ml) was reached. TH samples were filtrated and concentrated using a freeze-dryer (L101, Liobras, São Carlos, Brazil). The lyophylizated TH samples were diluted in ethanol until a final concentration of 30 mg/ml was reached.

PHDDT dilutions were made to identify sugars using Milli-Q water (EMD Millipore Corporation, Merck, Darmstadt, Germany) at a concentration of 200 mg/ml. Liquid TH was used without any further treatment. The glucose, maltose and lactose standards (Table 1) were diluted in water until a concentration of 10 mg/ml was reached.

Table 1. Standards used in TLC

Phenolics, antioxidants and volatiles	Origin
Bisdemethoxycurcumin	Sigma-Aldrich
Curcumin	(Darmstadt, Germany)
(≥94% curcuminoids; ≥80% curcumin)	
Demethoxycurcumin	
Volatile oil	[6]
Sugars	**Origin**
D (+) lactose	Dinâmica (Diadema, Brazil)
D-(+)-glucose (minimum 99.5%)	Sigma-Aldrich
D-(+)-maltose monohydrate	(Darmstadt, Germany)
(from potato, ≥99%)	

The mobile phase used to develop phenolics, antioxidants and volatiles was composed of chloroform, ethanol and glacial acetic acid at 95:05:01 (v/v) [14]. A mobile phase that consisted of ethyl acetate, acetic acid, methanol and water at 60:15:15:10 (v/v) was used to develop the sugars [15]. Table 2 provides the reagents and their origins.

Table 2. Reagents used to formulate the mobile phases

Phenolics, antioxidants and volatiles	Origin
Chloroform	Merck (Darmstadt, Germany)
Ethanol (99.5%)	Chemco (Hortolandia, Brazil)
Glacial acetic acid	Synth (Diadema, Brazil)
Sugars	**Origin**
Acetic acid (≥99.7%)	Sigma-Aldrich (Darmstadt, Germany)
Ethanol (99.5%)	Chemco (Hortolandia, Brazil)
Ethyl acetate	Dinâmica (Diadema, Brazil),
Methanol	Dinâmica (Diadema, Brazil).

2.3.2. Detection

The phenolics were detected using an NP (2-aminoethylborinate) spray reagent, according to the Wagner and Bladt [14], which was adapted by Albuquerque et al. [16].

Antioxidant compounds were detected by spraying a DPPH solution, in which 0.5 g of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was diluted in 250 ml of methanol [16].

Volatiles were detected by spraying a sulfuric vanillin (SV) reagent, using the formulation suggested by Krishnaswamy [17], in which 0.5 g of vanillin (3-hydroxy-4-methoxybenzaldehyde) was diluted in 20 ml of ethanol and 80 ml of sulfuric acid.

Before and after spraying with NP, DPPH and SV reagents, the plates were inserted into a UV chamber to visualize the compound bands that could not readily be observed.

The sugar detection methodology of Lewis and Smith [18], which was adapted by Fried and Sherma [15], was used. A 0.1 M sodium bisulfite (Dinâmica, Diadema, Brazil) solution was used to impregnate the plates and facilitate the resolution of the samples in the mobile phase. This solution was formulated by diluting 10.4 g of sodium bisulfite in 1 liter of Milli-Q water. The TLC plates were then pre-washed in a glass chamber containing this solution, air dried and activated in an oven (Tecnal, TE-385-1, Piracicaba, São Paulo) at 100°C for 30 minutes prior to spotting.

Carbohydrates are extremely hydrophilic compounds. Therefore, they strongly attach to adsorbents, such as silica gel, alumina and cellulose. Thus, highly polar solvents were necessary in the mobile phase of TLC development [18].

The sugar mobilities on silica gel primarily depend on the molecular weights and the number of hydroxyl groups. The resolution is improved by impregnating silica gel with weak acid salts or via the use of cellulose layers [18].

A sulfuric α-naphthol (SAN) reagent was used to detect sugars. This reagent was prepared by adding 5 g of α-naphthol to 33 ml of ethanol (solution A). Then, 21 ml from solution A were combined with 81 ml of ethanol, 8 ml of water and 13 ml of sulfuric acid. After spraying, the plates were heated for approximately 5 minutes at 100°C. The visualizing reagents (spray solutions) were formulated using the reagents listed in Table 3.

Table 3. Reagents used to formulate the spray solutions

Phenolics, antioxidants and volatiles	Origin
2-aminoethyl-diphenylborinate (99.8%)	Sigma-Aldrich
2,2-diphenyl-1-picrylhydrazyl	(Darmstadt, Germany)
3-hydroxy-4-methoxybenzaldehyde	Synth (Diadema, Brazil)
Sugars	**Origin**
1-naphtol (or α-naphthol)	Dinâmica (Diadema, Brazil)
Ethanol (99.5%)	Chemco (Hortolandia, Brazil)
Sulfuric acid	Exodo Científica
	(Hortolandia, Brazil)

3. Results and Discussion

3.1. Standards Fingerprints

A sufficiently strong TLC solvent causes a sample to shift into the R_F range of 0.2–0.8. In addition, if the correct selectivity is attained, the solvent will evenly distribute the

sample components throughout this range. If the sample contains a wide range of sample sizes, then the correct mobile phase will ensure the adequate separation of the major and minor components, rather than an even distribution throughout the R_F range [19]. Table 4 lists the standards used in this study and their R_F values.

Table 4. TLC plate standards with 8 cm elution heights and R_F values

Identity	Standard	Height (cm)	R_F (cm/cm)
BH	DDT (sample before hydrolysis)	5.52 – 7.04	0.69 - 0.88
C	Curcumin	5.52 – 7.04	0.69 - 0.88
DMC	Demethoxycurcumin	4.48 – 5.52	0.56 - 0.69
BDMC	Bisdemethoxycurcumin	3.04 – 4.48	0.38 – 0.56
LAC	Lactose	1.70	0.21
MAL	Maltose	2.30	0.29
GLU	Glucose	3.0	0.38
VO	Turmeric volatile oil	4.48 – 5.52	0.56 - 0.69

The TLC plate stationary phase used silica, which is a polar material. Lower polarity compounds are carried throughout the mobile phase, resulting in high R_F values. Higher polarity compounds are generally retained, resulting in lower R_F values [20].

The curcuminoids and volatile oil standards without a spray reagent appeared as yellowish spots in the visible (Figure 2A) and light-blue zones (Figure 2B) at a long wavelength, as well as in the black zone against a green background at a short wavelength (Figures 2C and 2F).

After spraying the UV-sensitive plates with NP, intense-orange zones were detected in the visible zone (Figure 2D) at 366 nm (Figure 2E).

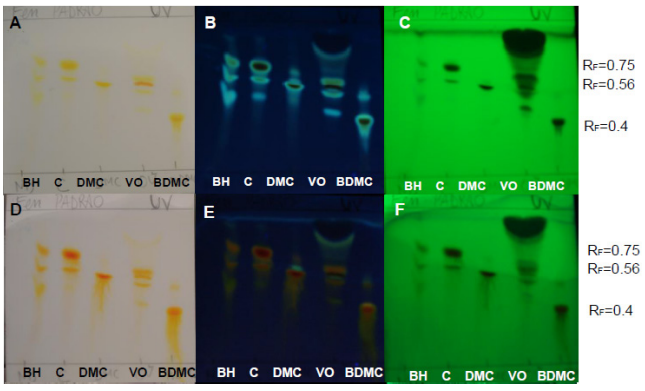

Figure 2. Curcuminoids standards, turmeric volatile oil and DDT solutions on UV-sensitive plates without a spray reagent (A, B and C) and sprayed with NP (D, E and F) at UV 366 nm and UV 254 nm

Curcuminoids and turmeric volatile oil displayed yellow spots in the visible zone after being sprayed with DPPH via UV-sensitive and non-sensitive plates (Figures 3A and 4G). DDT resulted in a very weak yellow spot, indicating that the sample possesses poor or no antioxidant activity (Figures 3A, 3B, 3C, 4G, 4H and 4I).

After spraying with SV, purple bands appeared on the turmeric volatile oil, indicating the presence of terpenes (Figure 3D) at R_F=0.63-0.75. The light-red color attributed to the volatile oil at R_F=0.38 (Figures 3D and 3E) is associated with the presence of thymol, which varies from red to purple. The same red color is visualized on bisdemethoxycurcumin after spraying with SV (Figure 3D). The color completely disappeared on the bisdemethoxycurcumin sample after 30 minutes and partially disappeared on the other standards in the visible zone (Figure 3G). Light-green spots are observed on the standards at R_F values of 0.38 and 0.4 at 366 nm. Light-blue spots are observed on the volatile oil at R_F =0.63 and R_F =0.74 (Figures 3H and 3I).

After treatment with the SV reagent, the monoterpene alcohols, esters, cineole, aldehyde citral and citronellal exhibited blue or blue-violet colors in the visible zone. The phenylpropane derivatives, including safrole, anethole, myristicin, apiol and eugenol, appeared as brown-red/violet spots, while thymol and carvone were red to red-violet. Piperitone displayed a typical orange color [14].

The oils from the *curcuma* species displayed seven to eight blue, red or violet-blue zones with an R_F of 0.3 at the solvent front. A prominent sesquiterpene zone can be see with an R_F of 0.8 at the solvent front [14].

Based on preliminary analyses, the standards used for the detection of volatile oil constituents can be well identified under UV-light using UV-sensitive TLC plates (Figure 3). The standards and samples used for the detection of sugars could not be visualized under UV-light. Therefore, UV-light and UV-sensitive plates were not used to detect sugars.

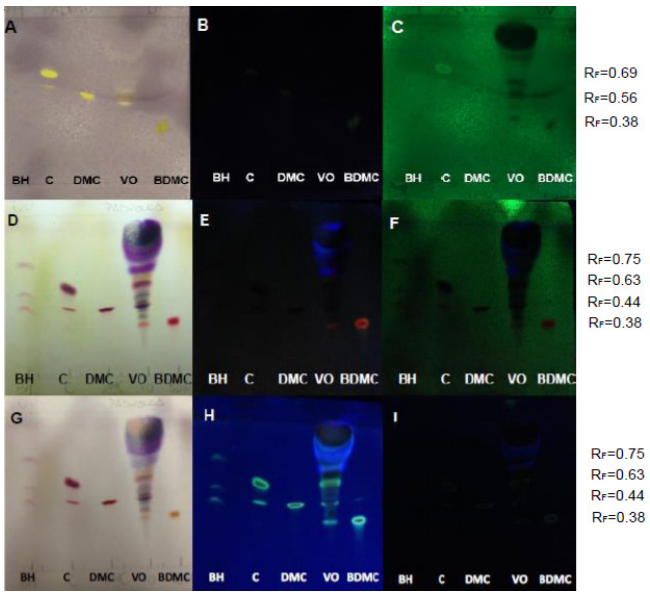

Figure 3. Curcuminoid standards, turmeric volatile oil and DDT solutions on UV-sensitive plates sprayed with DPPH (A, B and C) and SV immediately (D, E and F) and after 30 minutes (G, H and I) at 366 nm and 254 nm

According to Morlock et al. [21], the shortest possible wavelength yielded the best sensitivity when a UV detection analysis was performed. UV values of 200 or even 190 nm were preferred. However, these low UV wavelengths lack the selectivity required by complex food sample matrices.

DTT samples displayed three yellow zones in the visible spectrum (Figure 4A) and light green zones on the non-UV-sensitive plates (Figures 4B and 4C). Intense orange zones appeared after being sprayed with NP (Figures 4D, 4E and 4F).

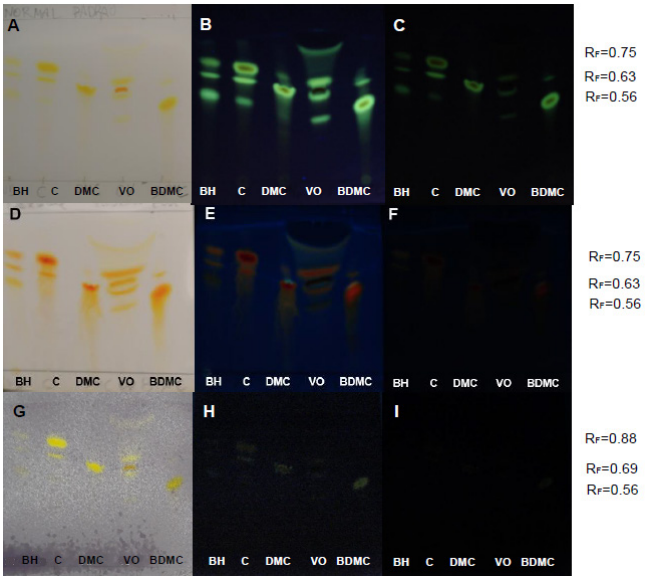

Figure 4. Curcuminoid standards, turmeric volatile oil and DDT solutions on non-UV-sensitive plates without a spray reagent (A, B and C), sprayed with NP (D, E and F) and sprayed with DPPH (G, H and I) at 366 nm and 254 nm

Kulkarni et al. [22] separated curcuminoids by TLC and obtained R_F values of 0.67 for curcumin, 0.6 for demethoxycurcumin and 0.51 for bisdemethoxycurcumin using chloroform: methanol (19:1) as the mobile phase.

Rafi et al. [23] detected R_F values corresponding to 0.05, 0.14 and 0.37 for curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively, using chloroform: dichloromethane (32.5:67.5) as the mobile phase.

Zhao et al. [24] used petroleum ether: ethyl acetate (90:10) as the mobile phase, which separated low polarity compounds from four *curcuma* species, and sesquiterpenoid standards provided a good resolution. TLC of *Curcuma xanthorriza* Roxb extract diluted in 70% ethanol with n-hexane:ethyl acetate (14:1) was developed using silica gel 60 F_{254} plates, resulting in R_F values that varied from 0.16 to 0.86 [25].

TLC plates with sugars standards are listed in Figure 5. A large and uncommon spot can be visualized after being sprayed with SAN and activated at 100°C for 5 minutes. The R_F calculations for each standard were based on the initial distance from the definitive spot formation. Various sugar standard concentrations were tested, yielding the same type of spot with various intensities.

No published studies were found that used the methodology adopted in this study. The type of stationary phase, salt solution and mobile phase likely influenced the shape of the spot.

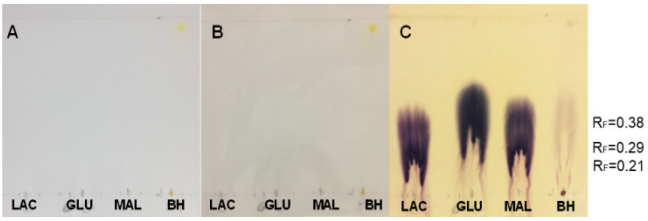

Figure 5. Sugar standard solutions and DDT samples on non-UV-sensitive plates before being sprayed (A), after being sprayed with SAN (B) and after activation at 100°C (C)

Morlock et al. [21] used the same mobile phase constituents employed in this study, excluding water, to analyze *Stevia* formulations, stating that the use of ethyl acetate as the major mobile phase component provided a fast separation of the bands, while the elution strength was adjusted with methanol and the addition of acetic or formic acid focused the zone to a sharp band.

3.2. Sample Fingerprints and Chemical Composition

Partial hydrolysis reactions occurred at three temperature levels (40, 70 and 100°C). The sample identities on the plates are listed as a function of the pressure in Table 5.

Table 5. Sample identities obtained by hydrolysis with PLE

Identity	Pressure (bar)
1	10
2	20
3	30
4	40
5	50
6	60
7	70

Three yellow zones were observed on the TLC plates after elution with chloroform, ethanol and glacial acetic acid (95:05:01) for DDT samples without a spray solution, as observed in Figures 6A, 7A and 8A. The R_F values of the DDT and TH samples ranged from 0.44 to 0.69, which are similar to the R_F values of the curcuminoid standards.

The obtained DDT and TH fingerprints can be used to qualitatively determine the active compound and sugar yields according to the temperature and pressure.

Light-blue fluorescent bands were detected on DDT samples against a dark blue background at 100°C and 366 nm using non-UV-sensitive plates (Figure 8B, non-UV-sensitive plate). Samples obtained at 40 and 70°C displayed light-blue and light-green zones (Figures 6B and 7B, non-UV-sensitive plates).

Green fluorescent zones were detected on the DDT samples obtained at 70°C at 366 nm (Figure 7B, UV-sensitive plate) using UV-sensitive plates. No visible differences were displayed by samples at 254 nm using UV-sensitive plates.

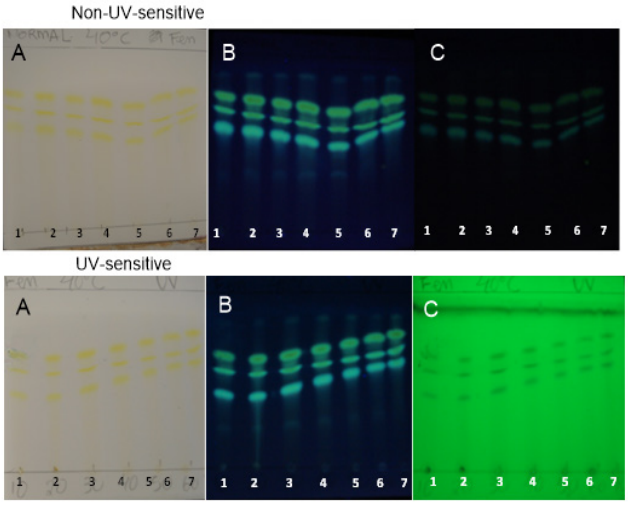

Figure 6. PHDDT samples obtained at 40°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

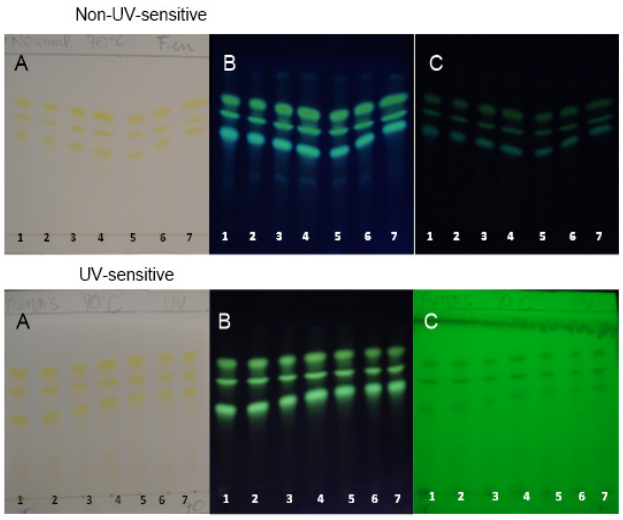

Figure 7. PHDDT samples obtained at 70°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

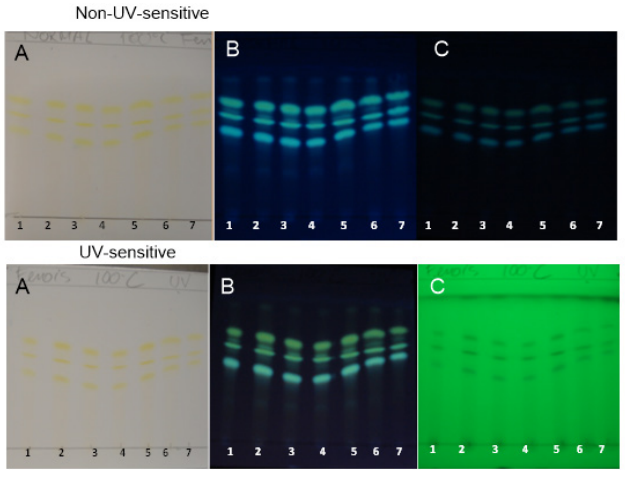

Figure 8. PHDDT samples obtained at 100°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

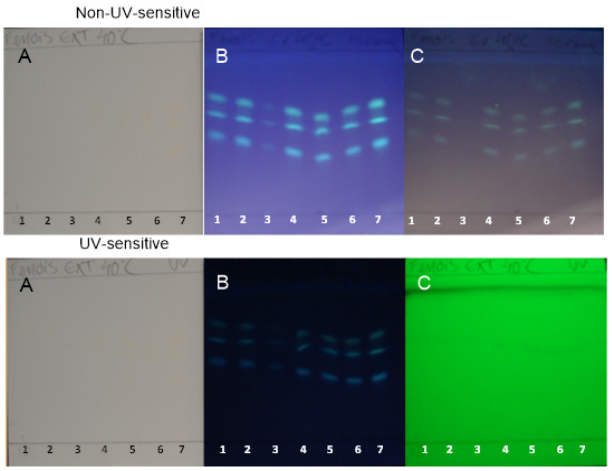

Figure 9. TH samples obtained at 40°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

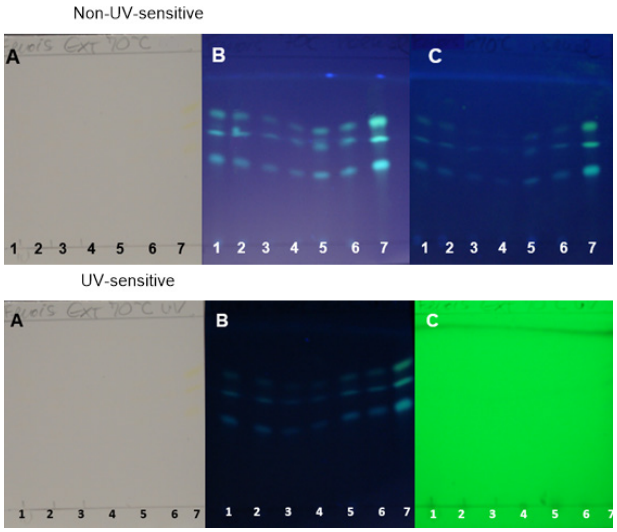

Figure 10. TH samples obtained at 70°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

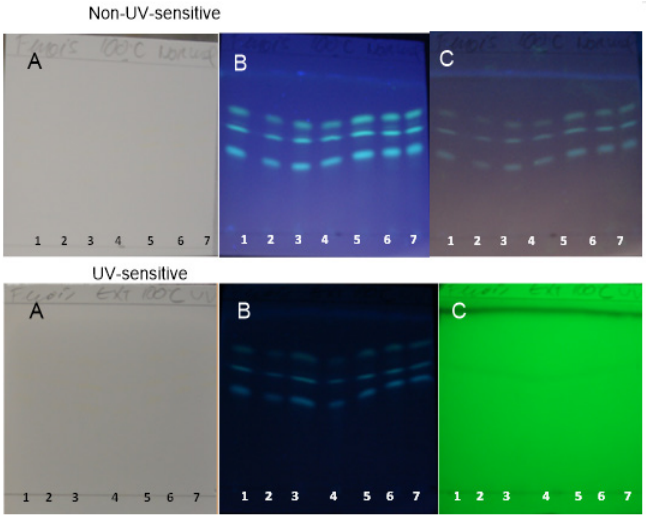

Figure 11. TH samples obtained at 100°C on non-UV-sensitive and UV-sensitive plates without a spray reagent at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

TH samples could not be identified in the visible spectrum regardless of the conditions. However, the TH samples were weakly visualized by UV light on non-UV-sensitive plates. Samples were only identified at 366 nm on UV-sensitive plates (Figures 9, 10 and 11).

3.3. Phenolic Compounds

Curcuminoids and volatile oil are the major bioactive constituents of turmeric. Curcuminoid analyses are essential for determining the quality of the turmeric plant material and processed products [26].

PHDDT and TH sample fingerprints displayed dark yellow and light yellow zones in the visible spectrum after being sprayed with NP (Figures 12-17). Dark-yellow to orange spots were visualized on PHDDT samples at 366 nm, while low intensity pale-yellow spots were visualized on TH samples at 366 nm.

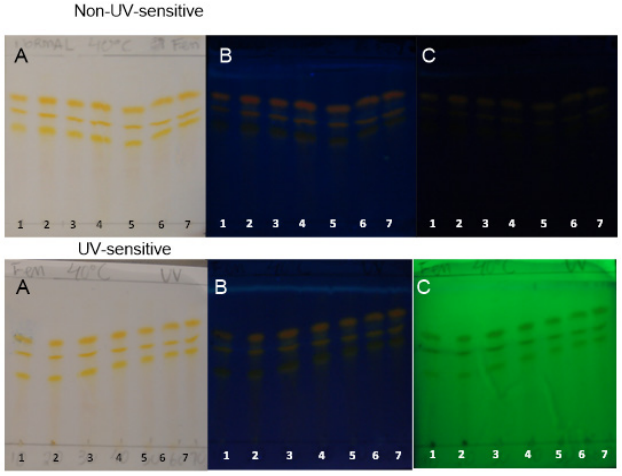

Figure 12. PHDDT samples after hydrolysis at 40°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

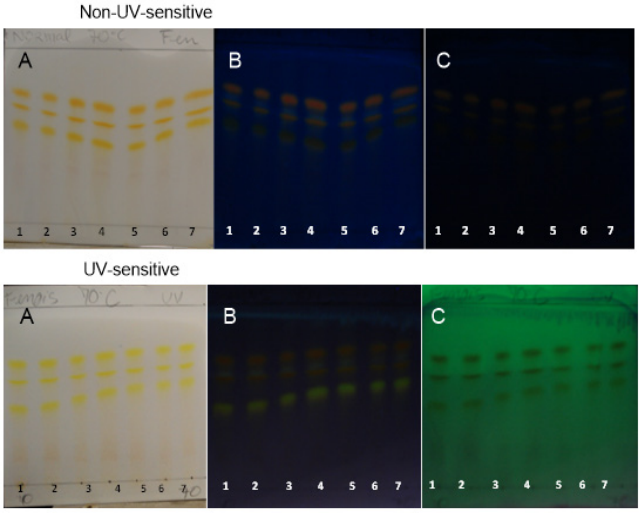

Figure 13. PHDDT samples obtained at 70°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

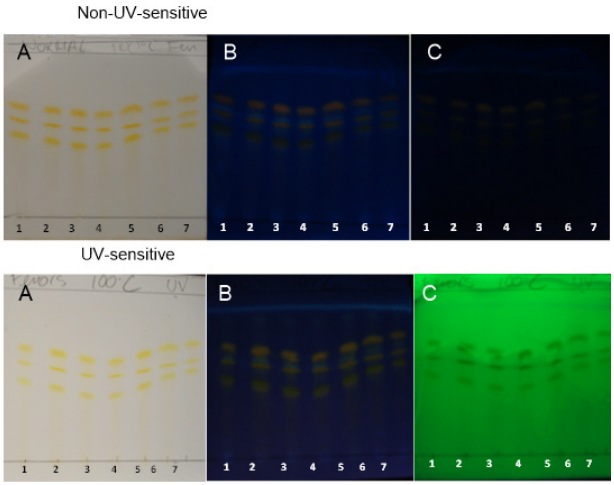

Figure 14. PHDDT obtained at 100°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

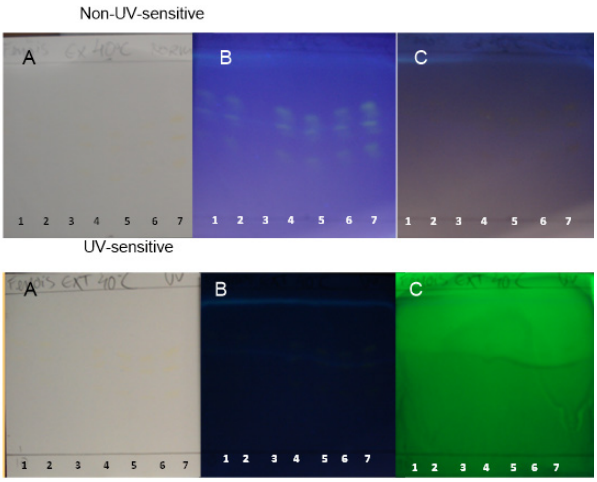

Figure 15. TH obtained at 40°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

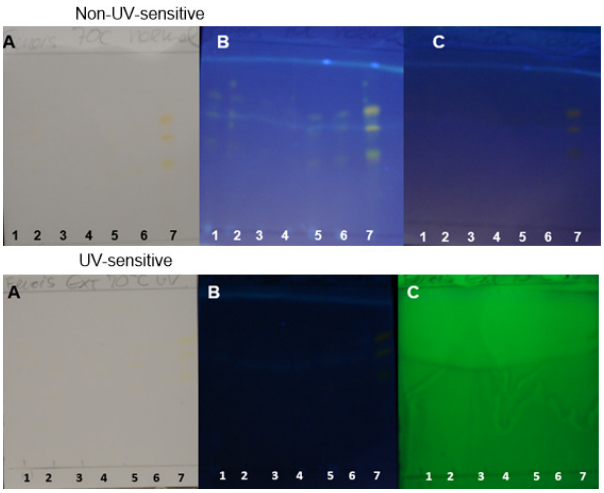

Figure 16. TH obtained at 70°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

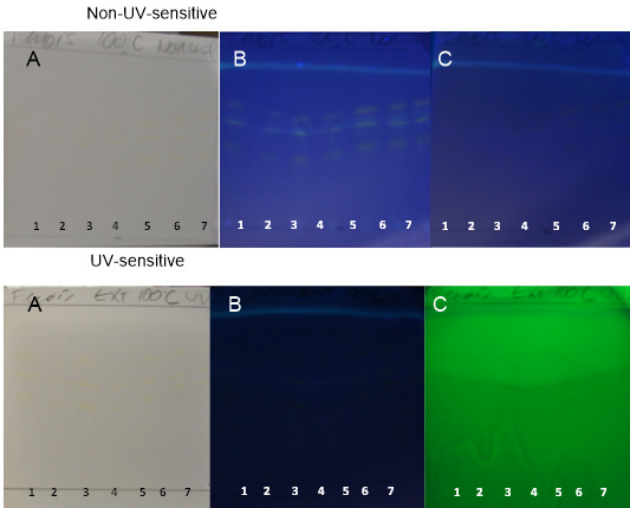

Figure 17. TH samples obtained at 100°C on non-UV-sensitive and UV-sensitive plates sprayed with NP at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

After chromatographic separation and spraying with NP/PEG, pomegranate juice samples were analyzed at 366 nm, displaying dark blue and light blue zones [27]. Annatto oil samples were visualized as orange, brown, black and light blue zones after spraying with NP at 366 nm [16].

It is relatively challenging to identify phenolic compounds using TLC. The most common strategy uses a set of reference compounds on the TLC plate. However, this approach incorporates inherent uncertainties, because two different compounds can have the same R_F value, requiring further characterization to establish compound identities at a higher confidence level. A higher confidence level can be achieved by scraping areas of the TLC plate where the compound of interest has migrated, followed by a solvent extraction of the matrix and detailed chemical analysis, such as gas chromatography or mass spectrometry [28].

The UV-sensitive plates in Figures 15-17 do not exhibit TH sample bands, suggesting that the samples contain few phenolics.

Definitive pressure influences are difficult to qualitatively analyze based on samples obtained under different conditions.

The temperature influenced the quality of the samples, as is evident in the differences among the fluorescences emitted by the samples at 366 nm.

3.4. Antioxidant Compounds

Antioxidant agents can be used for several cosmetic and medicinal applications [29]. Free radical scavenging compounds appeared as yellowish spots against a purple background, indicating a positive antioxidant activity due to the presence of an active antioxidant [30].

The TH samples were not visualized before or after DPPH detection at all temperature or pressure conditions and independent of the type of TLC plate used.

The PHDDT samples were not detected using UV-sensitive plates after being sprayed with DPPH. The antioxidant strength of PHDDT (Figures 18, 19 and 20) on UV-sensitive plates is classified as a weak activity. The antioxidant composition varied as the temperature increased.

The behaviors observed in the DDT and TH samples vary from the behaviors exhibited by other raw materials, such as bamboo leaves [31] and German Propolis [32], whose constituents were intensively detected by a DPPH solution and exhibited strong antioxidant activities.

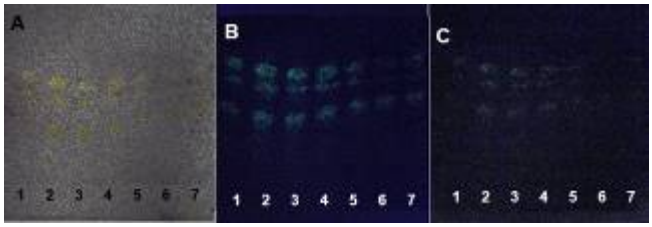

Figure 18. PHDDT samples obtained at 40°C on non-UV-sensitive plates sprayed with DPPH at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

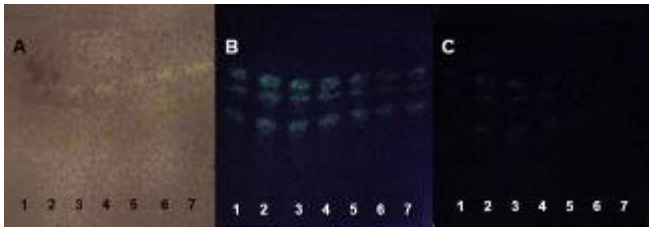

Figure 19. PHDDT samples obtained at 70°C on non-UV-sensitive plates sprayed with DPPH at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

Figure 20. PHDDT samples obtained at 100°C on non-UV-sensitive plates sprayed with DPPH at visible (A), UV 366 nm (B) and UV 254 nm (C) levels

3.5. Volatile Compounds

The SV can be used to detect steroids, volatile oils, terpenes, carotenoids, phenols, catechins, flavonoids, ginsenosides, fatty acids and antibiotics [33]. The SV fingerprints were detected using UV-sensitive plates.

PHDDT sample bands were only visualized at the visible level (Figures 21 and 22). TH samples were not visualized at the visible level or by UV-light after elution and being sprayed with SV.

Phenolic compounds with aromatic structures display intense absorption in the UV region of the spectrum associated with green, yellow, white to pale yellow, purple, pink, red, blue, grey, brown or black spots [34].

Dark red spots immediately appeared on DDT samples after being sprayed with SV (Figure 21) at R_{Fs} of 0.56-0.75, while light-red spots appeared at 0.44-0.5. The intensity of the coloration partially disappeared after 30 minutes

(Figures 22A, 22B and 22C).

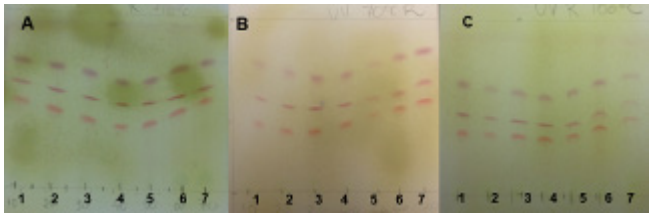

Figure 21. PHDDT samples obtained at 40 (A), 70 (B) and 100°C (C) after being sprayed with SV at the visible level

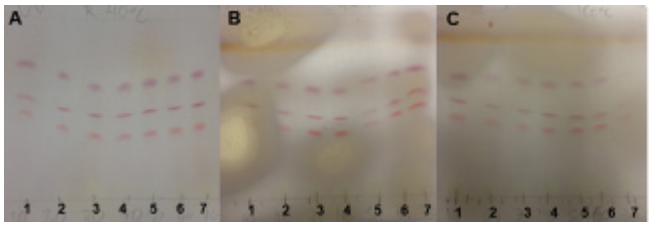

Figure 22. PHDDT samples obtained at 40 (A), 70 (B) and 100°C (C) 30 minutes after being sprayed with SV at the visible level

3.6. Sugars

The sugars detection via TLC uses furfuraldehyde in conjunction with numerous phenolic reagents, which give rise to different colors, e.g., α-naphtol yields a blue-violet coloration. The ketone and aldehyde groups of the reducing sugars preferentially react [35].

Carbohydrates attach to adsorbents. Thus, the mobile phase must be extremely polar. Buffering the silica gel with weak salt acids enhances the monosaccharide separation during TLC [35].

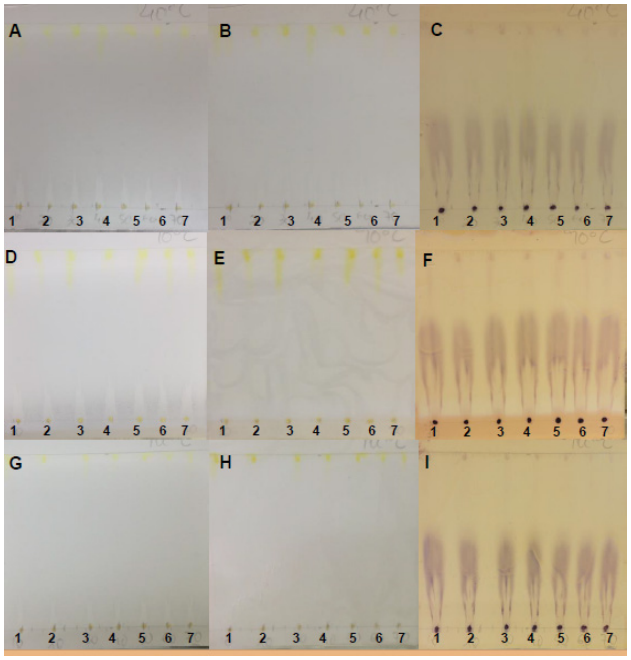

Figure 23. PHDDT samples obtained at 40 (A, B and C), 70 (D, E and F) and 100°C (G, H and I) on non-UV-sensitive plates before spraying (A, D and G), after spraying with a SAN reagent (B, E and H) and after activation at 100°C (C, F and H) at the visible level

Band separation did not occur on PHDDT samples (Figure 23), because the material is a source of high-molecular- weight sugars, such as starch [36].

TLC is commonly used to analyze low-molecular-weight sugars and their derivatives. Aniline diphenylamine - phosphoric acid, aniline phthalate, p-anisidine phosphate, sulfuric anisaldehyde and others are spray reagents that are used to detect sugars on TLC plates [37].

Carbohydrates are difficult compounds to separate from each other. They primarily differ based on the number of carbon atoms they possess, their chiral center configurations and their molecule sizes, i.e., their classification as di-, tri- or oligosaccharides. If two carbohydrates have any one of the three characteristics in common, they can be difficult to separate [38].

Two purple zones are clearly identified on TH samples at 40°C (Figure 24C) and 70°C (Figure 24F) at the visible level with R_F values of 0.24 (dark purple) and 0.36 (light purple), respectively. These R_F values are similar to the sugar standards used in this study. Only a weak purple zone with an R_F of 0.24 can be visualized on TH samples at 100°C (Figure 24I).

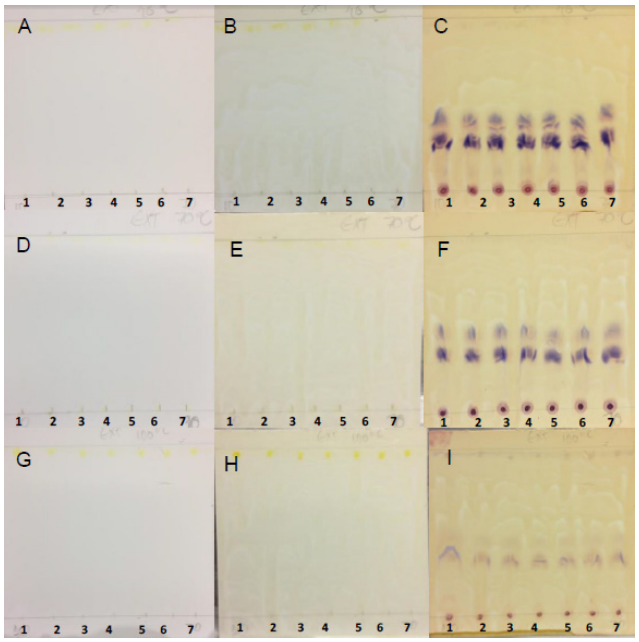

Figure 24. TH obtained at 40 (A, B and C), 70 (D, E and F) and 100°C (G, H and I) on non-UV-sensitive plates before spraying (A, D and G), after spraying with SAN (B, E and H) and after activation at 100°C (C, F and H) at the visible level

Only the process temperature influenced the composition (qualitative sugar analysis) of TH samples. The lowest sugar intensities were observed as the temperature increased, which can be attributed to the degradation of these constituents, resulting in furfural production.

The acid hydrolysis of cellulose and pentosans (xylans, arabinans and polyuronides) in plants and waste materials produces hydroxymethylfurfural and furfural, which are organic substances that serve as inputs for various industrial processes, including furfuryl alcohol and tetrahydrofuran preparation [39].

The sugar constituent bands of edible mushrooms displayed the best resolution using a acetonitrile: water (70:30) and 20% sulphuric acid solution as a spray solvent [40].

The hydrolysis products of glycosides were identified using ethyl acetate:2-propanol:water (65:30:10) and detected with a sulphuric naphtoresorcinol reagent in an enzymatic grape skin hydrolysis study based on endoglycosidase [41].

Okonkwo et al [42] identified sugar constituent bands in pineapple juice samples using butane-1-ol:acetone:water (4:5:1), resulting in R_F values of 0.19 and 0.3, which were associated with the R_F values of glucose (0.34) and fructose (0.17), concluding that the sample contained these two sugars.

An n-butanol:acetone:diethylamine:water (10:10:2:6 v/v) solvent system was used to identify sugars from the outer almond fruit skin, resulting in R_F values of 0.18 for lactose, 0.24 for maltose and 0.41 for glucose [43].

4. Conclusions

Turmeric is an important plant that is used as a medicine, condiment and cosmetic due to its wide range of bioactive substances. However, few studies have evaluated the functionality and processing feasibility of deflavored and depigmented turmeric.

This study explores a method that reuses waste turmeric from extraction processes via partial hydrothermal hydrolysis, generating partial-hydrolyzed deflavored and depigmented turmeric and turmeric hydrolysates products. The chemical profiles of these products were evaluated using thin layer chromatography, which analyzed phenolics, antioxidant, volatiles and sugars.

According to the TLC fingerprints, PHDDT and TH samples are predominant in phenolic compounds, presenting light-blue and light-green fluorescences at 366 nm and 254 nm without a spray reagent.

Establishing a comparison between these two products, PHDDT samples provide the most bioactive compounds, including curcuminoids, antioxidants and volatiles. However, TH is a considerable source of low-chain sugars when submitted to partial hydrolysis at 40°C.

Finally, this studied showed that waste turmeric can be reused for subsequent processes that generate new products with promising applicabilities.

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