applied sciences

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Exploration of the Potential Bioactive Molecules of Tamarillo (*Cyphomandra betacea*): Antioxidant Properties and Prebiotic Index

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Citation: Reyes-García, V.; Totosaus, A.; Pérez-Chabela, L.; Juárez, Z.N.; Cardoso-Ugarte, G.A.; Pérez-Armendáriz, B. Exploration of the Potential Bioactive Molecules of Tamarillo (*Cyphomandra betacea*): Antioxidant Properties and Prebiotic Index. *Appl. Sci.* **2021**, *11*, 11322. https://doi.org/10.3390/app112311322

Academic Editors: Piotr Latocha, Artur Wiktor and Barbara Łata

Received: 2 November 2021
Accepted: 22 November 2021
Published: 30 November 2021

Featured Application: Tamarillo is a fruit that contains several bioactive molecules with probed antioxidant properties, due to the relatively higher polyphenols content, as well as simple (mono-, di-, and oligo-saccharides) and complex (poly-saccharides as pectin) structural sugar-based molecules.

Abstract: Tamarillo is an alternative for the consumption of food with high added value through various technological methodologies with nutritional quality and low cost, generating an economic impact on society. The objective of this research was to evaluate the potential of tamarillo red variety, as a source of bioactive compounds, to generate scientific information on the importance of its chemical composition and antioxidant and prebiotic properties. Different analyses were carried out: spectroscopic methods (IR, UV, NMR) of pulp flour and epicarp flour, antioxidant properties, prebiotic activity, and bromatological analysis. The spectra obtained by FTIR, UV, and NMR allowed the identification of chemical structures associated with the inulin-like functional groups. Pulp flour showed the highest prebiotic activity with values of 1.49 for *Lactiacidbacillus. plantarum*. Total phenolic compounds content in pulp flour was 206.23 mg/100 g dry weight, with an acceptable antioxidant property (ABTS+ = 6.27 TEAC and DPPH= %AA of 91.74 at a concentration of 250.00 µg/mL, 131.26 of IC_{50} ascorbic acid). The results regarding tamarillo as a source of bioactive molecules with important physiological properties as an antioxidant and putative prebiotic indicate it is a good alternative for the formulation of functional foods.

Keywords: tamarillo (*Cyphomandra betacea*); antioxidant properties; ABTS; DPPH; FTIR; UV; H1 NMR; prebiotic activity

1. Introduction

Mexico possesses biodiversity or biological diversity [1] and has a wide variety of exotic fruits, which are traditionally consumed locally, representing an excellent opportunity for innovation and food security. However, given the diversity of exotic Mexican and native species, some of these fruits have not been studied in terms of their physiological properties and chemical composition. *C. betacea* is a high value-added product with a high antioxidant potential [2]. In Mexico, *C. betacea* is known as tamarillo and can be

found in the wild or in backyards. It is collected annually in the northeastern Sierra of the State of Puebla. *C. betacea* is given different names, such as tree tomato in Colombia and Ecuador; chile tomato in Spain; tamarillo in New Zealand, Canada, Brazil, and Malaysia; and tree tomato or chilto in Argentina and Chile. Currently, only three countries, Australia, Colombia, and New Zealand, commercially cultivate this fruit [3]. With the aim of finding sources of functional foods, many investigations have shown that *C. betacea* possesses various bioactive compounds and belongs to the *Solanaceae* family. It is characterized by its contents of antioxidants, such as polyphenols, anthocyanins, carotenoids, and vitamins A, C, B, and E [4,5]. These compounds present important physiological effects, related to their natural pigments [6,7]. Additionally, the prebiotic activity of *C. betacea* was reported [8], and the chemical characteristics of the pulp and mucilage covering the fruit seed, identifying hydrocolloids as an arabinogalactan protein associated with pectin, as well as hemicellulosic polysaccharides, producing short-chain fatty acids (SCFA), acetate, and propionate as a result of in vitro fermentation of mucilage and pulp hydrocolloids, respectively. The action of these bioactive compounds as a prebiotic can produce secondary metabolites associate with health [9].

Therefore, the objective of this research was to evaluate the potential of tamarillo red variety, as a source of bioactive compounds, in order to generate scientific information on the importance of its chemical composition and antioxidant and prebiotic properties.

2. Materials and Methods

2.1. Plant Material

Fresh samples of tamarillo red variety (Figure 1) were collected during the harvest period of November to January 2019 from the municipalities of Santiago Yaonahuac and Cuautempan belonging to the Northeast Sierra of the State of Puebla, Puebla. The specimens were deposited in the Herbarium-Hortorio CHAPA of the postgraduate program in Botany at the Colegio de Postgraduados, Montecillo, Texcoco, México. The samples were identified as *C. betacea* (Cav.) Sendtn and synonymy of *Solanum betaceum Cav.*, with CHAPA registration number 154 534.

Figure 1. Red variety of *C. betacea* fruit, collected in the Northeast Sierra of the State of Puebla, México.

2.2. Samples Preparation

Fruit was purchased at the local market in the community of Santiago Yaonahuac, Puebla. In total, 50 kg of fruit were refrigerated at a temperature of 5 to 8 °C. *C. betacea* was blanched at a temperature below boiling point for 5 min to facilitate peeling and separate the peel (epicarp) from the pulp. The pulp was then reduced to a size of 3×2 cm^2. The batches were divided into pulp and peel, using two methods for drying the samples: (i) lyophilization, in a freeze-drying system (FreeZone®, lyophilizer, Labconco-7343000, Kansas City, MO, USA) in which the equipment conditions were followed to obtain high efficiency in the process. The drying time was set according to the characteristics of the

samples, with a collector temperature of −50 °C and vacuum conditions of 0.22–0.05 mbar. After drying, the particle size of the samples was reduced, and two different flours were obtained: freeze-dried pulp flour (FPF) and freeze-dried epicarp flour (FEF). (ii) Convection dehydration, in a computerized convection oven, model HCU (San-Son®, Estado de México, México) was also used. The processing conditions for pulp included a temperature of 65 °C for 10 h, and for the shell, dehydration was performed at 65 °C for 4 h; the dehydrated samples obtained were reduced in particle size. The dry pulp flour obtained by convection (DPFC) and the dehydrated epicarp flour obtained by convection (DEFC) were crushed in a food processor (KRUPS GX4100 Electric Spice Herbs and Coffee Grinder with Stainless Steel Blades) from coarse to fine for 20 s. All the flours obtained by the methods were kept in airtight jars at room temperature in a dark place until their use for each analysis.

2.3. Preparation of Ethanolic Extracts

Ethanolic extracts were made from tamarillo of the FPF and FEF samples in a 1:2 ratio (samples-ethanol). The samples were macerated in ethanol (96% purity) for three days. The extracts were vacuum filtered with Whatman No. 4 filter paper and concentrated on a Büchi rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) under reduced pressure at 45 ± 2 °C.

2.4. Bromatological Analysis

The bromatological analysis of the tamarillo freeze-dried pulp flour and freeze-dried epicarp flour samples was carried out following the official methods of the AOAC [10], determining the percentage of moisture (Official Method 925.10), ash (Official Method 942.05), total ethereal extract (Official Method 920.39), protein (Official Method 984.13) using the conversion factor 6.25, and crude fiber (Official Method 991.43). Total dietary fiber was determined following the AOAC enzymatic-gravimetric method 985.29 and total sugars were determined by the method of Dubois [11]. All analyses were performed in triplicate on a dry basis.

2.5. Antioxidant Properties

Total polyphenol content was determined by the method proposed by Singleton and Rossi [12]. Folin–Ciocalteu's reagent was added to 1 mL of ethanolic extract of FPF and mixed with 8 mL of 0.7 M Na_2CO_3. The mixture was allowed to stand for 2 h at room temperature in the dark. The absorbance was measured at 765 nm and extrapolated with a catechol standard curve (0 to 100 mg/mL). Subsequently, in the ABTS and DPPH assays, the radical scavenging potential was evaluated only in the FPF sample according to the method described Re et al. [13]. The diammonium salt of 2, 2'-azino-bis(3-ethyl-6-benzothiazoneline) (ABTS) (Sigma Aldrich, St. Louis, MI, USA) and potassium persulfate of 2.45 µM were incubated at room temperature in the dark for 12 h. After the addition of 1.0 mL of diluted ABTS solution (A734 nm = 0. 700 ± 0.020) to 10 µL of diluted sample or Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchrom-2-carboxylic acid) standards in ethanol, the absorbance reading was taken exactly 1 min after the initial mixing and up to 10 min. The percent inhibition absorbance at 734 nm was calculated and plotted as a function of the antioxidant and Trolox concentration for the reference data of the standard. Subsequently, the method described by Kasote et al. [14] was followed. For this, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used at concentrations of 62.5–500 mg/mL, and ascorbic acid was used as a positive control.

2.6. Spectroscopic Methods

The IRs were obtained of the solid-state extracts of tamarillo freeze-dried pulp flour and freeze-dried epicarp flour employing a Cary 630 IR Spectrophotometer equipped with a FTIRARY 630 ATR Module (Agilent Technologies, Santa Clara, CA, USA) in the range 4000–600 cm^{-1}.

For UV spectroscopy, samples were dissolved in methanol and analyzed with a Cary 100 UV-Vis UV Spectrometer (Agilent Technologies, Santa Clara, CA, USA).

NMR spectroscopy ^{1}H was performed by dissolving and fractionating separately in increasing polarity order (chloroform, methanol, and deuterated water). Then, 20 mg of each of the samples were dissolved separately in 1 mL of deuterated chloroform, stirred, and filtered to determinate the NMR spectra in a Bruker Avance III spectrometer (Bruker BioSpin GmbH, Ettlingen, Germany) operating at 400.13 MHz for hydrogen and a reverse probe.

2.7. Prebiotic Activity Score

Three lactic acid bacteria (LAB) from the collection of the Popular Autonomous University of Puebla (UPAEP) were used, which were isolated from pulque (fermented Mexican beverage) and identified by MALDI-TOF mass spectrometry. Biotyper real-time classification was perrformed with software 2.2 (Bruker Daltonics, Billerica, MA). The isolated strains with probiotic potential were identified according to their score, *L. casei 2.23*, *L. plantarum 2.078*, and *L. paracasei 2.374*. The pathogen *E. coli* (ATCC 25922) was used as a negative control. The culture medium was prepared with 0.5% casein peptone, 0.3 yeast extract % w/v, and glucose as the control at 1.0% and 1.0 of prebiotic % w/v obtained from the samples of DPFC or DEFC of tamarillo; water was used to reach 100% of the medium. The prebiotic activity was determined according to the change in cell biomass after 18 h of LAB growth in 1% prebiotic and 1% glucose. The prebiotic activity score was defined as the relationship described by Huebner et al. [15], considering the growth of each bacterium during fermentation, according to Equation (1):

$$\left\{\frac{\text{Probiotic log CFUmL}^{-1}\text{at 8 h in prebiotic} - \text{Probiotic log CFUmL}^{-1}\text{at 0 h in prebiotic}}{\text{Probiotic log CFUmL}^{-1}\text{at 8 h in glucose} - \text{Probiotic log CFUmL}^{-1}\text{at 0 h in glucose}}\right\} - \left\{\frac{\text{Enteric log CFUmL}^{-1}\text{at 8 h in prebiotic} - \text{Enteric log CFUmL}^{-1}\text{at 0 h in prebiotic}}{\text{Enteric log CFUmL}^{-1}\text{at 8 h in glucose} - \text{Enteric log CFUmL}^{-1}\text{at 0 h in glucose}}\right\}, \quad (1)$$

To assess the effect of FDPF and FDEF as a carbon source on the prebiotic activity in vitro of the different employed lactic acid bacteria strains, a one-factor ANOVA was used, and Tukey's test was applied to determinate significant differences between the means analyzed in the Minitab 18 software (State College, PA, USA).

3. Results and Discussions

3.1. Bromatological Analysis

The proximal composition of the pulp and epicarp of *C. betacea*, shown in Table 1, is composed of approximately 86% moisture in pulp and 68% in epicarp. Morillas [16] reported 87.72% moisture in tamarillo, being a perishable food, as shown by recent studies claiming that the moisture content in fruits and vegetables is around 75% to 90% Brazil and Siddiqui [17]. Regarding the protein content, 0% was in the pulp and epicarp while 61.3% of the total carbohydrates was in the pulp and 20.5% in the epicarp. Further, 1.1% and 2.1% of the minerals and 4.04% and 8.58% of the total dietary fiber was in the pulp and epicarp, respectively. Prohens [4] showed protein values ranging between 1.5% and 2.5% in *C. betacea*. Tamarillo contains a great variety of minerals, with a greater presence of potassium, calcium, copper, iron, manganese, and magnesium. Vasco C, Avila J, Acosta-Quezada [5,18] noted that the high potassium content is similar to that of plantain. Acosta-Quezada [18] reported total sugars of 28.1% and 52.0% on a dry basis. Regarding total dietary fiber, Lister [19] reported that a single serving of *C. betacea* of approximately 60 g can contribute to the recommended daily intake (RDI) of minerals, with values in the range of 5.0%–7.2% for the red and yellow varieties. Regarding total dietary fiber, Morillas [16] reported values similar to those obtained in this study, with a value of 13.37%, while Mutalib [20] reported percentages of 4.10 and 6, respectively. *C. betacea* contributes 11% of the RDI. According to Vergara-Valencia et al. [21], *C. betacea* can be considered a nutritious fruit due to its balance in dietary fiber. In addition, these properties of solubility and

viscosity have profound effects on the functionality of dietary fiber during food processing and in the gastrointestinal tract [22]. Another property is that the adsorption of fat is part of the soluble dietary fiber for the purposes of stabilization of emulsions in the processing of high-fat foods, as well as to observe physiological effects in humans [23].

Table 1. Bromatological composition of tamarillo pulp and epicarp. *total polyphenol content and antioxidant properties of FPF flour.

Parameter [1]	Pulp	Epicarp
Moisture	86.75 ± 0.35	68 ± 0.00
Fat	0.18 ± 0.01	1.71 ± 0.17
Ash	1.1 ± 0.02	2.1 ± 0.04
Protein	0.00 ± 0.00	0.00 ± 0.00
Crude fiber	3.57 ± 0.09	30.12 ± 0.17
Total sugars	61.30 ± 0.21	20.51 ± 0.01
Soluble dietary fiber	0.12 ± 0.00	0.14 ± 0.01
Insoluble dietary fiber	3.92 ± 0.02	8.44 ± 0.03
* Total dietary fiber	4.07 ± 0.01	8.58 ± 0.00
Phenolic compounds [2]	206.23 ± 0.4	ND
Antioxidant capacity [3]	6.27 ± 0.01	ND
Antioxidant activity [4]	131.26 ± 0.00	ND

[1] All analysis were reported in dry weight. [2] mg of catechol equivalent per 100 g of dry sample. [3] ABTS assay, Trolox equivalent antioxidant capacity (TEAC), µmol/µmol Trolox. [4] DPPH assay, IC_{50} (µmol/µmol of ascorbic acid).

The difference in results is due to various factors, such as, for example, the analyzed crop, maturity index, and geographical and environmental conditions.

3.2. Antioxidant Properties

The yield of the ethanolic extracts of *C. betacea* FPF was 6.25. The result of the determination of phenolic compounds of the FPF ethanolic extract of *C. betacea* was 206.23 mg EAG/100 g dry weight. The antioxidant capacity of the FPF extract of *C. betacea*, by ABTS, showed a concentration of 6.27 µmol/Trolox to inhibit 50% of the free radicals. As for the percentage of antioxidant activity of the ethanolic extract of FPF in the DPPH method, the % AA was 91.74 with a concentration of 250.00 µg/m. It showed an IC_{50} of 131.26 µg/mL, which is lower than the control (IC_{50} of 780.60 µg/mL) as shown in Table 1. Different authors have reported the presence of phenolic compounds in *C. betacea*. Orqueda et al. [6] reported 223.80 mg/100 g of flavonoids in the pulp. Espin et al. [24] identified phenolic acids in the dried pulp (421.6 mg/100 g), in cultures of *C. betacea* in New Zealand. In the epidermis (peel) and pulp, they identified polyphenols ranging from 54.67 to 278.03 mg/100 g, mainly phenolic acids. The recommendation for flavonoid intake is between 250 and 400 mg/day, considering the seasonality of food sources [25]. The results of this study agree with those reported by Vasco et al. [5], with values of 4.2 to 10.3 µmol/Trolox of antioxidant activity, and are higher than those reported by Hurtado et al. [26] of 1.90 µmol/Trolox/g. Therefore, *C. betacea* showed a free radical inhibition capacity and antioxidant activity due to the presence of natural phytochemicals with antioxidant potential. Ordoñez et al. [27] attributed this antioxidant activity to the presence of flavonoids, polyphenols, and vitamins in the fruit. The values obtained were lower than those reported by Mutalib et al. [20], with an IC_{50} of 800 µg/mL in *C. betacea* pulp. The difference in the ABTS and DPPH values of the antioxidant compounds present in ethanolic extracts of FPF is due to the type of extractable compounds: hydrophilic and lipophilic [28]. Saura Calixto [29] showed that about 50% of the total dietary antioxidants, mainly polyphenols, cross the small intestine bound to dietary fiber, thus resulting in the transport of dietary antioxidants through the gastrointestinal tract, releasing the fiber matrix in the colon by the action of the bacterial microbiota, and producing metabolites and an antioxidant environment.

The presence of polyphenols in *C. betacea* was reported [25], identifying rosmarinic acid as a compound with important biological, antioxidant, anticancer, and diabetes control properties. These results are similar to those reported by [30], who demonstrated the presence of flavonoids in the red variety of *C. betacea*. Other compounds were identified [7], such as rutin, caffeic acid, and chlorogenic acid. According to Wan S. [3], *C. betacea* is a potential functional food due to its biological properties, antioxidant, anti-inflammatory, antiviral, antibacterial, antidepressant, and anticancer effects, in addition to its natural pigments, which are often associated with the prevention of chronic diseases [31].

3.3. Spectroscopic Methods

The FTIR spectra for both pulp flour and epicarp flour samples showed main observable differences reflected in the chemical composition around 1800–1600 cm^{-1}. In Figure 2, for the FTIR spectra of the tamarillo pulp and epicarp samples, the same typical bands can be observed but with a different magnitude because of the different compositions among the pulp and epicarp. Changes in the composition are reflected in several peaks assigned to different wavelength ranges for the contribution of specific regions: 3350 cm^{-1} for O–H stretching modes of water absorbing, −C−H stretching in fatty acids (2900 cm^{-1}), −C=O stretching of methyl esterified carbonyl (1745 cm^{-1}), asymmetric stretching of carboxylate anion −COO- (1630 cm^{-1}), symmetric stretching of carboxylate anion (1432 cm^{-1}), and C=O and C−C stretching of acids (1010 cm^{-1}), respectively [32]. The noticeable changes between 1300 and 800 cm^{-1} correspond to the typical fingerprint region similar to citrus pectin [7], since the presence of high methoxyl pectin in tamarillo pulp and low methoxyl in mucilage has been reported [33]. The results showed a similarity with the characteristic peaks of inulin of 3270–2929 cm^{-1} and 1025–985 cm^{-1} [34]. In the UV spectroscopy, the presence of carbohydrates was identified in tamarillo samples for both the pulp and epicarp, with absorption bands at 212 and 275 nm (values of 0.089 and 0.375 abs) for pulp and absorption bands at 210 and 328 nm (values of 0.145 and 0.803 abs) for epicarp (Figure 3). The results of UV showed that the peaks obtained are associated with the presence of monosaccharides as reported by Kaijanen et al. [35], where the maximum absorption peaks for xylose are from 245 to 255 nm and the UV spectrum for glucose, and the absorbance is close to a maximum of 270 nm and significantly low at 270 nm. Nonetheless, in tamarillo pulp, the presence of common monosaccharides from different polysaccharides is similarly fractionated depending on the extraction method, since in the water extraction procedure, mannose- and xylose-containing polysaccharides, major constituents of hemicelluloses, presented lower extractability [32]. Despite the extraction method, the UV analysis confirmed the presence of fermentable sugars, such as mono-, oligo-, and polysaccharides, in tamarillo pulp. There was an observable difference in pulp flour, which had more peaks, since pulp is the sweeter part of the fruit, than in epicarp flour, although other important components in epicarp, such as pigments, are present. Novel delphinidin 3-O-a-L-rhamnopyranosyl-(1→6)-β-Dglucopyranoside-3′-O-β-D-glucopyranoside as a minor constituent has been reported [36], and hence, tamarillo, as a tropical fruit, could be considered to be a good source of natural pigments with potential antioxidant activity. In the 1H RMN spectroscopy, the FDPF and FDEF samples showed signals between 3 and 4 ppm, which correspond to the various carbons of fructose (sugars), and signals at 5.0 ppm that correspond to an anomeric H, characteristic of sugars (Figure 4). The NMR spectra show the presence of fructose units and glucose units, as well as anomeric carbon at 5.44 ppm, corresponding to the α1-β1 proton of the D-glucopyranosyl unit, which is located at the beginning of the inulin chain [37]. do Nascimento [38,39] reported the presence of a galactose arabinose glucuronoxylan in the pulp of *C. betacea*, composed of major monosaccharides of glucose, arabinose, galactose, xylose, and uronic acids, showing an antinociceptive effect in inflammatory pain models. These results are associated with dietary fiber as the presence of pectins, being the main components of the soluble fraction of fiber in the pulp. Kou [40] showed that the phenolic compounds in *C. betacea* have high antioxidant potential and demonstrated inhibition of LDL oxidation in vitro and ROS production in PC12 cells.

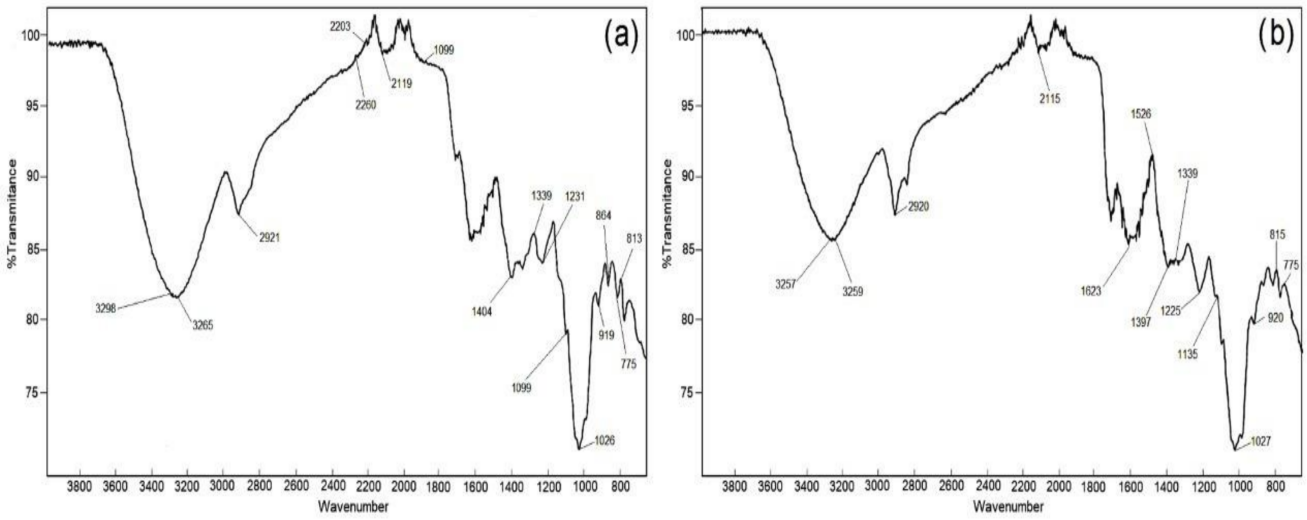

Figure 2. FTIR spectra of (**a**) tamarillo pulp flour and (**b**) tamarillo epicarp flour.

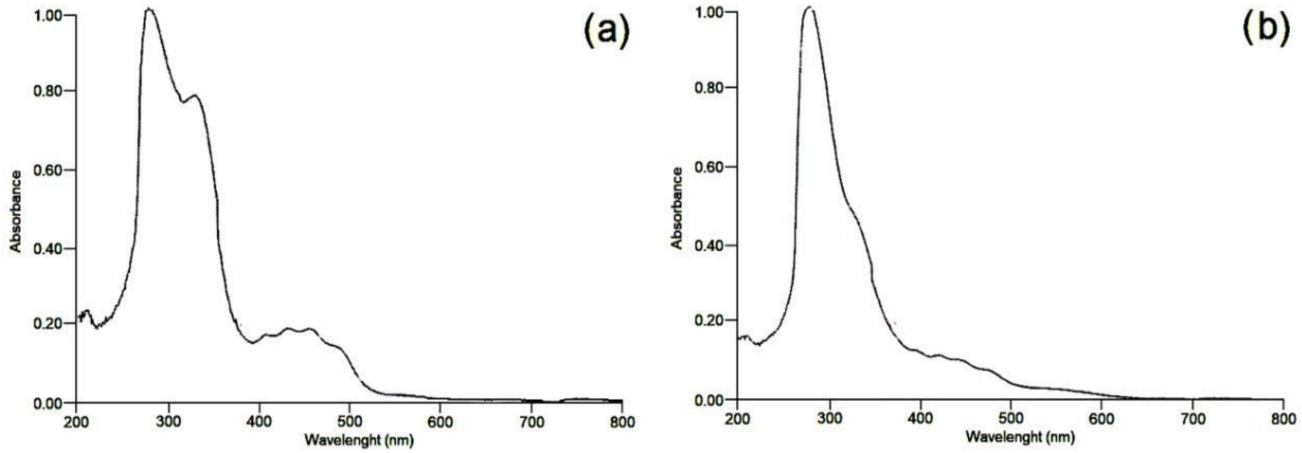

Figure 3. UV spectra of (**a**) tamarillo pulp flour and (**b**) tamarillo epicarp flour.

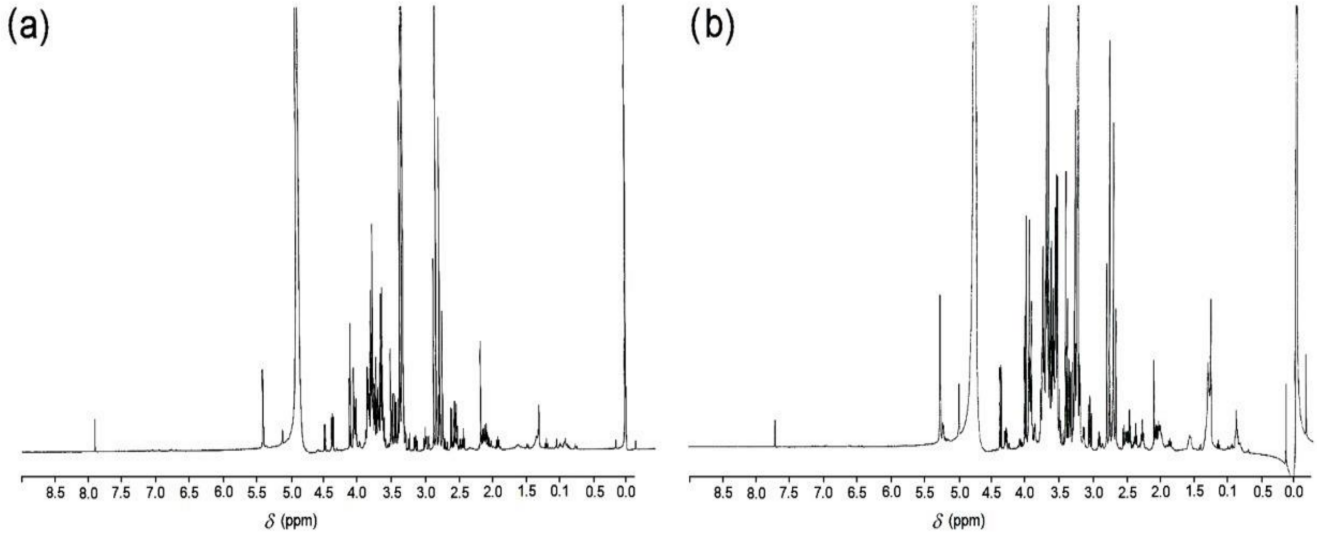

Figure 4. ^{1}H RMN spectra of (**a**) tamarillo pulp flour and (**b**) tamarillo epicarp flour.

3.4. Prebiotic Activity

In general, tamarillo pulp flour presented a higher prebiotic activity than tamarillo epicarp flour. *L. plantarum* showed significantly ($p < 0.01$) higher prebiotic activity for both pulp and epicarp flours as carbon sources, obtaining values of 1.49 and 1.30, respectively. Pulp flour presented positive values with all the lactic acid bacteria (Table 2). The prebiotic activity depends on the probiotic lactic acid bacteria's performance in the presence of a pathogenic strain, in order to be the dominant flora. In this study, under the employed experimental conditions, *L. plantarum* presented higher scores of 8 above 1), but in general, the employed strains presented a higher prebiotic activity score for tamarillo pulp flour than tamarillo epicarp flour. Although Diaz-Vela et al. [41] reported positive prebiotic activity values for *L. rhamnosus* GG with pineapple peel flour and cactus pear flour (0.19 and 0.21, respectively), it seems that tamarillo epicarp or peel presented lower fermentable carbohydrates than pulp. Nonetheless, it has been reported that tamarillo hydrocolloids are resistant to digestive enzymes and gastrointestinal conditions, indicating that they are available for fermentation by gut microbiota, producing short-chain fatty acids as well [33]. Pectic polysaccharides, as found in tamarillo pulp and epicarp, possess important biological activity [42]. In this view, tamarillo consumption, as a source of bioactive molecules with important physiological properties and as an antioxidant and putative prebiotic, is a good alternative for functional foods, since foods formulated with this fruit will present health benefits, as has been already reported, such as hyperlipidemia [43] or metabolic syndrome [30].

Table 2. Prebiotic activity index for both tamarillo pulp and epicarp flour with different lactic acid bacteria.

Substrate/Flour	*L. casei*	*L. plantarum*	*L. paracasei*	*p*
DPFC	0.08 ± 0.00^{c}	1.49 ± 0.01^{a}	0.33 ± 0.01^{b}	0.0001
DEFC	-0.35 ± 0.02^{b}	1.30 ± 0.01^{a}	-0.02 ± 0.01^{c}	0.0001
P	0.0001	0.5482	0.0002	

[a,b] Means with the same letter in the same column are not significantly ($p > 0.05$) different. DPFC= dehydrated pulp flour by convection, DEFC = dehydrated epicarp flour by convection.

4. Conclusions

This study identified for the first time the structures associated with prebiotics with similarity to inulin. In addition, the amount of antioxidants present in Mexican tamarillo is similar to those cultivated in other regions. The presence of bioactive components in tamarillo and its role as a prebiotic indicates that this fruit has potential for industrial applications in the area of health foods.

Author Contributions: Conceptualization, V.R.-G. and B.P.-A.; methodology, A.T., L.P.-C., Z.N.J., G.A.C.-U.; investigation, V.R.-G. and B.P.-A.; resources, B.P.-A.; writing—original draft preparation, V.R.-G. and B.P.-A.; writing—review and editing, B.P.-A., A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request.

Acknowledgments: Author Reyes Veronica thanks the grant for her graduate studies by Program for the Professional Development of Teachers (PRODEP) number. 511-6/18-7973, México. UPAEP Research Department.

Conflicts of Interest: The authors declare no conflict of interest.

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