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Hydrothermal–Microwave Processing for Starch Extraction from Mexican Avocado Seeds: Operational Conditions and Characterization

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Abstract: Avocado seeds are an agroindustrial residue widely produced in Mexico that are causing various environmental problems due to their accumulation. The evaluation of avocado residues to recover biopolymers by microwave-assisted extraction (MAE) and the characterization of avocado starch properties were studied in the present work. A central-composite design was used to optimize the MAE process. Moreover, a comparison was performed between MAE non-isothermal mode (NO–ISO) and conventional extraction. Starch optimization by MAE was obtained at 161.09 °C for 56.23 min with an extraction yield of 49.52% ± 0.69%, while with NO–ISO at 161 °C was obtained 45.75% ± 2.18%. Conventional extraction was 39.04% ± 2.22%. Compared with conventional starch, MAE starch showed similar proprieties and molecular spectra. In contrast, MAE starch showed high solubility, low water absorption capacity, a non-granular structure with small particle size (<2 μm) and polydispersity of fragments at different sizes of polymers. Therefore, MAE is a viable technology to extract the starch, and avocado seed can be considered an excellent starch source for the development of novel functional foods, contributing to promoting sustainability across the food chain.

Keywords: avocado seeds; microwave-assisted extraction; starch source

1. Introduction

Avocado (*Persea americana* Mill) is a native fruit of Mexico and Central America that is nowadays consumed worldwide due to its very complete nutritional content and many positive health benefits [1,2]. In 2018, avocado production in Mexico was more than two million tons, valued at more than five billion dollars, and representing more than 34% of worldwide production [3,4].

The avocado seed represents approximately 13–17% of the fruit. It is composed of many bioactive compounds, sugars, proteins and starches, which are the main compound of approximately 60% by dry weight [5–7]. However, avocado waste—like many fruit wastes—is a growing concern worldwide, as it represents an environmental, social and economic problems [8]. For the modern sustainable food industry, waste generated is one of the main concerns. Therefore, the recovery of fruit waste offers the possibility of obtaining ingredients with new nutritional and functional properties. Its management is also a crucial issue for the modern avocado industry.

Starch is a natural biopolymer and the main reserve of carbohydrates in plants as a form of glucose [9]. Starch is an important sustainable resource for various industries that can be obtained easily from fruits, seeds and tubers. The most common sources of commercial starch are cereals such as corn and wheat as well as the roots or tuberous of cassava and potato [9,10]. Tuberous plants have about 16–24% of starch in weight; the rest is water and traces of lipids and proteins. In contrast, the content of starch in cereals can be more than 60% and 10–20% of fibers, proteins and lipids, which is an important factor to take into consideration for manufacturing processes and further transformations [10,11]. Starch is an abundant, available, renewable, versatile and biodegradable polymer composed mainly of amylose, amylopectin and some minor components as protein, lipids and minerals that can be extracted with high purity [12,13]. Amylose is a linear polymer of α-1,4-linked glucans. Amylopectin is a larger molecule with highly α-1,6 branched chains [10,14–16]. Normaly, starches contain 75–80% amylopectin and 20–25% amylose, with some exceptions such as modified some starch that contains only amylopectin. The morphology, structure, size and relationship of amylose and amylopectin starch granules are variable depending on botanical source, stage of plant development and environmental conditions. These make starch granules a complex polysaccharide and variable [15]. Starch is a polymer with structural and physical properties that promote high demand, which is reflected in a growing market in recent years [17]. Thickening, gelling, stabilizing and binding properties make starch a widely used ingredient in food, cosmetic, pharmaceutical, textile, study, biodegradable materials and other industrial products. [15,16,18,19]. Currently, there is a great need to find new sources of starch that are natural and economically viable to use and reduce common sources and decrease the use of essential foods [20].

Some authors have reported works on the extraction and characterization of starch from avocado seeds, but using only by conventional extraction, such as crushing the seed with the addition of some salts such as sodium bisulfite, followed by several washes and filtrations to recover the starch, and a starch extraction yield about 20% dry weight [9,15,20]. Hydrothermal processing such as microwave-assisted extraction (MAE) is considered a green and safe technology to extract value-added compounds such as polyphenols and polymers such as pectin or starch due to its ease of use and the possibility of using only water as extraction solvent [21,22]. The microwave extraction mechanism is based on heating induced by microwave radiation, which creates a dipole moment caused by the agitation of water molecules within the extraction material, which sometimes causes pores or breakage of plant material and consequently a greater release of compounds [23,24]. This type of extraction is considered a technology with several advantages, such as short extraction times, higher extraction performance and less solvent consumption. For this reason, MAE technology is used on an industrial scale to obtain bioactive compounds—mainly polymers, antioxidants and oils rich in carotenoids or extracts of polyphenols—from plants and residues. Sometimes it is combined with other techniques such as ultrasound and pulsed electric field—or with some modifications to the equipment such as continuous MAE [24,25].

The objective of the present study was to evaluate and optimize the MAE conditions of avocado seed starch and characterize and promote an unconventional and viable source of starch for introduction in different industrial applications.

2. Materials and Methods

2.1. Raw Material

Avocado seeds were obtained from *Hass* variety in a ready-to-eat ripening stage from a local restaurant in Saltillo, Coahuila, Mexico. The seeds were washed to remove pulp residues and cut into small pieces with a kitchen knife for further freeze-drying (Labconco Freezone 4.5, Kansas City, MO, USA) [26]. Lyophilization was used to be able to create a homogeneous batch of avocado seed to find the optimal conditions of extraction, which was not achieved using fresh seed since great variability was obtained in the same treatments, derived from variations of starch content between seeds. on the other hand, the convection drying method decreased the starch extraction yield perhaps because the heating caused Maillard reactions on the surface of the seed pieces which created a caramel-like layer. Once lyophilized, the seeds were ground using a blender (Osterizer®, 10 velocities) for 5–10 s and sieved with mesh numbers 18 and 35 to obtain a particle size between 0.3–1 mm. The flours were stored at room temperature protected from light and air. The determination of the proximal composition of the avocado peel was determined by the standards of official methods of analysis (AOAC 1990) [27] to lipids, protein and ashes, 932.06, 925.09, 923.03 methods, respectively and 991.43 for insoluble and soluble fiber.

2.2. Avocado Starch Extraction by MAE

The starch was extracted by MAE (CEM Mars 6, USA) with temperature control in Teflon vessels of 70 mL (Xpress). The extraction was performed with a relationship of 1:20 (*w/v*), 20 mL of water with 1 g of the dry avocado seed, 2.45 GHz and 1200 W [28]. Each treatment was performed by triplicate. After extraction, the liquid phase was recovered by filtration and then the starch was precipitated with ethanol with a relationship 1:2 (*v/v*), after 12 h of precipitation, the starch was recovery by decantation and centrifugation at 4000× *g* by 10 min (Hermle Z326 K, Wehingen, Germany) and finally freeze-dried [29]. The starch yield was calculated by the following Equation (1):

$$Yield\ (\%) = \frac{mass\ of\ dry\ starch\ recovered}{mass\ of\ dry\ avocado\ seed} \times 100\% \quad (1)$$

Optimization of Starch Extraction

A central composite design (CCD) with a 99% confidence level was used to obtain optimal conditions for the highest starch extraction yield. For the optimization of starch extraction yield from avocado seeds, a response surface methodology was applied. Table 1 shows the independent variables of temperature (X1, °C) and time (X2, min) for three variation levels on starch extraction yield (%), obtained of a CCD with two factors and 3 replicates of the center point leading. Low and high factors were coded as −1 and +1; the center point was coded as 0. The data were analyzed by statistical software STATISTICA 7® to obtain the optimal condition (OC) and later validated by extraction with the conditions estimated by the software. The validation was performed by triplicate. The results were analyzed by analysis of variance (ANOVA), and the responses and variables (in coded unit) were correlated by response surface analysis to obtain the coefficients of Equation (2):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij}\, X_i X_j \quad (2)$$

In Equation (2), Y represents the response or dependent variable (starch yield extraction); β_0 is the interception coefficient; β_i, β_{ii} and β_{ij} are the coefficients estimated by the model and X_i and X_j are the coded levels of the independent variables (temperature and time).

Table 1. Experimental conditions used for starch extraction from avocado seed according to central composite design with two variables, temperature (X_1) and time (X_2) and results obtained for the response starch extraction yield (% (*w*/*w*)).

Assay	Variables				Starch Yield (% (*w*/*w*))
	X_1		X_2		
1	90	(−1)	30	(−1)	26.13 ± 0.87
2	90	(−1)	60	(1)	27.48 ± 1.87
3	90	(−1)	45	(0)	27.36 ± 4.92
4	180	(1)	30	(−1)	47.31 ± 0.85
5	180	(1)	60	(1)	44.82 ± 1.00
6	180	(1)	45	(0)	46.06 ± 0.81
7	135	(0)	30	(−1)	43.75 ± 0.28
8	135	(0)	60	(1)	45.48 ± 0.26
9	135	(0)	45	(0)	42.97 ± 0.53
10	135	(0)	45	(0)	44.15 ± 1.46
11	135	(0)	45	(0)	43.88 ± 3.83

The OC was extracted and validated in non-isothermal (NO–ISO) mode to decrease the energy expenditure of isothermal extraction and compare if significant differences are found.

2.3. Avocado Starch Extraction by a Conventional Method (CONV)

At present, the conventional process of starch extraction includes crushing of the vegetal material using water in a solution of salts as sodium metabisulfite or sodium bisulfite at 0.1–0.2% (*w*/*v*) [15,18,30–32], sodium hydroxide solutions 0.05–0.1% (*w*/*v*) [33,34] or extractions with enzymatic catalysts (xylanase protease) [35]. To extract starch from the avocado seed was followed the methodology described by Chel-Guerrero et al. [15], with some modifications. Seed powder was mixed with a sodium bisulfite solution (1500 ppm) at 1:5 (*w*/*v*) and was powdered in a blender (Oster) for 30 s twice. The mash was filtered through a sieve of fine muslin cloth of 150 mm mesh size to obtain the starch in a liquid fraction and separate the fibers. The recovered starch was washed three times with distilled water and then centrifuged at 2200× *g* for 10 min (Hermle Z326K, Wehingen, Germany). The starch was dried at 40 °C for 12 h in a convection oven. The starch yield was calculated by Equation (1). The resulting starch was powder and stored at room temperature in a sealed flask.

2.4. Starch Characterization

Starch characterization was performed to identify MAE modifications in avocado starch with OC and OC NO–ISO and compare with the starch obtained by conventional extraction.

2.4.1. Water Absorption and Solubility of Starch

Solubility, swelling power and water absorption patterns were measured at 60, 70, 80 and 90 °C following Chel-Guerrero et al. [15] with some modifications. Briefly, 20 mL of a 1% starch suspension (*w*/*v*) was prepared in a previously tared 50 mL centrifuge tube. The tubes were kept at a constant temperature (60, 70, 80 and 90 °C) in a water bath for 30 min with manual agitation at each 5 min. The suspension was then centrifuged at 2120× *g* for 15 min, the supernatant decanted and the swollen granules weighed. Water absorption capacity was measured using the same samples but was expressed as the weight of the gel formed per sample, divided by sample weight. Solubility and swelling power were calculated using the following Equations (3) and (4):

$$Solubility\ (\%) = \frac{dry\ weight\ at\ 120\ ^\circ C}{weigth\ of\ sample\ (g)} \times 400\% \quad (3)$$

$$Swelling\ power = \frac{weight\ of\ swollen\ granules\ (\mathrm{g})}{sample\ weight\ (\mathrm{g}) \times (100 - \%\ solubility)} \quad (4)$$

2.4.2. Scanning Electron Microscopy (SEM)

SEM analysis was performed in a scanning electron microscope Philips XL30, ESEM (environmental scanning electron microscope) with a GSED (gaseous secondary electrons detector) [36]. The samples were mounted in a metallic bracket and a gold/palladium coating was applied to provide conductivity. The micrographs were obtained with an accelerating potential of 30 kV under a low vacuum. Magnification of 750 and 7500 were selected for the study.

2.4.3. Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the samples were recorded on the spectrophotometer PerkinElmer Frontier with attenuated total reflectance (ATR). The analysis was conducted from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans [37].

2.4.4. Thermogravimetric Analysis (TGA)

The TGA spectra of the samples were recorded PerkinElmer TGA 4000 thermogravimetric analyzer. The samples (4–5 mg) were heated from 30 to 600 °C at 20 °C/min with a nitrogen gas flow of 20 mL/min [38].

2.4.5. Differential Scanning Calorimetry (DSC)

The thermal characteristics of avocado starch were determined as follows: Gelatinization onset (T_o), peak (T_p), conclusion (T_c) temperatures and melting enthalpies per gram of dry starch (ΔH) were measured in excess water (1:3 starch dry matter (dm): water) using differential scanning calorimeter (DSC) (Q2000, TA Instruments, New Castle, DE, USA) and results were analyzed with TA Universal analysis software©. Samples were heated from 25 to 250 °C at 5 °C/min. An empty pan was used as a reference. Calibration was performed with indium.

2.4.6. Wide-Angle X-Ray Scattering

The diffractogram was performed with powder starch containing 10% of moisture, an interval of 2θ angles ranged from 7° to 50° in the X-ray diffractometer (Panalytical Empyrean, Netherlands), at a rate of 4.3°/min operating at a power of 40 kv/30 mA [39]. The crystallinity degree was calculated by the following Equation (5):

$$Cd = \frac{Ac}{Ac + Aa} \times 100\% \quad (5)$$

where *Cd* means the relative grade of crystallinity; *AC* means the crystallinity area of X-ray diffractogram and *Aa* means to an amorphous area of X-ray diffractogram.

2.4.7. Molecular Exclusion Chromatography

Starch molecular weight distribution profiles were determined by using a double gel permeation chromatography (GPC) column, PL Aquagel–OH MIXED-H 8 µm, 300 × 7.5 mm (Agilent Technologies, Stockport, UK) in HPLC Agilent Technologies 1260 Infinity II with a refractive index detector, adapted from Nie et al. [40]. An aqueous solution of starch 0.5% (*w/v*) was prepared and mechanically stirred for 1 h at room temperature. Two milliliters of solution were filtered with a 0.45 µm filter, to inject 50 µL in the column at 40 °C using deionized water as eluent at a flow rate of 1 mL/min. Aqueous SEC startup kit of Polyethylene oxide/glycol (Agilent Technologies) was used as a calibration curve.

2.5. Statistical Analyses

Statistical analyses were performed to calculate the average tendency and deviation. To compare the yields of starch extraction from avocado seed and optimize the conditions of extraction analysis of variance (ANOVA) and Tukey's test with a 99% confidence level were run in the STATISTICA 7® software (StatSoft, Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Microwave-Assisted Extraction of Avocado Starch

The dry avocado seed used contains 6.20 ± 0.01% of moisture, 3.63 ± 0.04% of lipids, 3.06 ± 0.15% of proteins, 20.65 ± 0.03% of insoluble fiber and 64.61 ± 4.79% of soluble fiber. Starch extraction from avocado seed was very effective using MAE technology coupled to the process of obtaining by hot filtration and ethanol precipitation, generating yields of 47% (*w/w*). Table 1 shows the values of starch extraction yields of each treatment. The degree of solubilization/extraction of starch increased for higher heating temperatures, ranged between 42.97% and 47.31% at 135 and 180 °C and decreasing of 47.31% to 44.82% at 180 °C with the increasing the extraction time and decrease to 26–27% at the conditions of less temperature (90 °C). Chel-Guerrero et al. [15] showed a yield of 20% in wet basis from *Hass* avocado using a conventional extraction with saline solutions, that means a lower yield of extraction than the yield obtained by MAE, considering that the seed has about 50% of moisture, in wet basis the yield of MAE will be about 25%, which is a superior yield.

3.2. Statistical Analysis and Optimization of Starch Extraction

Table 1 shows the experimental data of the treatments of the experimental design. The analysis of variance (ANOVA) was carried out to obtain, *F*-value, *p*-value and lack of fit of the model and the independent variables, temperature and time, X_1 and X_2, respectively. The ANOVA results showed in Table 2, revealed that the second-order polynomial model was found to be for prediction of starch extraction yield response within the range of experimental variables. The determination coefficient of the model was $R^2 = 0.9949$, indicating a high adjustment of the model and only 0.51% of the total variation was not explained by the proposed model. The current model showed no significant lack of fit, which means a good adjustment of the model.

Table 2. Analysis of variance (ANOVA) for optimization of the yield of starch extraction from avocado seeds, model as a function of temperature (X_1) and time (X_2).

Source	Sum of Squares	df	Mean Square	*F*-Value	*p*-Value
Model	703.87	5	140.77	196.75	0.0001 *
x_1	545.41	1	545.41	759.45	0.000001 *
x_1^2	146.39	1	146.39	203.83	0.00003 *
x_2	0.06	1	0.06	0.08	0.78802
x_2^2	0.24	1	0.24	0.33	0.58952
x_1 x_2	3.69	1	3.69	5.15	0.07259
Residual	3.59	5	0.72		
Lack of fit	2.81	3	0.94	2.45	0.3027
Pure error	0.76	2	0.38		
Cor total	707.2153	10			
R^2	0.9949				
Adj R^2	0.9899				
C.V.	2.12				

* *p*-value < 0.01 indicate model terms are significant.

It was found that the response of temperature was significant (p-value < 0.01) with a linear and quadratic effect, which shows that the temperature is the factor with effect to extract/solubilize the starch from an avocado seed. The high effect of temperature in the extraction of starch can be explained by the effect of high temperature on gelation of the starch (>90 °C), which increases the solubility of the starch in water, therefore thus increases the yields of extraction. This effect is observable in Table 2 since at a low temperature (90 °C) the yield of starch extraction is lower compared with that obtained at higher temperatures (135 and 180 °C). The yield of extraction between 90 and 135 or 180 °C increases 1.8 times.

The time did not have a significant effect on the model, but it was evident at a temperature of 180 °C that with increasing time of extraction from 30 to 60 min, the yield of starch extraction decreases, which indicates that at this temperature the starch is degrading maybe into oligosaccharides, glucose or other degradation compounds derived from the effect of self-hydrolysis caused by the high temperature.

The second-order polynomial model of the optimization of starch extraction yield (SEY) as a function of the factor with statistical significance, temperature (X_1), is expressed in the next Equation (6):

$$Y\ (\%) = 43.92 + 9.54X_1 - 7.60{X_1}^2 \quad (6)$$

The second-order polynomial of Equation (1) was plotted a three-dimensional response surface to obtain the optimum condition to starch extraction from avocado seed (Figure 1). The starch extraction yield was estimated to be 46.72% at optimum condition (temperature: 161.09 °C, time: 56.23 min). With the validation of optimum condition, performed by triplicate, a yield of starch extraction of 49.52 ± 0.69% was obtained, showing that the model has a good fit for avocado starch extraction.

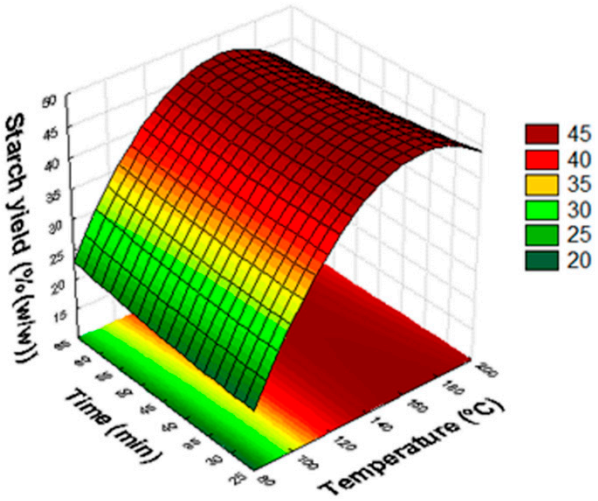

Figure 1. Response surface and contour plot showing the optimization of starch extraction by effects of temperature (X_1) and time (X_2).

3.3. Avocado Starch Extraction MAE NO–ISO and Conventional Extraction

By comparing the starch extraction yields of the three methodologies tested, MAE obtained the best yields. The yield of starch extraction by OC NO–ISO was slightly lower (45.75%), when compared to the extraction with OC (49.52%) but was not statistically different when compared by the Tukey's test with 99% confidence level. With the conventional extraction, the yield of starch extraction was lower (39.04%) and statistically different from the methods of extraction by MAE, indicating the high efficiency of the extraction promoted by microwave radiation. To select the condition of starch extraction by MAE (OC or OC NO–ISO) is necessary for an economic study to evaluate the energetic costs with the benefits of obtaining more starch or less starch with a low energetic cost. As MAE treatments have no statistical difference in extraction yields can be cost-effectively using OC NO–ISO

condition because the low extraction time, only heating time (about 12 min), allows more extractions and more starch extraction, when compared with OC in the same time of extraction.

3.4. Starch Characterization

3.4.1. Scanning Electron Microscopy (SEM)

Figure 2 shows the scanning electron micrographs recorded for avocado seed flour (A), the avocado starch extracted by MAE with OC and OC NO–ISO (B and C) and by conventional extraction (D) and corresponding fiber residues (1). The size reported to the starch granules of the avocado seed is between 5–35 μm [15,18]. Starch can be classified into four categories by the size: large (>25 μm), medium (10–25 μm), small (5–10 μm) and very small (<5 μm) [20,41].

Native starch granules of avocado seed flour and the starch obtained by the conventional method shown in Figure 2A,D, respectively, are within this size range (5–25 μm) with a clear, regular elliptical shape with smooth surfaces and classified as medium and small granules. The starch obtained by MAE have not regular shape and the same size, showing a degraded structure in an agglomerated form. With OC the starch obtained shows a pressing shape as in the same way if it sees the resulting fiber residues while the starch obtained by OC NO–ISO shows very small oval shape (<0.5 mm) and in fiber, residues is possible to see the wells where the starch granules were and some residue of starch. The effect of degradation of the starch structure by microwave radiation has also been demonstrated by Fan et al. [42] showing that starch granules lost their birefringence and the granules ruptured completely at 80 °C. A similar effect was reported by Xie et al. [43] showing that treatment for 20 s with final temperatures of 95 °C caused serious deformation, fracture, and collapse of most starch granules. Analyzing Figure 2D1, it is possible to see starch granules after extraction with the fiber residues explaining the low extraction yield of conventional extraction and demonstrating the efficiency of starch extraction of MAE.

3.4.2. Solubility, Swelling Power (SP) and Water Absorption Capacity (WAC) of Starch

Avocado starch patterns of solubility, swelling power (SP) and water absorption capacity (WAC) are shown in Figure 3. Solubility, SP and WAC are directly correlated with increases in temperature. The continuous rising of starch swelling, as the effect of the increase in temperature, is caused by the rupture of intermolecular bridges (hydrogen bond cleavage) in amorphous zones and allows a progressive and irreversible water absorption. The molecular organization of starch granules is irreversibly destroyed with gelatinization and increases the starch solubility due to the hydrogen bond cleavage and water fixation [13,15].

The CONV starch shows a normal behavior with temperature increases (60–90 °C) showing an increase of solubility of 12.8 to 24.3%, WAC of 6.7 to 13.2 g/g and SP of 7.7 to 17.4 g/g. Similar results were presented by dos Santos et al. [12], Chel-Guerreo [15] and Silva et al. [26] with avocado seed starch obtained by conventional methods. Contrary to the MAE starches have completely different behavior when compared with CONV starch and other authors. The MAE starches have a high solubility and increase with the temperature of 60 to 90 °C, the solubility increases of 86.3% and 93.2% to 93.6% and 96.8%, in OC and OC NO–ISO starches, respectively. Consequently, the starches have a low WAC, between 1–2%, decreasing with the increase in temperature as a consequence of the increase of solubility. The high SP of MAE starches, which had no significant fluctuation with temperature, can be explained by the MAE effect in the structure of the starch. As shown in Figure 2, the MAE starch has a small size and probably these starches are small structures of amylose and amylopectin with many cleaved bonds that promote a rapid water bond and a high SP, due to the relation between a large number of water molecules by starch particle, however, the WAC is low due to the low quantity of these particles associate to the high solubility and the small particles gelatinized when compared with large size of a gelatinized granule of CONV starch.

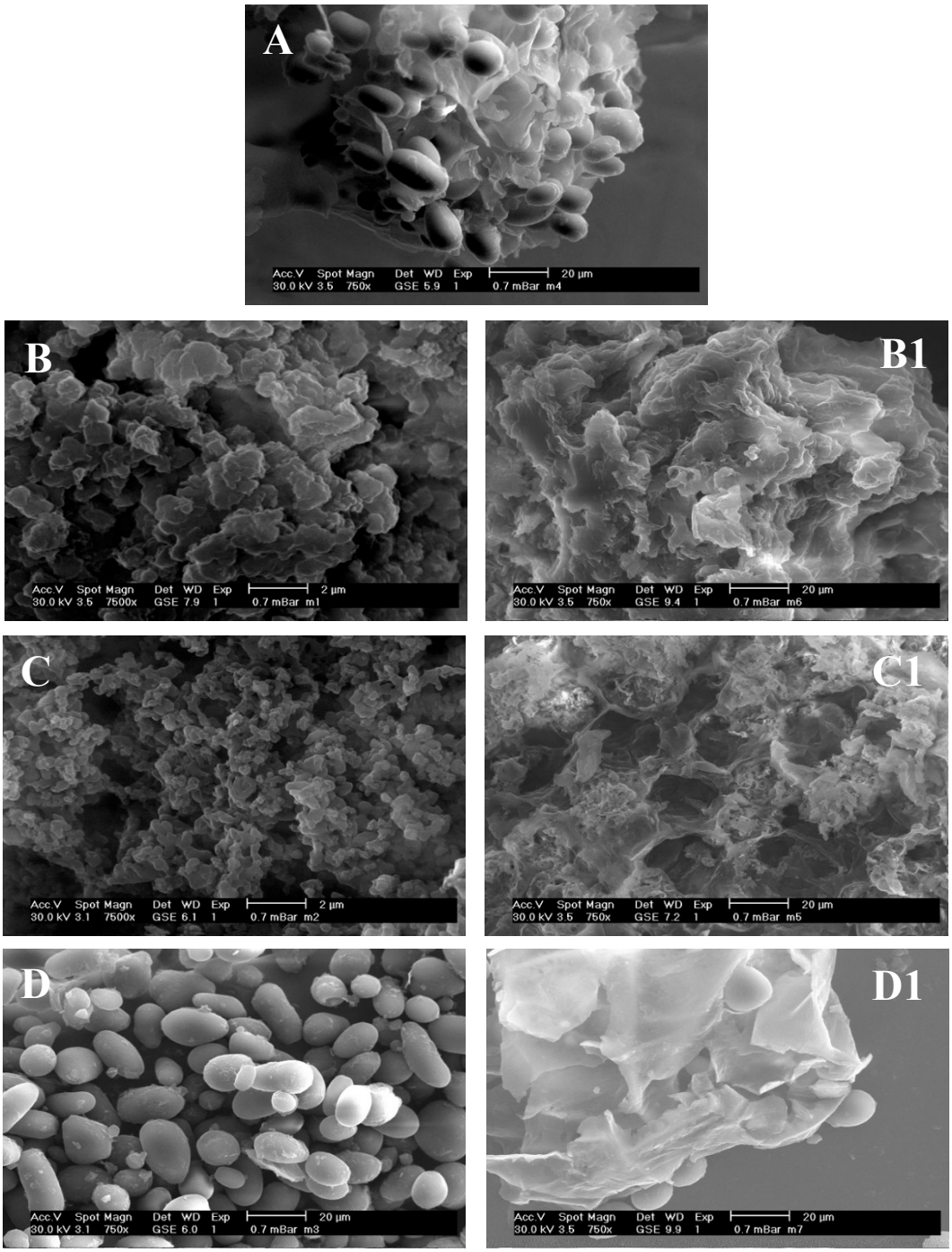

Figure 2. Scanning electron micrograph of (**A**) avocado seed flour, (**B**) avocado starch extracted by microwave-assisted extraction (MAE) with optimal condition (OC), (**C**) with OC MAE non-isothermal mode (NO–ISO) and (**D**) conventional method (CONV), and (**B1**,**C1**,**D1**) respective residues resulting from each extraction.

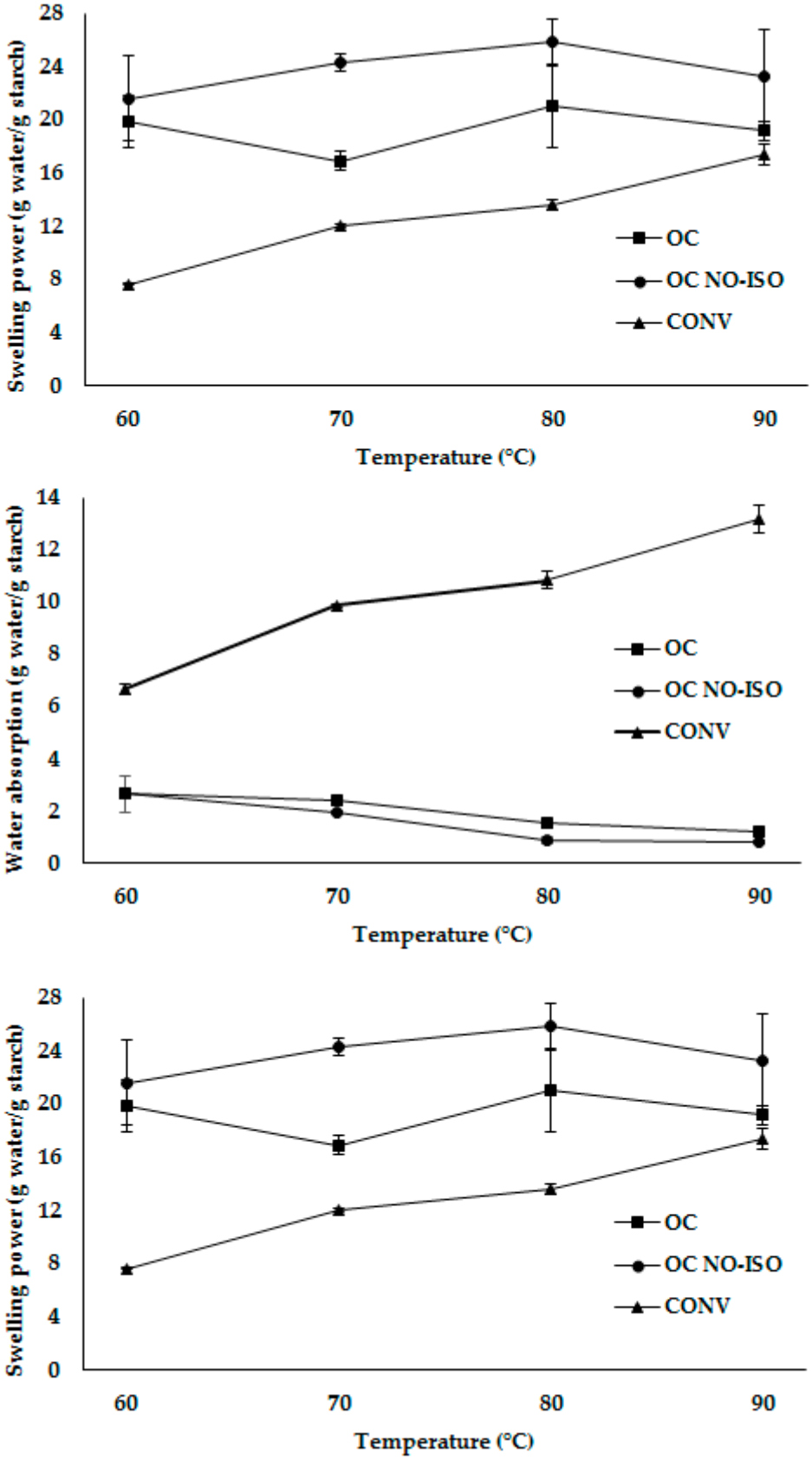

Figure 3. Solubility, water absorption and swelling power patterns of avocado starch extracted by MAE (OC and OC NO–ISO) and conventional method (CONV).

3.4.3. Thermogravimetric Analysis

TGA analysis showed changes in a weight loss of the sample with temperature increase and was performed to determine the thermal stability of the starches. As shown in Table 3, it can be observed three mass loss during the heating process of starches, which is characteristic from starch [18,44]. The first weight loss starts about 70 °C to MAE starch and about 120 °C to CONV starch was the result of starch dehydration. The difference showed between starches can be explained by the moisture of starches, that MAE starches have more moisture content and because they are not granular in shape it may allow a loss of water at lower temperatures. The second mass loss corresponds to the decomposition of organic matter. In this step occurred a great mass loss, which means that it has a great number of compounds with similar thermal properties, which is characteristic of a homopolysaccharide as the starch. The starch decomposition with temperatures exceeding 300 °C, consists of depolymerization of the starch, formation of pyrodextrins followed by the degradation of macromolecules in levoglucosan, furfural and volatile products and finally carbon residues that is the last mass loss. MAE starches start the second mass loss of about 280 °C and CONV starch at 300 °C. This difference in temperature can be explained by the depolymerization of the starch generated with the extraction of microwaves, which begin to degrade faster at lower temperatures. Thermally, the starches are similar and these results were observed by other authors with starches from avocado seed and other botanical sources [9,18,44–47].

Table 3. Differential scanning calorimetry values and temperature to each mass loss stages in Thermogravimetric Analysis (TGA) analysis of MAE avocado starch optimal condition (OC), optimal condition non-isothermal mode (NO–ISO) and conventional extraction (CONV).

Parameters	OC Starch	OC NO–ISO Starch	CONV Starch
DSC			
T_0 (°C)	36.86	46.62	35.94
T_m (°C)	47.43	51.96	60.26
T_f (°C)	58.35	63.29	74.33
ΔH (J/g)	113.00	218.90	562.0
TGA			
1st	70.33	77.96	115.23
2nd	280.5	280.4	299.3
3rd	348.28	343.08	355.74

Gelatinization onset (T_o), peak (T_p), conclusion (T_c) temperatures and melting enthalpies (ΔH).

3.4.4. Differential Scanning Calorimetry

Starch experiments an order–disorder phase transition called gelatinization when it is heated in excess of water. Gelatinization properties of starch, such as Onset Temperature (T_o), Melting Temperature (T_m), Conclusion Temperature (T_f) and Enthalpy Increment (ΔH) were measured by Differential Scanning Calorimetry (DSC) and are presented in Table 3. All three samples showed one endothermic peak on the DSC thermograms, however, these peaks are located at different temperatures evidencing the effect of the MAE procedures on the starch granule structure. On one hand, the melting temperature determined for a starch obtained by conventional extraction was 60.26 °C, this value is like the reported by Chel-Guerrero et al. [15] for native starch.

In another hand, the starches obtained by OC and OC NO–ISO presented lower melting temperatures (47.43 °C and 51.96 °C, respectively), this temperature decrement could be explained in terms of granules structure damage, as was previously mentioned in SEM studies (Figure 2), the starch obtained by MAE have not regular shape and the same size, showing a degraded structure in an agglomerated form, in this sense, we can say that starch is already retrograded. In other words, granule structure has lost and new structure stabilized trough double helices is formed, these low melting temperatures for retrograded starches was previously reported by (Vamadevan & Bertoft,

2018), evidencing that avocado starch probably contains amylopectin with DP > 50 and lacks long internal chains (Type 1).

Another interesting factor, is the change registered for the transition enthalpy, as can be seen in Table 3. Starch endothermic enthalpy can be related to the loss of double-helical order rather than the crystalline register (Cooke & Gidley, 1992), in this sense, it seems that the forces holding starch granules are greater in the starch obtained through CONV than MAE starches. This values for enthalpy, are similar to the reported for Silva et al. [26] for native avocado starch.

3.4.5. Fourier-Transform Infrared Spectroscopy (FTIR)

Figure 4 shows the infrared spectroscopy of the starches obtained by the different methodologies, showing a normal starch spectra profile for all samples analyzed. Starch is composed mainly of amylose and amylopectin that are polysaccharides composed of monomers of glucose and water. Starch has two characteristic regions of the spectrum, the -OH and -CH stretching vibration of the glucose unit, 3650–3000 cm^{-1} and the region of major adsorption bands or region of carbohydrate vibrations between 1200–800 cm^{-1}, known as fingerprint region [37,47]. The band at 2926 cm^{-1} corresponds to C–H stretching associated with the ring methane hydrogen atoms. The absorptions between 1638 and 1300 cm^{-1} were associated with H_2O-bending vibration. The intensities of bands at 1149, 1077 and 996 cm^{-1} are associated with C–O and C–C stretching with some C–O–H contributions [13,48]. The spectra of the starches obtained are quite similar to some differences in the intensities of main bands identified and these differences of intensities can be explained by the differences in the degree of humidity of the samples or some modifications induce by MAE [49].

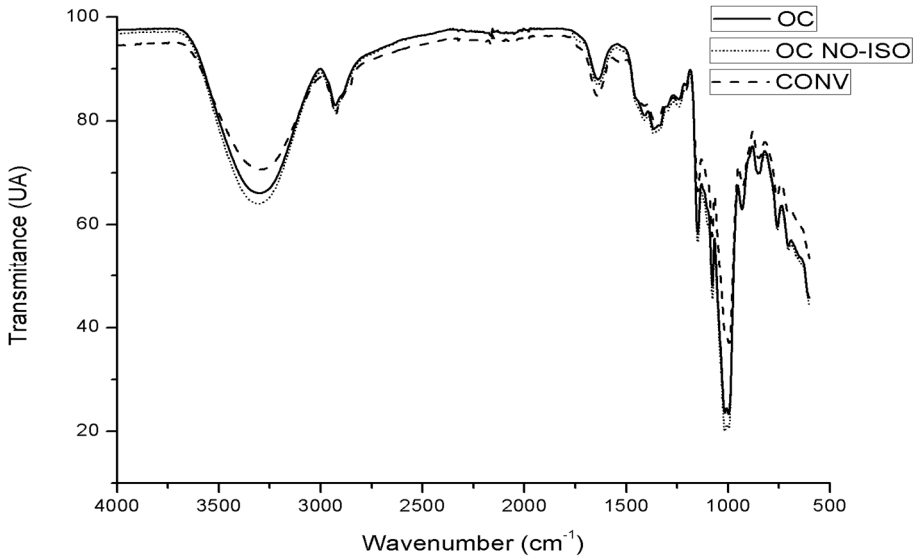

Figure 4. FTIR spectra of MAE avocado starch of optimal condition (OC), optimal condition non-isothermal mode (NO–ISO) and conventional extraction (CONV).

3.4.6. X-Ray Diffraction (XRD)

The X-ray diffraction patterns of the avocado starches are shown in Figure 5. The X-ray diffraction patterns of the starch obtained by MAE have the same peaks (13° and 20° 2θ) and similar intensity indicating a similar crystallinity (24.15 and 25.40), though the starch extracted with CONV has a different diffraction pattern with peaks of 17°, 20° and 22° 2θ and different crystallinity (12.19) which are similar results reported by Lacerda et al. [9]. The X-ray diffraction patterns of avocado starch indicate that the amorphous structure is more dominant than the crystalline structure. Diffraction patterns of granules starches have been classified in 3 types, A, B and C. The results obtained indicate that the starch obtained is type A because the peaks identified 17°, 20° and 22° 2θ [13,50]. MAE starches

and CONV starch have a different pattern of diffraction indicating that they have a different structure or organization of the chains of amylose and amylopectin, creating a different level of crystallinity and changes in amorphous zone.

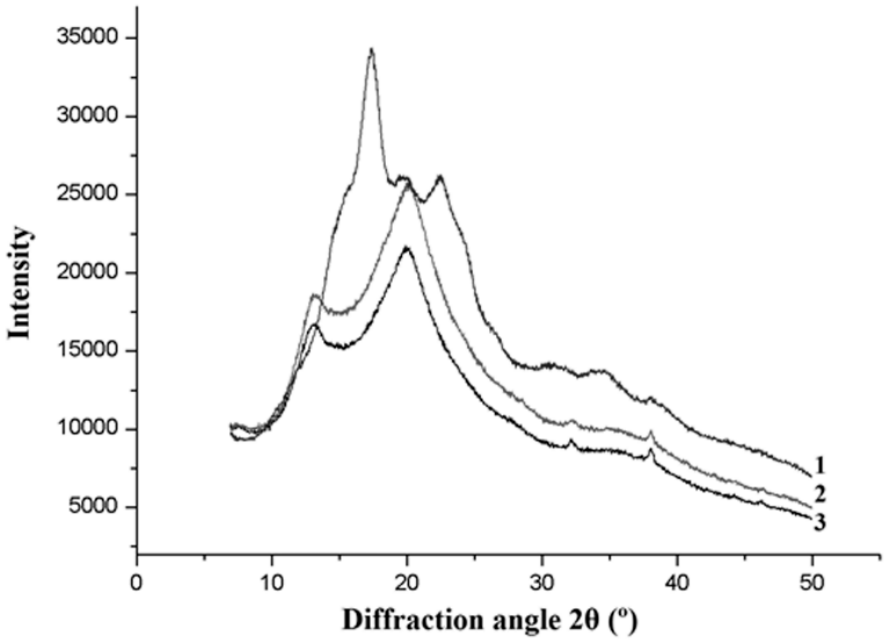

Figure 5. X-ray diffraction patterns of avocado starch extracted by the conventional method (1) and avocado starch extracted by MAE with OC NO–ISO (2) and OC (3).

3.4.7. Molecular Exclusion Chromatography (GPC)

Figure 6 shows the GPC chromatograms and Table 4 shows the molecular weight (MW) of main peaks identified in starches obtained by MAE and CONV. As expected, by analysis of SEM results, it was found that the starches obtained by MAE have a family of particles with different and more size groups. Starch extracted with OC has seven groups of MW between 411 and 1.91×10^8 g/mol, indicating a high fragmentation of granular structure of native starch, generating more fragments of starch that OC NO–ISO because the autohydrolysis by the effect of more time of extraction. To starch obtained with OC NO–ISO was identified five molecular weights between 533 and 1.65×10^5 and in CONV starch only was identified four molecular weights between 324 and 2.23×10^6 with low intensity indicating a low concentration of these sizes.

Table 4. Molecular weights and polydispersity of MAE avocado starch of optimal condition (OC), optimal condition non-isothermal mode (NO–ISO) and conventional extraction (CONV).

	OC		OC NO–ISO		CONV	
Peak	**Mp (g/mol)**	**Pd**	**Mp (g/mol)**	**Pd**	**Mp (g/mol)**	**Pd**
1	190,732,143 *	1.128	165,387	1.020	2261,719 *	1.037
2	27,003,383 *	1.201	120,484	1.011	38,172	1.021
3	512,653	1.194	78,030	1.053	16,601	1.086
4	262,398	1.036	3866	1.281	324	1.062
5	57,883	1.065	533	1.155		
6	5165	1.386				
7	441	1.049				

* Value calculated by extrapolation of the calibration curve. Pd: polydispersity.

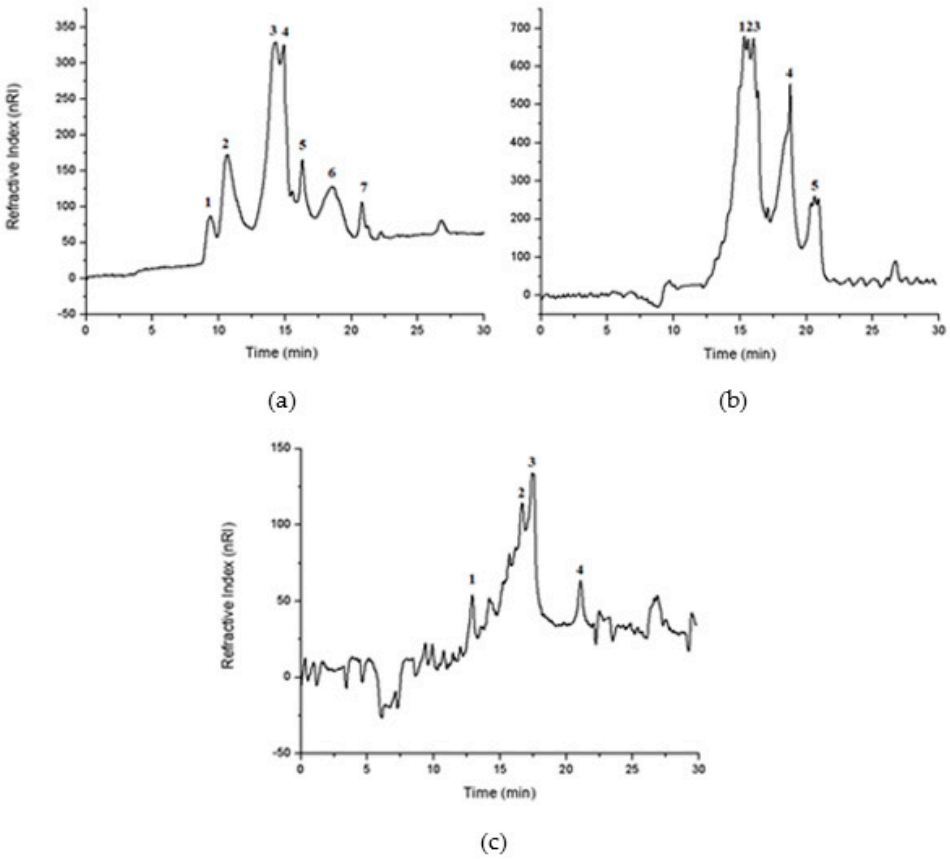

Figure 6. Double gel permeation chromatography (GPC) chromatograms of starch extracted by (**a**) MAE with OC, (**b**) with OC NO–ISO and (**c**) starch isolated by CONV.

Autohydrolysis is a method widely used to extract added value compounds from lignocellulosic materials as hemicellulose, lignin, oligosaccharides and others. These compounds are obtained by the deconstruction of the linkages and disruption of the cross-linked structure of lignocellulosic biomass by the effect of the autoionization of water into hydronium ions and acetyl group hydration [51–54]. A similar effect may have occurred in the structure of MAE starch by disruption of α-1–4 and 1–6-linked glucans of amylose and amylopectin structures.

4. Conclusions

Avocado seeds are a currently unused agroindustrial residue that can be utilized as an unconventional starch source. MAE technology has been shown to be a simple, efficient and rapid extraction process for the extraction of starch from avocado seeds, using only water as a solvent. However, it did induce some differences in structure and properties of starch as the solubility and water absorption capacity, the granule structure, the molecular weight of the starch and generation of chains of starch with different sizes and a structural organization that show a different diffractogram of the conventional starch. These modifications, for example, the starch chain of small size, can provide a new application to MAE starch, maybe in biotechnological applications (nanoparticle formulation, production of oligomers with bioactivity) or for example, the high starch solubility can provide good aqueous dispersion in food applications. The world of starch applications is huge and the starch modifications are so vast that they create innumerable uses. Avocado seed starch has a good potential in food industry applications as candies, meat products, drinks, sauces, bread products and many more applications as pharmaceutical products, biodegradable polymers for food packaging to decrease the use of plastics.

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