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Optimization of the conditions of alkaline extraction of tomato peels and characterization of tomato peel extracts obtained under atmospheric and oxygen free conditions

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Abstract: This study aims to optimize the extraction conditions to obtain the highest yield, to characterize tomato peel extract (TPE) under optimized conditions, and also to determine the effect of ambient oxygen on the properties of TPE. Optimisation were performed at three temperatures (60 °C, 80 °C, 100 °C) and three periods (2, 4, 6 h) by the response surface methodology. The properties of the extract under atmospheric and oxygen-free conditions (AC, OFC) were analysed to determine whether the characteristics of both extracts changed depending on the presence of oxygen; moreover, the morphological, chemical, thermal, biochemical, and antimicrobial properties were analysed. The maximum yield was 31.3% at 100 °C/6 h. A quadratic model was used to create the best fit. Both TPE samples exhibited similar morphological structure, similar weight losses at three stages of TGA curve, similar band assignments in FTIR spectra. GC-MS analysis showed that both samples mainly consisted of cutin in abundance of 70.45% and 68.14% for AC and OFC, respectively. OFC had higher total phenolic content possibly depending on the absence of oxygen. AC and OFC extracts exhibited substantial antimicrobial activity against *S. aureus, E. coli, C. albicans,* and *A. brasiliensis* with a MIC value of 100 µg TPE/ mL.

Key words: Alkaline extraction, characterization, optimization, response surface methodology, tomato industry wastes, tomato peels.

INTRODUCTION

Tomatoes (*Lycopersicum esculentum*) are one of the most widely consumed fruits in the world, and they are consumed either raw or as processed food. Approximately 25% of the world's tomato production is used in production of juice, sauce, soup, paste, puree, ketchup, and dried and canned foods (Szabo et al. 2019a). However, tomato processing generates several wastes such as seeds, pulp, and peels that are mainly associated with environmental pollution. These tomato wastes constitute approximately 5%-30% of the tomatoes by weight (Szabo et al. 2018, Saini et al. 2018, King & Zeidler 2004) and cannot be utilised for several reasons, such as non-digestibility, consumer disinterest, and processing inconvenience. Therefore, the bulk of the waste is used in landfilling and must be discarded yearly. Only a small fraction of these wastes can be reused as animal feed; for the production of fertilizers and biogas after being composted or dried (Nincevic Grassino et al. 2020, Cifarelli et al. 2016).

Tomato fruit comprises the exocarp, endocarp, and mesocarp layers; tomato peel is found in the exocarp, which is the outermost layer of the fruit tissue (Tamasi et al. 2019). Tomato peels contain several components such as cutin, cellulose, hemicellulose, lignin, and waxes (Benitez et al. 2018) and also several phytochemicals such as carotenoids (Albanese et al. 2014), polyphenols, sterols, terpenes and tocopherols (Kalogeropoulos et al. 2012). Cutin is an important component of tomato peel as it makes up 45%-80% of tomato peel (Benitez et al. 2018). Cutin is an amorphous polyester consisting of long-chain 16 or 18 carbon polyhydroxy fatty acids linked by cross-ester bonds (Benitez et al. 2018, Heredia 2003), and the specified fatty acids are predominant in the structure of cutin (Philippe et al. 2016, Parsons et al. 2013). The viscoelastic structure and tunable mechanical properties of cutin which also depend on the presence of other components of tomato peel enable most of the proposed industrial applications to be performed in packaging (Scavee 2018). Waxes (approx. 2% of tomato peel by weight) build up the minor component of tomato peel (Benitez et al. 2018). By acting as polymer fillers, waxes exert a cross linking effect and increase the rigidity of cutin matrix (Dominguez et al. 2011, Takahashi et al. 2012). Besides, waxes (especially the intracuticular waxes that contain short chain fatty acids) make a significant contribution to the barrier properties of cutin (Vogg et al. 2004, Leide et al. 2007). All these arguments reveal that they might be used as reinforcing agents in packaging for the improvement of mechanical and barrier properties of other materials. The other tomato peel components consist of cell wall polysaccharides which may be utilized in the design of functional foods, new biomaterials and carriers for drugs and bioactive substances (Albuquerque et al. 2016). Thanks to the biodegradability and versatile applicability of all its components, tomato peels also provide a great opportunity in the food sector to produce foods with highly added value and food packaging material originated from natural sources.

Extraction of several components, which either have high nutritive value or substantial health promoting effects from agricultural and industrial wastes has been considered as the preliminary stage of waste valorization. The feasibility of extraction on industrial scale depends on the attainment of high efficiency which means maximum yield with minimum input (energy, material, labor and investment costs). Therefore, the selected method must be simple, economic and adaptable to industrial applications. An optimization process is usually required to analyze the extraction conditions that most satisfactorily meet the specified targets and ensure to realize whether they are attainable or not also on industrial basis. Conventional extraction methods such as maceration using either organic solvents or acid/alkali treatment are mainly based on effective mass transfer created by chaotic movement of vortexes with continuous agitation and excess of heat (Mosca et al. 2018) and can be applied particularly for plant matrices where the solvent easily penetrates and diffuses into the solid waste. An increase in temperature favors the solubility of the compounds and matrix-analyte interactions are more easily broken (Cussler 2009). In some cases, where solvent-solute interactions are weakened due to the complex and heterogenous structure of the solid waste, the use of harsh chemicals (oxalic acid, hydrogen peroxide, sodium methoxide, sodium carboxylate, acidified organic solvents, lithium aluminium hydride) might be necessary for the cleavage of esterified and etherified bonds (Heredia-Guerrero et al. 2017, Cifarelli et al. 2016, Kolattukudy 2001) and also to overcome the cohesive energies of tightly bound macromolecules (polymers, polysaccharides, lipids, polyphenols) and facilitate the liberation of the desired components. Alkali treatment is an effective method used for the separation of skins from the fruit since alkali solution first dissolves cuticular waxes, then

penetrates into the skin and continues to diffuse homogenously into the fruit (Ayvaz et al. 2016). This method is applicable for the extraction of proteins from rice residues (Zhang et al. 2018); pectin from sweet potato peels (Abang-Zaidel et al. 2017); flavonoids, phenolic acids from peels of kiwi fruits (Sun-Waterhouse et al. 2009) and several cereal wastes (Kim et al. 2006, Verma et al. 2009, Nenadis et al. 2013) and also preferable due to reasonable material costs (Heredia-Guerrero et al. 2017).

Although a number of investigations have been performed so far with respect to the extraction of major polysaccharides from tomato peel (Szymanska-Chargot et al. 2017) and isolation of cutin from tomato pomace (Benitez et al. 2018), no prior investigation focused on the optimization and/or modelling of conditions for extraction of tomato peels. Furthermore, the so far reported studies concentrated on the isolation and fractional separation of each component from tomato wastes. This approach could envisage neither the synergistic relationship between several components (cutin-waxes) nor the interdependence of their functionality (polysaccharides-bound phenolics; cutin-polysaccharides) associated with their co-existence. Taking into account all these aspects and the main objective of this study, a less complicated but more integrative alkali extraction method, that eliminates dewaxing and other sequential separation steps, seems to be more appropriate to tomato peels compared to the fractional separation of each component. Ultimately, a further examination and evaluation is essential to understand how the above described aspects will affect the structural, thermal, chemical and functional properties of tomato peel extract (TPE).

Tomato peels also contain several phenolic compounds such as caffeic, chlorogenic, p-coumaric, ferulic and rosmarinic acids, quercetin and rutin (Cetkovic et al. 2012). However, there are some limitations in the industrial valorization of these phenolic compounds such as chemical instability under environmental and processing conditions such as pH, long exposure to light, oxygen and heat (Nishimoto-Sauceda et al. 2021). Oxidation, hydrolysis and polymerization are the main reactions stimulating the degradation of these compounds under the presence of oxygen, light and heat (Nishimoto-Sauceda et al. 2021). It was reported that some flavonoids such as quercetin and rutin present in plant derived materials might be decomposed under the presence of oxygen and long exposure periods to heat (Wang et al. 2016, Kalinová et al. 2018). Thermal treatments at 70-95 °C for long periods led to the reduction in the antioxidant capacity due to the degradation of citrus flavonoids under the presence of dissolved oxygen (M'hiri et al. 2017). These factors have a strong influence on the decrease in the bioavailability of phenolic compounds (Nishimoto-Sauceda et al. 2021). For this reason, investigation of the extraction of phenolic compounds under oxygen free conditions is necessary to reveal any possible difference in the extraction yield of phenolic compounds caused by oxygen free conditions. Additionally, we have not encountered any study that investigated the extraction of tomato peels under oxygen-free conditions.

Therefore, this study aimed i) to optimize the extraction conditions of tomato peels by the response surface methodology (RSM) to maximize the extraction yield; ii) to determine the morphological, thermal, chemical, biochemical, antimicrobial, and antioxidant properties of tomato peel extracts (TPE) obtained both under atmospheric condition (AC) and oxygen-free condition (OFC).

MATERIALS AND METHODS

We purchased the following chemicals from Sigma Aldrich (Missouri, USA): Chloroform, methanol, N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA, Supelco), Folin-Ciocalteu, ABTS (2,2 azinobis-3 ethylbenzothiazoline-6 sulfonic acid diammonium salt), DPPH (1,1diphenyl-2 picyrlhydrazyl) reagents, potassium persulfate, gallic acid, Trolox standards, sodium carbonate, hexane, sodium hypochlorite, ethanol (99.96% purity), methanol (99.99% purity), acetonitrile (99.99% purity), and formic acid (99.99% purity). The purge gases (argon and nitrogen) were supplied by Linde AG (Dublin, Ireland). Further, we purchased Tryptic Soy Agar; Mueller–Hinton Agar and Broth; Sabouraud Dextrose Agar and Broth; 2, 3, 5 triphenyltetrazolium chloride, and dimethyl sulfoxide (DMSO) from Merck Millipore (Burlington, USA).

Sample preparation

Fresh tomato pomace was supplied by a tomato paste processing factory (TUKAŞ Inc., Izmir, Turkey). The tomato pomace was initially exposed to a sedimentation process, which separated the seeds from the skins by using containers filled with water. After removing the tomato seeds, the skins and fibres were dried in a cabinet dryer (Lab T2-Eksis, Turkey) at 60 °C for approximately 8 h (until reaching a constant weight) at an air flow rate of 1 m/s. The dried tomato peels were then milled using a hammer mill (Brook Crompton 2000 Series, UK) and sieved with a pore diameter of 500 µ (Retsch, Germany). The dried peels were then stored at -20 °C in vapour-proof, airtight packages until the extraction process started.

Alkaline extraction of tomato peels

The extraction was carried out using the procedure described in literature (Cifarelli et al 2016, Benitez et al. 2018). In each experiment, the samples were treated with NaOH solution (3% w/v) at a solvent: solute ratio of 10:1. After the filtration, the residue was washed twice with excess distilled water, and the supernatant was combined with the filtrate. Subsequently, the supernatant was acidified with 3 M of HCl until the pH of the solution reached 4.3. The samples were then centrifuged at 4000 rpm for 15 min thrice by dialyzing with alkaline water (pH=8.45) until the pH reached 6.5. This process was performed to remove acid insoluble lignin from the TPE (Mussatto et al. 2007). Afterwards, the precipitates were freeze-dried using a freeze dryer (Christ, Alpha 1-2 LD plus, Sweden). The precipitates were then weighed immediately and stored at -18 °C until the analysis started.

Optimization of extraction process

The effect of two independent variables (temperature and time) on the yield of TPE was examined by the RSM. The independent variables were selected as three levels of 60 °C, 80 °C and 100 °C for the temperature; 2, 4 and 6 h for the extraction time. When it is not feasible to predict the relationship between independent variables and the responses, RSM is a helpful tool for obtaining the best fit to this relationship.

The face-centred central composite design (CCD) was used in RSM; 19 runs (4 factorial points and 4 axial points each with two replicates and one central point with three replicates) were determined using the Design-Expert software (Version 7.0, Stat-Ease Inc., Minneapolis, USA).

$$X_k = \frac{x_k - x_i}{x_k} \quad (1)$$

$$y = \beta_0 + \sum_{i=0}^{k} \beta_i X_i + \sum_{i=0}^{k} \beta_{ii} X_i^2 + \sum_{i\leq 1}^{k} \sum_{i=1}^{k} \beta_{ij} X_i X_j + \ldots + e \quad (2)$$

The experimental results of the CCD were evaluated using Equation (1) and (2) where *y* denotes the predicted response, β_0 the constant coefficient, βii the linear coefficient, βij the strength of interaction between variables *i* and *j*, *k* the number of factors, and *e* the random error (Liu et al. 2011, Khor & Abdullah 2012)

The RSM results showed the optimized temperature and time for maximizing the extraction yield. To analyze whether there would be any difference in terms of morphological, thermal, chemical, and functional properties (antioxidant and antimicrobial) of the extracts when no oxygen was present during the extraction, the extractions were performed under both AC and OFC at the optimized temperature and period with two replicates and two repetitions. OFC was provided by using argon gas purged permanently through the reflux condenser into the reaction chamber during the extraction. TPE were further investigated for their characterization in terms of their morphological, thermal, chemical, antioxidant, and antimicrobial properties.

Approximate composition analysis

The approximate composition analysis of TPE was performed gravimetrically as described by Szymanska-Chargot et al. (2017) and Benitez et al. (2018). Briefly, certain amount of TPE (4 g) was treated hexane/methanol mixture (3:1 v/v) to remove waxes. Then, the dewaxed TPE was treated with boiling water for 10 min to recover polyphenols and water soluble pectin. In the following stage, the residue of the earlier stage after filtration was acidified with 1 M and 0.5 M HCl for 30 min at 85 °C twice to separate acid soluble pectin. The solution was filtered and the residue was treated with 1 M NaOH for 30 min at 85 °C three times. The supernatant was acidified with 3 M HCl and freeze-dried to yield cutin. The residue of the final stage was treated with NaOCl (1.5%) at 95 °C to separate lignin from cellulose fraction, where the supernatant contained lignin and the residue contained cellulose. All the fractions including the supernatants in each stage and the final residues were dried until constant weight. The ratio of each component in the sample was calculated based on the Equation (3).

$$\text{Yield (\%)} = \frac{w_f}{w_i} \times 100 \quad (3)$$

where w_i presents the initial weight of the analyzed sample; w_f represents the final weight of the dried fraction.

Morphological characterization

Scanning electron microscope (SEM) analysis were performed using Thermo Scientific Apreo S (USA) operating at 1-30 kV with a resolution between 0.7 and 1.2 nm. The device was equipped with a

Schottky electron source module. The samples were coated with gold (12 nm thickness) using a coating assembly (Leica EM ACE600, Germany). Digital image analysis was performed using Thermo Scientific AutoScript™ 4 Software—Python.

Thermal characterization

Thermogravimetric Analysis (TGA)

The mass loss (%) in TPE during progressive heat application between 25 °C–1000 °C was measured at a thermal increment of 20 °C/min using the Perkin Elmer Diamond TG/TGA (USA) instrument under a nitrogen atmosphere with a flow rate of 100 mL/min A total of 10 mg of powdered sample was implemented in an alumina crucible onto the sampling unit of the instrument. The change in mass was recorded, and a curve was obtained by plotting percentage mass loss against temperature to indicate the degradation zone of the extracts. Weight loss (Δw) and derivative thermograms (DTG) were analyzed using the Pyris Series software.

Differential Scanning Calorimetry (DSC)

DSC analyses were performed by the Perkin Elmer-DSC 4000 (USA) instrument from 0 °C to 300 °C under nitrogen flow at a flow rate of 40 ml/min Accurately weighed samples (5 mg) were installed in the pans. Subsequently, a heating–cooling–heating process was conducted. The first step involved heating from 0 °C to 300 °C, the second step involved cooling from 300 °C to 0 °C, and the final step involved heating from 0 °C to 300 °C by increments of 20 °C/min The graph of temperature versus change in the heat flux was plotted. The exact temperatures and the integrated areas of several peaks presented in the graphs were calculated by the instrument to identify any process (depolymerization, phase alteration, crystallinity, formation of other compounds, glass transition) that might have occurred during the extraction.

Chemical characterization

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra were provided using the Perkin Elmer Spectrum 100 (USA) instrument with an ATR auto sampling unit. Each sample was applied on ATR as powder. The spectra were collected over the range 4000-650 cm^{-1}. For each sample, spectral scans were provided at a spectral resolution of 0.5 cm^{-1}.

Fatty acid composition

The analytical composition of TPE was determined by gas chromatography mass spectrometry (GC-MS) based on a previously reported method (Benitez et al. 2018). The analysis was performed with an equipment (Agilent 7890 B with 5977A Series GC/MSD, USA) using an HP-5 MS ultra-inert column (60 m × 0.25 mm, ID, 0.25 µm film thickness). The oven temperature was adjusted to 50 °C for 2 min and subsequently increased to 90 °C at 30 °C/min The oven was kept at the latter temperature for 2 min and raised to 250 °C with an increment of 8 °C/min afterward. Finally, the oven was kept at 250 °C for 15

min Helium (1 ml/min) was used as the carrier gas. The compounds were identified by comparing their mass spectra and retention times with those of the standards. The cutin's monomer (10, 16 dihydroxy hexadecanoic acid) was identified by comparing its mass spectra with that reported in the literature (Benitez et al. 2018) and analyzing its mass fragmentation patterns. Quantification was performed by computing the total peak areas. The sum of the peak areas was normalized and expressed as molecular percentages. The percentual abundances were based on area integration without quantification by calibration since cutin's monomer (10, 16 dihydroxy hexadecanoic acid) has no commercial standard.

Functional properties

Total phenolic content

Total phenolic content (TPC) of the TPE were determined according to Folin–Ciocalteu's method (Xu & Chang 2007). Gallic acid was used as the standard and the results were given as gallic acid equivalents (GAE). Briefly for the extraction of phenolic compounds, the dried TPE was treated with ethanol (96 % v/v), then 50 µL of the mixture was vortexed for 30 seconds after the addition of 250 µL Folin Ciocalteu reagent and 3 mL of distilled water. 750 µL of sodium carbonate solution (7% w/v) was added to the mixture and vortexed for another 30 seconds. Then 950 µL of distilled water was added to each sample and stirred gently. The mixture was allowed to stand at room temperature in the dark for 2 hours. The absorbance of the mixture was measured at 765 nm using a spectrophotometer (Cary 60 UV-VIS, Agilent Technologies, USA). A calibration curve of gallic acid in ethanol (96%) at different concentrations versus the absorbances was plotted. The TPC for each sample was calculated using the linear function of this curve. The results were expressed in terms of mg GAE/100 g of dried TPE.

Trolox Equivalent Antioxidant Capacity (TEAC)

To determine the TEAC, the ABTS method was used as previously described (Re et al. 1999). The freeze-dried TPE were first treated with ethanol (96% v/v) for 20 min at 1000 rpm. The absorbance at 734 nm was measured using the Carry 60 UV-VIS spectrophotometer. The calibration curve was obtained by measuring the absorbance of the Trolox solutions at different concentrations and plotting a graph concentration against percentage inhibition, which was calculated by Equation (4).

$$\%\ inhibition\ = \frac{\left(A_b - A_s\right)}{A_b} \times 100 \tag{4}$$

where A_b denotes the absorbance of the blank (initial absorbance value of ABTS reagent), and A_s denotes the absorbance of the sample. The results were expressed in terms of µM Trolox/100 g dried TPE.

DPPH radical scavenging activity

The experiment was based on a method reported in the literature (Kumaran & Joel Karunakaran 2006, Tezcan et al. 2009). The absorbance was measured at 517 nm against ethanol, and subtracted from the blank values. Quantification was performed based on the calibration curve of Trolox in ethanol (10,

15, 20, 30, 40, 60, and 80 mg/L). The radical scavenging activity was calculated using Equation (5). The antioxidant capacities were expressed in terms of µM Trolox/100 g dried TPE.

$$\%\ reduction\ in\ DPPH\ =\ \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (5)$$

Phenolic composition analysis by LC-QTOF-MS

LC-QTOF-MS is a commonly used method for the putative identification of plant polyphenols in complex mixtures and secondary metabolites in semi polar nature (Moco et al. 2006) because of its simplicity and higher accuracy compared with devices such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) (Brock et al. 2020). Because the major component of TPE cutin is soluble in ethanol (Cifarelli et al. 2016), freeze-dried tomato peels were extracted with ethanol (% 99.96). The extracts were further diluted with methanol (% 99.99) before the injection to the Zorbax-SB-C18 column (4.6 × 100 mm, 3.5 µm) of the instrument (Agilent 6530 Accurate Mass QTOF/LC-MS, USA) equipped with an auto sampler G1367E and electrospray ionization source (ESI-Dual AJS) operating in both negative and positive ion modes. The column and injection temperatures were set at 30 °C and 4 °C, respectively. A volume of 5 µL was injected using a binary pump (Agilent, G1312B, USA) operating with a draw speed of 0.2 mL/min The acquisition mode was adjusted to an m/z range of 100 to 2000 with a scan rate of 10 spectra/sec. The gas and sheath gas temperatures were set to 300 °C and 350 °C, respectively. The gas flow rate was 11 L/min Isocratic elution was performed with a solvent composition of 30% of A (deionized water acidified with formic acid (1%)) and 70% of B (acetonitrile acidified with formic acid (1%)) at a solvent flow rate of 0.5 mL/min for a period of 20 min.

Antimicrobial activity

Preparation of the culture suspensions

The antimicrobial activity of TPE was determined using several bacteria including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and the fungi *Candida albicans* and *Aspergillus brasiliensis.*

The bacterial cultures were incubated on Tryptic Soy Agar at 37 °C for 18-24 h; the yeast culture was incubated on Sabouraud Dextrose Agar at 25 °C for 48 h. The density of the cultures was adjusted to 0.5 McFarland using a densitometer (Grant Inst, Cambridge, UK). For the cultures of *A. brasiliensis*, commercial spore suspension (10^8 microorganisms) was used (Biomerieux, France).

Minimum Inhibitory Concentration (MIC) Method (Liquid Microdilution Method)

The MIC values of TPE were determined by broth microdilution assay according to the Clinical and Laboratory Standards Institute. For bacteria and fungi, 100 µL of Mueller–Hinton Broth and 100 µL of Sabouraud Dextrose Broth were transferred, respectively, to each well of a 96-well microplate; 100 µL of the sample was transferred to the first well. After mixing the sample with the medium, 100 µL was taken and transferred to the next well. In this way, a 1:2 sequential dilution of the extract was

obtained up to the 8th well. Then, 10 µL of culture suspensions were inoculated into all wells at a final concentration of 10^6. To measure the growth of microorganisms at the end of the incubation, the dye (2, 3, 5-triphenyltetrazolium chloride) with a concentration of 0.5% was added in a volume of 20 µl to each well. After 30 min, the samples in the micro wells in which color changes were observed were regarded as positive as they indicated the growth of microorganisms. The MIC value was determined subsequently. To determine whether microorganisms grew in the micro wells without color changes or turbidity, the samples were taken and inoculated on the Mueller–Hinton Agar and Sabouraud Dextrose Agar.

Time-Kill assay

Time-kill assay was conducted by using a previously reported method (ASTM E2315-16 2016). Extract solutions prepared at × 10 of MIC concentration were inoculated from culture suspensions with a final concentration of 10^6 for bacteria and yeast and 10^5 for *A. brasiliensis*. The samples were incubated at 25 °C and 100 rpm in an orbital shaking incubator. To measure microbial growth, 0.1 mL aliquot was serially diluted with phosphate buffer solution (pH=7.4) after 0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 6 h, 12 h, and 24 h incubation. Samples and dilutions of 0.1 mL were spread on appropriate agar plates and incubated. After the incubation period, the numbers of colonies on the agar plates were counted, and log10 reductions were calculated. The tests were duplicated.

Statistical Data Analysis

By using Design-Expert, a one-way ANOVA was performed to identify the significance of the effects of the independent variables on the response. The TPC, TEAC, and DPPH antioxidant capacities and the antimicrobial activity of the TPE were investigated in duplicate twice. The mean values of the associated antioxidant capacities were recorded with their standard deviations (±). ANOVA was performed on the variation in the antioxidant capacities and TPC of the extracts. The differences in the antioxidant capacities and total phenolic content between the AC and OFC samples were examined using student t-test ($p < 0.05$).

RESULTS AND DISCUSSION

Optimization of Extraction Process

The results of each experimental run designed by Design Expert program were shown in Table I. The yield of extraction ranged from 10.20 to 32.04 g /100 g dry peel. The highest yield based on the average of two replicates (31.31 ± 0.73 g/100 g) of dry peel under AC was observed at 100 °C for 6 h. The optimal conditions are within the range of the performed experiments.

Two independent variables including extraction temperature and time were examined and optimized individually using CCD (Table II). The predicted regression coefficient was close to the experimental regression coefficient as summarized in Table II, indicating that the suggested optimized parameters were highly suitable for TPE.

Table I. RSM design and the responses for the model optimization of the extraction yield of tomato peels.

Run	Temperature (°C)	Time (h)	Extraction yield (g/100 g dry peel)
1	60.00	2.00	10.20
2	60.00	4.00	11.85
3	80.00	2.00	13.30
4	80.00	6.00	23.45
5	100.00	4.00	24.85
6	80.00	4.00	16.00
7	100.00	6.00	32.04
8	100.00	6.00	30.58
9	100.00	2.00	20.45
10	100.00	2.00	20.50
11	80.00	2.00	16.20
12	60.00	6.00	17.94
13	60.00	6.00	16.26
14	80.00	4.00	19.00
15	60.00	4.00	12.65
16	60.00	2.00	13.30
17	100.00	4.00	25.90
18	80.00	4.00	17.15
19	80.00	6.00	22.20

The model for the predicted extraction yield might be expressed as presented in Table II with respect to the multiple regression analysis. The quadratic model was the best fit for the extraction yield as it had the highest coefficient of determination (adjusted R^2 = 0.9603) compared with that of the other models (linear, two factorial interaction and cubic revealing adjusted R^2 of 0.9125, 0.9335, and 0.9575, respectively). The p and F values summarized in Table II, also imply that the model was significant. A lack of fit was non-significant ($p > 0.05$), further indicating the significance of the model. Design-Expert proposed a solution of 31.59 g/100 g dry peel as the response and 100 °C and 6 h as the optimized conditions with a high desirability (D = 0.979). The significance of each variable was determined using the F-test and p-value listed in Table II.

All parameters studied in this framework (temperature, time, temperature-time interaction, square of temperature, square of time) were found to be statistically significant on the yield of TPE ($p < 0.05$). Temperature and time may be regarded as having the greatest effect on yield because they exhibited considerably lower p-values compared with those of the other factors. Moreover,

Table II. ANOVA table indicating the statistical data of extraction parameters for tomato peels.

Source of variation	Sum of Squares	Degree of Freedom	Mean of Squares	F	p
Model[c]	663.15	5	132.63	88.16	< 0.0001[a]
A-temperature	433.44	1	433.44	288.11	< 0.0001
B-time	196.18	1	196.18	130.40	< 0.0001
AB	15.04	1	15.04	10.00	0.0075
A^2	8.51	1	8.51	5.66	0.0334
B^2	8.21	1	8.21	5.46	0.0362
Residue	19.56	13	1.50		
Lack of Fit	1.84	3	0.61	0.35	0.7934[b]
Pure Error	17.72	10	1.77		
Overall	682.71	18			

Equation = + 24.02507 - 0.39419 × *A* - 3.45866 ×B + 0.034281 × *A* × *B* + 3.4847 × 10^{-3} × A^2 + 0.34223 × B^2
[a] statistically significant at α=0.05; [b] statistically insignificant at α=0.05; [c] Adjusted R^2 = 0.9603; Predicted R^2 = 0.9375; Adequate Precision = 29.171; *A: Temperature (°C); B: Time (h)

temperature and time as a single factor, their interaction (AB), square of temperature, and square of time variables were observed to have a positive influence on the extraction yield. Consequently, an increase in extraction temperature and time within the upper and lower boundaries of this work could also lead to a rise in the extraction yield (Figure 1). The results indicated that higher temperatures and longer extraction times promoted the extraction thereby contributing significantly to the extraction yield of TPE.

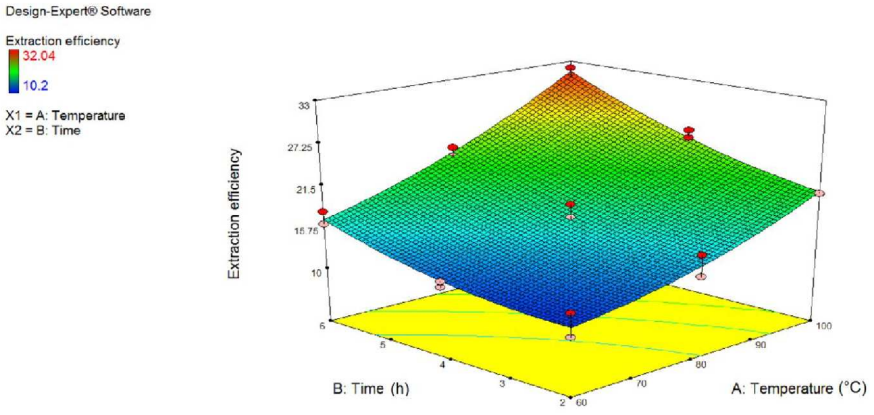

Figure 1. Effect of time and temperature on the extraction yield of tomato peels.

The extraction was performed under OFC at 100 °C for 6 h and the extraction yield was determined as 29.7 ± 0.6 g/100 g dry peel. This yield was slightly lower than the yield of AC (31.31 g/100 g dry peel) which was found statistically not significant ($p > 0.05$). The reason for the decline in the yield

was considered the absence/ lack of volatile oxidation by-products with low molecular weight. These products were further discussed in the results of fatty acid composition analysis.

Approximate composition analysis

The analysis revealed that TPE obtained at 100 °C for 6 h contained several weight fractions including cutin (32.79% ± 2.43), hemi-cellulose (28.87% ± 6.53), acid soluble pectin (10.97% ± 0.23), water soluble pectin and polyphenols (2.32% ± 0.38), lignin (11.48% ± 1.96), cellulose (11.16% ± 2.26), and waxes (2.42% ± 0.03) based on dry weight.

Morphological characterization

The images of SEM analysis are presented in Figure 2a-b for AC and OFC samples, respectively. As can be observed in both images, both TPE samples exhibited an irregular structure with an appreciable roughness; the particles on the surface had a non-uniform shape distribution. A wrinkled structure between the clusters is also visible in both samples. However, a non-homogenous distribution of finer particles having slightly smoother surfaces was observed on the OFC samples; the tortuosity was less apparent in the OFC samples. The roughness on the surface of tomato peels might depend on the presence of oxidation products (Heredia-Guerrero et al. 2012), which may explain the rougher surface of the morphology of the AC samples. Round-shaped objects might also indicate the presence of epicuticular waxes (Heredia-Guerrero et al. 2012).

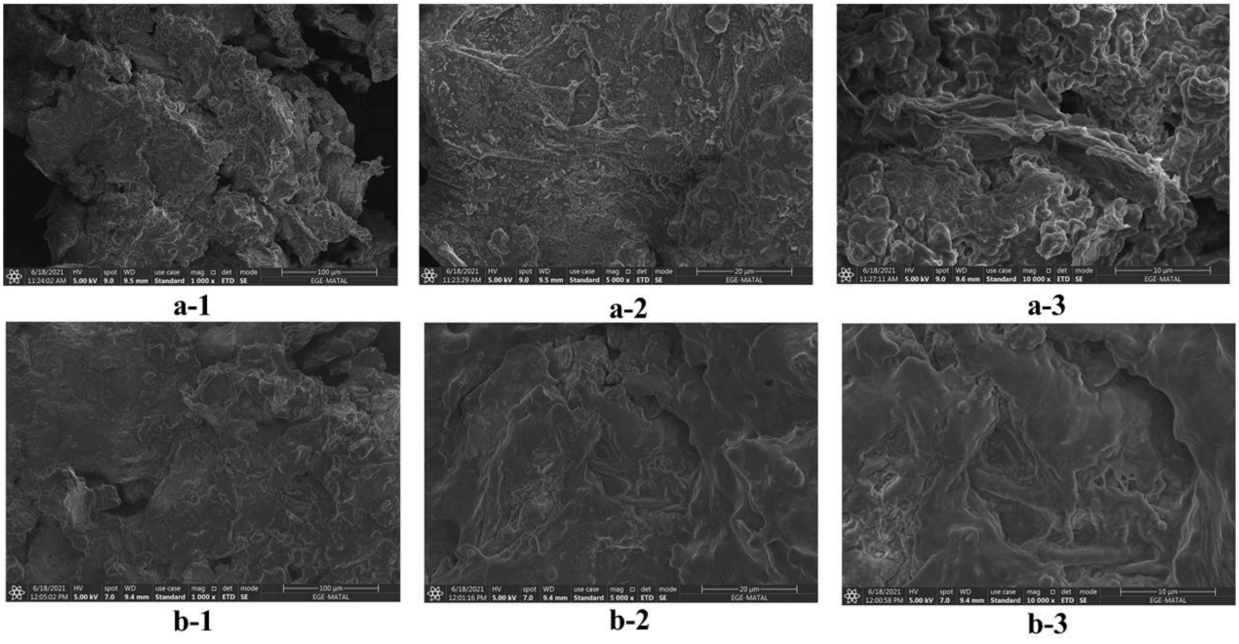

Figure 2. SEM images of the tomato peel extracts under a) atmospheric conditions: magnified by 1000 × (a-1); 5000 × (a-2); 10000 × (a-3); b) oxygen-free conditions: magnified by 1000 × (b-1); 5000 × (b-2); 10000 × (b-3).

Thermal characterization

TGA and DTG analyses

TGA and DTG analyses were performed to investigate the thermal stability and determine the decomposition stages of TPE. Figure 3 illustrates that the TGA curve of tomato skin extracts exhibited similar weight losses at three stages for both samples of AC and OFC. The thermal degradation of tomato peels has been reported to occur in three stages under atmospheric conditions (Midhun Prasad & Murugavelh 2020). The first weight loss corresponded to the loss from the adsorption of water in equilibrium with the atmosphere, which was considerably more noticeable in AC than in OFC; both extracts were stable until 200 °C. The second stage was observed between 200 °C and 500 °C, where a substantial decline in mass was noticed and the molecular breakdown of TPE occurred. The final stage was considered as the progress of pyrolysis reaction initiated after 500 °C where the change in the mass loss substantially decreased and plateaued; no appreciable weight loss was observed above this temperature, as also reported in a previous study (Toscano et al. 2015). This stage might be attributed to the bio-oil formation from tomato peels, composed of liquid, char, and gas fractions as reported by (Midhun Prasad & Murugavelh 2020).

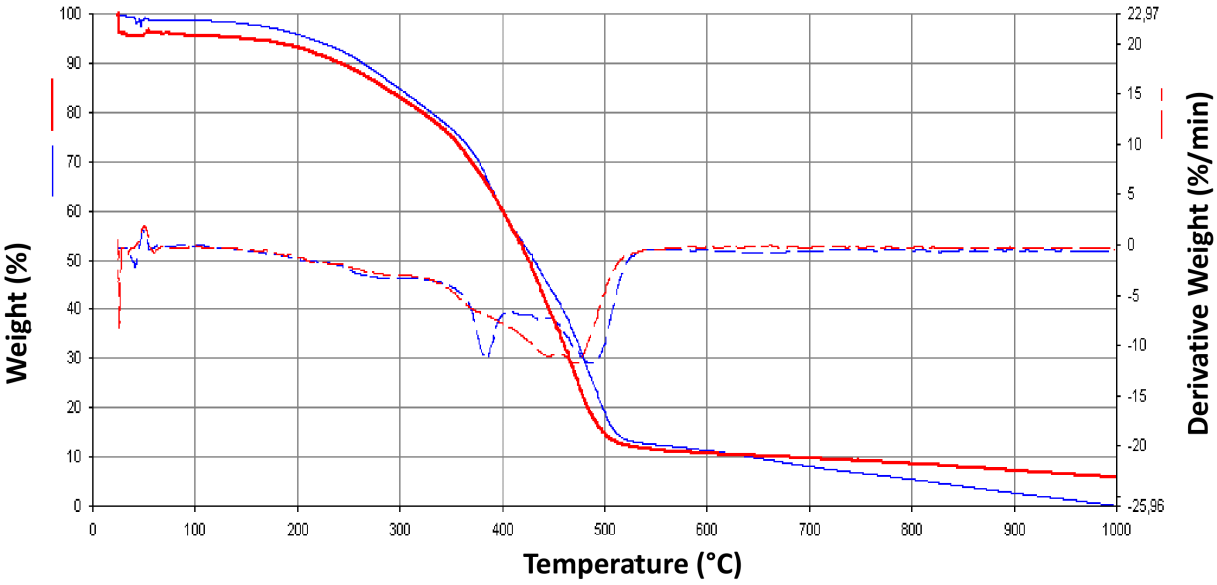

Figure 3. TGA (continuous line) and DTG (dashed line) curves of tomato peel extracts under the atmospheric (red) and oxygen-free (blue) conditions.

The results of the DTG analysis are illustrated in Figure 3. As indicated by the results, both extracts exhibited a multi-stage weight loss starting at approximately 400 °C; the phenomenon might be associated with the formation of a complex biomass structure. These findings are consistent with previously reported data (Cifarelli et al. 2016). The substantial mass losses that occurred after 400 °C in both samples might be associated with the CO_2 release caused by the depolymerization of aromatic compounds rich in lignin (Montoya et al. 2015, Baldwin et al. 2012). The peaking of these components at 428 °C and 449 °C might be due to lignin and non-lignocellulosic material (oils and waxes), respectively (Mangut et al. 2006). On the other hand, several studies have reported the losses

peaking at 300 °C and 500 °C in DTG curves as the degradation of hemicellulose and cellulose in the tomato pomace, respectively (Mangut et al. 2006, Aksit & Genccelep 2021, Brachi et al. 2016). However, it has been shown that the thermal decomposition of tomato wastes involved in complicated pyrolysis (Aksit & Genccelep 2021) with a wide range of peaks of lignin, hemicellulose, and cellulose which might partially overlap (Toscano et al. 2015, Sebio-Punal et al. 2012).

DSC analysis

As shown in Figures 4-5, two main endothermic peaks were detected in DSC thermograms of both extracts. The first peak at 52.5 °C (enthalpy change, 52.83 J/g) for the samples of atmospheric conditions corresponded to the melting point of cuticular waxes. The melting point of cuticular waxes was found 47 °C (enthalpy change, of 8.34 J/g) in OFC samples. The results are in agreement with literature (Luque et al. 1995). The second peak was detected at 176 °C and 170 °C in the AC and OFC samples, respectively. This peak could belong to lignin as lignin has a melting point of 170 °C (Patel & Parsania 2018, Nassar & McKay 1984), and 5.85% of the tomato pulp consists of lignin (Szymanska-Chargot et al. 2017).

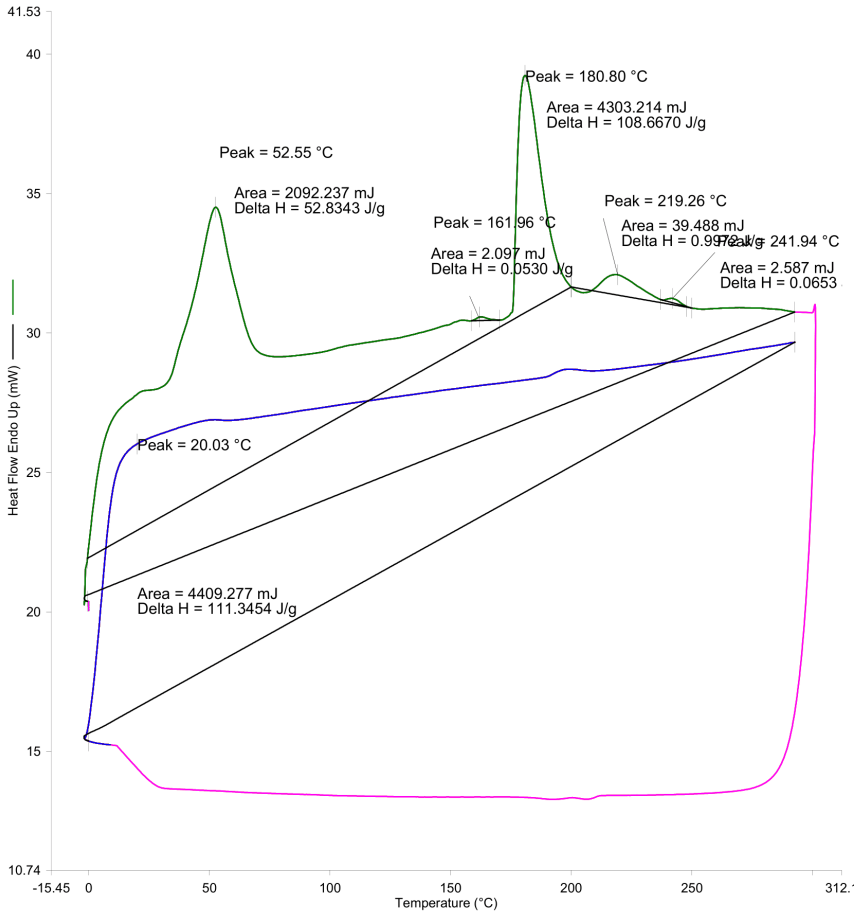

Figure 4. DSC thermogram of tomato peel extracts under atmospheric conditions: green line and blue line represent thermograms of the sample and reference material respectively.

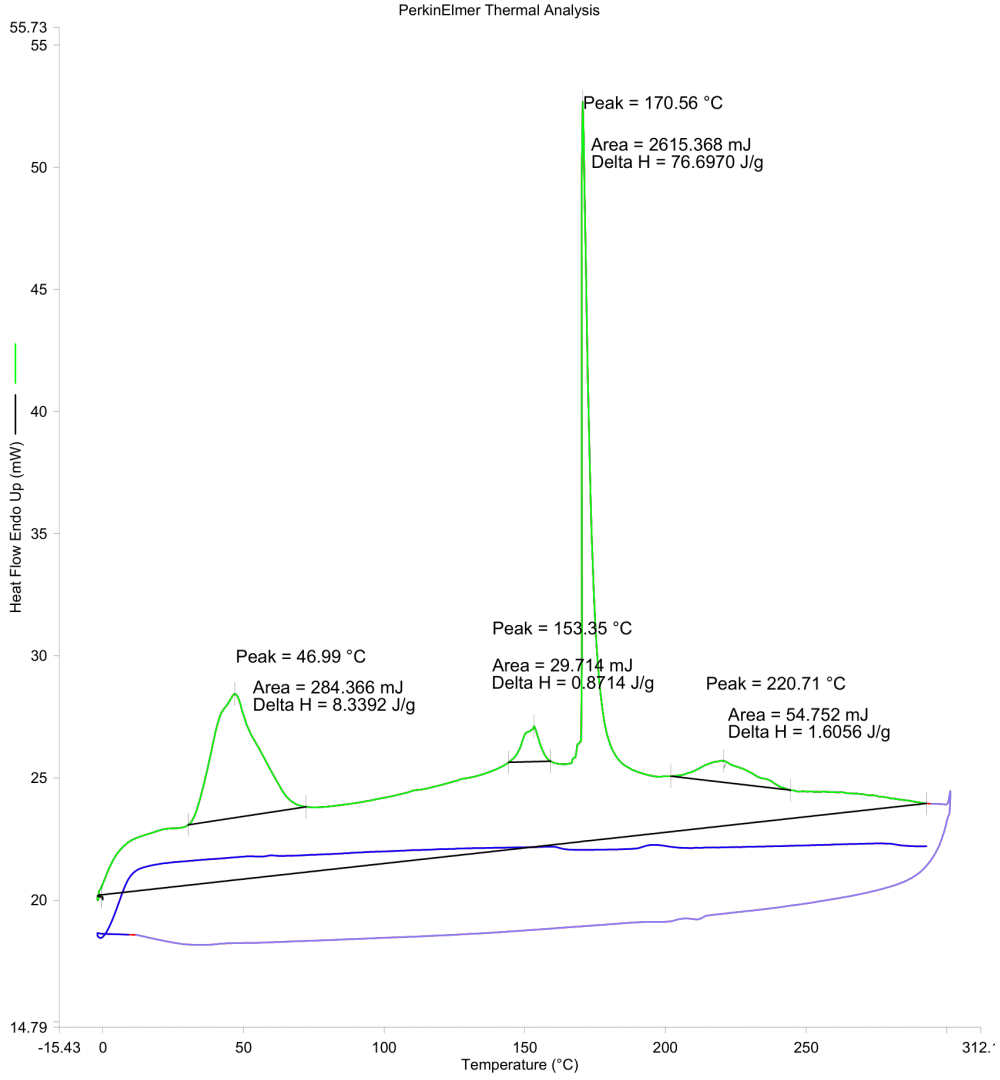

Figure 5. DSC thermogram of tomato peel extracts under oxygen-free conditions: green line and blue line represent thermograms of the sample and reference material respectively.

Additionally, the third endothermic peak with a weak intensity was depicted at 160 °C and 153 °C for AC and OFC samples, respectively. This peak might indicate the presence of α-D galacturonic acid, which is the main component of pectin with a melting point of 159 °C (Anonimous 2021). Alkaline extraction conditions cause the formation of labile pectin molecules in the backbone (galacturonic acid), resulting in the decomposition of pectin (Abang-Zaidel et al. 2017). Moreover, the FT-IR findings of the present study also confirm the presence of pectin in TPE. It has also been noted that the heat-alkaline treatment of tomato peels might cause a partial degradation of pectin through β-elimination (Diaz et al. 2007). Thus, it can be presumed that the polymer matrix extracted under both conditions was mainly composed of cutin while containing other components such as lignin and pectin, which might have enhanced the thermal stability of TPE.

Chemical characterization

FT-IR analysis

As shown in Figure 6, FT-IR spectra of both AC and OFC samples were highly similar in terms of band assignments. The spectra also enabled the identification of bands in relation to the chemical structure of the hydroxy acids peculiar to cutin as well as the presence of phenolic compounds in addition to the cell wall polysaccharides. The first peak at a band of approximately 3300 cm^{-1} in both samples can be attributed to the presence of O-H stretching (Benitez et al. 2018, Cifarelli et al. 2016, Midhun Prasad & Murugavelh 2020). A previous study has also reported the presence of aromatic compounds with a C-H stretch between the bands of 860-680 cm^{-1} in both samples (Midhun Prasad & Murugavelh 2020). Out-of-plane bending (C-O) and (C-C) in phenolic compounds were observed at 835 cm^{-1} and 834 cm^{-1} for AC and OFC, respectively. The main differences between the spectra of the samples of AC and OFC are the bending CH_2 at 721 cm^{-1} that is absent in the AC sample and the bending CH_2 at 1462 cm^{-1} that is absent in the spectra of the OFC sample. These bendings indicate the presence of long-chain compounds (Heredia-Guerrero et al. 2014). The bands ascribed to the carbonyl stretch of carboxylic acid to ester were va(C-O-C) band at 1168 and 1105 cm^{-1} in the AC and vs(C-O-C) at 1169 and 1119 cm^{-1} in the OFC samples. The results are consistent with those of Benitez et al. (2018) and Cifarelli et al. (2016).

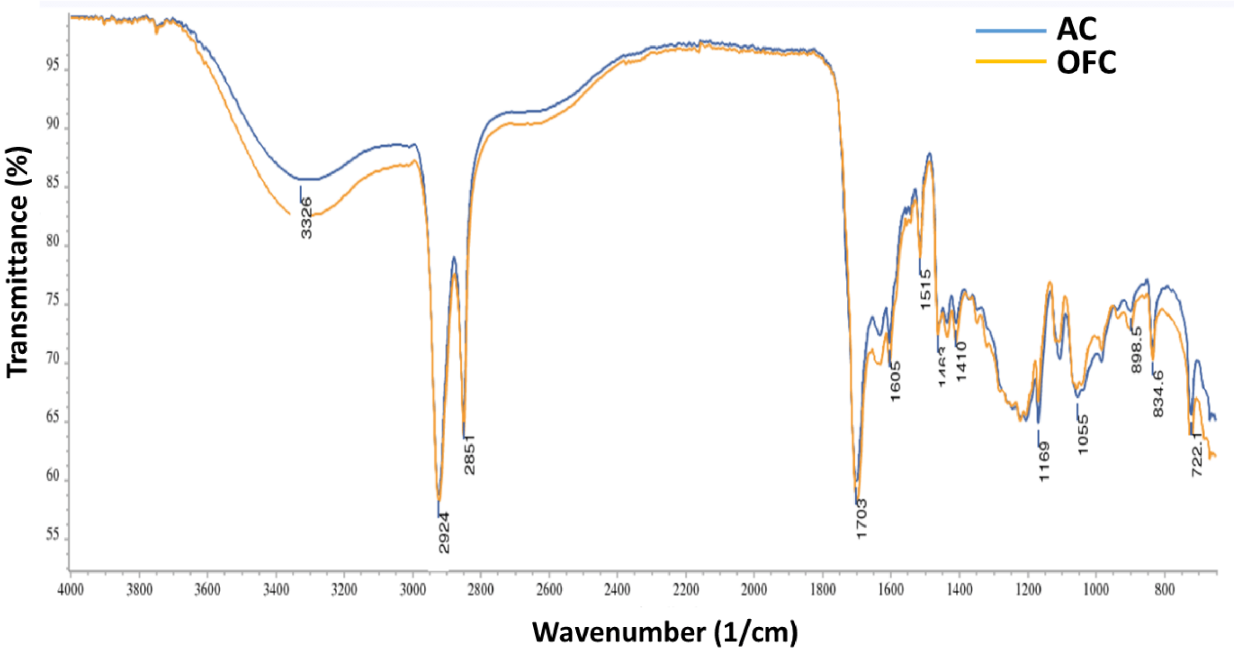

Figure 6. FTIR spectrum of AC and OFC extracts.

Additionally, the band assigned to the (C=O) stretching asymmetric carboxylic acid at 1279 cm^{-1} was not observed in the AC sample. Previous studies have reported primary and secondary stretching of (C-O) as well as the bending of (O-H) in polysaccharides (Benitez et al. 2018, Cifarelli et al. 2016), as found in this present study. Studies have also reported the bands ascribed to stretching (C-O) as well as (C-C) at 1243 and 1229 cm^{-1} in pectin (Benitez et al. 2018, Szymanska-Chargot & Zdunek

2013). These bands were also observed in our study. It was reported that bands at 1045 and 1059 could be assigned to C-O and O-H deformations of secondary alcohols which may be evident for the presence of cellulose in the TPE (Fengel 1993). Several investigators also confirmed these findings and stated that cellulose was the main polysaccharide present in the cuticle by pointing at the possible depolymerization of cellulose into glucose (Villena et al. 2000). It was revealed that pectin, cellulose and hemicellulose were detected in similar relative amounts (each fraction around 25% of the total polysaccharides present in the cuticles) by the X-ray diffraction pattern (Lopez-Casado et al. 2007). Table III illustrates the assignments of the signals in the FT-IR spectra with respect to the chemical structure of the compounds present in TPE.

Table III. Assignments of the signals in the FTIR spectra with respect to the chemical bonding structure of the compounds present in the tomato peel extracts.

Peaks	FTIR / cm^{-1}		Assignment[b]
	AC	OFC	
1	3343	3326	str O-H---O
2	2924	2924	str asymmetric CH_2
3	2851	2850	str symmetric CH_2
4	1701	1698	str C=O free acid group
5	1629	1643	str C=C double bonds of phenolic acids
6	1605	1604	str C-C aromatic
7	1558	-	str C-C aromatic conjugated with C=C
8	1515	1515	str C-C aromatic conjugated with C=C
9	1466	1464	str. C-C aromatic in phenolic compounds
10	1463	-	bend CH_2
11	1436	1436	str C-C aromatic in phenolic compounds
12	1410	1410	bend (O-H) carboxylic acid
13	1343	1364	NA[e]
14	1331	1336	ring vibration of pectin[c]
15	1309	1314	wagging CH_2[f]
16	-	1279	(C-O) streching asymmetric carboxylic acid
17	1253	1250	bend (O-H) of primary and secondary of possible polysaccharides
18	1243	1229	str (C-O) of pectin[c]
19	1217	1223	str (C-C) chain
20	1203	1203	NA[e]
21	1168	1169	str. asymmetric and symmetric C-O-C ester
22	1105	1119	str. asymmetric and symmetric C-O-C ester
23	1045	1057	bend of (O-H) primary and secondary or str. (C-O) in polysaccarides
24	1033	1029	str. (C-O); (C-C) pectin[d]
25	950	929	bend (O-H) carboxylic acid
26	902	898	bend C1-H β anomeric link of cellulose, xyloglucane[f,g]
27	852	-	Out of plane bending (C-O) and (C-C) aromatic
28	835	834	Out of plane bending (C-O) and (C-C) aromatic
29	-	721	bend CH_2

[a] Peaks are displayed in Figure 6; [b] reported by Cifarelli et al. (2016); [c] reported by Szymanska-Chargot & Zdunek (2013); [d] reported by Benitez et al. (2018); [e] Data not available; [f] reported by Toscano et al. (2015); [g] reported by Kacuráková et al. (2002).

Fatty acid composition

The GC-MS analysis showed that both samples mainly consisted of cutin (10, 16 dihydroxy hexadecanoic acid) in abundance of 70.45% and 68.14% for AC and OFC samples, respectively, as presented in Supplementary Material – Table SI.

Other components such as unsaturated and saturated fatty acids were also found in lower or minor abundances. The molecular abundance of cutin was substantially higher than that reported as 43.3% (Benitez et al. 2018) and lower than that reported as 92.2% (Rodriguez et al. 2020). These differences might be associated with the extraction conditions, extraction method, and concentration of the solvent used during the extraction; the differences might also be associated with the difference in compositions of the raw materials used (tomato pomace and tomato peel). On the other hand, a previous study has reported an abundance of 62% (Cifarelli et al. 2016), which is close to the result of the present study for cutin at the same extraction conditions. The other significant components in both extracts were in a descending order of molecular abundance (%) as follows: p-coumaric acid > linoleic acid > palmitic acid > oleic acid. Cifarelli et al. (2016) also reported the presence of these compounds.

No significant difference was observed in terms of the total abundance of unsaturated fatty acids and saturated fatty acids between the two extraction conditions. However, substances that might not be observed in the samples of OFC were detected in minor abundances in the samples of AC. These substances included several oxidation by-products (e.g., succinic and malic acids) and some phenolics (e.g., p-hydroxybenzoic and cinnamic acids).

Functional properties

Total phenolic content analysis

The TPC results showed substantial values of 3535.0 ± 183.2 and 4432.6 ± 43.8 mg GAE/100 g dried powder for samples of AC and OFC, respectively. The difference between the results was statistically significant ($p < 0.05$). This result further indicates that phenolic substances can be preserved better under OFC as expected. Unfortunately, no data has been found in the literature with respect to the total phenolic content of tomato peels treated with an alkaline solution. Several studies only investigated the total phenolic content of tomato peels extracted with organic solvents, such as methanol and ethanol, and the total phenolic content was determined as 10.08 ± 0.18 to 903.5 ± 7.1 mg GAE/100 g dried extract in these studies (Szabo et al. 2019a, Tamasi et al. 2019, Valdez-Morales et al. 2014). However, some other investigators found that the TPC of tomato peels ranged between 1688.16 ± 12.47 and 3878.91 ± 1.42 mg GAE/100 g dry extract (Nincevic Grassino et al. 2020). The highest TPC was obtained after a 12 h extraction with 70% of ethanol, whereas after a 6 h extraction (the same extraction period in our study), the TPC was determined as 2866.54 ± 6.51 mg GAE/100 g, which is lower than the result of our study.

These findings suggest that the TPC of tomato peels may vary widely based on the extraction conditions (type of extraction method, solvent, solvent/solute ratio, extraction temperature, extraction period, etc.). Our results demonstrated that the alkaline treatment in the extraction process provided a higher amount of total phenolic content compared with the results of studies that used organic

solvents in the extraction process. This may be due to the alkaline treatment at a high temperature that enabled the extraction of the lipophilic part as well as hydrophilic part which is easily obtained with organic solvents. Because the lipophilic fraction of TPE consists of the bound phenolics that are covalently linked to cell wall carbohydrates such as lignin, cellulose, and hemicellulose (Perea-Domínguez et al. 2018), and this fraction might be released more easily by alkaline hydrolysis (Liyana-Pathirana & Shahidi 2006). Thus, the bound phenolics that are mostly abundant in tomato by-products, constitute 67% of the total phenolics and were not extractable by aqueous ethanol but can be extracted by alkaline hydrolysis (Perea-Domínguez et al. 2018). The efficacy of alkaline method in extraction of bound phenolics was also reported by several authors in different fruits and vegetables (Padayachee et al. 2012, Wang et al. 2019, Aarabi et al. 2015). This was probably caused by alkaline hydrolysis which might have facilitated the cleavage of ester bonds between phenolic acids and polysaccharides that prevented the loss of phenolic acids (Kim et al. 2006). Aarabi et al. (2015) reported a more than three times higher TPC in sugar cane with 0.5 M NaOH extraction for 6 h compared to that yielded by methanolic extraction and stated that some phenolic compounds could be more efficiently extracted due to the hydrolysis of covalent esteric bonds. It was also found that alkaline extraction was the most ideal method for the extraction of bound phenolics present in mulberry leaves (Wang et al. 2021).

Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH Radical Scavenging Activity

The TEAC values of TPE were determined as 7203 ± 71.5 to 7298 ± 212 µM Trolox/100 g dried extract in ABTS assay and 2793.1 ± 21.2 and 2949.2 ± 148.4 µM Trolox/100 g dried extract in DPPH assay for AC and OFC samples, respectively. No significant difference was found between these extraction conditions in both assays ($p > 0.05$). However, no data have been reported in the literature with respect to the ABTS and DPPH assays of tomato peels treated with an alkaline solution. Several studies investigated the antioxidant capacity of tomato peels extracted with organic solvents such as methanol and ethanol by using different extraction methods. Valdez-Morales et al. (2014) found the TEAC values of the peel extracts of four different tomato cultivars between 47.9 and 405.7 µmol TE/ 100 g dw and the DPPH antioxidant capacity 51.4-181.4 µmol Trolox/100 g dw by ultrasound assisted extraction at 80 °C for 1 h using methanol. In another study, the antioxidant activity of ten different tomato peel varieties extracted by ultrasonication with 80% of ethanol for 15 min was between 120 ± 2 and 250 ± 3 µmol Trolox/100 g dw (Szabo et al. 2019a). These results indicate that the antioxidant capacity of tomato peels might vary widely depending on the extraction process, conditions, and type of cultivar of tomato fruit.

It was found that the alkaline extraction of bound phenolics that cannot be extracted with organic solvents (Perea-Domínguez et al. 2018), also contribute more to the antioxidant activity compared with the free and esterified fractions (Liyana-Pathirana & Shahidi 2006). The elevated temperature and long extraction time combined with alkali treatment enabled the extraction of the lipophilic fraction of TPE. This fraction includes the phenolic compounds which were bound to cell wall polysaccharides such as lignin, cellulose and hemi-cellulose covalently (Navarro-Gonzalez et al. 2011). These compounds can be easily extracted by alkali (Perea-Domínguez et al. 2018). Hence, the majority of the scavenged

components originating from bound phenolics present in AC and OFC samples might have caused higher antioxidant capacities in this study compared with previous studies.

Phenolic composition analysis by LC-QTOF-MS

The LC-QTOF-MS analysis indicated that both extracts of AC and OFC have a considerably similar phenolic profile. In this study, 16 of the same metabolites including several phenolic acids, flavonoids, and stereo glycoalkaloids were detected for AC and OFC as presented in Table SII. Naringin and α-tomatine could only be detected in AC samples, whereas rutin could only be detected in OFC samples. Seven metabolites were putatively assigned in the positive ion mode while twelve metabolites were detected in the negative ion mode. The phenolic acids were mostly detected in the negative ion mode whereas several stereo alkaloids were detected in the positive ion mode. This result is consistent with the results of a comprehensive investigation covering the database of the metabolomics present in the tomato peels and fleshes of 96 different tomato cultivars by LC-MS (Moco et al. 2006). In contrast to the findings of Moco et al. (2006), more flavonoids could be putatively assigned in the negative ion mode in our study.

Moreover, Tamasi et al. (2019) detected appreciable amounts of caffeic acid and ferulic acid, small amounts of α-tomatine, and trace amounts of p-coumaric acid and rutin by HPLC-MS/MS on the skins of several tomato cultivars, and these phenolic compunds were also detected in our samples. Other studies confirmed the presence of caffeic acid and rutin (Carrillo-López & Yahia 2013), p-coumaric and ferulic acids (Carrillo-López & Yahia 2013, Luthria et al. 2006) in the skins as in our study. In the cell wall structure of TPE, alkali labile cross linkages of etherified ferulic acids and coumaric acids might have been formed between two polymers (lignin and pectin) (Torre et al. 2008). The presence of these cross-linkages might explain the rapid solubilization of both phenolic acids by alkaline treatment (Noor Hasyierah et al. 2011). It was also reported that alkaline extraction led to the conversion of chlorogenic acid into caffeic acid (Carrillo-López & Yahia 2013), which might explain the absence of chlorogenic acid in the LC-MS analysis of our study.

Antimicrobial activity

MIC method

Both samples of AC and OFC indicated substantial antimicrobial activities. No significant difference was found between the MIC values of both extracts ($p > 0.05$). The results corresponding to the MIC values of TPE are presented in Table SIII.

Both samples of AC and OFC were most effective against S. *aureus*, E. *coli*, C. *albicans*, and A. *brasiliensis* with a MIC value of 100 µg TPE/ mL. The antimicrobial effect of both samples against L. *monocytogenes* (MIC= 200 µg/ mL for both samples) was less than the effect against the above-described microorganisms. On the other hand, no antimicrobial effect could be observed against B. *cereus* in both extracts within the range of the investigated concentrations (3.13-400 µg/ mL).

The literature lacks information about the antimicrobial activity of tomato peels extracted under alkaline conditions. Tomato peels had an antimicrobial activity ranging from 2500 to 5000 µg/ mL

in terms of MIC against *S. aureus*, E. *coli*, and L. *monocytogenes* (Szabo et al. 2019a). Other similar results have been reported against the same microorganisms as the MIC value of tomato pomace ranging from 625 to 2500 µg/ mL (Szabo et al. 2019b) and of the tomato seeds ranging from 5000 to 20000 µg/ mL (Taveira et al. 2010). Compared with these values, our results indicate a much higher antimicrobial activity of tomato peels. Hence, several components with high antimicrobial potential that could not be achieved by organic solvents could be extracted from tomato peels through alkaline treatment.

Time-Kill assay

To determine the bactericidal and fungicidal effect of TPE on the microorganism kinetics, time-kill assay was applied. The results of each microorganism are illustrated in Figure 7. Overall, the inhibition of the bacteria took a shorter time compared with that of the fungi. After 3 h of exposure, the inhibition of the S. *aureus* growth reached a level of over 5 log. As L. *monocytogenes* is more resistant than other bacteria, a reduction over 5 log units could only be achieved after 6 h. While OFC extracts managed to inhibit and resulted in a reduction of over 5 log in the growth of *C. albicans* after 12 h, the AC extracts could exhibit almost the same effect on the same microorganism only after 24 h. On the other hand, a reduction of over 4 log was achieved in the growth of A. *brasiliensis* after 24 h by the inoculation of both extracts. Nevertheless, the antifungal effect of the TPE was significant as the spores of A. *brasiliensis* are resistant to several chemicals. Consequently, TPE has stronger bactericidal effects than antifungal effects.

CONCLUSION

Tomato peels are an important waste in the tomato industry, and it is crucial to investigate the properties of tomato peels to evaluate them in value-added applications. This paper described the optimization of the alkaline extraction method, which is the preferred method for the extraction of tomato peels. The alkaline extraction method was demonstrated to depend strongly on temperature and time and could be modelled by a quadratic effect of these factors as the best fit approach. The extraction yield was determined as 31.31 ± 0.73 g/100 g dry peel.

Slight differences were observed in the morphological, chemical, thermal, biochemical, and antimicrobial properties of both samples of AC and OFC. Namely, both extracts were thermally stable until 200 °C, after which the degradation of tomato peels proceeded rapidly in a multi-stage process where the increase in the mass loss reached a maximum between 300 °C and 500 °C. The extracts seemed to have a similar chemical structure with respect to FT-IR analysis although noticeable differences existed in the bands of the CH_2 bending and in the (C=O) asymmetrical stretching of carboxylic acids.

The alkaline extraction process with heat showed a substantial amount of antioxidant capacity and considerably high antimicrobial activity. Both extracts exhibited extremely high total phenolic content and antioxidant capacity, which might be attributed to prolonged heating and alkaline treatment. The total phenolic content of the samples extracted under OFC compared to the AC was higher; however, both extracts exhibited nearly the same antioxidant capacities. Alkaline

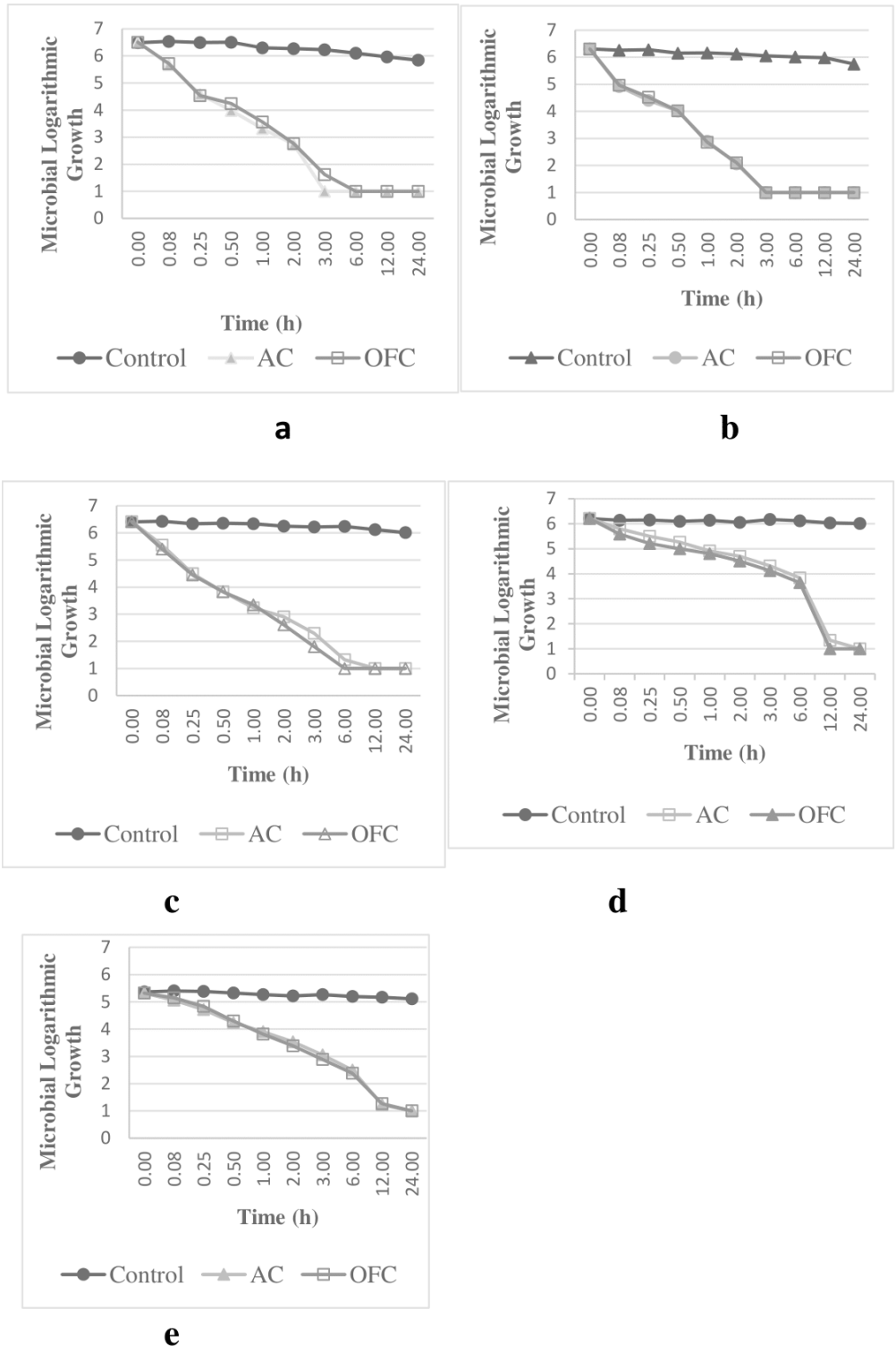

Figure 7. Microbial growth of S. *aureus* (a), E. *coli* (b), L. *monocytogenes* (c), *C. albicans* (d), A. *brasiliensis* (e) in tomato peel extract inoculated medium during an incubation period of 24 hours.

treatment enabled the extraction of bound phenolics in appreciable amounts, which might have also increased the antioxidant capacity. Both extracts exhibited various metabolites (several phenolic acids, flavonoids, and stereo glycoalkaloids) and significant antimicrobial activity against some gram-positive and gram-negative bacteria as well as some pathogenic fungi that may cause food spoilage. Due to the high antioxidant and antimicrobial activity, TPE can be used as a functional ingredient in producing composite food packaging material in future applications.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Tables SI, SII, SIII.

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