Food and Bioproducts Processing

Valorization of agro-food by-products and their potential therapeutic applications

--Manuscript Draft--

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	Marta Perez-Fernandez
	Sandra Garcia-Juiz
	Jesus Osada
	Olga Martin Belloso
Abstract:	Agro-food industries generate a huge amount of fruit and vegetable wastes. These residues are composed by structural parts (leaves, peels, pulps, seeds, roots and stems) after the extraction of their juices, and they are rich in bioactive compounds such polyphenols, protein, carbohydates, fibre, lignin, lipids and minerals. Therefore, these wastes constitute a source of bioactive compunds and their valorization leads to a circular economy in which industry and society benefit. Between the component of the residues, polyphenols compounds may contribute to prevent or treated cancer, neurodegenerative and cardiovascular diseases, among others. The aim of this work was the study of the potential therapeutic effect of fruit and vegetable extracts (peach, apple, cucumber and red pepper) obtained by ultrasounds, on different lines of cancer cells (colon, liver and breast) and on oxidative stress in a healthy colon. The results showed that extracts rich in polyphenols had an antiproliferative effect against tumor cells and their action was improved by encapsulation in maltodextrin. Encapsulation also increased the protection against oxidative stress in intestinal cells. This study opened the gate to use the extracts from fruit and vegetable wastes as pharmaceutical excipients, food additives, nutraceutical products or functional foods for therapeutic purposes and prevention of diseases related to oxidative stress.
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May 13, 2021

María Jesús Rodriguez Yoldi

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Dear editor of Food and Bioproducts Proccesing journal,

First of all, thank you very much for giving us the opportunity to revise and resubmit the work for publication.

We are resubmitting for publication our paper in relation to Valorization of agro-food extracts, entitled "Valorization of agro-food by-products and their potential therapeutic applications".

We have considered all points raised by the reviewers in the revised manuscript and addressed the responses to the reviewers. Please, you can find below the reply to reviewer**s'** comments. The corrections were made directly in the manuscript and they appear in the text highlighted in yellow. We hope that these responses will be acceptable for publication in your journal.

If you need further information, do not hesitate to contact us.

Yours sincerely,

MJ Rodriguez Yoldi, PhD.

ORCID iD: 0000-0002-3595-7668

Date: Apr 26, 2021
To: "Maria Jesus Rodriguez Yoldi" mjrosyol@unizar.es
From: "Food and Bioproducts Processing" fbp@elsevier.com
Subject: Decision on submission to Food and Bioproducts Processing
Manuscript Number: FBP-D-21-00204

By-products of agro-food industries with potential therapeutic applications

Dear Mrs Rodriguez Yoldi,

Thank you for submitting your manuscript to Food and Bioproducts Processing.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by May 16, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/fbp/, and navigate to the "Submissions Needing Revision" folder.

Food and Bioproducts Processing values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Fotios Spyropoulos

Subject Editor

Food and Bioproducts Processing

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The fruit and vegetable wastes were kindly provided by a juice company with which we collaborated through the project European Interreg-SUDOE REDVALUE (SOEI/PI/EO123)) entitled "Technological alliance to complete the agro-industrial and forestry production cycle". This company produces juices with those fruits and vegetables that belonged to an area of northern Spain (Lleida).

The material used in the present work was a residual material not marketable which was discarded. In order to enhance their valorization and reduce their environmental and economic impact in the agro-food industry, the company was searching new approaches and applications for those fruit and vegetable wastes.

Besides, in page 13, Conclusion Section, lines 42-46, the authors claim that "No correlation was found between the total content of polyphenols in the extracts and their effect on cancer cells since the action of these bioactive compounds also depend on the type of polyphenols present and the synergistic or additive effects between them." However, there is not an analysis correlating the type of polyphenols present in the studied extracts and their effect on cancer cells. Is it a novel methodology to revalue the bioactive compounds from fruits and vegetables wastes for their potential application in the pharmaceutical and food industries with health benefits? or is it just an evaluation of extracts obtained from wastes of peach, apple, cucumber and red pepper? Therefore, in my opinion, the aim of the manuscript should be clearly stated and supported to enhance and highlight the relevance of the work.

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Thank you very much for the comment. All experiments were performed in triplicate.

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Highlights

. Bioactive compounds of agro-food waste could have anticancer effects.

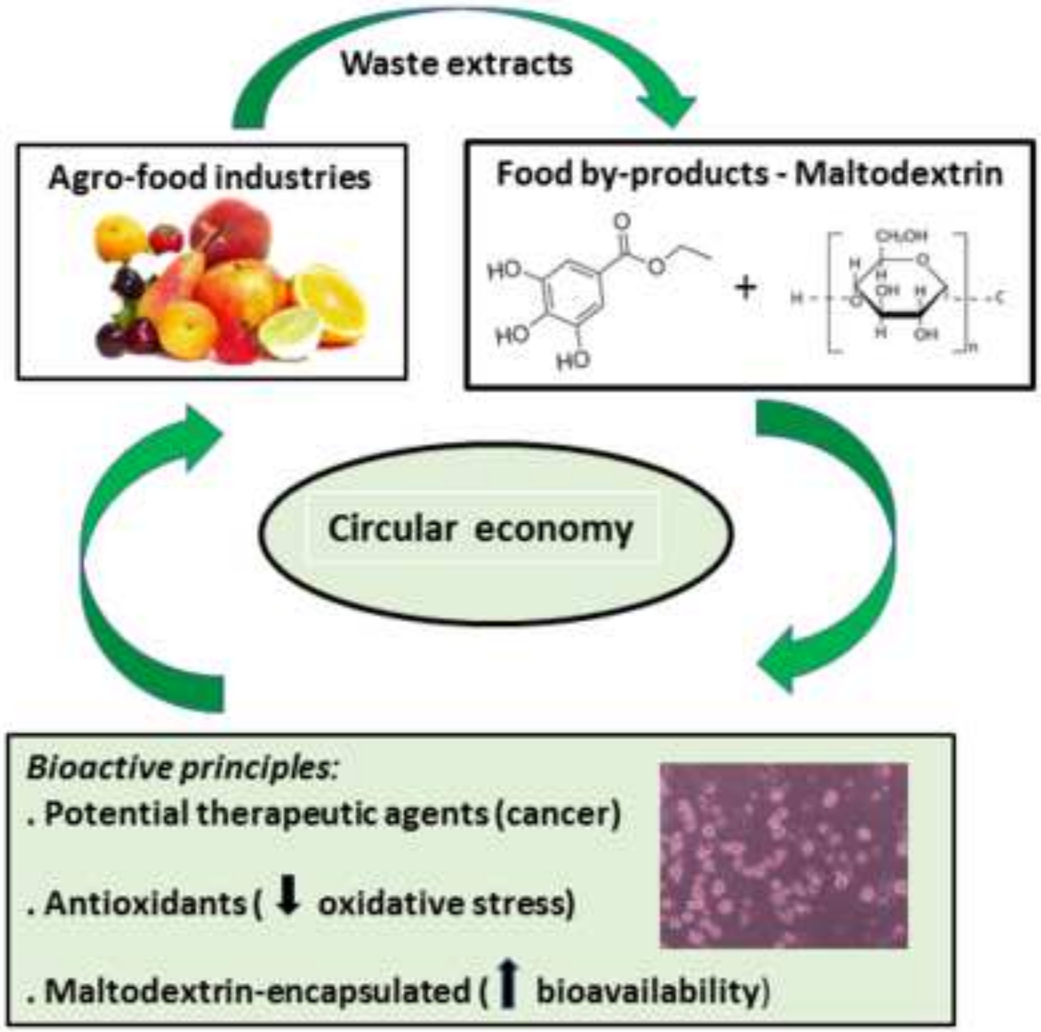
. Maltodextrin plant extracts increase the bioavailability of bioactive compounds.

. Maltodextrin waste extracts protect the intestinal barrier against oxidative stress.

. The agroindustry by-products could be used for therapeutic purposes.

. Fruit and vegetable wastes could be revalued in food industries.

Graphical Abstract



Valorization of agro-food by-products and their potential therapeutic applications

Inés Mármol[a], Javier Quero[a], Raquel Ibarz[b], Pedro Ferreira-Santos[c], Jose A. Teixeira[c], Cristina M.R. Rocha[c], Marta Pérez-Fernández[d], Sandra García-Juiz[d], Jesús Osada[e, f], Olga Martín-Belloso[b*] and María Jesús Rodríguez-Yoldi[a, f*]

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Received: date; Accepted: date; Published: date

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Keywords: antioxidants, cancer, maltodextrin, valorization, fruit and vegetable wastes

1. Introduction

The transformation of raw materials in the agro-food industry generates a significant amount of waste in the form of shells, skins, stems, seeds, and pulps among others. These discarded materials are costly disposed in landfills or incinerators causing negative effects on the environment. Nevertheless, agro-food waste products can contain health-beneficial compounds with a clear potential market, due to their low price, abundant and easy access (Jimenez-Moreno et al., 2020; Machado et al., 2021; Montenegro-Landívar et al., 2021; Quero et al., 2021; Ruiz Rodriguez et al., 2021; Tylewicz et al., 2018; Villacis-Chiriboga et al., 2021).

Furthermore, a high daily intake of fruits and vegetables is associated with a reduced risk of illnesses related to marked pro-oxidant and pro-inflammatory status such as atherosclerosis, diabetes and cancer (Tylewicz et al., 2018). In the specific case of cancer, the Mediterranean and similar diets, which are characterized by high consumption of plant-derived foodstuffs, have been related to a decrease in the incidence of colorectal and breast cancer, among others (Mármol et al., 2018). The chemopreventive effect of fruits and vegetables seems to be mediated by their content in fiber and phenolic compounds. Fiber reduces exposure time of carcinogens on the gastrointestinal tract, decreases the total amount of secondary bile salts, which are known to be pro-tumorigenic agents, and its role as prebiotic enhances the population of bacterial strains producers of butyrate (Mármol et al., 2018). Moreover, phenolic compounds, a heterogeneous group of phytocompounds, offer great interest due to their beneficial properties for human health (Ganesan and Xu, 2017; Gascon et al., 2018; Jimenez-Moreno et al., 2019; Jimenez et al., 2016; Khan and Mukhtar, 2018; Piccolella et al., 2019b; Quero et al., 2021; Quero et al., 2020; Tylewicz et al., 2018). Besides that role in prevention, plant-derived extracts might display a role on the management of cancer treatment as well. Certain phytocompounds such as taxifolin (Razak et al., 2018), quercetin (Hashemzaei et al., 2017) and curcumin (Bianchi et al., 2018) have shown a strong antitumor potential on both *in vitro* and *in vivo* cancer models. Given that whole plant extracts are composed by a complex mixture of potential anti-carcinogenic agents, and considering that each phenolic compound displays affinity for one or more different cellular targets, an additive or synergistic effect among all of them has been observed (Lewandowska et al., 2014; Mertens-Talcott et al., 2003; Seeram et al., 2005). Therefore, the use of whole plant extracts might result on reinforced clinical benefits in comparison to the administration of single isolated agents (Jimenez et al., 2016). Besides, non-edible parts such as bark or peel show similar content in polyphenols, thus suggesting their potential application also in clinical practice (Marmol et al., 2019), opening the gate for the valorization of agroindustry residues as source of phenolic compounds (Baci et al., 2019; Grillo et al., 2019; Jimenez-Moreno et al., 2019; Jimenez-Moreno et al., 2020; Montenegro-Landívar et al., 2021; Piccolella et al., 2019a; Quero et al., 2021).

Another aspect requiring further technological developments is the loss of biocompounds caused by inadequate processing in the conventional extraction procedures (maceration, shaking, soxhlet, among others) (Alirezalu et al., 2020; Araujo et al., 2021; Ruiz Rodriguez et al., 2021; Sumere et al., 2018; Villacis-Chiriboga et al., 2021). Ultrasound Assisted Extraction (UAE) has been successfully used to obtain thermolabile compounds, such as polyphenols (Chemat et al., 2011; Montenegro-Landívar et al., 2021). UAE produces the disruption of the membrane wall cells caused by the compression and rarefaction waves (from 20 kHz to 100 MHz), facilitates the release of extractable biocompounds, and enhances the heat and mass transfer, becoming a reproducible, selective, productive, and eco-friendly technology (Jambrak and Herceg, 2014). In addition, the simultaneous dehydration and coating of the agroindustrial food waste extracts also preserves them from the environmental damage, becoming more thermally stable as well as facilitating the release of bioactive compounds (Medina-Torres et al., 2013; Souza et al., 2018; Yousefi et al., 2011). Encapsulation by spray drying is the most common and cheapest technique, which confers mechanical stability of dehydrated extracts, maintaining quality, and protecting compounds from oxidative processes, compared to other drying methods (Desobry et al., 1997; Mahdavi et al., 2014; Popovic et al., 2021). Maltodextrin is habitually used as wall material for encapsulating biocompounds by spray drying in the food industry, due to their bland flavour, high solubility in water, colourless solutions, and low viscosity (Ferreira-Santos et al., 2021; Gibbs et al., 1999).

Given the large amount of waste obtained in agri-food companies that are discarded, containing bioactive compounds with potential therapeutic applications, it seemed interesting to find new ways to their valorization. Therefore, this study evaluated the potential of bioactive compounds, presented in agroindustrial bagasse (pulp and skin) extracts of apple, peach, cucumber and red pepper obtained by UAE from agro-food juice company, on the antiproliferative activity in three cancer cell models: human colorectal adenocarcinoma Caco-2 cell line, human breast adenocarcinoma MCF-7 cell line and human hepatocellular carcinoma HepG2 cell line. In addition, it was tested the protective effect of red pepper toward a model of the intestinal barrier (differentiated Caco-2 cells) upon hydrogen peroxide insult. Furthermore, it is important to take in consideration that the bioactive compounds can undergo changes when they are exposed to adverse conditions and therefore their encapsulation in maltodextrins by spray drying to protect them from oxidative processes was also evaluated.

2. Materials and Methods

2.1. Chemicals

Maltodextrin (dextrose equivalent 14-17 (DE14-17)), Folin-Ciocalteu reagent, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), dimethyl sulfoxide (DMSO, ≥99.9%), sulforhodamine B sodium salt, trichlroacetic acid, 2′, 7′-dichlorofluorescin diacetate and all standard markers for HPLC were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and ultra-pure water was used throughout the experiments.

2.2. *Raw material preparation and characterization*

Mediterranean fruits and vegetables wastes (apple, peach, cucumber, and red pepper) were obtained from an Agro-food Company (Indulleida S.A. Lleida, Spain). The agro-food wastes (AFW) were the bagasse (pulp and skin) resulting from the juice extraction process of the selected fruits and vegetables. The material used in the present work was a residual material not marketable which was discarded. In order to enhance their valorisation and reduce their environmental and economic impact in the agro-food industry, the company was searching new approaches and applications for those fruit and vegetable wastes

Chemical summative analyses were determined using official protocols (National Renewable Energy Laboratory (NREL) and AOAC, in accordance to Ferreira-Santos *et al.* (Ferreira-Santos et al., 2020) and included ethanol and water extractives (NREL/TP-510-42619), carbohydrates and lignin (NREL/TP-510-42618), fiber, lipids, protein, ash (NREL/TP-510-42622), moisture and mineral content. The mineral content was determined by inductively coupled plasma atomic emission spectrometry (Optima 8000 ICP-OES, PerkinElmer, Waltham, MA, USA), after digestion with HNO_3. Fat content was determined according to the official AOAC method (nº 920.39). Total proteins content estimated by using the N×6.25 conversion factor, was performed using a Kjeldahl distillator (Kjeltec 8400 Analyzer, FOSS, Hilleroed, Denmark) by quantification of Nitrogen after digestion. Moisture was determined gravimetrically using a moisture analyzer (MAC 50/1/NH, RADWAG, Radom, Poland). Total dietary fiber content was determined by the AOAC 985.29 gravimetric method using a Megazyme® assay kit (Megazyme Ltd., distributed by José Manuel Gomes dos Santos, Lisbon, Portugal). All experiments were performed in triplicate.

2.3. Extraction process from Mediterranean fruit and vegetables wastes

In order to recover bioactive extracts from Mediterranean wastes, a portion of 20 g of each waste using 200 g of extractor (70:30 ethanol:water (w/w) solvent ratio) were placed into a plastic beaker and homogenised at 1600 rpm for 2 min with an Ultra-Turrax T25-Basix mixer (IKA, Staufen, Germany). The UAE treatments were performed to maximize the antioxidant phenolic content, according to Plazzotta *et al.* (Plazzotta et al., 2020), using an ultrasonic processor (Hielscher Ultrasonic Procesor GmbH, mod UP400S, Teltow, Germany), with a Titanium tip H14 at 24 kHz and nominal amplitude of 125 µm for 120 s. Suspensions were centrifuged after cooled to room temperature and the resulting extracts were stored at -18 °C in darkness until further analysis.

2.4. Encapsulation process for the extracts

The agro-food waste extracts obtained from UAE treatments, were evaporated using a rotary evaporator Büchi model Rotavapor R-3000 (Büchi Laboratoriums Technik, Switzerland) at final ethanol:water ratio concentration of 20:30 (w/w). The encapsulation process by spray drying was carried out mixing 1 g dw of each AFW-E with 15 g of maltodextrin and homogenized at 1600 rpm for 5 min with an Ultra-Turrax T25-Basix mixer (IKA, Staufen, Germany). The Mini Spray Dryer Büchi model B-191 (Büchi Laboratoriums Technik, Switzerland) was used with compressed air at 6 bar. All samples were atomized at a liquid feed flow rate of 1 mL/h, a constant nozzle rate of 600 L/h and maximum aspiration rate. Air inlet temperature was set at 140 °C.

2.5. Encapsulated and non-encapsulated AFW characterization

2.5.1. ξ- Potential

The surface charge at the interface of the agro-food waste extracts encapsulated (AFW-EE) and non-encapsulated (AFW-E) in the aqueous solution were measured by phase-analysis light scattering (PALS) with a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd., Worcestershire, UK).

2.5.2. Particle size

The particle size of the AFW-E and AFW-EE were analyzed by Static Light Scattering instrument Master-Sizer 3000 (Malvern Instrument Ltd, US) using bidistilled water as dispersion agent.

2.5.3. Phenolic analyses

The total phenolic content (TPC) was determined spectrophotometrically following the method proposed by Singleton and Rossi (Singleton and Rossi, 1965), which is based on the chemical reduction of Folin–Ciocalteu reagent, with some modifications. For all analyses, 5 µL of AFW-E or AFW-EE were mixed with 15 µL Folin–Ciocalteu reagent, 60 µL of Na_2CO_3 (75 g/L). The prepared solution was kept at 15 °C for 5 min. Absorbance was measured at 700 nm by an UV/vis spectrophotometer (Synergy HT, BioTek Instruments, Inc., Winooski, VT, USA). Gallic acid (0–500 mg/L) was used for calibration of the standard curve. Results were expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/ 100 g dw). All experiments were performed in triplicate.

Identification and quantification analysis of individual phenolic compounds were performed as described previously Ferreira-Santos *et al.* (Ferreira-Santos et al., 2019) using a Shimadzu Nexera X2 UPLC chromatograph equipped with Diode Array Detector (DAD) (Shimadzu, SPD-M20A, Duisburg, Germany). Separation was performed on a reversed-phase Aquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size; from Waters) at 40 °C. The HPLC grade solvents used were water/formic acid (0.1%) and acetonitrile as eluents and the flow rate was 0.4

mL/min. Phenolic compounds were identified by comparing their UV spectra and retention times with that of corresponding standards. Quantification was carried out using calibration curves for each compound analyzed using concentrations` between 2.5-250 mg/L (2.5, 5, 10, 25, 50, 100, 200 and 250 mg/mL). In all cases, the coefficient of linear correlation was $R^2 > 0.994$. Compounds were quantified and identified at different wavelengths (250–329 nm). All experiments were performed in triplicate.

2.5.4. Antioxidant potential

The antioxidant potential was measured using two different assays differing their mechanisms of the antioxidant action (Cilek et al., 2012). The $ABTS^{+}$ radical scavenging activity was measured spectrophotometrically (Synergy HT, BioTek Instruments, Inc., Winooski, VT, USA) according to the method proposed by Al-Duais *et al.* (Al-Duais et al., 2009). This method measures the ability of antioxidants to scavenge the stable radical cation $ABTS^{\bullet+}$ (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)), a blue-green chromophore with maximum absorption at 734 nm. Trolox (0–0.55 mmol/L) was used for calibration of the standard curve. The results were expressed as millimols of Trolox per 100 grams of dry weight of fraction (mmol Trolox/100 g dw). FRAP assay measures the antioxidant capacity when the ferric complex (Fe^{3+}-TPTZ) is reduced to a blue intense ferric complex (Fe^{2+}-TPTZ) using a spectrophotometric instrument (Tsao et al., 2003) at 593 nm. Ferric sulphate (II) heptahydrate (0–2 mmol/L) was used for calibration of the standard curve. The results were expressed as millimols of iron sulphate (II) heptahydrate per 100 grams of dry weight of fraction (mmol Fe^{2+}/100 g dw). All experiments were performed in triplicate.

2.5.5. ATR-Fourier Transform Infrared Spectroscopy

Chemical groups and bonding arrangement of constituents present in the AFW were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) using an ALPHA II-Bruker spectrometer (Ettlingen, Germany) with a diamond-composite attenuated total reflectance (ATR) cell. The measurements were recorded with a wavenumber range from 4000 to 400 per cm, with a resolution of 4 per cm and 64 scans per sample.

2.6. Biological assays

2.61. Cell culture

Human colorectal adenocarcinoma Caco-2 cells were kindly provided by Dr. Edith Brot-Laroche (Université Pierre et Marie Curie-Paris 6 UMR S872, Les Cordeliers, France). Human breast adenocarcinoma MCF-7 cells were kindly provided by Dr. Carlos J. Ciudad and Dr. Verònica Noé (Departamento de Bioquímica y Fisiología, Facultad de Farmacia, Universidad de Barcelona, Spain). Human hepatocellular carcinoma HepG2 cells were kindly provided by Dr. María Angeles Alava (Departamento de Bioquímica y Biología Molecular, Universidad de Zaragoza, Spain). All cell lines were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were grown in Dulbecco's Modified Eagles medium (DMEM) (Gibco Invitrogen, Paisley, UK) supplemented with 20% fetal bovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 µg/mL) and 1% amphotericin (250 U/mL). Culture medium was replaced every two days and cells were passaged enzymatically with 0.25% trypsin-1 mM EDTA and sub-cultured on 25 cm^2 flasks at a density of $2 \cdot 10^4$ cells/cm^2.

Experiments on undifferentiated Caco-2 cells were performed 24 h post-seeding. For assays on differentiated Caco-2 cells, MCF-7 and HepG2 cells were cultured on 96-wells plates under standard culture conditions for 10 to 15 days, until reaching 80% confluence as confirmed by optic microscopy observance.

2.6.2. Cell proliferation assay and IC_{50} calculation

Cells were grown in 96-wells plates at a density of 4000 cells per well and incubated overnight at standard culture conditions. Then, cells were exposed to a range of concentrations of extracts 250-4000 µg/mL for non-encapsulated extracts (AFW-E) on undifferentiated Caco-2 and HepG2 cell lines; 2000-6000 µg/mL for MCF-7 cell line; 500-8000 µg/mL for differentiated Caco-2 cells; 62.5-1000 µg/mL for encapsulated extracts (AFW-EE) on each cancerous cell line and 500-8000 µg/mL for differentiated Caco-2 cells) for 72 h. Changes in cell proliferation were analyzed by sulforhodamine B assay (SRB) (Skehan et al., 1990), performed as previously described by Jiménez et al. (Jimenez et al., 2016), Cells were fixed with 500 g/L trichlroacetic acid (1 h, 4 °C), washed with distilled water and stained with 4 g/L of sulforhodamine B (20 min, room temperature). The plates were then washed with 20 mL/L acetic acid to remove unbound dye. Protein-bound dye were extracted with 10 mmol/L Tris base. The results were obtained by measuring absorbance with SPECTROstar Nano (BMG Labtech, Bad Friedrichshall, Germany). Finally, the IC_{50} value was calculated under all conditions tested. IC_{50} represents the concentration of compound that halves cell proliferation or viability. All experiments were performed in triplicate.

2.6.3. Determination of intracellular reactive oxygen species (ROS) levels

The production of ROS was assessed using the dichlorofluorescein (DCF) assay (Ruiz-Leal and George, 2004). Caco-2 cells were grown in 96-wells plates at a density of 4000 cells per well and, after overnight incubation under standard culture conditions, were exposed to AWF-E for 3 h. Thereafter, determination of total intracellular ROS levels was performed as previously described by Sánchez-de-Diego *et al.* (Sanchez-de-Diego et al., 2017). Thus, cells were washed twice with PBS and 100 µL of 20 mM DCFH-DA (dichloro-dihydro-fluoresceindiacetate) were added to each well. Cell were incubated 1 h at 37 °C and washed twice with PBS; finally, 100 µL of PBS were added. The formation of the fluorescence oxidized derivative of DCF was monitored at an emission wavelength of 535 nm and an excitation of 485 nm in FLUOstar Omega (BMG Labtech) multiplate reader. A measure at time “zero” was performed, cells were then incubated at 37 °C in the multiplate reader, and generation of fluorescence was measured after 20 min. ROS levels were expressed as a percentage of fluorescence compared to the control. The obtained values of fluorescence intensity are considered as a reflection of total intracellular ROS content and were normalized with percentage of cell viability determined by SRB. All experiments were performed in triplicate.

2.7. Statistical analysis

All assays were performed at least three times. Data are presented as mean±SD. Means were compared using one-way analysis of variance (ANOVA). Significant differences at $p<0.05$ were compared using a Bonferroni’s Multiple Comparison Test. The statistical analyses and the graphics were performed using the GraphPad Prism Version 5.02 software for Windows (GraphPad Software San Diego, CA, USA).

3. Results and Discussion

3.1. Chemical characterization of studied Mediterranean fruit and vegetable wastes

The chemical composition of the Mediterranean agro-food wastes used in this study is summarized in Table 1. The results show that the major fraction of the fruits and vegetables composition are carbohydrates, representing around 31.2%, 32.2%, 23.7% and 28.2% for apple, cucumber, red pepper and peach wastes, respectively. The monomeric composition shows a predominance of the cellulose fraction in all matrices. These fruit and vegetable wastes are rich in dietary fibre (approximately 20% of total composition in all residues). In this work, the water and ethanol soluble extracts represent 29.5%, 22%, 20.1% of apple, red pepper and cucumber and a lower amount (approx. 11%) for peach waste, respectively. The lignin content is around 15% except for the cucumber waste that has 10% less lignin than the other fruits and vegetables wates studied in this work. Other constituents such a protein represent 15.8%, 10.3%, 8.3% and 6.1% for cucumber, red pepper, peach and apple wastes, respectively. Lipid fraction (fat) is between 5.3 to 1.4% of total composition of AFW, and the ash content is below 6% for all wastes. The elemental analysis (inorganic substances) of studied AFW showed that the most relevant minerals, determined by plasma atomic emission spectrometry, are potassium, calcium, magnesium, phosphorous and sodium (see Table 1). These results are in accordance with other studies of nutritional compositions of commonly consumed fruits and vegetables (Septembre-Malaterre et al., 2018). Authors reported that foods of vegetable origin are characterized by low caloric intake (due to their high water and low fat content) and it is mostly composed of high content of carbohydrates, fibers, minerals and micronutrients of interest (*e.g.* polyphenols) (Septembre-Malaterre et al., 2018).

Table 1. Proximal (chemical and nutritional) and elemental (minerals) composition of Mediterranean agro-food wastes (AFW) used in this study.

AFW	Apple	Cucumber	Red pepper	Peach
Proximate composition (g/100 g *dry material*)				
Total extractives*	29.50 ± 0.40	20.07 ± 1.02	21.98 ± 1.31	11.06 ± 0.34
Protein	6.06 ± 0.07	15.84 ± 0.03	10.32 ± 0.00	8.28 ± 0.22
Total carbohydrates**	31.19 ± 2.15	32.24 ± 0.70	23.69 ± 0.45	28.16 ± 1.40
Dietary Fibre	25.28 ± 0.98	22.48 ± 1.12	19.95 ± 1.14	22.23 ± 2.24
Lignin	15.44 ± 0.32	4.48 ± 0.03	14.35 ± 0.00	14.63 ± 0.25
Lipids	1.41 ± 0.09	2.44 ± 0.08	5.33 ± 0.08	1.97 ± 0.04
Ash	1.61 ± 0.01	6.53 ± 0.01	4.08 ± 0.07	1.58 ± 0.10
Moisture	5.00 ± 0.00	5.08 ± 0.00	5.96 ± 0.01	5.07 ± 0.01
Elemental composition (mg/100 g *dry material*)				
Potassium	601.1 ± 22	2348.3 ± 68.2	1884 ± 2.8	517 ± 2.5
Calcium	135 ± 5.0	623 ± 24	130 ± 1.5	211 ± 1.2
Magnesium	44 .3 ± 3.2	235± 10.4	78.2 ± 1.2	33.6 ± 1.5
Phosphorous	12.3 ± 0.20	35.9 ± 0.02	258 ± 22.1	53.7 ± 9.3
Sodium	20.6 ± 1.3	69.8 ± 4.4	25.3 ± 7.0	20.1 ± 0.8
Zinc	1.3 ± 0.00	2.9 ± 0.03	2.2 ± 0.00	12.3 ± 0.2
Iron	2.6 ± 0.01	5.7 ± 0.01	3.4 ± 0.01	2.1 ± 0.00

*Ethanol and water extractives; ** Glucose, fructose and sucrose content.

3.2. Phenolic composition and antioxidant activity in fruit and vegetable extracts

Fruits and vegetables include in their composition bioactive compounds that are important for human health, being polyphenols among them (Ganesan and Xu, 2017; Gascon et al., 2018; Jimenez-Moreno et al., 2019; Jimenez et al., 2016; Khan and Mukhtar, 2018; Piccolella et al., 2019b; Quero et al., 2021; Quero et al., 2020; Tylewicz et al., 2018). TPC presented in the different tested bagasses turned out to be variable, given the different origin of the fruits and vegetables processed in the agro-food industry. The highest concentration was found in red pepper extracts (692.4 ± 31 mg GAE/100 g dw) followed by apple (533.2 ± 8 mg GAE/100 g dw), cucumber (285.7 ± 3 mg GAE/100 g dw) and peach (256.9 ± 2 mg GAE/100 g dw) extracts. Table 2 shows the main phenolic compounds identified in each case and ten phenolic compounds were identified in the extracts of fruits and vegetables. It is possible to observe that apple wastes had more variety of phenolic compounds and the most representative were chlorogenic, ellagic and ferulic acids. In cucumber, red pepper and peach extracts the major compounds were ellagic acid and taxifolin.

After the spray drying encapsulation process, the phenolic compounds presented in the AWF-EE were reduced (TPC: 564.3 ± 11 mg GAE for red pepper, 454.1 ± 1 mg GAE for apple, 213.8 ± 1 mg GAE for cucumber and 223.0 ± 2 mg GAE for peach, per 100 gram of dry material). The most affected compounds were ferulic acid, which was not detected, and taxifolin, which was detected approximately 3 times less in all extracts after encapsulation (Table 2).

Table 2. Identification and quantification of phenolic compounds presented in the encapsulated (AFW-EE) and non-encapsulated (AFW-E) extracts from Mediterranean fruits and vegetable wastes.

Phenolic compounds (mg/100 g)	Apple	Cucumber	Red pepper	Peach
		Non-encapsulated		
Chlorogenic acid	9.37 ± 0.35	4.91 ± 0.08	n.d.	n.d.
p-coumaric acid + epicatechin	n.d.	9.80 ± 0.81	n.d.	3.45 ± 0.23
Rosmarinic acid	8.28 ± 0.12	n.d.	7.15 ± 0.03	n.d.
Ellagic acid	16.48 ± 1.87	37.54 ± 0.07	32.10 ± 2.55	28.26 ± 0.39
Naringenin	8.46 ± 0.28	n.d.	1.52 ± 0.04	2.87 ± 0.41
Hesperidin	n.d.	1.16 ± 0.03	n.d.	3.37 ± 0.00
Resveratrol	3.96 ± 0.25	n.d.	n.d.	n.d.
Ferulic acid	14.10 ± 0.62	n.d.	3.02 ± 0.12	n.d.
Rutin	4.67 ± 0.65	n.d.	5.88 ± 0.16	n.d.
Taxifolin	6.57 ± 3.96	28.01 ± 0.01	29.61 ± 0.20	4.44 ± 0.46
		Encapsulated		
Chlorogenic acid	6.57± 0.12	2.61 ± 0.06	n.d.	n.d.
p-coumaric acid + epicatechin	n.d.	3.12 ± 0.36	n.d.	n.d.
Rosmarinic acid	6.82 ± 0.03	n.d.	4.23 ± 0.11	n.d.
Ellagic acid	12.33 ± 2.77	31.86 ± 0.13	29.19 ± 1.05	20.48 ± 0.39
Naringenin	6.21 ± 0.47	n.d.	1.20 ± 0.18	1.76 ± 0.02
Hesperidin	n.d.	0.77 ± 0.07	n.d.	1.92 ± 0.14
Resveratrol	2.28 ± 0.44	n.d.	n.d.	n.d.
Ferulic acid	n.d.	n.d.	n.d	n.d.
Rutin	1.29 ± 0.10	n.d.	3.43 ± 0.06	n.d.
Taxifolin	2.90 ± 0.65	8.21 ± 0.16	10.66 ± 0.35	1.62 ± 0.20

n.d.: not detected; AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)

FTIR measurements were carried out to identify the presence of major functional groups in the *Mediterranean* fruit and vegetable extracts. The ATR-FTIR spectra of apple, cucumber, red pepper and peach extracts, recorded in the 400-4000 cm^{-1} region, are shown in Figure 1. For both fruit and vegetable extracts, prominent absorption bands at 3600-3000 cm^{-1} could be assigned to absorption bands of O–H stretching of phenolic and aliphatic structures. Absorption peaks at 2917 and 2849 cm^{-1} were characteristic of C–H asymmetric and symmetric vibration, respectively, and could be attributed to hydrophobic phenolic compounds extracted with ethanol (Ferreira-Santos et al., 2020). In the spectrum of fruit extracts (with the exception of cucumber extract), medium bands at 1732 cm^{-1} was attributed to the carbonyl C-O in ester groups (e.g. esterified pectins). The presence of bands at 1605 and 1323 cm^{-1} were attributed to aromatic skeletal vibrations and 1233 cm^{-1} peaks to skeletal vibrations of aromatic rings with C-O stretching. Bands to 1440 and 1015 cm^{-1} were assigned to pectins, polysaccharides and sugars. Peaks between 864 to 775 cm^{-1} showed the stretching and bending vibrations of –CH from aromatic rings, related to phenolic compounds. The vibrational bands corresponding to the bonds O–H, C–H, C=C ring, C–OH and C–C ring were derived from flavonoids and polyphenols (Firdaus et al., 2017). These results were in accordance with our results (phenolic composition) and previous research by other authors that showed a presence of pectins, lignin, sugars and high content of phenolic compounds in fruits and vegetable extracts (Canteri et al., 2019; Simonovska et al., 2016).

The encapsulated extracts were analyzed by ATR-FTIR, but the spectrum was identical to the maltodextrin spectrum (data not shown).

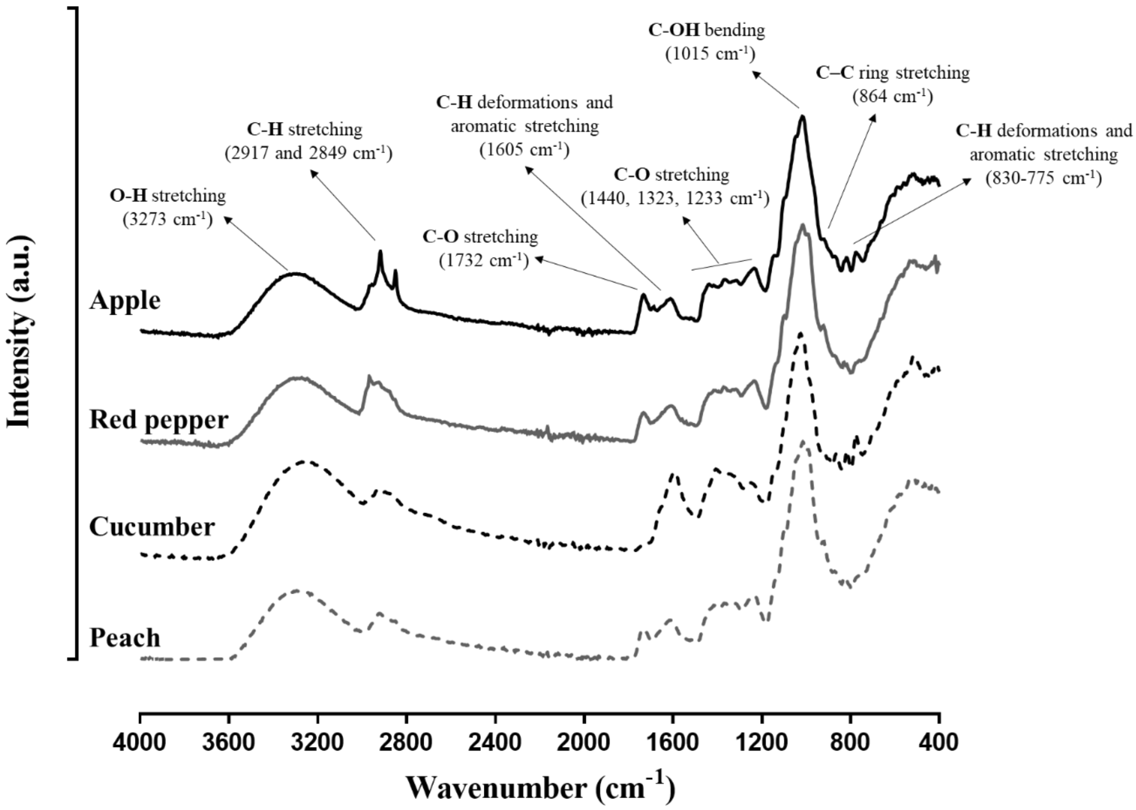

Figure 1. ATR-FTIR spectra of Mediterranean fruit and vegetable extracts.

Regarding the antioxidant capacity measured by ABTS and FRAP assays, vegetables showed greater antioxidant capacity than fruits, the highest value was obtained in red pepper (Table 3). This seemed to corrolate well with its high content in taxifolin. Also relevant may be the important pigment (namely carotenoids) content in red pepper, which may account for the significant differences between the two tested vegetables.

Table 3. Antioxidant potential of the extracts from Mediterranean fruits and vegetable wastes encapsulated (AFW-EE) and non-encapsulated (AFW-E), measured by $ABTS^{+}$ and FRAP assays.

AFW	**Antioxidant potential**	
	$ABTS^{+}$ $\left(\frac{mg\ Trolox}{100\ g\ dry\ weight}\right)$	**FRAP** $\left(\frac{mg\ Fe^{2+}}{100\ g\ dry\ weight}\right)$

Apple-E	1.73 ± 0.09	2.94 ±0.19
Apple-EE	1.14 ± 0.02	1.38 ± 0.05
Cucumber-E	2.49 ± 0.13	5.07 ± 0.23
Cucumber-EE	1.63 ± 0.16	3.55 ± 0.41
Red pepper-E	2.34 ± 0.14	7.00 ± 0.51
Red pepper-EE	2.03 ± 0.23	5.23 ± 0.41
Peach-E	1.96 ± 0.12	2.40 ± 0.40
Peach-EE	1.02 ± 0.36	1.68 ± 0.74

AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)

3.3. Antiproliferative effect of the extracts from Mediterranean fruit and vegetable wastes

Polyphenols are the most abundant antioxidants in the human diet. They can be beneficial for human health thanks to their antioxidant, immunomodulatory, anticancer and antibacterial actions. Raw fruits and vegetables are a good source of polyphenols. However, due to their seasonal nature, they are often processed industrially. Consequently, a significant amount of by-products (peel, pulp, seeds, stones, stem) are produced that contain valuable bioactive compounds, such as flavonoids, anthocyanins and phenolic acids . (Baci et al., 2019; Grillo et al., 2019; Jimenez-Moreno et al., 2019; Jimenez-Moreno et al., 2020; Montenegro-Landívar et al., 2021; Piccolella et al., 2019a; Quero et al., 2021; Quero et al., 2020; Tylewicz et al., 2018). Besides that role in prevention, plant-derived wastes might display a role on the management of cancer treatment as well

Due to the tumor growth inhibition properties showed by extracts of fruits and vegetables (D'Angelo et al., 2017; Jimenez-Moreno et al., 2019; Noratto et al., 2014; Quero et al., 2021), it seemed interesting to determine if AFW displayed antiproliferative activity as well. In addition, the extracts encapsulation influence on the anticancer effect was also evaluated, since the encapsulation enhances the solubility of bioactive compounds, increasing the antioxidant power, and improves the nutraceutical delivery into cancer cells (Popovic et al., 2021). Thus, both AFW-E and AFW-EE were tested onto a panel of human cancer cell lines that included colorectal adenocarcinoma Caco-2, hepatocellular carcinoma HepG2 and breast adenocarcinoma MCF-7. In order to analyze the potential selectivity of the AFW-E and AFW-EE, all of them were also tested on differentiated Caco-2 cells as a model of the intestinal barrier. The obtained results were expressed as IC_{50} values and are summarized in Table 4. Cell models were also incubated with the wall material agent, maltodextrin, to evaluate the effect of the carbohydrate delivery vehicle; since the IC_{50} values obtained exceeded 8000 μg/mL on each cancer cell line (data not shown); the antiproliferative effect was considered to be a consequence of the presence of plant extracts rather than to the vehicle itself.

Table 4. IC_{50} values of the extracts of Mediterranean fruits and vegetable wastes encapsulated (AFW-EE) and non-encapsulated (AFW-E) obtained on differentiated and undifferentiated Caco-2, HepG2 and MCF-7 cell lines after 72h incubation.

AFW	**IC_{50} (μg/mL)**			
	Undifferentiated Caco-2 cell line	**HepG2 cell line**	**MCF-7 cell line**	**Differentiated Caco-2 cell line**
Apple-E	856 ± 171	6291 ± 562	16513 ± 3250	9228 ± 242
Apple-EE	205 ± 92	152 ± 4	2480 ± 50	9741 ± 1181
Cucumber-E	1169 ± 94	1572 ± 11	5116 ± 38	6446 ± 406
Cucumber-EE	574 ± 142	543 ± 60	2455 ± 316	5276 ± 1046
Red pepper-E	1252 ± 164	2791 ± 4	5643 ± 292	10096 ± 2181

Red pepper-EE	619 ± 46	643 ± 23	3330 ± 92	14570 ± 922
Peach-E	1034 ± 101	4272 ± 156	9946 ± 2059	7408 ± 1330
Peach-EE	337 ± 130	364 ± 30	5078 ± 472	6750 ± 1068

AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)

Undifferentiated Caco-2 and HepG2 cell lines showed a better response to treatment with both AFW-E and AFW-EE than MCF-7 cells. This might be explained by the fact that MCF-7 cell line is an estrogen receptor-positive breast cancer model and some authors have noticed that phytoestrogens contained in plant extracts might boost the growth of these cancer cell models instead of inhibiting it (Marmol et al., 2017). These results are in agreement with Sun *et al.* (Sun and Liu, 2008) regarding to the greater sensitivity to cell death induced by apple phytochemicals on the estrogen receptor-negative MDA-MB-231 cell line than on MCF-7 cell line.

AFW-EE resulted in a significant increase in the antiproliferative effect versus the AFW-E on each cancer cell line. According to the data from Table 4, upon encapsulated extracts gained anionic charge, a more negative ξ potential, along with a reduced particle size. An increase in the anionic charge of AFW-EE might enhance their cellular uptake (Kang and Ko, 2015), which is mediated by phagocytic and/or endocytic mechanisms depending on the type of cancer cell (Gonzalez-Ballesteros et al., 2019; Xie et al., 2014). On the other hand, the size of AFW-EE is also considered a key factor on particles uptake since the smaller ones are known to access more efficiently to tumor cells (Gonzalez-Ballesteros et al., 2019; Kang and Ko, 2015). Furthermore, smaller particles might have a more promising future on chemotherapy due to the enhanced permeability and retention effect (EPR), according to which these particles are retained more easily in tumor microenvironment (Ngoune et al., 2016). Taken together, the anionic charge and smaller size of capsules and controlled release of active agents of our AFW-EE in comparison to those AFW-E extracts might be the reason of the observed protection of bioactives and the highest antiproliferative effect.

Table 5. ξ potential and particle size values of the extracts from Mediterranean fruits and vegetable wastes encapsulated (AFW-EE) and non-encapsulated (AFW-E).

AFW	ξ Potential (mV)	Particle size (μm)
Apple-E	-2.20 ± 0.10	20.1 ± 0.6
Apple-EE	-15.53 ± 3.56	1.4 ± 0.4
Cucumber-E	-2.26 ± 0.73	23.2 ± 5.5
Cucumber-EE	-23.37 ± 2.20	2.4 ± 0.8
Red Pepper-E	-0.67 ± 0.56	38.7 ± 2.8
Red-Pepper-EE	-33.2 ± 0.99	2.0 ± 0.2
Peach-E	-3.45 ± 0.32	54.6 ± 12.2
Peach-EE	-19.85 ± 0.92	1.9 ± 0.2

AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)

To determine the Selectivity Index (SI) of the AFW extracts in the different cancer cells tested, the IC_{50} of the different cancerous cell lines was compared to the IC_{50} in non-cancerous cells (differentiated Caco-2) (Table 4) as previously described by Badisa *et al.* (Badisa et al., 2009). The results are summarized in Table 6. All AFW displayed low SI values on MCF-7 cell line, which might be indicative of poor selectivity, and therefore all extracts could be discarded as potential anticancer agents in MCF-7 cells. On the other hand, undifferentiated Caco-2 and HepG2 cells showed comparable SI values for non-encapsulated extracts, being slightly higher those obtained on undifferentiated

Caco-2 cells (ranging between 5 and 10 in comparison to 1 to 10 for HepG2 cells). AFW-EE enhanced the SI of each sample, being the greatest increase for apple extracts (4.4-up fold and 43.46-up fold compared to non-encapsulated apple extracts on undifferentiated Caco-2 and HepG2 cells, respectively) and the lowest for cucumber encapsulated extracts (1.67-up fold and 2.37-up fold compared to non-encapsulated cucumber extracts on undifferentiated Caco-2 and HepG2 cells respectively).

Table 6 . Selectivity index (SI) values of the extracts from Mediterranean fruits and vegetable wastes encapsulated (AFW-EE) and non-encapsulated (AFW-E) obtained on differentiated Caco-2 cells compared to undifferentiated Caco-2 cells.

AFW	SI value		
	Undifferentiated Caco-2 cell line	HepG2 cell line	MCF-7 cell line
Apple-E	10.78	1.47	0.56
Apple-EE	47.38	63.88	3.93
Cucumber-E	5.51	4.10	1.26
Cucumber-EE	9.19	9.71	2.15
Red pepper-E	8.06	3.62	1.79
Red pepper-EE	23.52	22.66	4.38
Peach-E	7.16	1.73	0.74
Peach-EE	19.99	18.50	1.33

AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)

Some fruit and vegetables-derived extracts have been related to an increase on the trans-epithelial electrical resistance (TEER) of differentiated Caco-2 cells, which is in turn correlated with the abundance of tight junction proteins and consequently the whole integrity of the intestinal epithelium model. In this line, Vreeburg *et al.* (Vreeburg et al., 2012) found that apple extracts up-regulated the expression of numerous genes involved on the formation of monolayer Caco-2 tight junctions and enhanced TEER as well. As previously discussed, AFW-EE might contribute to a more efficient cellular uptake of the AFW-E, so it is not surprising that encapsulated extracts display a higher protective effect on the intestinal barrier model, thus increasing SI value.

Therefore, bioproducts rich in polyphenols, obtained from the wastes of apple, peach, cucumber and red pepper, had the potential to be used for therapeutic purposes in colon and liver cancers. In addition, its encapsulation with maltodextrin demonstrated to protect the bioactive compounds and enhanced their action.

3.4. Protective effect of the red pepper extract on a model intestinal barrier

Chronic oxidative stress on the mucosal barrier is strongly correlated with gastrointestinal tract malignancies such as inflammatory bowel disease (IBD) or colorectal cancer (Bhattacharyya et al., 2014; Mármol et al., 2018). The sources of pro-oxidant agents are wide, from drugs and compounds present in food, to intestinal pathogens (Chalouati et al., 2015; Lee and Kang, 2019; Utzeri and Usai, 2017). In this context, dietary intervention with antioxidant supplementation has been suggested as a novel therapeutic approach to reduce oxidative stress-related damage of the intestinal barrier (Bertani et al., 2021). Thus, by-products, food waste, or bagasse have bioactive components, like polyphenols, applicable in the therapy of diseases such as IBD (Machado et al., 2021). In this way, intake of red raspberries successfully reversed the induction of colitis on a mice model through a reduction in pro-inflammatory markers along with an increase in the expression of enzymes involved on antioxidant defense (Bibi et al., 2018). Likewise, food by-products obtained from artichoke and grape stem showed an antioxidant effect on Caco-2 cells after H_2O_2-insult (Jimenez-Moreno et al., 2019; Quero et al., 2021).From a molecular point-of-view, Catanzaro *et al*. (Catanzaro et al., 2015) found that incubation of differentiated Caco-2 cells with *Boswellia serrata* extracts protected against H_2O_2-induced damage and maintained monolayer integrity by avoiding the disassembly of tight junctions. Since red pepper contained the highest antioxidant potential (Table 4) and TPC (692.4 ± 31 mg GAE/100 g dw), of all the extracts tested, the protective effect of encapsulated red pepper extract and non-encapsulated on differentiated Caco-2 cells upon hydrogen peroxide insult was evaluated (Fig. 2). This cell line spontaneously acquires the phenotypic features of non-cancerous enterocytes after reaching confluence (differentiated cells). Monolayer Caco-2 cells formed tight junctions and presented the cylindrical polarized morphology of enterocytes, expressing functional microvilli on the apical membrane (Sambuy et al., 2005; van Breemen and Li, 2005; Zeller et al., 2015). Therefore, differentiated Caco-2 cells have been established as an acceptable *in vitr*o intestinal barrier model.

High intracellular ROS levels were related to the initiation, development, and progression of cancer, since free radicals lead to malignant transformation and damage lipids, proteins, ans nucleic acids (Sambuy et al., 2005). Thus, differentiated Caco-2 cells were incubated for 3 h with two non-toxic red pepper concentrations (1000 and 2000 μg/mL), according to the previously obtained IC_{50} value (Table 4), and then exposed to 500 μM H_2O_2 for 20 minutes. A significant reduction of ROS levels induced by encapsulated red pepper extracts was observed comparable to negative control, whereas no significant changes were noticed for non-encapsulated extracts (Fig. 2.A). These results suggested that encapsulation with maltodextrin as a wall material agent lead to a more efficient uptake of the antioxidant phytocompounds found in red pepper extracts on the cells, thus increasing the protective effect of the extracts. Similar results were observed in Caco-2 cells with pytosterols (Gies et al., 2020) and in prostate cancer cells with curcumin (Yallapu et al., 2010),. The protective effect of encapsulated red pepper extracts also seemed to be closely related to concentration, since the lowest one tested did not display a significant reduction in ROS levels in comparison to H_2O_2-treated cells (data not shown). In addition, the protective effect of encapsulated red pepper extracts resulted in an amelioration of the deleterious effect induced by hydrogen peroxide on differentiated Caco-2 cells viability (Fig. 2.B).

These results obtained with red-pepper encapsulated extracts suggest that they could have a potential application in the management of gastrointestinal diseases related to oxidative stress since the antioxidant capacity of plant extract is strongly correlated with its clinical application in these diseases (Almeer et al., 2018; Kumar et al., 2019).

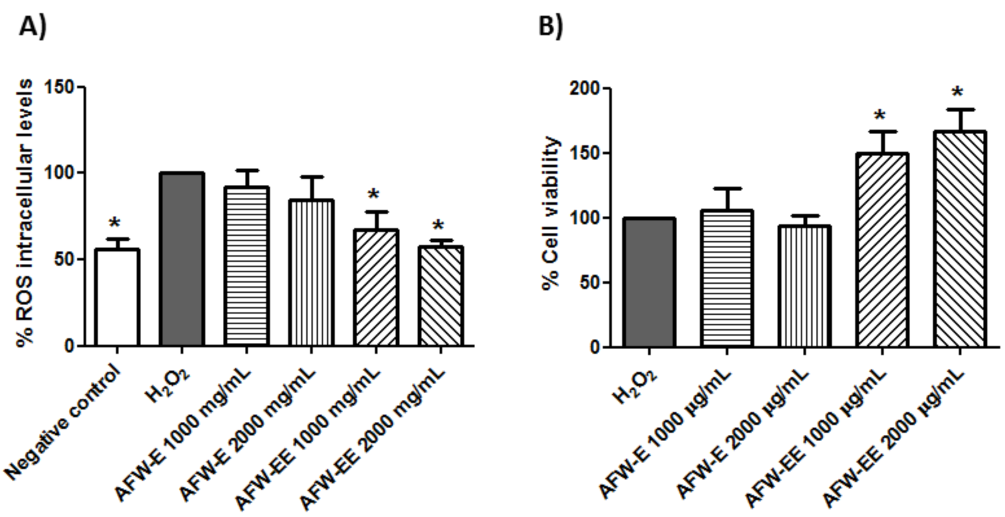

Figure 2. Effect of pre-incubation with encapsulated red pepper extracts and non-encapsulated on differentiated Caco-2 cells under hydrogen peroxide insult. A) Measurement of ROS levels of differentiated Caco-2 cells pre-incubated for 3 h with two concentrations (1000 and 2000 µg/mL) of encapsulated red pepper extracts and non-encapsulated and then exposed to 500 µM H_2O_2 for 20 minutes. *$p<0.05$ vs H_2O_2-treated cells. B) Measurement of cell viability of differentiated Caco-2 cells pre-incubated 3 h with two concentrations (1000 and 2000 µg/mL) of encapsulated red pepper extracts and non-encapsulated, then exposed to H_2O_2 for 20 minutes and incubated overnight at 37°C. *$p<0.05$ vs H_2O_2-treated cells. *AFW-EE: agro-food waste extracts encapsulated; AFW-E: non-encapsulated extracts (acronym from agro-food waste extracts)*

4. Conclusions

The bioactive potential of apple, peach, cucumber and red pepper by-products obtained from agro-food industry has been evaluated on the treatment of three types of cancer (colon, liver and breast) and on the prevention of intestinal oxidative stress. The agro-food extracts showed *in vitro* antioxidant potential and different phenolic profiles for each studied AFW. On the one hand, both AFW-E and AFW-EE showed antitumor potential toward a panel of cancer cell lines (Caco-2, MCF-7 and HepG2). Moreover, encapsulation within maltodextrin was found to enhance the antiproliferative effect of every tested extract without compromising its toxicity to non-cancerous tissue. There does not seem to be a correlation between TPC in the extracts and their effect on cancer cells, since the action of these bioactive compounds also depend on the type of polyphenols presented and the synergistic or additive effects between them. Furthermore, maltodextrin-encapsulated red pepper extracts were found to be able to protect a model of the intestinal barrier (differentiated Caco-2 cells) against oxidative stress. Taken together, results suggested that vehiculation of food by-products in maltodextrin might enhance their therapeutic potential since the encapsulation could help to improve the bioavailability of bioactive compounds by increasing the cellular uptake and it could suggest a pharmacological formulation. In addition, based on the content of bioactive compounds and the biological activity of fruit and vegetable by-products, these residues could also be used as natural additives in food and their encapsulation could be a promising alternative for the use of agro-industrial waste as ingredient in food industry.

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The authors declare no conflits of interest