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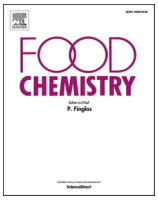

Influence of the packaging systems on the phenolic profile and antioxidant properties of wine pomace used as seasoning in chicken meat

Victor Gutierrez-Gonzalez, Mª Dolores Rivero-Perez, Gisela Gerardi, Pilar Muñiz, Mª Luisa González-SanJose, Isabel Jaime, Mónica Cavia-Saiz *

Department of Biotechnology and Food Science, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos, 09001 Burgos, Spain

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ABSTRACT

White wine pomace products (wWPP) represent an innovative strategy as a functional food ingredient to be used as a seasoning both for their technological and functional properties. Nevertheless, the bioactive compounds of wWPP used as a seasoning could be modified during storage. The seasoning in the meat, regardless of the storage method used, modified its phenolic profile and in its bioaccessible fractions, while maintaining a high total antioxidant capacity and total polyphenol content. The contact of the seasoning with the meat can be considered safe as it does not show cytotoxicity in the Caco-2 cells. Additionally, the ability to modulate the cell oxidative stress of the bioaccessible fractions and the potential benefits on microbiota by the colonic fermentation fraction, suggest its potential use as a functional ingredient, without being affected by storage. These results are novel and may help to establish the value of this product as a functional ingredient.

1. Introduction

The winery industry generates large amounts of wine pomace, also known as grape pomace which has been considered as an alternative for the production of value-added products, especially for the food industry. Around 20–30 % of the weight of the grapes represents the wine pomace, the main by-product of the winemaking process (Garcia-Lomillo et al., 2017). In fact, the wine pomace is an important source of bioactive compounds (polyphenols and fiber) and modulates many biological activities, providing health benefits related to endothelial dysfunction, hypertension, hyperglycemia, etc (Gerardi et al., 2021).

The processing of grape by-products to obtain wine pomace products with a high content of bioactive compounds using environmentally friendly and economically affordable procedures, such as techniques that avoid the use of organic solvents (González San José, et al., 2015) are a novel alternative to obtain functional food ingredients that can combine preservative, antimicrobial, flavoring and potentially health properties (Baroi et al., 2022; Ferrer Gallego et al., 2022; Gerardi et al., 2021). In this sense, our research group have obtained a seasoning from winemaking by-products that are rich in dietary fiber, minerals and polyphenols such as proanthocyanidins, flavonoids, phenolic acids and stilbenes (Del Pino-García et al., 2015; García-Lomillo et al., 2014) with antioxidant, anti-inflammatory effects and antimicrobial activities that modulate various redox signal pathways and reduce endothelial dysfunction (Del Pino-García et al., 2016a; Gerardi et al., 2020b, 2021). Furthermore, there is growing concern among consumers about the link between dietary habits and health problems, which has prompted changes in the food industry. These changes include the addition of nutritional value, fortification of dietary fiber content, or the replacement of artificial additives. Artificial additives are primarily used to prevent lipid and protein oxidation and preserve food quality, but they have been reported to have potential damaging side effects. As a result, consumers have become increasingly interested in finding products that utilize natural plant extracts, which are rich in antioxidants. Several studies have tested the efficacy of plant extracts (Rather et al., 2016) and compared them to artificial additives (Ghorbani Gorji et al., 2019), demonstrating their ability to reduce storage degradation. This has led to the commercialization of multiple natural additives.

Wine pomace is one of the main natural additives under study for its use as a seasoning. In previous studies, we evaluated the effect of red wine pomace as a seasoning to improve microbial stability, product quality, and maintain sensory properties, thanks to its high polyphenol and fiber content (García-Lomillo et al., 2014; Ortega-Heras et al., 2020). However, the addition of red wine pomace as a seasoning

* Corresponding author at: Plaza Misael Bañuelos, Facultad de Ciencias, Departamento de Biotecnología y Ciencia de los Alimentos, 09001 Burgos, Spain.
E-mail addresses: victor.gutierrez@ubu.es (V. Gutierrez-Gonzalez), drivero@ubu.es (M.D. Rivero-Perez), mggerardi@ubu.es (G. Gerardi), pmuniz@ubu.es (P. Muñiz), marglez@ubu.es (M.L. González-SanJose), ijaime@ubu.es (I. Jaime), monicacs@ubu.es (M. Cavia-Saiz).

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increased the color of the foods. For this reason, white wine pomace is a good alternative for use as a seasoning, as it has a high content of phenolic compounds and health properties (Gerardi et al., 2020b).

The potential of wine pomace as a functional additive could be modified by the storage methods. The atmosphere or vacuum storage conditions, temperature and time can modify the antioxidant properties of the wine pomace product and the properties of the product added as seasoning (Łopacka et al., 2016). Although there are no reports, it is expected that the content of bioactive compounds of pomace could be altered during storage when used as seasoning as its polyphenols characteristics to react with free radicals to avoid oxidation of the food molecules (Gottardi et al., 2016). Several studies have determined how modified atmosphere packaging (MAP) and vacuum packaging (VP) change the polyphenol content of foods seasoned with a plant extract (Kurek & Krzemińska, 2020). Other studies have determined the changes of the pomace alone stored in different conditions (Nemetz et al., 2021), but no studies have been found that determine how the storage conditions alter the pomace while it is being used as seasoning. This determination is important because the wine pomace is not only promising as seasoning to improve food quality during storage, but it has the potential to be a functional ingredient in on itself. Therefore, its composition and antioxidant activity after storage is also relevant.

The properties of seasoning can be modified by gastrointestinal digestion and gut microbial fermentation. There is some evidence to show that of digestion process could modified the polyphenol profile of the wine pomace. In this regard, wine pomace is also rich in diet fiber, which cannot be absorbed in the gut, but it is highly fermentable. In this sense, the main fermentation end products are short-chain fatty acids (SCFAs), mainly butyrate, acetate and propionate acid; which are able to induce immune function, epithelial cell stability and lipid metabolism (Gerardi et al., 2020b; Holscher, 2017).

In view of the above, we hypothesized that the bioactivity of white wine seasoning products, stored in contact with chicken meat, would make them suitable for use as a functional ingredient. Therefore, the aim of the present study was to evaluate the antioxidant properties of white wine pomace used as a seasoning in chicken meat subjected to different packaging methods, vacuum and modified atmosphere, in order to establish its potential use as a functional ingredient. For this purpose, an in vitro digestion and fermentation of white wine seasoning products was carried out. The effect of the gastrointestinal products on viability and biomarkers of oxidative stress in Caco-2 cells were also evaluated. The influence on the microbiota and the SCFAs profile was also analyzed.

2. Materials and methods

2.1. White wine pomace products (wWPPs)

The white wine pomace product (wWPP) used as seasoning in this study was prepared at the University of Burgos from seedless white wine pomace from the vinification of *Vitis vinífera* L. cv. Verdejo. It was kindly provided by several wineries located in Burgos (Spain). wWPP underwent a process of dehydration, grinding and heat treatment for its microbiological stabilization, according to previous studies referenced in the work (García-Lomillo et al., 2014) and patent ES2524870 B2 Spain (González SanJose et al., 2015). The main characteristics, nutrient composition, (poly)phenol content, and "in vitro" antioxidant capacity have been described previously (Gerardi et al., 2020a).

2.2. Seasoned chicken breast preparation

The chicken meat was purchased from a local supermarket and 100 g fillets were prepared in duplicate for each of the stored batches. The fillets were seasoned with 2 % salt and 3 % wWPP product and stored under modified atmosphere packing method (MAP) (70 % N_2/30 % CO_2) with 0,1–0,4 % O_2 and under vacuum-packing method (VP) at 4 °C for 7 and 15 days. The wWPP used as seasoning before being added to the meat constitutes the control pomace (C). After 7 or 15 days, the seasonings were scrapped and recovered from the chicken to analyze them. The recovered seasonings are the white wine pomace seasoning (wWPS).

2.3. "In vitro" gastrointestinal digestion

In order to mimic the physiological process, "in vitro" gastrointestinal digestions of the seasoning obtained from chicken breasts stored under MAP and VP for 7 and 15 days were performed, according to a described method by Minekus et al. (2014). An initial oral phase was performed by incubating 300 mg of seasoning samples pooled from chicken replicates in 6 mL simulated salivary fluid (pH 7.0) containing 75U/mL α-amylase (EC 232.565.6 from the porcine pancreas) for 2 min at 37 °C. Then for the gastric phase, pH was adjusted to 1.5 with 1 M HCl and one volume of pepsin solution (final concentration of 500 U/ml, EC 232.629.3 from the porcine gastric mucosa) was added and incubated for 2 h at 37 °C in an orbital shaker. At that point, 1 M $NaHCO_3$ solution was added to adjust pH to 7.5 and the intestinal phase started after the addition of 10 mL of 0.1 M sodium phosphate buffer (pH 7.5) containing pancreatin (EC 232.468.9 from the porcine pancreas) and bile salts (final concentration in the sample of 100 U/mL and 10 mM respectively). The intestinal digestion was carried out during 2 h at 37 °C in an orbital shaker (100 rpm). The resultant gastrointestinal digested solution was centrifuged (5300 g, 10 min), the supernatant and the pellet were both freeze dried (Labconco, MO, USA) and stored at − 20 °C until use. This supernatant is the bioaccessible gastrointestinal fraction (GI-WPS) and the residue is the non-bioaccessible gastrointestinal fraction.

2.4. "In vitro" colonic fermentation

"In vitro" colonic fermentation was performed under anaerobic conditions at 37 °C for 24 h using rat caecal content on the non-bioaccessible gastrointestinal fraction following a previous method (Minekus et al.,2014) and modified by Del Pino-García (2016b). 100 mg of the non-bioaccessible gastrointestinal fractions were added to 8 mL of a sterile anaerobic medium and incubated overnight at 4 °C. The next day 100 mg of the caecal content diluted in sterile anaerobic medium was added and anaerobiosis was achieved by adding CO_2. After 24 h, fermentation was stopped with 1 mL NaOH 1 N, the samples were centrifuged. The supernatant and the pellet were both freeze-dried and stored at −20 °C until use. The supernatant obtained is the fermented bioaccessible fraction (F-WPS), available for absorption at the large intestine, and the residue is the non-bioaccessible fermented fraction.

2.5. Characterization of wine seasoning products and bioaccessible gastrointestinal and fermented fractions

Phenolic content and "in vitro" total antioxidant capacity of the wine seasoning before and after storage, and their corresponding bioaccessible fractions after digestion and fermentation (WPP, WPS, GI-WPS and F-WPS), were analyzed using QUENCHER methods (Del Pino-García et al., 2015). In addition, the detailed phenolic composition of the seasoning products, and the gastrointestinal and fermented fractions were analyzed by HPLC.

2.5.1. QUENCHER Folin-Ciocalteu assay (Q-FC)

Total polyphenol content was determined by incubating 1 mg of the samples with 0,1 mL of Folin-Ciocalteu reagent, and after 2 min, 2 mL of Na_2CO_3 75 g/L solution and Milli-Q water up to 5 mL were added (Singleton & Rossi, 1965). The samples were incubated for 1 h in an orbital shaker and then centrifuged (11900 g 10 min). The absorbance of the supernatant was measured spectrophotometrically at 750 nm.A calibration curve was determined with gallic acid and results were expressed as g of gallic acid equivalents (GAE)/ 100 g sample.

2.5.2. QUENCHER ABTS assay (Q-ABTS)

Q-ABTS (2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid) method was adapted from the assay developed by Del Pino-García (2015). The stock solution of 7 mM $ABTS^{\cdot+}$ was diluted with milli-Q water to an absorbance of 0.7–0.9 al 734 nm. -ABTS determination of the antioxidant activity was performed incubating 1 mg of the samples with 12 mL of dilute ABTS•+ radical solution, in the dark with continuous shaking for 30 min. After centrifugation at 12,000 rpm for 3 min, the absorbance of the supernatant was measured spectrophotometrically at 734 nm. A calibration curve was constructed with Trolox and the results were expressed as mmol Trolox equivalent (TE)/ 100 g sample.

2.5.3. QUENCHER FRAP assay (Q-FRAP)

The Q-FRAP (ferric reducing/antioxidant power) was assessed by incubating 6 mL of FRAP reagent, freshly prepared each day, with 1 mg of the samples for 30 min in a shaking water bath at 37 °C in the dark, followed by a 11900 g 3 min centrifugation. Absorbance was measured at 593 nm and results were expressed as mmol Fe (II) E/ 100 g sample. A $FeSO_4$ calibration curve was used.

2.5.4. HPLC phenolic compound analysis

Polyphenols were extracted from wWPP with MeOH acidified with 1% formic acid (5 g/ 20 mL) at 25 °C for 24 h, according to a previous method with slight modifications (Del Pino-García et al., 2017). The wWPS had more moisture therefore a different ratio was used (2.5 g/ 5.5 mL). Both wWPP and wWPS extracts were diluted 40:60 in water before the injection. The bioaccessible fractions were diluted in water (10 mg sample/ mL water) and injected. Seasonings and bioaccessible fractions were analyzed following a method previously described (Pérez-Magariño et al., 2008). Identification and quantification of phenolic acids, flavan-3-ols and flavonols were carried out using analytical reversed-phase HPLC with an Agilent 1100 series HPLC system (Agilent Technologies Inc., Palo Alto, CA, USA) coupled to a diode array detector and a Spherisorb3® ODS2 reversed phase C18 column (250 mm × 4.6 mm, 3 μm particle size; Waters Chromatographic S.A., Barcelona, Spain). The operational flow was 0.6 mL/min and the injection volume was 200 μL. The mobile phases: A, water:glacial acetic acid (98:2, v/v); B, water:acetonitrile:glacial acetic acid (78:20:2, v/v/v); C, acetonitrile. The solvent gradient used was: 0–25 min, linear gradient from 0 to 100% to 25–75% of B in A; 25–60 min, linear gradient from 25 to 75% to 70–30% of B in A; 60–100 min, linear gradient from 70 to 30% to 100–0% of B in A; 100–120 min, 100% B; 120–130 min; linear gradient from 0 to 100% to 100–0% of C in B; 130–140 min, 100% C; 140–150 min; linear gradient from 100 to 0% to 0–100% of C in A. The eluent was monitored at 254, 280, 320, 360, and 520 nm. The samples were injected in triplicate and calibration was performed by injecting mixes of the standards also in triplicate at different concentrations. Peak identification was performed by comparing the retention times and diode array spectral characteristics of the standards and the samples. The results were expressed in μg of phenolic compound/ g sample.

2.6. Cell culture and treatment

Human colon adenocarcinoma cell line Caco-2 (ATCC® HTB-37™) was purchased from the American Type Culture Collection (ATCC, Barcelona, Spain). Cells were cultured as a monolayer in Eagle in Minimum Essential Medium (MEM) supplemented with 20% (v/v) heat inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL Penicillin, 100 μg/mL Streptomycin, 1% non-essential amino acids, and 0,5 μg/mL Amphotericin B. Cell cultures were incubated at 37 °C and 90 % humidity in a 5% CO_2 and 95% air atmosphere. The culture medium was changed every 2 or 3 days and subculture was performed at 70–80 % confluence. Caco-2 for the experiments were seeded at a $2x10^4$ cells/ cm^2 density and were grown in a complete medium for 24 h. Then, the treated-cells were exposed to 0.07 μg GAE/mL of GI-WPS for 24 h. Non-treated control cells were only incubated with a complete medium. All experiments were carried out as three independent assays.

2.7. Cell viability assessment

Cell viability was determined on Caco-2 cells using the MTT method. Caco-2 cells were cultured at a density of 10^4 cells in 150 μL per well on a 96-well plate for 24 h and treated with the bioaccessible gastrointestinal fractions (0.07 μg GAE/mL) for another 24 h. Subsequently, 40 μL of MTT solution (5 mg/mL) was added and cells were incubated for 2 h at 37 °C. After incubation for 2 h with the MTT solution, the medium was carefully aspirated, MTT formazan crystals were dissolved in 100 μL of DMSO, and the optical density was measured at 570 nm using a microplate reader (Varioskan Lux, Life Technologies Holdings Pte. Ltd, ThermoFisher Scientific, Singapore). The results were expressed as a percentage of cell viability compared to control non-treated cells.

2.8. Intracellular reactive oxygen species (ROS)

Intracellular ROS levels determination was performed by measuring the fluorescence intensity of the 2′,7′-dichloro-dihydro-fluorescein diacetate (H_2DCF-DA) probe. H_2DCF-DA is enzymatically hydrolyzed by intracellular esterases to originate non-fluorescent H_2-DCF, which is then rapidly oxidized to originate highly fluorescent dichlorofluorescein (DCF) in the presence of ROS. The DCF fluorescence intensity is proportional to the amount of intracellular ROS formed. Caco-2 cells were cultured at a density of $2x10^4$ cells per well on a 96-well plate for 24 h and incubated with the bioaccessible gastrointestinal fractions for 24 h. The cells were washed with a colourless external medium (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2{\cdot}.6H_2O$, and 10 mM HEPES), the fluorescence was measured to establish a blank for each well, and then they were preloaded with 100 μL of DCFH-DA 20 μM for 30 min at 37 °C. After incubation, DCFH becomes DCF due to intracellular oxidants. The cells were washed and 100 μL of external medium or 2.5 mM of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP) were added. ABAP was used to induce oxidative stress. Fluorescence was measured every 30 min for 6 h at an excitation wavelength of 480 nm and an emission wavelength of 530 nm in a microplate reader (Varioskan Lux, Life Technologies Holdings Pte. Ltd, ThermoFisher Scientific, Singapore). Results were expressed as ROS levels percentages respect to non-treated cells (NT), using the equation: Treatment/C [%] = $\Delta Fsample(t_{30} - t_0)/\Delta FNT(t_{30} - t_0)$.

2.9. Assessment of oxidative stress cell biomarkers

Caco-2 cells were cultured at $2x10^4$ cells/cm^2 density for 24 h. Then, the treated-cells were exposed for 24 h to the bioaccessible gastrointestinal fractions. After the treatment period, the cells were scraped and centrifuged (1,500 g, 5 min, 25 °C). The cell pellets were resuspended in 1 mL of PBS and frozen at −80 °C for further analyses of different oxidative stress cell biomarkers. 100 μL of the suspension cells were acidified with 10 μL of 20 % perchloric acid (PCA), centrifuged for 5 min at 4100 g 4 °C, and the supernatant was stored at −80 °C for the glutathione ratio analysis.

2.9.1. Glutathione oxidized/reduced (GSSG/GSH) ratio analysis

GSSG/GSH ratio was determined using a previous method (Del Pino et al., 2016a). Aliquots of the cell suspensions collected with PCA (2 % final concentration) were centrifuged (6500 g, 5 min, 4 °C) and the supernatants were neutralized with triethanolamine (TEAM). 10 μL of the samples were added to 190 μL of assay mix (potassium phosphate buffer 0.1 M pH 7 with EDTA 1 mM, 5,5′-Dithiobis (2-nitrobenzoic acid) 0.6 mM, NADPH 0.3 mM and glutathione reductase). The kinetic spectrophotometric assay was carried out by measuring absorbance at 410 nm every 2.5 min for 20 min. Glutathione disulfide (GSSG) was measured using the same method after derivatizing the samples with 2-

vinilpiridine, and GSH was estimated by subtracting GSSG from total GSH. Total GSH and GSGG calibration curves were obtained and the results were expressed as the GSSG/GSH ratio.

2.9.2. Malondialdehyde (MDA) assessment

MDA levels in Caco-2 cells were determined by HPLC-DAD. Briefly, 45 μL of the previously sonicated cell suspension were incubated with 15 μL of Milli-Q water and 15 μL 3 M NaOH at 60 °C for 30 min. After this, 75 μL of 6 % (v/v) H_3PO_4 and 75 μL of 0.8 % (w/v) thiobarbituric acid (TBA) were added, which forms a Schiff base adduct with the MDA, and incubated at 90 °C for 45 min. 50 μL of MDA-TBA2 complex formed was injected into an Agilent 1100 Series HPLC systems (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with a diode array detector. The column was a Spherisorb ODS2 reversed phase C18 (250 mm x 4.6 mm, 3 mm particle size, Waters Chromatography S.-A., Barcelona Spain). The mobile phase was a mixture of 65% of 50 mM potassium phosphate buffer pH 6.8 and 35% Methanol. The flow rate was maintained isocratically at 0.8 mL/min, the absorbance at 532 nm was monitored, and the total run time was 10 min. Concentrations of MDA were calculated from calibration curves obtained using 1,1,3,3-tetramethoxypropane (TMP) as standard and expressed as μM of MDA equivalents.

2.9.3. Carbonyl groups (CGs) assessment

The carbonyl groups in cell proteins were assayed in sonicated cell lysates using a previous method (Levine et al., 1990). 250 μL of 0.2 % (w/v) 2,4-dinitrophenylhydrazine (DNPH) diluted in 2 M HCl were mixed with 50 μL of the sonicated cell suspensions. After incubation for 1 h, proteins were precipitated with 250 μL of 20 % trichloroacetic acid (TCA) and incubated 15 min at 4 °C. The samples were centrifuged at 6500 g for 3 min to discard the supernatant. The pellets were washed 3 times with ethanol: ethyl acetate (1:1 v/v). Finally, the pellet was resuspended on 200 μL of 6 M guanidine acidified and the presence of carbonyl groups was measured spectrophotometrically at 373 nm. The carbonyl concentration was calculated using a molar absorption coefficient of 22.000 M^{-1} cm^{-1}. The carbonyl groups levels were then normalized by the protein content of each cell homogenate, expressing the final results as nmol CGs/mg of protein. Protein content was determined using the Bradford method.

2.10. Microbiota analysis

Total DNA was isolated from the froze-dried non-bioaccessible fermented fractions using the QIAamp Mini DNA kit (Qiagen, West Sussex, UK) following manufactureŕs instructions. Eluted DNA was treated with RNase and DNA concentration was measured spectrophotometrically by using a NanoDrop (BioTek, Vermont, USA). Six groups of bacteria were analyzed by qPCR: All bacteria, *Bacteroides*, *Lactobacilli*, *Bifidobacterium*, *Eubacterium rectale/Clostridium coccoides* and *Clostridium leptum*. The 16S rDNA specific primers used are listed in Supplementary Table 1.

The qPCR conditions for All Bacteria, *Bacteroides, Bifidobacterium* and *Lactobacilli* consisted of a first step at 50 °C for 2 min, followed by 10 min at 95 °C for initial denaturing and 40 cycles of 15 s at 95 °C, 1 min at 60 °C and 1 min at 72 °C for denaturing, annealing and product elongation respectively. For *Eubacterium rectale/Clostridium coccoides* and *Clostridium leptum,* qPCR conditions included a first step of 5 min at 94 °C for denaturing and 40 cycles with a sequence of 20 s at 94 °C, 20 s at 50 °C and 1 min at 72 °C for denaturing, annealing and product elongation. Proper amplification was verified by melting curve analysis. The results were expressed as log copy number per ng of DNA.

2.11. Analysis of fecal contents of short chain fatty acids (SCFAs)

The concentrations of acetic, propionic and butyric acids in the bioaccessible fermented seasoning fractions were measured by a previously described method (Del Pino et al., 2017). The pH of the F-WPS was adjusted to 2–3 with 5 mM HCl and 50 μL was mixed with 50 μL of the internal standard (1 mM valeric acid in 0.1% orthophosphoric acid solution). The mixture was analyzed with a gas chromatograph Agilent 7890 A (Agilent Technologies, Inc., Santa Clara, California, USA) equipped with a flame ionization detector (FID) and a DB-FFAP column (30 m × 0,32 mm, 0,5 μm film thickness, Agilent Technologies). Using the "spitless mode", 1 μL of the solution was injected at 200 °C. The temperature was increased 10 °C/min after 30 s at 100 °C to 200 °C, it was maintained for a min and increased again 25 °C/min up to 230 °C, then maintained for 5 min. The FID detector was programmed at 230 °C and used Helium as vehicle gas with a 1 mL/min flow. Peak identification was based on comparison of retention times with the respective SCFAs standards. Calibration curves were obtained for each SCFA standard and used for quantitative analysis. The results were expressed as mM of SCFA.

Table 1
Total antioxidant capacity (TAC) and total polyphenols of white wine pomace seasoning (wWPS), the bioaccessible gastrointestinal fractions (GI-WPS) and the bioaccessible fermented fractions (F-WPS). Values represents mean (n = 3) ± SD. Significant differences between the samples in different storage conditions (control, modified atmosphere and vacuum) are indicated in the rows by Latin letters and the differences between the seasonings and their fractions are indicated in columns by Greek letters for each test and storage condition. ANOVA Variance test ($p < 0.05$). wWPS: white wine pomace seasoning; GI-WPS: bioaccessible gastrointestinal digested fraction; F-WPS: bioaccessible fermented fraction; Control: white wine seasoning or its fractions after being in contact with the chicken breasts for 30 min; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric reducing antioxidant power; TE: Trolox equivalent; GAE: Gallic acid equivalent.

	Control	Modified Atmosphere		Vacuum	
		7 days	15 days	7 V days	15 days
wWPS					
Q-ABTS (mmol TE/ 100 g WPS)	0.46 ± 0.07$^{a\alpha}$	1.69 ± 0.14$^{cd\alpha}$	1.22 ± 0.21$^{b\alpha}$	1.53 ± 0.10$^{c\alpha}$	1.79 ± 0.06$^{d\alpha}$
Q-FRAP (mmol Fe (II)E/100 g WPS)	7.13 ± 0.60$^{d\beta}$	2.69 ± 0.48$^{b\alpha}$	4.02 ± 0.29$^{c\beta}$	1.62 ± 0.21$^{a\alpha}$	1.71 ± 0.23$^{a\alpha}$
Total polyphenols (g GAE/100 g WPS)	0.60 ± 0.06$^{b\alpha}$	0.06 ± 0.01$^{a\alpha}$	0.02 ± 0.01$^{a\alpha}$	0.04 ± 0.02$^{a\alpha}$	0.04 ± 0.02$^{a\alpha}$
GI-WPS					
Q-ABTS (mmol TE/ 100 g GI-WPS)	14.59 ± 0.90$^{\beta}$	15.12 ± 0.52$^{\gamma}$	15.08 ± 0.24$^{\gamma}$	15.02 ± 0.40$^{\gamma}$	14.73 ± 0.11$^{\gamma}$
Q-FRAP (mmol Fe (II)E/100 g GI-WPS)	2.51 ± 0.39$^{c\alpha}$	2.14 ± 0.13$^{b\alpha}$	1.95 ± 0.08$^{ab\alpha}$	2.06 ± 0.14$^{b\alpha}$	1.69 ± 0.14$^{a\alpha}$
Total polyphenols (g GAE/100 g GI-WPS)	0.93 ± 0.02$^{cd\beta}$	0.79 ± 0.05$^{b\gamma}$	0.97 ± 0.02$^{d\gamma}$	0.89 ± 0.02$^{c\beta}$	0.33 ± 0.02$^{a\beta}$
F-WPS					
Q-ABTS (mmol TE/ 100 g F-WPS)	15.30 ± 1.01$^{c\beta}$	12.88 ± 0.04$^{ab\beta}$	11.97 ± 0.34$^{a\beta}$	12.84 ± 0.31$^{ab\beta}$	13.55 ± 0.16$^{b\beta}$
Q-FRAP (mmol Fe (II)E/100 g F-WPS)	30.79 ± 1.04$^{c\gamma}$	25.02 ± 1.10$^{b\beta}$	23.74 ± 0.27$^{b\gamma}$	23.99 ± 0.48$^{b\beta}$	21.74 ± 0.59$^{a\beta}$
Total polyphenols (g GAE/100 g F-WPS)	0.59 ± 0.05$^{b\alpha}$	0.54 ± 0.03$^{ab\beta}$	0.74 ± 0.06$^{c\beta}$	0.90 ± 0.06$^{d\beta}$	0.47 ± 0.08$^{a\gamma}$

2.12. Data presentation and statistical analysis

Statistical analysis was performed using StatGraphics® Centurion 18.1.13 (Statpoint Technologies Inc., Warrenton, Virginia, USA). Data were expressed as means ± standard deviation of independent experiments performed in triplicate and One-way analysis of variance (ANOVA), Fisher's least significant difference (LSD) multiple range test,

was used to determine significant differences ($p < 0.05$) between data. The multivariate analysis was performed by applying principal component analysis (PCA).

3. Results and discussion

White wine pomace products (WPP) are a winery by-product with a high polyphenol content, which presents healthy properties and can be used as an additive in food, specially recommended for meat seasoning (Gerardi et al., 2020b). In this study we have evaluated the bioaccessibility of wWPP used as a seasoning (wWPS) in chicken breast stored under modified atmosphere (MAP) and vacuum (VP) conditions for 7 and 15 days.

To date, there has been no evaluation of whether wWPP used as seasoning in meat showed its healthy properties, which is important to determine their use as a functional ingredient. Therefore, in this study we evaluated whether the wWPP used as seasoning (wWPS) in chicken breast and stored under modified atmosphere (MAP) and vacuum (VP) conditions for 7 and 15 days maintained in its bioaccessible fractions gastrointestinal digestion and colonic fermentation) its phenolic profile, antioxidant capacity, cell preventive oxidative damage in Caco-2 cells and the capacity to modulate the microbiota intestinal.

3.1. Phenolic profile and antioxidant capacity of white wine pomace seasoning (wWPS) and the effect of packaging conditions

No previous study had analyzed the phenolic profile of a seasoning and its bioaccessible fractions after being used as a chicken breast seasoning. The phenolic profiles of the control pomace and the wWPS used as meat seasoning vacuum and modified atmosphere packaging are presented in Supplementary Table 2.

With the exception of gallic acid, the contents of all phenolic acids decreased with storage in both packaging methods compared to the control. The results showed that the gallic acid content of the seasoning in contact with the meat increased significantly ($p < 0.05$) by 97 % after 7 days and by 92 % after 15 days storage under modified atmosphere conditions in comparison with the control sample. Vacuum-packed samples also showed an increase in the levels of gallic acid of 43 % at 7 days and of 106 % after 15 days. The increase in gallic acid observed during storage could be due to the degradation of polymers, hydrolysable tannins called gallotannins, present in the pomace (Fernandes et al., 2022). An increase in epicatechin levels was also observed after 15 days of vacuum (97.3 ± 2.85 μg/g in control vs 127 ± 0.77 μg/g in 15 days of vacuum). The other phenolic compounds analyzed, catechins, procyanidins and flavonols, showed a significant ($p < 0.05$) decreased in the seasoning in both storage conditions. Similar results were observed by others authors in a study on strawberry purees, which showed a decrease in protocatechuic acids and quercetin concentrations during the first weeks of storage in a nitrogen atmosphere (Kadivec et al., 2013). These authors observed an increase in gallic acid and a maintenance of catechin levels are observed after 6 weeks of storage. In another study, it has been observed that the storage in a nitrogen atmosphere containing 2 % of O_2 reduced the accumulation of phenolic compounds in lettuce and broccoli by about 35 % (Jamie and Saltveti, 2002). This suggests that phenolic compounds are relatively unstable during processing and can easily undergo numerous reactions and modifications.

The chemical changes and bioaccessibility of wWPS bioactive compounds in the gastrointestinal tract are key to determining their bioavailability. Furthermore, the bioactive compounds enclosed in the indigestible fraction of the gastrointestinal tract reach the large intestine for the gut microbiota. These gastrointestinal and colonic fermentation processes can lead to the release and modification of wWPS components. The phenolic profiles of their bioaccessible gastrointestinal (GI-WPS) and fermented fractions (F-WPS) are presented in the Supplementary tables, 3 and 4 respectively. The results showed that the storage process also modifies the composition of the fractions after digestion and colonic fermentation, regardless of the packaging method. When each fraction was analyzed in relation to its control fraction, a significant decrease ($p < 0.05$) in most phenolic acids and monomeric flavan-3-ol was observed. In addition, there was an increase in flavonols in the gastrointestinal samples, but no detectable flavonols in the post-fermentation samples.

A Principal Component Analysis (PCA) (Fig. 1) allows to separate wWPS based on their phenolic profile, as well as the corresponding digested and fermented fractions. It was used to summarize the effect of the storage method and digestion processes in all of the phenolic profiles. The first component (PC1) explains 48.2 % of the variance, while the second (PC2) adds 28.2 %. The distribution showed differences between the wWPS and the stored wWPS and between the gastrointestinal and fermentation fractions and their corresponding control. However, no differences were observed between storage methods and between storage times, especially in the wWPS. Therefore, storage of the seasoning in contact with the meat, regardless of the method used, modifies their phenolic profile and the similar form of their digested and fermented fractions.

The next step is to analyze whether these changes in the phenolic profile affect to a greater or lesser impact on the antioxidant and bioactive potential of the seasonings. For this purpose, the antioxidant capacity (Q-ABTS and Q-FRAP) and the total polyphenol content (Q-FC) of the samples were analyzed (Table 1). When comparing the pomaces, both total polyphenols and the antioxidant capacity according to the Q-FRAP method were significantly higher ($p < 0.05$) in the control wWPP than in those used as chicken breast seasoning (wWPS). However, with the Q-ABTS method, a significant increase ($p < 0.05$) was observed in the stored samples with respect to the control, which can be attributed to the presence of substances in the meat (amino acids, dipeptides, L-carnitine, glutathione, taurine and creatine) which could also have migrated or interacted with the seasoning, increasing Q-ABTS values (Serpen et al., 2012). These authors also observed the inability of meat to increase Q-FRAP values, which would explain the decrease in Q-FRAP values in the seasoning after storage, compared to the control. Also, a decrease of antioxidant properties detected by Q-FRAP assay over time has been reported for foods stored with wWPS in vacuum conditions for longer periods of time, so our results are in line with this trend (Sánchez Alonso et al., 2008).

Table 2
Microbiota composition of the caecal content used for the colonic fermentation of the seasonings assessed by qPCR. Values represent mean (n = 3) ± SD. Significant differences between caecal content used for fermentation and the non-bioaccessible F-WPS obtained after the colonic fermentation of the seasonings are indicated by Latin letters for each bacteria species present. ANOVA Variance test ($p < 0.05$). Control: microbiota of the caecal content used for the colonic fermentation without seasoning.

	Non-Bioaccesible F-WPS				
	Control	Modified atmosphere		Vacuum	
		7 days	15 days	7 days	15 days
All Bacteria	7.36 ± 1.79^{a}	9.45 ± 0.08^{b}	9.35 ± 0.22^{b}	10.16 ± 0.12^{b}	9.52 ± 0.01^{b}
Bacteroides spp.	4.10 ± 0.75	4.54 ± 0.06	4.55 ± 0.09	4.51 ± 0.09	4.18 ± 0.08
Lactobacillus spp.	8.52 ± 0.09^{a}	10.19 ± 0.85^{c}	9.60 ± 0.05^{c}	9.53 ± 0.02^{bc}	8.64 ± 0.04^{ab}
Bifidobacterium	21.48 ± 0.04^{a}	24.19 ± 1.55^{b}	23.27 ± 0.69^{ab}	22.80 ± 1.11^{ab}	23.58 ± 0.37^{b}
Clostridium leptum	8.82 ± 1.14^{b}	6.15 ± 0.60^{a}	8.26 ± 0.94^{b}	8.59 ± 0.17^{b}	8.20 ± 0.29^{b}
Eubacterium rectale/ Clostridium coccoides	9.07 ± 0.97^{c}	6.29 ± 1.87^{a}	7.81 ± 0.28^{b}	8.98 ± 0.05^{c}	7.61 ± 0.81^{ab}

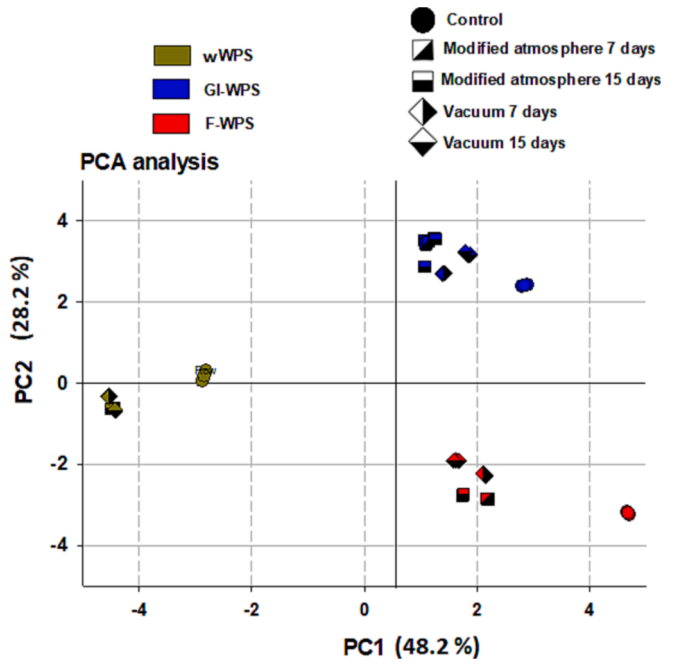

Fig. 1. Principal component analysis (PCA) of the phenolic profiles of the non-stored and stored seasonings, gastrointestinal and fermented bioaccessible fractions. Principal component analysis of all of the polyphenols characterized by reversed phase HLPC. Dot represent the individual replicates of the phenolic profile of the samples. PCA test. PC1: Principal component 1; PC2: Principal component 2. WPS: white wine pomace seasoning; GI-WPS: bioaccessible gastrointestinal digested fraction; F-WPS: bioaccessible fermented fraction; Control: white wine pomace seasoning or its digested fractions before being stored with meat.

Gastrointestinal digestion and colonic fermentation of the wWPS results in changes in the total antioxidant capacity of the seasoning such as can be observed when we compare digested seasoning with undigested seasoning (Table 1). Q-ABTS capacity in gastrointestinal fraction (GI-WPS) and colonic fermentation fraction (F-WPS) is 30-fold higher in control samples and 8-fold in the stored samples (MA and V) compared to undigested samples (WPS). In both control and storage samples, Q-FRAP activity is lower in the GI-WPS fraction and higher in F-WPS fraction compared to the undigested fraction. Thus, the Q-FRAP capacity in the control samples is 2.8-fold lower in the GI-WPS and 4.3-fold higher in the F-WPS, this change being lower in the storage samples except for the treatment with vacuum at 7 days where a 1.6-fold increase was observed. The total polyphenols in the digestion fractions of control wWPS resulted in an increased in the GI-WPS but not in F-WPS and significantly lower values were observed in control samples after storage but not in GI-WPS and F-WPS. In control samples the increase in GI-WPS is 1.5-fold and in storage samples, it varies according to technique and time. Changes in the antioxidant capacity of wine pomace products resulting from in vitro digestion were previously observed by our research group in red wine pomace products, where a tendency towards higher antioxidant capacity was observed in different types of samples obtained from wine pomace (Del Pino-García R. et al. 2016b).

On the other hand, the type and time of storage modified the total antioxidant capacity of the digested fractions. The Q-ABTS capacity of the gastrointestinal fractions of seasoning (GI-WPS) stored under modified atmosphere and vacuum not change compared to the control GI-WPS. Regarding Q-FC the lowest values were observed after 15 days of vacuum storage (0.93 ± 0.02 g GAE/100 g GI-WPS in control vs 0.33 ± 0.02 g GAE/100 g GI-WPS). However, the Q-FRAP values decreased significantly ($p < 0.05$) in the GI fractions of the seasoning storage, with lower values under vacuum conditions. The Q-FRAP values are in agreement with those of Pesic et al., (2019) who observed a 25 % decrease in Q-FRAP values in gastrointestinal digested samples of grape skin added to turkey meat in comparison with the control grape skin. This study explains the relationship between phenolic acids and Q-FRAP values, so that the decrease in phenolic acids after storage can explain the decrease in Q-FRAP in the seasoning and in their fractions.

The fermented fractions of wine pomace seasoning (F-WPS) stored under atmosphere modified and vacuum show a reduction ($p < 0.05$) in Q-ABTS and Q-FRAP values compared to the control F-WPS, regardless of the packaging method. However, an increase in Q-FC values was observed in the samples stored under MAP 15 days and under VP 7 days, showing the highest amount of phenolic acids.

Despite the changes described, both seasonings and their in vitro digested fractions maintained a phenolic profile, as well as high total antioxidant capacity and total polyphenol content, after storage for either 7 or 15 days under both packaging methods. These results indicate the potential use of wWPP as a meat seasoning.

3.2. Effect of the gastrointestinal fractions of wine pomace product seasoning (GI-WPS) stored under MAP and VP on Caco-2 cell viability and oxidative stress

The effects of the bioaccessible gastrointestinal fractions of the GI-WPS (under MAP and VP) on cell survival were assessed to ensure non-cytotoxicity. The results presented in Fig. 2A showed non-cytotoxic effects of all GI fractions, which did not reduce the viability compared to not treated cells. Similar results with gastrointestinal digestion fractions of WPP was observed in previous studies (Del Pino-García, et al., 2016a).

Oxidative stress due to high ROS production or to insufficient ROS removal by endogenous or exogenous antioxidant mechanisms can result in DNA, lipid and protein damage associated with functional cell alterations. In the current study, the exposure of Caco-2 cells to GI-WPS fractions did not increase ROS levels in the Caco-2 cells (Fig. 2B). Furthermore, to evaluate the preventive effect of the GI-WPS against oxidative stress, we evaluated the effect in Caco-2 cells treated with 2.5 mM ABAP which causes a marked increase in ROS levels (Fig. 2C). Interestingly, when cells were treated with the GI-WPS fractions, a significant ($p < 0.05$) reduction in ROS levels of approximately 66% was observed. No differences were observed among the different GI-WPS samples. The results could be due to the ROS-modulating effects of phenolic compounds and metabolites present in the GI fractions, such as phenolic acids and flavonoids, gallic acid, epicatechin, myricetin or quercetin, which are present in high concentrations in these bioaccessible fractions.

Oxidative stress levels are controlled by endogenous and exogenous ROS scavenging systems, such as intracellular GSH. Intracellular GSH is the representative endogenous non-enzymatic mechanisms that protects against oxidative damage. The ratio between oxidized glutathione (GSSG) and reduced glutathione (GSH) constitutes a good biomarker of the redox state in biological systems (Lee et al., 2012). The ratio of oxidized/reduced glutathione (GSSG/GSH) (Fig. 3A) was analyzed. Changes in the GSSG/GSH ratio were observed in the cells treated with the GI-WPS fraction of the seasoning in contact with chicken breast and storage under MAP and VP. The GSSG/GSH ratio of the cells treated with GI-WPS fractions of MAP samples of 7 days did not change but the treatment with the obtained of the sample stored for 15 days reduced the GSSG/GSH levels compared to not-treated cells. On the other hand, the GI-WPS fractions of the VP samples had a higher GSSG/GSH ratio than the non-treated cells. An increase in this ratio indicates a higher consumption of GSH or a lower availability of NADPH under conditions of oxidative stress. Polyphenols have been showed to activate endogenous cellular systems through antioxidant responsive elements (AREs) present in the promoter regions of many oxidative stress-inducible genes, including the limiting enzyme in glutathione synthesis, which could explain the increase in total GSH levels in the cells treated with GI-WPPS (Masella et al., 2005). Previous studies on the effect of WPP on GSH in endothelial cells and muscle cells had already reported an increase in

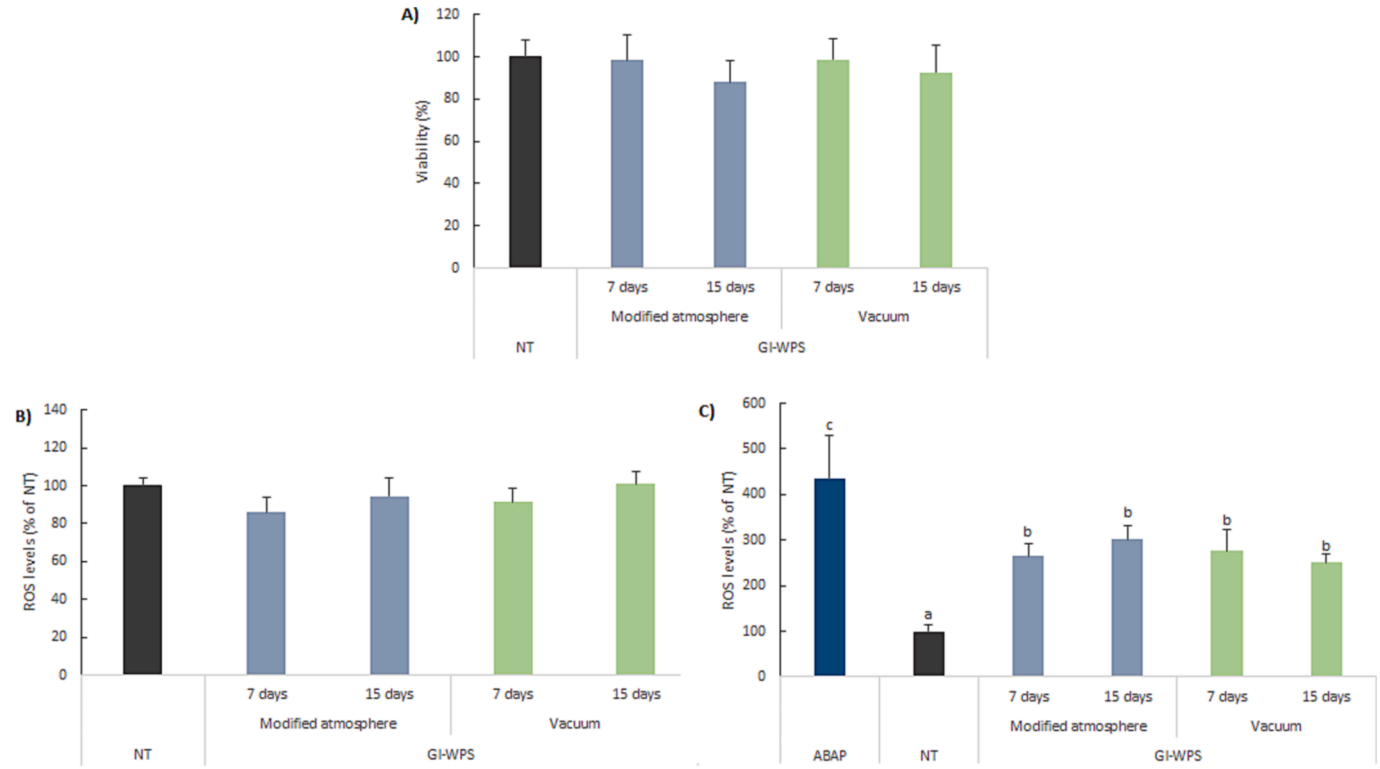

Fig. 2. Viability and ROS levels of Caco-2 cells treated with the bioaccessible gastrointestinal fractions (GI-WPS) of the seasonings before and after being stored with chicken breasts in a vacuum and modified atmosphere conditions. (A) Percentage of Caco-2 viability via MTT assay, (B) Intracellular ROS production of Caco-2 cells in normal conditions, (C) Intracellular ROS production of Caco-2 cells in oxidative stress conditions caused by ABAP 2.5 mM. Values represent mean (n = 3) ± SD. ANOVA Variance test ($p < 0,05$). Significant differences in ROS production of Caco-2 between different treatments are indicated by Latin letters. NT: non-treated cells; ABAP: 2,2′-azobis(2-methylpropionamidine) dihydrochloride; GI-WPS: bioaccessible gastrointestinal digested fraction.

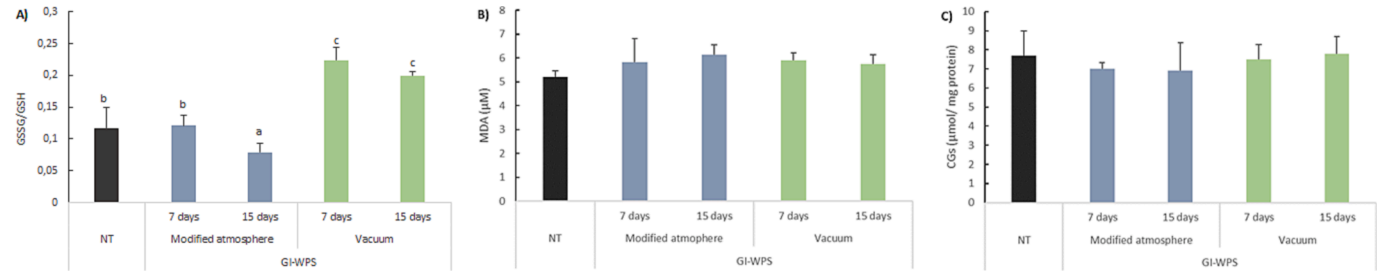

Fig. 3. Cell stress biomarkers of Caco-2 cells incubated with the bioaccessible gastrointestinal fractions (GI-WPS) of the seasonings before and after being stored with chicken breasts in a vacuum and modified atmosphere conditions. (A) Intracellular redox status (GSSG/ GSH), (B) Lipid oxidation status, MDA levels (C) Protein oxidation status, CGs levels. Values represents mean (n = 3) ± SD. Significant differences in Caco-2 between different treatments are indicated by Latin letters. ANOVA Variance test ($p < 0.05$). NT: non-treated cells; GI-WPS: bioaccessible gastrointestinal digested fraction; GSH: Reduced glutathione; GSSG: oxidized glutathione; MDA: Malondialdehyde; CGs: Carbonyl groups.

glutathione (Del Pino et al., 2016a; Goutzourelas et al., 2014), so this seems to be one of the molecular mechanisms responsible for the enhancement of antioxidant activity by the wine pomace product in cells.

Biomarkers of oxidative stress are relevant biomolecules that indicate cellular damage caused by ROS (Marrocco et al., 2017). Therefore, to ensure the potential antioxidant effect of the GI-WPS, several biomarkers of oxidative stress were evaluated in Caco-2 cells after treatment with the bioaccessible fractions. We assessed malondialdehyde levels in Caco-2 cells treated with the GI-WPS of the stored samples (WPS). MDA is a product of the peroxidation of polyunsaturated fatty acids used as a biomarker of lipid peroxidation, and MDA equivalents were evaluated by reverse-phase HPLC analysis (Fig. 3B). No effects were observed in Caco-2 cells treated with the GI-WPS compared to non-treated cells. To determine protein oxidation, we quantified carbonyl groups in the proteins via spectrophotometry (Fig. 3C). The GI-WPS did not significantly modify protein oxidation compared to not treated cells. Furthermore, the ability of the bioaccessible fraction to protect against lipid oxidation and protein damage was not affected by the different packaging methods.

In conclusion, the packaging conditions of the seasoning in contact with chicken breast did not modify the antioxidant properties of the gastrointestinal fraction in Caco-2 cells and it was even able to modulate the total content of the redox system GSSG/GSH and reduce the production of ROS in response to oxidative stress.

3.3. Effect of the wine pomace product-seasoning (WPPS) on the microbiota composition and SCFAs

Microbiota from rat caecal content was used in the colonic

fermentation to mimic the process of the human intestine. The microbiota is able to modify the phenolic compounds and the dietary fiber of the seasoning, and at the same time, the microbiota composition is changed by the wine pomace products. The microbiota plays a key role in human health; changes in the composition and function of the gut microbiota affect not only food digestion but also permeability, metabolism and immune response (Gomaa, 2020), so we aimed to study the probiotic effects of the wWPPS.

Most of the polyphenols in wine pomace products are highly polymerized or combined with dietary fiber, making the action of intestinal microbiota is essential for their metabolism (Gerardi et al., 2020a; Gil-Sánchez et al., 2018). Therefore, the potential functional effects of the consumption of wine pomace products will be due, at least in part, to the action of all these microbial-derived metabolites.

The changes in the microbiota obtained after the fermentation of the WPS of non-bioaccessible gastrointestinal fractions were analyzed and compared. Table 2 shows the microbiota in the colonic fermentation fraction (F-WPS) and a sample of the caecal content used in a colonic fermentation without wine pomace product sample, which was used as a control. The F-WPS of the MAP and VP samples significantly increased All bacteria group. Also was observed that F-WPS of MAP samples (7 and 15 days) and the VP sample of 7 days increased *Lactobacillus* spp. and both MAP conditions and 15 days of VP increased *Bifidobacterium*. Previous studies have also reported an increase in *Bacteroides* spp., *Bifidobacterium* and *Lactobacillus* spp. and a decrease in *Clostridium leptum* in "in vivo" studies with a polyphenol rich diet (Gerardi et al., 2020b; Plamada & Vodnar, 2022). The increase in *Bacteroides* by the treatment with seasoning could be a desirable effect due to their higher number of glycan-degrading enzymes, these enzymes are required for the cleavage of the glycosidic linkages of polyphenols and provoke changes in the polyphenol content (Pathak et al., 2018). With regard to *C. leptum* and Eubacterias the treatment with F-WPS of 7 days under MAP conditions reduced both and the fractions of 15 days MAP and 15 days VP only decreased the presence of *Eubacterias*. This is a positive effect, inhibiting the potentially pathogenic bacteria. In fact, some studies have shown a significant increase in *Clostridium leptum* in individuals with an high-cardiometabolic risk (Vetrani et al., 2020; Gerardi et al., 2020b).

Additionally, colonic fermentation of wWPS promotes the production of short-chain fatty acids. They are partly responsible for the reported beneficial effects of dietary fiber and polyphenols present in the wWPP (Saura-Calixto et al., 2010). The main SCFAs present in fermented white pomace are acetic, propionic and butyric acids (Del Pino et al., 2017). The SCFA measured and the ratio between them in the F-WPS of the control wWPP and the stored seasoning samples are showed in Fig. 4. Acetic acid showed the highest concentration in all F-WPS samples, which is consistent with previous reports (O'Keefe, 2016). When comparing the acetic acid in the F-WPS control sample with the F-WPS stored seasoning, a significant difference ($p < 0.05$) was found. The levels of butyric acid in the F-WPS fraction of the control sample were also significantly higher ($p < 0.05$) than in the F-WPS stored. This could be due to the reduction in *Clostridium leptum* and Eubacterias observed in the microbiota study, as both of these groups are reported to produce butyrate in the gut (Kabeerdoss et al., 2013; Vermeiren et al., 2012).

The SCFA ratio of the stored seasonings was similar, with a higher concentration of propionic acid than in the control samples. Propionic acid is involved in the lipid and glucose metabolism in the host, acting as a gluconeogenic agent and playing an important role in the competitive regulation of lipogenesis (Ríos-Covián et al., 2016). *Bacteroides thetaiotaomicron*, present in the human gut has been reported to produce propionic acid (Adamberg et al., 2014), mainly in long fermentations, which is consistent with our results on the modification of the microbiota by the samples.

4. Conclusions

White wine pomace used as seasoning for chicken meat maintains its antioxidant properties when stored for 7 or 15 days in vacuum or modified atmosphere packaging. Vacuum and modified atmosphere packaging of the wWPs in contact with chicken breast does not adversely affect the polyphenol content and antioxidant capacity of in vitro digested fractions (gastrointestinal and colonic fermentation). The contact of the seasoning with the chicken breast can be considered safe as it does not show cytotoxicity in the gastrointestinal Caco-2 cells. Furthermore, the beneficial effect of the colonic fermentation fraction on the microbiota and its ability to modulate the cell oxidative stress suggest its potential use as a functional ingredient, without being affected by the storage process. However, the present study also has certain limitations, and further studies are needed to evaluate the interactions with the food matrix and the effect of the cooking on the seasoning composition.

In conclusion, these results are novel and may help to establish the value of this product as a natural seasoning for chicken breast.

CRediT authorship contribution statement

Victor Gutierrez-Gonzalez: Methodology, Investigation, Formal analysis, Writing – original draft. **Mª Dolores Rivero-Perez:** Methodology, Investigation. **Gisela Gerardi:** Methodology, Investigation, Writing – review & editing. **Pilar Muñiz:** Conceptualization, Supervision, Project administration, Funding acquisition, Formal analysis, Writing – review & editing. **Mª Luisa González-SanJose:** Conceptualization, Methodology, Investigation. **Isabel Jaime:** Supervision, Project

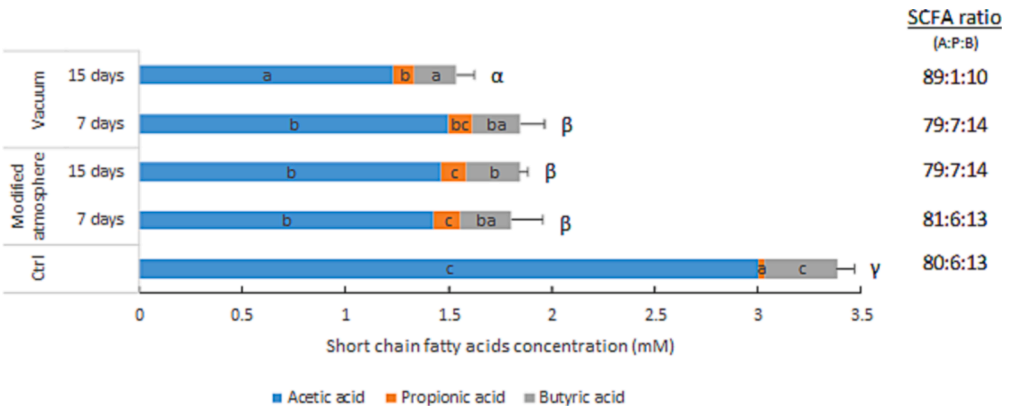

Fig. 4. Content of short-chain fatty acids (SCFAs) in the bioaccessible fermented fractions (F-WPS). Values represents mean (n = 3) ± SD. Significant differences between fractions are expressed by Latin letters for specific acids and by Greek letters for the total SCFAs added. ANOVA Variance test ($p < 0.05$). F-WPS: bioaccessible fermented fraction; Control: F-WPS of the seasoning before storage; A: acetic acid; P: propionic acid; B: butyric acid.

administration, Funding acquisition. **Mónica Cavia-Saiz:** Conceptualization, Supervision, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136625.

References

Adamberg, S., Tomson, K., Vija, H., Puurand, M., Kabanova, N., Visnapuu, T., Jõgi, E., Alamäe, T., & Adamberg, K. (2014). Degradation of Fructans and Production of Propionic Acid by Bacteroides thetaiotaomicron are Enhanced by the Shortage of Amino Acids. *Frontiers in Nutrition, 1*, 21. https://doi.org/10.3389/fnut.2014.00021

Baroi, A. M., Popitiu, M., Fierascu, I., Sărdărescu, I.-D., & Fierascu, R. C. (2022). Grapevine Wastes: A Rich Source of Antioxidants and Other Biologically Active Compounds. *Antioxidants (Basel, Switzerland), 11*(2), 393. https://doi.org/10.3390/antiox11020393

Del Pino-García, R., García-Lomillo, J., Rivero-Pérez, M. D., González-SanJosé, M. L., & Muñiz, P. (2015). Adaptation and Validation of QUick, Easy, New, CHEap, and Reproducible (QUENCHER) Antioxidant Capacity Assays in Model Products Obtained from Residual Wine Pomace. *Journal of Agricultural and Food Chemistry, 63*(31), 6922–6931. https://doi.org/10.1021/acs.jafc.5b01644

Del Pino-García, R., Gerardi, G., Rivero-Pérez, M. D., González-SanJosé, M. L., García-Lomillo, J., & Muñiz, P. (2016). Wine pomace seasoning attenuates hyperglycaemia-induced endothelial dysfunction and oxidative damage in endothelial cells. *Journal of Functional Foods, 22*, 431–445. https://doi.org/10.1016/j.jff.2016.02.001

Del Pino-García, R., González-SanJosé, M. L., Rivero-Pérez, M. D., García-Lomillo, J., & Muñiz, P. (2016). Total antioxidant capacity of new natural powdered seasonings after gastrointestinal and colonic digestion. *Food Chemistry, 211*, 707–714. https://doi.org/10.1016/j.foodchem.2016.05.127

Del Pino-García, R., González-SanJosé, M. L., Rivero-Pérez, M. D., García-Lomillo, J., & Muñiz, P. (2017). The effects of heat treatment on the phenolic composition and antioxidant capacity of red wine pomace seasonings. *Food Chemistry, 221*, 1723–1732. https://doi.org/10.1016/j.foodchem.2016.10.113

Fernandes, T. A., Antunes, A. M. M., Caldeira, I., Anjos, O., de Freitas, V., Fargeton, L., Boissier, B., Catarino, S., & Canas, S. (2022). Identification of gallotannins and ellagitannins in aged wine spirits: A new perspective using alternative ageing technology and high-resolution mass spectrometry. *Food Chemistry, 382*, Article 132322. https://doi.org/10.1016/j.foodchem.2022.132322

Ferrer-Gallego, R., & Silva, P. (2022). The Wine Industry By-Products: Applications for Food Industry and Health Benefits. *Antioxidants, 11*(10), Article 10. https://doi.org/10.3390/antiox11102025.

García-Lomillo, J., & González-SanJosé, M. L. (2017). Applications of wine pomace in the food industry: Approaches and functions. *Comprehensive Reviews in Food Science and Food Safety, 16*(1), 3–22. https://doi.org/10.1111/1541-4337.12238

García-Lomillo, J., González-SanJosé, M. L., Del Pino-García, R., Rivero-Pérez, M. D., & Muñiz-Rodríguez, P. (2014). Antioxidant and Antimicrobial Properties of Wine Byproducts and Their Potential Uses in the Food Industry. *Journal of Agricultural and Food Chemistry, 62*(52), 12595–12602. https://doi.org/10.1021/jf5042678

Gerardi, G., Cavia-Saiz, M., & Muñiz, P. (2021). From winery by-product to healthy product: Bioavailability, redox signaling and oxidative stress modulation by wine pomace product. *Critical Reviews in Food Science and Nutrition, 1–23*. https://doi.org/10.1080/10408398.2021.1914542

Gerardi, G., Cavia-Saiz, M., Rivero-Pérez, M. D., González-SanJosé, M. L., & Muñiz, P. (2020a). The dose-response effect on polyphenol bioavailability after intake of white and red wine pomace products by Wistar rats. *Food & Function, 11*(2), 1661–1671. https://doi.org/10.1039/c9fo01743g

Gerardi, G., Cavia-Saiz, M., Rivero-Pérez, M. D., González-SanJosé, M. L., & Muñiz, P. (2020b). Wine pomace product modulates oxidative stress and microbiota in obesity high-fat diet-fed rats. *Journal of Functional Foods, 68*, Article 103903. https://doi.org/10.1016/j.jff.2020.103903

Ghorbani Gorji, S., Calingacion, M., Smyth, H. E., & Fitzgerald, M. (2019). Effect of natural antioxidants on lipid oxidation in mayonnaise compared with BHA, the industry standard. *Metabolomics: Official Journal of the Metabolomic Society, 15*(8), 106. https://doi.org/10.1007/s11306-019-1568-4

Gil-Sánchez, I., Cueva, C., Sanz-Buenhombre, M., Guadarrama, A., Moreno-Arribas, M. V., & Bartolomé, B. (2018). Dynamic gastrointestinal digestion of grape pomace extracts: Bioaccessible phenolic metabolites and impact on human gut microbiota. *Journal of Food Composition and Analysis, 68*, 41–52. https://doi.org/10.1016/j.jfca.2017.05.005

Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: A review. *Antonie Van Leeuwenhoek, 113*(12), 2019–2040. https://doi.org/10.1007/s10482-020-01474-7

González-Sanjosé, M. L., García-Lomillo, J., Del Pino-García, R., Rivero, M. D., & Muñiz-Rodríguez, P. (2015). Sazonador de origen vegetal con propiedades conservantes, sustitutivo de la sal, y procedimiento de obtención del mismo. Patent ES2524870 B2 Spain.

Gottardi, D., Bukvicki, D., Prasad, S., & Tyagi, A. K. (2016). Beneficial Effects of Spices in Food Preservation and Safety. *Frontiers in Microbiology, 7*, 1394. https://doi.org/10.3389/fmicb.2016.01394

Goutzourelas, N., Stagos, D., Demertzis, N., Mavridou, P., Karterolioti, H., Georgadakis, S., Kerasioti, E., Aligiannis, N., Skaltsounis, L., Statiri, A., Tsioutsiouliti, A., Tsatsakis, A. M., Hayes, A. W., & Kouretas, D. (2014). Effects of polyphenolic grape extract on the oxidative status of muscle and endothelial cells. *Human & Experimental Toxicology, 33*(11), 1099–1112. https://doi.org/10.1177/0960327114533575

Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes, 8*(2), 172–184. https://doi.org/10.1080/19490976.2017.1290756

Jamie, P., & Saltveit, M. E. (2002). Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharvest Biology and Technology, 26*(1), 113–116. https://doi.org/10.1016/S0925-5214(02)00006-6

Kabeerdoss, J., Sankaran, V., Pugazhendhi, S., & Ramakrishna, B. S. (2013). Clostridium leptum group bacteria abundance and diversity in the fecal microbiota of patients with inflammatory bowel disease: A case–control study in India. *BMC Gastroenterology, 13*, 20. https://doi.org/10.1186/1471-230X-13-20

Kadivec, M., Može Bornšek, Š., Polak, T., Demšar, L., Hribar, J., & Požrl, T. (2013). Phenolic Content of Strawberry Spreads during Processing and Storage. *Journal of Agricultural and Food Chemistry, 61*(38), 9220–9229. https://doi.org/10.1021/jf4035767

Kurek, M. A., & Krzemińska, A. (2020). Effect of modified atmosphere packaging on quality of bread with amaranth flour addition. *Food Science and Technology International, 26*(1), 44–52. https://doi.org/10.1177/1082013219864197

Lee, R., Margaritis, M., Channon, K., & Antoniades, C. (2012). Evaluating Oxidative Stress in Human Cardiovascular Disease: Methodological Aspects and Considerations. *Current Medicinal Chemistry, 19*(16), 2504–2520. https://doi.org/10.2174/092986712800493057

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A.-G., Ahn, B.-W., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology, 186*, 464–478. https://doi.org/10.1016/0076-6879(90)86141-H

Łopacka, J., Półtorak, A., & Wierzbicka, A. (2016). Effect of MAP, vacuum skin-pack and combined packaging methods on physicochemical properties of beef steaks stored up to 12days. *Meat Science, 119*, 147–153. https://doi.org/10.1016/j.meatsci.2016.04.034

Marrocco, I., Altieri, F., & Peluso, I. (2017). Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxidative Medicine and Cellular Longevity, 2017*, 6501046. https://doi.org/10.1155/2017/6501046

Masella, R., Di Benedetto, R., Varì, R., Filesi, C., & Giovannini, C. (2005). Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione-related enzymes. *The Journal of Nutritional Biochemistry, 16*(10), 577–586. https://doi.org/10.1016/j.jnutbio.2005.05.013

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Nemetz, N. J., Schieber, A., & Weber, F. (2021). Application of Crude Pomace Powder of Chokeberry, Bilberry, and Elderberry as a Coloring Foodstuff. *Molecules, 26*(9), 2689. https://doi.org/10.3390/molecules26092689

O'Keefe, S. J. D. (2016). Diet, microorganisms and their metabolites, and colon cancer. *Nature reviews. Gastroenterology & hepatology, 13*(12), 691–706. https://doi.org/10.1038/nrgastro.2016.165

Ortega-Heras, M., Villarroel, E., Mateos, S., García-Lomillo, J., Rovira, J., & González-SanJosé, M. L. (2020). Application of a seasoning obtained from red grape pomace as a salt replacer for the elaboration of marinated chicken breasts: Study of their physical-chemical and sensory properties and microbiological stability. *CyTA - Journal of Food, 18*, 122–131. https://doi.org/10.1080/19476337.2019.1709558

Pathak, S., Kesavan, P., Banerjee, A., Banerjee, A., Celep, G. S., Bissi, L., & Marotta, F. (2018). Chapter 25—Metabolism of Dietary Polyphenols by Human Gut Microbiota and Their Health Benefits. En. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.),

Polyphenols: Mechanisms of Action in Human Health and Disease (Second Edition, pp. 347–359). Academic Press. https://doi.org/10.1016/B978-0-12-813006-3.00025-8.

Pérez-Magariño, S., Ortega-Heras, M., & Cano-Mozo, E. (2008). Optimization of a Solid-Phase Extraction Method Using Copolymer Sorbents for Isolation of Phenolic Compounds in Red Wines and Quantification by HPLC. *Journal of Agricultural and Food Chemistry, 56*(24), 11560–11570. https://doi.org/10.1021/jf802100j

Pešić, M. B., Milinčić, D. D., Kostić, A.Ž., Stanisavljević, N. S., Vukotić, G. N., Kojić, M. O., Gašić, U. M., Barać, M. B., Stanojević, S. P., Popović, D. A., Banjac, N. R., & Tešić, Ž. L. (2019). In vitro digestion of meat- and cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected? *Food Chemistry, 284*, 28–44. https://doi.org/10.1016/j.foodchem.2019.01.107

Plamada, D., & Vodnar, D. C. (2022). Polyphenols—Gut Microbiota Interrelationship: A Transition to a New Generation of Prebiotics. *Nutrients, 14*(1), 137. https://doi.org/10.3390/nu14010137

Rather, S. A., Masoodi, F. A., Akhter, R., Rather, J. A., & Shiekh, K. A. (2016). Advances in use of Natural Antioxidants as Food additives for improving the Oxidative Stability of Meat Products. *Madridge Journal of Food Technology, 1*(1), 10–17. https://doi.org/10.18689/mjft-1000102

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Frontiers in Microbiology, 7*, 185. https://doi.org/10.3389/fmicb.2016.00185

Sánchez Alonso, I., Jiménez Escrig, A., Saura Calixto, & Borderías, A. J. (2008). Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT - Food Science and Technology, 41*(1), 42–50. https://doi.org/10.1016/j.lwt.2007.02.002

Saura-Calixto, F., Pérez-Jiménez, J., Touriño, S., Serrano, J., Fuguet, E., Torres, J. L., & Goñi, I. (2010). Proanthocyanidin metabolites associated with dietary fibre from in vitro colonic fermentation and proanthocyanidin metabolites in human plasma. *Molecular Nutrition & Food Research, 54*(7), 939–946. https://doi.org/10.1002/mnfr.200900276

Serpen, A., Gökmen, V., & Fogliano, V. (2012). Total antioxidant capacities of raw and cooked meats. *Meat Science, 90*(1), 60–65. https://doi.org/10.1016/j.meatsci.2011.05.027

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Vermeiren, J., Van den Abbeele, P., Laukens, D., Vigsnæs, L. K., De Vos, M., Boon, N., & Van de Wiele, T. (2012). Decreased colonization of fecal Clostridium coccoides/Eubacterium rectale species from ulcerative colitis patients in an in vitro dynamic gut model with mucin environment. *FEMS Microbiology Ecology, 79*(3), 685–696. https://doi.org/10.1111/j.1574-6941.2011.01252.x

Vetrani, C., Maukonen, J., Bozzetto, L., Della Pepa, G., Vitale, M., Costabile, G., Riccardi, G., Rivellese, A. A., Saarela, M., & Annuzzi, G. (2020). Diets naturally rich in polyphenols and/or long-chain n-3 polyunsaturated fatty acids differently affect microbiota composition in high-cardiometabolic-risk individuals. *Acta Diabetologica, 57*(7), 853–860. https://doi.org/10.1007/s00592-020-01494-9