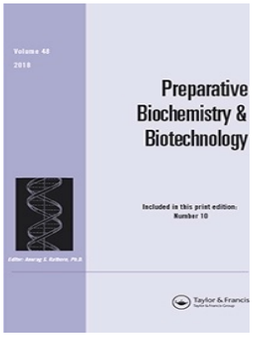


Preparative Biochemistry & Biotechnology

ISSN: 1082-6068 (Print) 1532-2297 (Online) Journal homepage: https://www.tandfonline.com/loi/lpbb20

Anti-glycation effect and the α-amylase, lipase, and α-glycosidase inhibition properties of a polyphenolic fraction derived from citrus wastes

Annayara C. F. Fernandes, Ádina L. Santana, Isabela M. Martins, Debora K. T. Moreira, Juliana A. Macedo & Gabriela A. Macedo

To cite this article: Annayara C. F. Fernandes, Ádina L. Santana, Isabela M. Martins, Debora K. T. Moreira, Juliana A. Macedo & Gabriela A. Macedo (2020): Anti-glycation effect and the α-amylase, lipase, and α-glycosidase inhibition properties of a polyphenolic fraction derived from citrus wastes, Preparative Biochemistry & Biotechnology, DOI: 10.1080/10826068.2020.1737941

To link to this article: https://doi.org/10.1080/10826068.2020.1737941

Published online: 11 Mar 2020.

PREPARATIVE BIOCHEMISTRY & BIOTECHNOLOGY
https://doi.org/10.1080/10826068.2020.1737941

Anti-glycation effect and the α-amylase, lipase, and α-glycosidase inhibition properties of a polyphenolic fraction derived from citrus wastes

Annayara C. F. Fernandes[b], Ádina L. Santana[a], Isabela M. Martins[c], Debora K. T. Moreira[c], Juliana A. Macedo[c], and Gabriela A. Macedo[c]

[a]Food Innovation Center, University of Nebraska-Lincoln, Lincoln, NE, USA; [b]Bioprocessos LES Laboratory, School of Food Engineering, University of Campinas, Campinas, Brazil; [c]University of Campinas, Campinas, Brazil

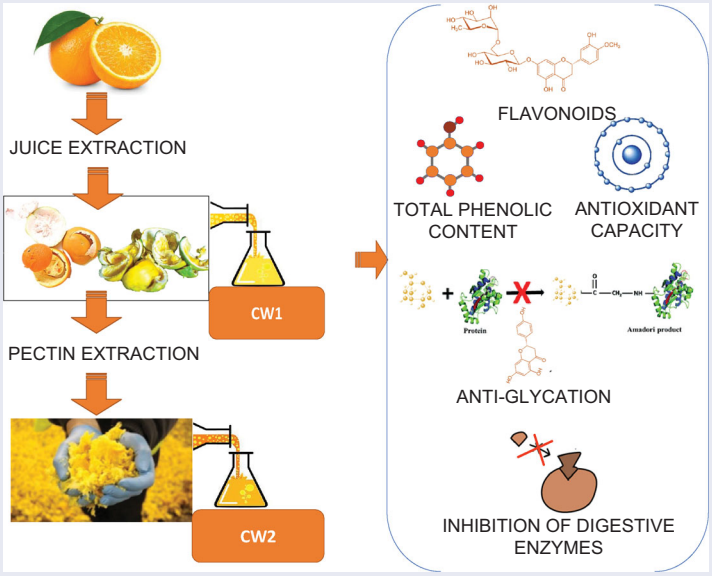
ABSTRACT

The advanced glycation end products (AGEs) constitute a wide variety of substances synthesized from interactions between amino groups of proteins and reducing sugars, which excess induces pathogenesis of chronic diseases. Brazil is the major producer of citrus, a low-cost source of hesperidin, which is a polyphenol recognized for its capacity to inhibit AGEs formation. This is the first work to evaluate the effects of a polyphenolic fraction derived from citrus wastes on the antiglycation and on the inhibition properties of digestive enzymes on the possibility to process these wastes in high value-added products. At concentrations of 10, 15 and 20 mg/mL inhibition of AGEs was higher than 60%. The extracts were able to inhibit by 76% the activity of pancreatic lipase and by 98% the activity of α-glucosidase. For the α-amylase the inhibition capacity was lower than 50%. Strong correlation was obtained among anti-glycation with polyphenolic content and antioxidant capacity.

KEYWORDS

Antiglycation; antioxidant; biowaste; flavonoids; inhibition of digestive enzymes

GRAPHICAL ABSTRACT



Introduction

Protein glycation, also known as the Maillard reaction, involves various complex reactions between the carbonyl group of a reducing sugar and the amino group of a protein. During the initiation step of the reaction, a reducing sugar attaches itself to the free amino group of amino acids, peptides or proteins to produce an unstable Schiff base, which produces relatively stable ketoamines known as Amadori products. The Amadori products are degraded, resulting in the formation of oxoaldehydes, which form the Advanced Glycation End Products (AGEs).[1]

The AGEs are toxic when present in the human body for a long time by inducing the onset of various pathologies like Alzheimer's disease[2] diabetes mellitus,[3] and obesity.[4]

Type 2 diabetes (T2D) and obesity are chronic noncommunicable diseases that can lead to multiple complications

CONTACT Ádina L. Santana adina.santana@gmail.com; Annayara C. F. Fernandes annayaraferreira@hotmail.com; Gabriela Macedo macedoga@gmail.com Food Innovation Center, University of Nebraska-Lincoln, 1901, N 21st street, Lincoln, NE 68588, USA.

and death. The inhibition of digestive enzymes, including α-amylase, α-glucosidase, and pancreatic lipase have been considered a therapeutic alternative to treat obesity and symptoms associated with T2D,[5] like blurry vision, slow healing of cuts and wounds, and patches of dark skin.

Researches on the development of natural and novel AGEs inhibitors increased in order to supply the urgent demand to retard or prevent premature aging and complications caused by several diseases. Polyphenols are plant-based secondary metabolites considered as potential glycation inhibitors because of their ability to scavenge free radicals, to capture reactive dicarbonyl compounds, and to regulate gene expression.[6]

The consumption of citrus fruits has been associated to retard the formation of AGEs because of effect to scavenge reactive dicarbonyl species (deoxyglucosone, glyoxal, and methylglyoxal). The reactive dicarbonyl species react with amines and thiol groups, resulting in the formation of AGEs.[7,8]

The polyphenols typically presented in *Citrus* species are flavanones, a subgroup of compounds derived from flavonoids like hesperidin, hesperitin, naringenin, that showed a strong capacity to inhibit the formation of AGEs.[9,10]

Brazil is the world's largest producer of citrus, approximately 17 million tons in 2016–2017.[11] Most of the Brazillian production of citrus is destined to produce juice. After the extraction of juice, the remaining solids (about 50% of the fruit weight) are discarded, resulting in large amounts of waste.[12]

Citrus peel is the primary residue product of juice processing, and it is generally sold as a raw material for pectin extraction or for animal origin food. However, the main problem also faced by the pectin industry is the generation of a considerable residue volume.[13] In this context, the development and implementation of processes capable of converting agroindustrial residues into several value-added products is a fundamental factor to generate lower environmental impact.

The agroindustrial wastes of citrus have been considered a low cost and feasible source of flavonoids, a class of polyphenols associated with strong antioxidant activity. The flavonoids detected in the extracts of citrus wastes are composed of naringin, hesperidin, naringenin, and hesperitin.[14,15]

To the best of our knowledge, this is the first wok to evaluate the effects of industrial citrus residues to inhibit glycation and digestive enzymes, on the possibility to obtain low-cost products with strong bioactive potential and to valorize citrus wastes.

Material and methods

Reagents

Folin Ciocalteu, arginine and fructose reagents were purchased from Dinâmica Química Contemporânea (Diadema, Brazil). Bovine serum albumin (BSA) was purchased from Thermo Fisher Scientific (Waltham, MA). Gallic acid, narirutin, hesperidin, naringenin, hesperetin, tangeretin, methylglyoxal (MGO), 2,2-diphenyl-1-picr1ylhydrazyl (DPPH), 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 4,6-tripyridyl-s-triazine (TPTZ), were all purchased from Sigma-Aldrich (St. Louis, MO). Monobasic and dibasic potassium phosphate, sodium carbonate and hydrochloric acid were obtained from LabSynth (Diadema, Brazil). Methanol was purchased from JT Baker (Center Valley, PA). Ferric chloride was purchased from Vetec Química (São Paulo, Brazil). Fluorescein and sodium acetate were purchased from Ecibra (São Paulo, Brazil).

Extraction of polyhenolic fraction from citrus wastes

The CP Kelco (Limeira, Brazil), a company specialized in pectin production donated two different types of dried citrus wastes. The crude citrus waste (CW1) consists of the citrus bagasse, derived from juice extraction, but with the presence of pectin, and citrus waste devoided of pectin (CW2) consists of the waste bagasse after removal of pectin.

The raw materials were milled and sieved (10-mesh screen, Bertel, Brazil). The obtaining of the polyphenolic fraction was based on the method of Nakajima et al., with adaptations. Ten grams of the raw material were solubilized in 125 mL of 50% ethanol (ethanol/water, 50/50, v/v), and the solution was inserted on a stackable shaker (MaxQ 8000, Thermo Scientific, MA) at 200 rpm and 25 °C for 1 h. Afterward, the solution was placed in ultrasonic bath (Unique, USC 1800 A, São Paulo, Brazil) at 155 W and 60 Hz. at 25 °C for 15 min, centrifuged (Beckman J2-21 centrifuge, Beckman-Coulter, Inc. Fullerton, CA) at 3000 rpm for 10 min, and filtered through a Whatman No.1 filter paper. Finally, the extract containing the polyphenolic fraction was rotary evaporated (Marconi, Sao Paulo, Brazil) at 40 °C to remove ethanol, followed by freeze drying (Liobras, Sao Paulo, Brazil) to remove the remaining water in the extracts.

Characterization of extracts

Total phenolic content (TPC)

TPC was evaluated with the use of Folin–Ciocalteu reagent.[16] Gallic acid was used as standard, and a calibration curve was constructed (16–300 µg/mL). Results were expressed as mg gallic acid equivalent (GAE)/mg of lyophilized extract.

Determination of polyphenolic compounds by high-performance liquid chromatography (HPLC-DAD)

The polyphenols narirutin, hesperidin, naringenin, hesperetin, and tangeretin present in the extracts of citrus wastes were detected by High-Performance Liquid Chromatography coupled with a diode array detector (HPLC-DAD).

A Dionex UltiMate 3000 (Germany) liquid chromatographer equipped with a C-18 Acclaim® column (Dionex, 3 µm, 120 Å, 4.6 × 150 mm) at 30 °C, was used.

The mobile phases consisted of water acidified with 0.1% formic acid (A), and methanol acidified with 0.1% formic

acid (B). The elution gradient at 0.6 mL/min consisted of 90% A (0–5 min), 20% A (5–80 min), 90% A (80–85 min), and 90% A. Results were expressed as mg/g extract.

DPPH assay

Free radical scavenging of DPPH was evaluated according to Brand-Williams et al.[17] A calibration curve of Trolox standard (15–300 µmol/mL) against DPPH was plotted to calculate the antioxidant capacity of extracts. Results were expressed in µmol Trolox equivalent/mg of extract.

ORAC assay

The oxygen reduction antioxidant capacity (ORAC) assay was performed according to Prior et al.[18] The samples were incubated at 37 °C and fluorescence from the extracts was detected at the wavelengths of 485 nm (excitation) and 520 nm (emission), respectively. A standard curve was plotted with Trolox (1.5–1500 µmol/mL) to determine the antioxidant capacity of extracts. Results were expressed in µmol Trolox equivalent/mg extract.

FRAP assay

The antioxidant capacity of extracts with the use of ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain.[19] The samples were incubated at 37 °C and absorbance were detected at 593 nm. A calibration curve with Trolox (15–1500 µmol/mL) was used to calculate antioxidant capacity. and expressed in µmol Trolox equivalent/mg extract.

Antiglycation potential

Bovine serum albumin-fructose (BSA-FRU) model

This assay was performed according to Wang et al.[20] Potassium phosphate buffer (50 mM and pH 7.4) was used as a solvent. The solvent contained 0.02% sodium azide to prevent microbial growth.

Solutions composed of fructose and bovine serum albumin (BSA) were prepared at 1.5 M and 30 mg/mL, respectively. The extracts from citrus waste were diluted at concentrations of 1, 5, 10, 15, and 20 mg/mL). The assay was conducted by mixing 500 µL of fructose solution with 500 µL of extracts in 10 mL polyethylene tubes and incubated at 37 °C for 2 h.

Afterward, 500 µL of BSA solution (1.5 M) was added to the test tubes, which were incubated at 37 °C for 6 days. The phosphate buffer was used as a control parameter. The fluorescence emitted by AGEs was monitored at 360 nm as (excitation) and 460 nm (emission) with the use of a 96-well fluorescence microplate reader (NOVOstar, BMG LABTECH, Germany). The inhibition in AGE formation, expressed in percentage was calculated by Eq. (1).

$$Inhibition(\%) = \left[1 - \left(\frac{F_E}{F_C}\right)\right] \times 100 \qquad (1)$$

Where F_C is the fluorescence emitted by control and F_E is the fluorescence emitted by extract.

Bovine serum albumin-methylglyoxal (BSA-MGO) model

To evaluate glycation inhibition by BSA-MGO model the solvent used was a 0.02% sodium azide potassium phosphate buffer (50 mM and pH 7.4). Methylglyoxal (MGO) and BSA were diluted in the solvent at the concentrations of 60 mM and 30 mg/mL, respectively. The extracts were diluted at 1, 5, 10, 15, and 20 mg/mL into 10 mL polyethylene tubes.

Afterward, 500 µL of methylglyoxal solution was mixed with 500 µL of diluted extracts and kept at 37 °C for 2 h. Then, 500 µL of BSA solution (60 mM) was added and the tubes were incubated at 37 °C for 6 days.[20] The fluorescence emitted by AGEs was monitored at 360 nm as (excitation) and 460 nm (emission) with the use of a NOVOstar 96-well fluorescence microplate reader (BMG LABTECH, Germany). The inhibition in the AGE formation was calculated according to Eq. (1).

Arginine-methylglyoxal (ARG-MGO) model

The potassium phosphate buffer (50 mM and pH 7.4) containing 0.02% sodium azide was used to prepare the solutions of MGO (60 mM), arginine (60 mM), and extracts (1, 5, 10, 15, and 20 mg/mL).

The mixtures of 500 µL of 60 mM MGO and 500 µL of each extract solution were incubated at 37 °C for 2 h. Afterward, 500 µL of arginine (ARG) solution was added to the mixture and the tubes were incubated at 37 °C for 6 days. Potassium phosphate buffer was used as control.[20]

The fluorescence emitted by AGEs was monitored at 360 nm as (excitation) and 460 nm (emission) with the use of a NOVOstar microplate reader (BMG LABTECH, Germany). The antiglycation capacity was calculated as described Eq. (1).

Inhibition of digestive enzymes activities

α-Amylase

The inhibition of α-amylase activity assay was performed by mixing 50 µL of extracts with 100 µL of starch solution (1 g of starch solubilized in 100 mL of Tris-HCl buffer, pH 7.0) and further incubation of this mixture at 37 °C for 5 min. Afterward, 50 µL of α-amylase was added to the mixture and incubated at 37 °C for 10 min.

The reaction was interrupted by adding 200 µL of 3,5-dinitrosalicylic acid (DNS, formulated by solubilizing 1 g of DNS in 50 mL of water), followed by incubation in boiling water bath (100 °C) for 5 min, and inserted into an ice bath. Afterward, 1.6 mL of distilled water was added, vortex-agitated, and then 200 µL of the solution was added into 96-well plates, and the absorbance was detected at 540 nm. Distilled water was used as control.[21]

All measurements were performed in triplicate. The inhibition percentage was calculated according to the Eq. (2).

Table 1. The TPC and antioxidant capacity of extracts derived from crude citrus waste (CW1) and pectin depleted citrus waste (CW2) for the DPPH, ORAC, and FRAP assay.

Extract	TPC (mg GAE/mg of lyophilized extract)	DPPH (μmol Trolox equivalent/mg of lyophilized extract)	ORAC (μmol Trolox equivalent/mg of lyophilized extract)	FRAP (μmol Trolox equivalent/mg of lyophilized extract)
CW1	$35.21^{a} \pm 0.82$	$125.52^{b} \pm 4.42$	$896.05^{a} \pm 58.82$	$220.00^{a} \pm 3.27$
CW2	$45.57^{b} \pm 2.83$	$40.45^{a} \pm 1.31$	$1482.17^{b} \pm 66.38$	$245.00^{b} \pm 10.38$

Note: Different letters on the same column show statistically significant difference at $p < 0.05$.

Table 2. Content of flavanones (mg/g of lyophilized extract) detected by HPLC in the extracts from crude citrus waste (CW1) and the pectin depleted citrus waste (CW2).

Phenolic compounds	Narirutin	Hesperidin	Naringenin	Hesperetin	Tangeretin
CW1	$3.83^{a} \pm 0.30$	$24.65^{a} \pm 1.46$	$0.12^{a} \pm 0.01$	$0.01^{a} \pm 0.01$	$0.19^{a} \pm 0.01$
CW2	$6.83^{b} \pm 0.74$	$87.64^{b} \pm 3.19$	$0.30^{b} \pm 0.02$	$0.62^{b} \pm 0.19$	$1.07^{b} \pm 0.11$

Note: Different letters on the same column show statistically significant difference at $p < 0.05$.

$$Inhibition(\%) = \left[\frac{(Abs_C - Abs_E)}{Abs_C}\right] \times 100 \qquad (2)$$

Where Abs_C is the absorbance of control and Abs_E is the absorbance of extract.

α-Glucosidase

The inhibition of α-glucosidase enzymatic activity assay was determined with the use of a reaction mixture consisting of 50 μL of extract, 50 μL of substrate (5 mM p-nitrophenyl-α-D-glucopyranoside solution), and 100 μL of α-glucosidase solution (10 mg/mL, from *Saccharomyces cerevisiae*, Sigma-Aldrich, Darmstadt, Germany), solubilized in a potassium phosphate buffer (100 mM and pH 6.9). The reaction was conducted at 37 °C for 10 min and absorbance was detected at 405 nm.[21] Distilled water was used as control and the inhibition percentage was calculated according to Eq. (2).

Pancreatic lipase

The capacity of extracts to inhibit lipase activity was evaluated by using p-Nitrophenyl palmitate (p-NPP) as substrate, and porcine pancreatic lipase, based on procedures described elsewhere.[22] The substrate solution was formulated by dilution of p-npp in isopropanol and TRIS/HCl buffer (pH 8.8) containing 0.1% gum Arabic and 0.4% Triton.

An aliquot of extracts (125 μL) was mixed with 125 μL of substrate solution and incubated at 37 °C for 5 min. Afterward, 250 μL of the enzyme (diluted in TRIS/HCl buffer pH 8.0) was added, and the mixture was incubated at 37 °C for 20 min. After incubation, the samples were centrifuged at 15,000 rpm for 5 min and absorbance was detected at 405 nm. All tests were performed in triplicate.

Statistical evaluation

The results were expressed as the mean ± standard deviation (SD). All measurements were performed in triplicate. Minitab software was used to calculate the analysis of variance (Tukey test, $p \leq 0.05$), to evaluate differences between the control and the samples. Correlations between variables were quantified by the correlation coefficient r.

Pearson's correlation coefficients (r) were calculated to determine the correlation among the antiglycation assays with the phenolic composition (TPC and polyphenols detected by HPLC) and antioxidant capacity.

Results and discussion

Characterization of extracts

TPC of citrus wastes is provided in Table 1. The TPC similarly like the polyphenolic profile detected by HPLC (Table 2) was higher in the extract from citrus waste devoided of pectin (CW2).

This result is justified by the pretreatment done in the raw material during the industrial processing to remove pectin. Pectin extraction is commonly done with the use of acidic solutions formulated with hydrochloric or nitric acid) and temperatures around 80 °C.[23] Pretreatment breaks the cell walls in plants and induces the formation of large cavities and intercellular spaces that allow the remaining compounds like the polyphenols to be easily extracted.[24]

The removal of a pectic fraction of fruits with the enzyme pectinase increased the extraction of polyphenols from citrus wastes, similarly like those reported to the wastes of apple,[25] guarana[26,27] and citrus.[15]

Polyphenolic fractions of extracts from CW1 and CW2 consisted of flavanones hesperidin, narirutin and tangeretin, narigenin and hesperitin. Hesperidin was by far, the most abundant polyphenol detected in CW1 (24.65 mg/g extract) and CW2 (87.64 mg/g extract), respectively (Table 2), similarly like those reported by Garg et al.[25] and Khan et al.[26] Pereira et al.[28] detected flavanones from extracts indigenous to different residues of Brazilian oranges, reporting that the most abundant flavanone found was hesperidin (11.49–44.23 mg/g extract) and lowest content found was tangeretine (0–1.93 mg/g extract).

Low content of naringenin was found in both CW1 and CW2 extracts similarly to those detected previously in extracts obtained from citrus wastes.[14,15,29]

The TPC found in extracts (35.21 and 45.57 mg GAE/mg extract or 3521 and 4557 mg GAE/g raw material) were approximately 100 times higher the than the 40 mg GAE/g raw material detected in waste orange extracts[30] and the 46.63 mg GAE/kg raw material found in the orange seed

essential oil.[31] The antioxidant capacity for the ORAC assay was higher than the 542.93 µmol equivalent Trolox/mg extract detected in citrus wastes biotransformed with tannase.[32] For the FRAP method, CW2 extract showed higher antioxidant capacity (245.00 ± 10.38 µmol Trolox equivalent/mg extract) which value was comparable with the content detected in blue butterfly pea flower.[33]

DPPH and FRAP assays were higher than those found in methanolic extracts of *Tribulus terrestris*,[34] and the extracts of rosemary and oregano.[35]

The DPPH scavenging capacity of CW1 extract (125.52 µmol Trolox equivalent/mg extract) was 3 times higher than that of CW2 extract (Table 1) and was next to the 129.17 µmol Trolox equivalent/mg extract detected in autoclaved citrus extracts.[32] Even considering that CW2 is a waste derived from the juice processing and pectin removal, the capacity of hydrogen donation to DPPH radical from this extract (40.45 µmol Trolox equivalent/mg extract) is relevant and higher than other materials like the 15.6 µmol Trolox equivalent/mg extract detected in waste guarana extracted with supercritical carbon dioxide.[26]

The high antioxidant capacity of extracts was correlated to the flavanones content (Table 3). Previous work reported that the presence of phenolic compounds is associated with the high antioxidant activity in citrus fruits.[36]

In this context, the results obtained support the hypothesis that citrus residues present high antioxidant activity and can be a viable source of flavanone extraction.

Effects of waste citrus extracts on antiglycation

To evaluate the inhibitory effect of extracts of citrus residues on protein glycation, three glycation inducing reaction systems were performed (BSA-FRU, BSA-MGO, and ARG-MGO). Glycation was monitored for 6 days at 37 °C. The period of 6 days was selected because after 5 days of incubation the production of AGEs remains stable and the fluorescence emitted by the AGEs are developed more slowly.[37–39] Elosta et al.[40] evaluated the antiglycation capacities of aged and fresh garlic extracts at concentrations ranging between 5 and 15 mg/mL for 8 days and observed that after 4 days of incubation the formation of AGEs stabilized.[40]

The antiglycation effect of citrus extracts was dose-dependent. The AGEs inhibition increased at concentrations up to 10 mg/mL. A stable behavior was observed at concentrations higher than 10 mg/mL (Figures 1 and 2).

For the BSA-FRU assay the extracts diluted at concentration of 10 mg/mL was associated to inhibit the AGEs by approximately 55% (CW2, Figure 1) and 30.3% (CW1, Figure 2).

The BSA has a high concentration in serum, and it is highly sensitive to glycation and is 90% analogous to human serum albumin.[41] The BSA-FRU model system serves to evaluate protein glycation degree at an accelerated rate, representing some of the complications of hyperglycemia and diabetes.[20]

Reactive dicarbonyls such as glyoxal (GO) and methylglyoxal (MGO) are crucial intermediates for AGEs formation *in vivo*.[42] Results of BSA-MGO assay indicated that CW2 extract inhibited s the formation of AGEs. The maximum inhibition of AGEs by MGO elimination for this assay (62%) was attributed to the CW2 extract (Figure 1), which effect was higher than the inhibitory effect of 40.5% from black raspberry extract at 100 µg/mL.[43]

These results suggest that CW2 and CW1 efficiently inhibit fluorescent AGEs formation in part by decreasing glycation and reducing levels of reactive precursors (such as MGO) for glycation.

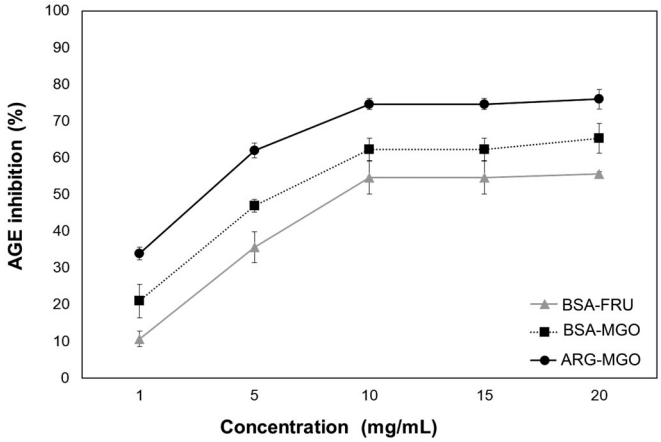


Figure 1. Anti-glycation capacity of the extract from citrus wastes depleted of pectin (CW2) in the BSA-FRU, BSA-MGO, and ARG-MGO models. Values (mean ± standard deviation) are expressed as glycation inhibition percentage.

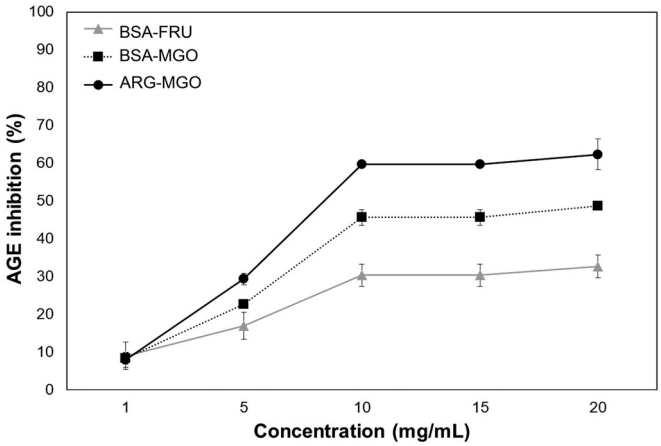


Figure 2. Anti-glycation capacity of the extract from crude citrus wastes (CW1) extract in BSA-FRU, BSA-MGO and ARG-MGO models. Values (mean ± standard deviation) are expressed as glycation inhibition percentage.

Table 3. Pearsons's correlation among anti-glycation capacity with the flavanones, the TPC and antioxidant capacity.

	Narirutin	Hesperidin	Naringenin	Hesperetin	Tangeretin	Total Phenolic Content	DPPH	ORAC	FRAP
BSA-Fructose	0.923	0.967	0.937	0.978	0.989	0.943	n.s.	0.957	0.797
BSA-MGO	0.887	0.972	0.965	0.943	0.980	0.889	n.s.	0.959	0.782
ARG-MGO	0.930	0.995	0.990	0.889	0.965	0.918	n.s.	0.980	0.919

Note: n.s.: non-significant.

During glycation, arginine reacts with MGO dicarbonyl, causing irreversible protein modification.[44] In this context, we used the ARG-MGO model to evaluate antiglycation capacity of citrus residue to prevent the connection of MGO to arginine. The main findings indicate that the highest inhibition percentage was found in the arginine-MGO model, where CW2 and CW1 were inhibited by the formation of AGEs by 76% and 62%, respectively.

The antiglycation effects from citrus wastes showed a strong correlation with the polyphenolic profile presented in the extracts (Table 3). The CW2 extract showed the highest antiglycation because of its flavonoids content, from which hesperidin is presented at the highest concentration (Table 2). Several health benefits were attributed to flavanones, including antioxidant, anti-inflammatory, anticarcinogenic and neuroprotection.

A recent study indicated that the flavanone hesperidin is able to reduce fluorescent AGEs formation, because of the ability of these compounds to inhibit the formation of carbonyl groups, as well as proteins aggregations and aldose reductase activity.[45] The aldose reductase converts glucose into sorbitol, which is then converted to fructose. The high level of aldose reductase expression may induce toxicity.[46]

Hesperidin and hesperetin (10 µM) have shown to inhibit the formation of AGEs by 65.57% and 35.6%, respectively.[47]

Previous studies suggested that polyphenols have stronger inhibition against glycation,[48,49] corroborating the results obtained in our study. It is worth mentioning that our work is the first to demonstrate the role of citrus residue devoided of pectin (CW2) extract and citrus residue (CW1) extract in glycation control.

Considering that the AGEs are known to induce the development of inflammatory responses for various diseases, many efforts have focused to control the formation of AGE with the use of bioactive compounds derived from natural sources.

Effects of waste citrus extracts on the inhibition of digestive enzymes

A recent study reported that phenolic compounds play a key role in inhibiting α-amylase, α-glucosidase and lipase activity.[5] The enzymes α-amylase and α-glucosidase are involved in starch breakdown and absorption of glucose in the intestine.[50] The inhibition of these enzymes reduces glucose digestion and absorption, and consequently, decreases postprandial hyperglycemia,[51] which is crucial to control T2D.[52]

Both CW1 and CW2 extracts inhibited α-amylase by less than 50%. Otherwise, both extracts inhibited α-glucosidase by 98% (Figure 3).

It has been reported that the inhibition of digestive enzyme activity by polyphenols is a reasonable approach to control hyperglycemia and calorie intake in patients with T2D, by reducing the absorption of sugars and fatty acids from the intestine.[53]

The hypoglycemic effect of citrus has been extensively studied. For instance, red blood orange extract inhibited the

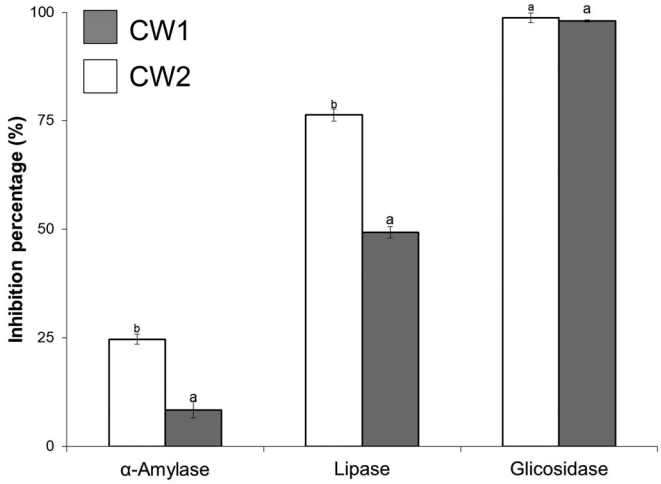


Figure 3. Effects of extracts derived from citrus wastes (10 mg/mL) on the inhibition against the activities of α-amylase, α-glucosidase and pancreatic lipase. Different letters within the same figure show statistical difference ($p < 0.05$).

α-amylase activity by 32.3%.[50] The extracts of lime basra at 0.5–2 ng/mL inhibited the α-amylase activity by 80%.[54]

Tundis et al.[55] showed that high inhibition against α-amylase and α-glucosidase from the extracts of *Poncirus trifoliata* (L.) was attributed to the flavonoids like neoeriocitrin, which showed to inhibit more effectively α-amylase activity, and didymin, which showed the highest inhibition against the α-glycosidase activity.

Owira and Ojewole[56] also found that *Citrus paradisi* juice reduced significantly blood glucose levels with no negative alterations on plasma insulin levels for 1.5 h.

Shen et al.[57] concluded that citrus flavanones (hesperidin, naringin, neohesperidine and nobiletine) inbibited the α-amylase activity during digestion assay in HepG2 cells (a human liver cancer cell line).

Lipase is a key enzyme involved in the digestion of triacylglycerols. Its inhibition is considered one of the most effective strategies to treat obesity, because of inhibited hydrolysis of triacylglycerol in fatty acids and monoacylglycerol, resulting in the reduction of their absorption in the human intestine.[58] Both CW1 and CW2 extracts inhibited considerably the pancreatic lipase by 76% (Figure 3).

Mollace et al.[59] observed that bergamot extract ameliorated the symptoms related to hyperlipidemic and hyperglycemic disorders in Wistar rats and in humans, because of enhancement in high-density lipoprotein (HDL) cholesterol levels and reduction in low-density lipoprotein (LDL) cholesterol, triglyceride, and glycemia levels.

Our results show that citrus wastes are low cost and potential inhibitors against pancreatic lipase, α-amylase, and α-glycosidase, and may act as dietary adjuvants to control glycemia, body weight and reduce obesity.

Correlation among anti-glycation capacity with the phenolic profile and antioxidant capacity

Pearson's coefficient factor higher than 0.7 was obtained, which is associated with a strong correlation among the

antiglycation capacity of extracts with the flavanones analyzed by HPLC the assays, TPC and antioxidant capacity assays, except for the DPPH free radical, which was not significant (Table 3).

The inhibition of AGE with the use of the BSA-FRU model showed the highest correlation with the phenolic composition (flavanones, TPC). Regarding the effects of flavanones for this model, hesperetin (the aglycone form of hesperidin) and tangeretin, which is presented in low content in citrus wastes, showed a higher correlation than the hesperidin, which represents the highest fraction detected.

Besides the similar antioxidant capacity of hesperidin and hesperetin, hesperetin was able to provide neuroprotection in rats by inhibition of lipid peroxidation, while the hesperidin was inactive.[60] This is a factor that may explain the correlation values obtained for the BSA-FRU model.

Another explanation is regarding the proportion of the target compound presented in the extracts. Considering the concentration, this behavior was similar to those reported for mandarin (*Citrus reticulate*) fruits, which highest concentration showed the lowest correlation with anticancer activity,[61] suggesting that the highest proportions may induce toxicity and consequences reduced inhibition of AGEs.

Antioxidant capacity for the ORAC and FRAP assays were better correlated to the ARG-MGO model (Table 3).

Conclusions

Brazil is the largest producer of citrus worldwide. The processing of citrus to extract juice, essential oil, and pectin generates tons of underutilized solid materials. This is the first study to show citrus wastes as a low-cost antiglycant agent, and a potential inhibitor of digestive enzymes on the possibility to process these materials as novel functional foods.

The extracts from crude citrus (CW1), derived from juice processing and from the citrus waste depleted of pectin (CW2) contain a significant fraction of flavanones, which content showed a strong correlation with antioxidant capacity and with the antiglycation assays performed in this work. In addition, the extracts evaluated inhibited enzymes involved in the digestion of carbohydrates and lipids.

The extract obtained from citrus residue depleted of pectin (CW2) showed the highest antioxidant, antiglycation and inhibitory capacity and α-amylase and lipase inhibitory activities, which indicates that the pretreatment done in industrial pectin extraction of citrus contributed to form intercellular spaces in the plant matrix, which contributed to allowing the availability of polyphenols for extraction. Our results support further *in vivo* studies to evaluate the potential of these residues to inhibit AGE and digestive enzymes.

Abbreviations

AGEs	advanced glycation end products
BSA	bovine serum albumin
CW1	citrus residue
CW2	citrus residue devoided of pectin
GAE	gallic acid equivalent
MGO	methylglyoxal
T2D	Type 2 diabetes

Disclosure statement

The authors declare no conflict of interest.

Funding

The present work was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundação de Amparo à Pesquisa do Estado de São Paulo.

References

[1] Wu, C.-H.; Huang, S.-M.; Lin, J.-A.; Yen, G.-C. Inhibition of Advanced Glycation Endproduct Formation by Foodstuffs. *Food Funct.* **2011**, *2*, 224–234.

[2] Vicente Miranda, H.; El-Agnaf, O. M.; Outeiro, T. F. Glycation in Parkinson's Disease and Alzheimer's Disease. *Mov. Disord.* **2016**, *31*, 782–790.

[3] Ahmed, N. Advanced Glycation Endproducts—Role in Pathology of Diabetic Complications. *Diabetes Res. Clin. Pract.* **2005**, *67*, 3–21.

[4] Boyer, F.; Vidot, J. B.; Dubourg, A.; Rondeau, P.; Essop, M.; Bourdon, E. Oxidative Stress and Adipocyte Biology: Focus on the Role of AGEs. *Oxid. Med. Cell. Longev.* **2015**, *2015*, 1–9.

[5] Spínola, V.; Llorent-Martínez, E. J.; Castilho, P. C. Polyphenols of Myrica Faya Inhibit Key Enzymes Linked to Type II Diabetes and Obesity and Formation of Advanced Glycation End-Products (In Vitro): Potential Role in the Prevention of Diabetic Complications. *Food Res. Int.* **2019**, *116*, 1229–1238.

[6] Khangholi, S.; Majid, F. A. A.; Berwary, N. J. A.; Ahmad, F.; Aziz, R. B. A. The Mechanisms of Inhibition of Advanced Glycation End Products Formation through Polyphenols in Hyperglycemic Condition. *Planta Med.* **2016**, *82*, 32–45.

[7] Shao, X.; Chen, H.; Zhu, Y.; Sedighi, R.; Ho, C.-T.; Sang, S. Essential Structural Requirements and Additive Effects for Flavonoids to Scavenge Methylglyoxal. *J. Agric. Food Chem.* **2014**, *62*, 3202–3210.

[8] Bellier, J.; Nokin, M.-J.; Lardé, E.; Karoyan, P.; Peulen, O.; Castronovo, V.; Bellahcène, A. Methylglyoxal, a Potent Inducer of AGEs, Connects between Diabetes and Cancer. *Diabetes Res. Clin. Pract.* **2019**, *148*, 200–211.

[9] Khan, M. K.; E-Huma, Z.; Dangles, O. A Comprehensive Review on Flavanones, the Major Citrus Polyphenols. *J. Food Comp. Anal.* **2014**, *33*, 85–104.

[10] Wang, Y.; Wang, L.; Xu, G.; Wei, D. Hesperidin Exerts the Gestational Diabetes Mellitus via AGEs-RAGE Signalling Pathway. *Int. J. Pharmacol.* **2019**, *15*, 604–615.

[11] Food and Agricultural Organization. FAOSTAT, 2019. Statistics division, food and agriculture organization of the United Nations, Rome, Italy. Available at: http://www.fao.org/faostat/en/#data/QC.

[12] Okino Delgado, C. H.; Fleuri, L. F. Orange and Mango by-Products: Agro-Industrial Waste as Source of Bioactive Compounds and Botanical versus Commercial Description—a Review. *Food Rev. Int.* **2016**, *32*, 1–14.

[13] Rafiq, S.; Kaul, R.; Sofi, S. A.; Bashir, N.; Nazir, F.; Nayik, G. A. Citrus Peel as a Source of Functional Ingredient: A Review. *J. Saudi Soc. Agricult. Sci.* **2018**, *17*, 351–358.

[14] Barbosa, P. P. M.; Ruviaro, A. R.; Macedo, G. A. Comparison of Different Brazilian Citrus by-Products as Source of Natural Antioxidants. *Food Sci. Biotechnol.* **2018**, *27*, 1301–1309.

[15] Ruviaro, A. R.; Barbosa, P. d.-P. M.; Macedo, G. A. Enzyme-Assisted Biotransformation Increases Hesperetin Content in Citrus Juice By-Products. *Food Res. Int*. **2018**, *124*, 213–221.

[16] Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Viticult*. **1965**, *3*, 144–158.

[17] Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT Food Sci. Technol*. **1995**, *28*, 25–30.

[18] Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (Oxygen Radical Absorbance Capacity (ORAC(FL))) of Plasma and Other Biological and Food Samples. *J. Agric. Food Chem*. **2003**, *51*, 3273–3279.

[19] Benzie, I.; Strain, J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem*. **1996**, *239*, 70–76.

[20] Wang, W.; Yagiz, Y.; Buran, T. J.; Nascimento Nunes, C. d.; Gu, L. Phytochemicals from Berries and Grapes Inhibited the Formation of Advanced Glycation End-Products by Scavenging Reactive Carbonyls. *Food Res. Int*. **2011**, *9*, 2666–2673.

[21] Kwon, Y. I.; Apostolidis, E.; Shetty, K. Inhibitory Potential of Wine and Tea Against A-Amylase and A-Glucosidase for Management of Hyperglycemia Linked to Type 2 Diabetes. *J. Food Biochem*. **2008**, *32*, 15–31.

[22] de Souza Mesquita, L.; Caria, C.; Santos, P.; Ruy, C.; da Silva Lima, N.; Moreira, D.; da Rocha, C.; Murador, D.; de Rosso, V.; Gambero, A.; et al. Modulatory Effect of Polyphenolic Compounds from the Mangrove Tree Rhizophora mangle L. on Non-Alcoholic Fatty Liver Disease and Insulin Resistance in High-Fat Diet Obese Mice. *Molecules* **2018**, *2114*, 23.

[23] Oreopoulou, V.; Tzia, C. Utilization of Plant by-Products for the Recovery of Proteins, Dietary Fibers, Antioxidants, and Colorants. In *Utilization of by-Products and Treatment of Waste in the Food Industry*. Springer, 2007; pp. 209–232.

[24] Liu, Z.; Esveld, E.; Vincken, J.-P.; Bruins, M. E. Pulsed Electric Field as an Alternative Pre-Treatment for Drying to Enhance Polyphenol Extraction from Fresh Tea Leaves. *Food Bioproc. Tech*. **2019**, *12*, 183–192.

[25] Oszmiański, J.; Wojdyło, A.; Kolniak, J. Effect of Pectinase Treatment on Extraction of Antioxidant Phenols from Pomace, for the Production of Puree-Enriched Cloudy Apple Juices. *Food Chem*. **2011**, *127*, 623–631.

[26] Santana, Á. L.; Queirós, L. D.; Martínez, J.; Macedo, G. A. Pressurized Liquid- and Supercritical Fluid Extraction of Crude and Waste Seeds of Guarana (*Paullinia cupana*): Obtaining of Bioactive Compounds and Mathematical Modeling. *Food Bioprod. Proc*. **2019**, *117*, 194–202.

[27] Santana, Á. L.; Macedo, G. A. Effects of Hydroalcoholic and Enzyme-Assisted Extraction Processes on the Recovery of Catechins and Methylxanthines from Crude and Waste Seeds of Guarana (Paullinia Cupana). *Food Chem*. **2019**, *281*, 222–230.

[28] Pereira, R. M.; López, B. G.-C.; Diniz, S. N.; Antunes, A. A.; Garcia, D. M.; Oliveira, C. R.; Marcucci, M. C. Quantification of Flavonoids in Brazilian Orange Peels and Industrial Orange Juice Processing Wastes. *Agric. Sci*. **2017**, *08*, 631–644.

[29] Coll, M.; Coll, L.; Laencina, J.; Tomas-Barberan, F. Recovery of Flavanones from Wastes of Industrially Processed Lemons. *Z. Lebensm. Unters. Forsch*. **1998**, *206*, 404–407.

[30] Sir Elkhatim, K. A.; Elagib, R. A.; Hassan, A. B. Content of Phenolic Compounds and Vitamin C and Antioxidant Activity in Wasted Parts of Sudanese Citrus Fruits. *Food Sci. Nutr*. **2018**, *6*, 1214–1219.

[31] İnan, Ö.; Özcan, M. M.; Aljuhaimi, F. Effect of Location and Citrus Species on Total Phenolic, Antioxidant, and Radical Scavenging Activities of Some Citrus Seed and Oils. *J. Food Proc. Preserv*. **2018**, *42*, e13555.

[32] Nakajima, V. M.; Madeira, J. V., Jr.; Macedo, G. A.; Macedo, J. A. Biotransformation Effects on anti Lipogenic Activity of Citrus Extracts. *Food Chem*. **2016**, *197*, 1046–1053.

[33] Mehmood, A.; Ishaq, M.; Zhao, L.; Yaqoob, S.; Safdar, B.; Nadeem, M.; Munir, M.; Wang, C. Impact of Ultrasound and Conventional Extraction Techniques on Bioactive Compounds and Biological Activities of Blue Butterfly Pea Flower (*Clitoria ternatea* L.). *Ultrason. Sonochem*. **2019**, *51*, 12–19.

[34] Rajurkar, N. S.; Hande, S. M. Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. *Ind. J. Pharm. Sci*. **2011**, *73*, 146–151.

[35] Fernandes, R. P. P.; Trindade, M. A.; Tonin, F. G.; Lima, C. G.; Pugine, S. M. P.; Munekata, P. E. S.; Lorenzo, J. M.; de Melo, M. P. Evaluation of Antioxidant Capacity of 13 Plant Extracts by Three Different Methods: Cluster Analyses Applied for Selection of the Natural Extracts with Higher Antioxidant Capacity to Replace Synthetic Antioxidant in Lamb Burgers. *J. Food Sci. Technol*. **2016**, *53*, 451–460.

[36] Sun, Y.; Qiao, L.; Shen, Y.; Jiang, P.; Chen, J.; Ye, X. Phytochemical Profile and Antioxidant Activity of Physiological Drop of Citrus Fruits. *J. Food Sci*. **2013**, *78*, C37–C42.

[37] Povichit, N.; Phrutivorapongkul, A.; Suttajit, M.; Chaiyasut, C.; Leelapornpisid, P. Phenolic Content and In Vitro Inhibitory Effects on Oxidation and Protein Glycation of Some Thai Medicinal Plants. *Pak. J. Pharm. Sci*. **2010**, *23*, 403–408.

[38] McPherson, J. D.; Shilton, B. H.; Walton, D. J. Role of Fructose in Glycation and Cross-Linking of Proteins. *Biochemistry* **1988**, *27*, 1901–1907.

[39] Kim, H. Y.; Kim, K. Protein Glycation Inhibitory and Antioxidative Activities of Some Plant Extracts In Vitro. *J. Agric. Food Chem*. **2003**, *51*, 1586–1591.

[40] Elosta, A.; Slevin, M.; Rahman, K.; Ahmed, N. Aged Garlic Has More Potent Antiglycation and Antioxidant Properties Compared to Fresh Garlic Extract In Vitro. *Sci. Rep*. **2017**, *7*, 39613.

[41] Wautier, J. L.; and Guillausseau, P. J. Advanced Glycation End Products, their Receptors and Diabetic Angiopathy. *Diabetes Metab*. **2001**, *27*, 535–544.

[42] Li, X.; Zheng, T.; Sang, S.; Lv, L. Quercetin Inhibits Advanced Glycation End Product Formation by Trapping Methylglyoxal and Glyoxal. *J. Agric. Food Chem*. **2014**, *62*, 12152–12158.

[43] Ma, H.; Johnson, S. L.; Liu, W.; DaSilva, N. A.; Meschwitz, S.; Dain, J. A.; Seeram, N. P. Evaluation of Polyphenol Anthocyanin-Enriched Extracts of Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry for Free Radical Scavenging, Reactive Carbonyl Species Trapping, anti-Glycation, anti-β-Amyloid Aggregation, and Microglial Neuroprotective Effects. *Int. J. Mol. Sci*. **2018**, *19*, 461.

[44] Mesías, M.; Navarro, M.; Martínez-Saez, N.; Ullate, M.; Del Castillo, M.; Morales, F. Antiglycative and Carbonyl Trapping Properties of the Water Soluble Fraction of Coffee Silverskin. *Food Res. Int*. **2014**, *62*, 1120–1126.

[45] Patil, K. K.; Gacche, R. N. Inhibition of Glycation and Aldose Reductase Activity Using Dietary Flavonoids: A Lens Organ Culture Studies. *Int. J. Biol. Macromol*. **2017**, *98*, 730–738.

[46] Ramasamy, R.; Goldberg, I. J. Aldose Reductase and Cardiovascular Diseases, Creating Human-Like Diabetic Complications in an Experimental Model. *Circ. Res*. **2010**, *106*, 1449–1458.

[47] Dhanya, R.; Jayamurthy, P. In Vitro Evaluation of Antidiabetic Potential of Hesperidin and Its Aglycone Hesperetin under Oxidative Stress in Skeletal Muscle Cell Line. *Cell Biochem. Funct*. **2020**, 1–9.

[48] Stefek, M. Natural Flavonoids as Potential Multifunctional Agents in Prevention of Diabetic Cataract. *Interdisciplin. Toxicol*. **2011**, *2*, 69–77.

[49] Poojary, M. M.; Greco, I.; Jansson, T.; Rauh, V.; Sørensen, J.; Lund, M. N. *Green Tea Polyphenols decrease the formation of advanced glycation end products in UHT milk*. In: 3rd Young

AGErs Symposium Proceedings, Wageningen University & Research, Wageningen, Netherlands, July 12–13. 2018.
[50] Alu'datt, M. H.; Rababah, T.; Alhamad, M. N.; Al-Mahasneh, M. A.; Ereifej, K.; Al-Karaki, G.; Al-Duais, M.; Andrade, J. E.; Tranchant, C. C.; Kubow, S.; et al. Profiles of Free and Bound Phenolics Extracted from Citrus Fruits and Their Roles in Biological Systems: Content, and Antioxidant, anti-Diabetic and anti-Hypertensive Properties. *Food Funct*. **2017**, *8*, 3187–3197.
[51] Kim, Y.-M.; Jeong, Y.-K.; Wang, M.-H.; Lee, W.-Y.; Rhee, H.-I. Inhibitory Effect of Pine Extract on Alpha-Glucosidase Activity and Postprandial Hyperglycemia. *Nutrition* **2005**, *21*, 756–761.
[52] Balasubramaniam, V.; Mustar, S.; Khalid, N. M.; Rashed, A. A.; Noh, M. F. M.; Wilcox, M. D.; Chater, P. I.; Brownlee, I. A.; Pearson, J. P. Inhibitory Activities of Three Malaysian Edible Seaweeds on Lipase and α-Amylase. *J. Appl. Phycol*. **2013**, *25*, 1405–1412.
[53] Chethan, J.; Kumar, P.; Prakash, H. Antidiabetic and Antihypertensive Potential of Selected Asteraceae Plant Species. *Am. J. Adv. Drug Deliv*. **2014**, *2*, 355–363.
[54] Şeker Karatoprak, G.; Yücel Aşık, Ç.; Çakır, A.; Köngül Şafak, E. In vitro pharmacological screening of antioxidant, cytotoxic and enzyme inhibitory activities of Citrus aurantifolia Linn. Dried fruit extract. *Int. J. Env. Health Res*. **2020**, 1–10.
[55] Tundis, R.; Bonesi, M.; Sicari, V.; Pellicanò, T. M.; Tenuta, M. C.; Leporini, M.; Menichini, F.; Loizzo, M. R. *Poncirus trifoliata* (L.) Raf.: Chemical Composition, Antioxidant Properties and Hypoglycaemic Activity via the Inhibition of α-Amylase and α-Glucosidase Enzymes. *J. Funct. Foods* **2016**, *25*, 477–485.
[56] Owira, P.; Ojewole, J. Grapefruit Juice Improves Glycemic Control but Exacerbates Metformin-Induced Lactic Acidosis in Non-Diabetic Rats. *Meth. Find Exp. Clin. Pharmacol*. **2009**, *31*, 563–570.
[57] Shen, W.; Xu, Y.; Lu, Y.-H. Inhibitory Effects of Citrus Flavonoids on Starch Digestion and Antihyperglycemic Effects in HepG2 Cells. *J. Agric. Food Chem*. **2012**, *60*, 9609–9619.
[58] Xu, B. J.; Han, L. K.; Zheng, Y. N.; Lee, J. H.; Sung, C. K. In Vitro Inhibitory Effect of Triterpenoidal Saponins from Platycodi Radix on Pancreatic Lipase. *Arch. Pharm. Res*. **2005**, *28*, 180–185.
[59] Mollace, V.; Sacco, I.; Janda, E.; Malara, C.; Ventrice, D.; Colica, C.; Visalli, V.; Muscoli, S.; Ragusa, S.; Muscoli, C.; et al. Hypolipemic and Hypoglycaemic Activity of Bergamot Polyphenols: From Animal Models to Human Studies. *Fitoterapia* **2011**, *82*, 309–316.
[60] Cho, J. Antioxidant and Neuroprotective Effects of Hesperidin and Its Aglycone Hesperetin. *Arch. Pharm. Res*. **2006**, *29*, 699–706.
[61] Fayed, S. A. Antioxidant and anticancer activities of Citrus reticulate (Petitgrain Mandarin) and Pelargonium graveolens (Geranium) essential oils. *Res. J. Agric. Biol. Sci*. **2009**, *5*, 740–747.