Antioxidant capacity in the bioavailable fraction as an indicator for selecting wholesomeness strawberry varieties in breeding programs

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Corresponding Author:	M Teresa Ariza Fernandez IFAPA Malaga, Málaga Spain
First Author:	Elsa Martinez
Order of Authors:	Elsa Martinez
	Lucia Cervantes
	Carmen Soria
	Tamara Forbes-Hernández
	Patricia Reboredo-Rodríguez
	Maurizio Battino
	M Teresa Ariza Fernandez
Abstract:	Strawberry phenolic compounds are responsible of many health benefits and are one of the major contributors to antioxidant capacity. In this study, different phenolic compounds as well as antioxidant capacity (TEAC) were determined spectrophotometrically and by HPLC on strawberry fruits of different genotypes. In addition, fruits of five selected genotypes were subjected to simulated in vitro digestion to determine their bioaccessibility and bioavailability. Digested extracts from the two most contrasting genotypes were further used to evaluate in vitro bioactivity on HepG2 cells. The main results showed that cultivar's raw fruits differed in their amount of phenolic compounds and antioxidant capacity. Besides, the pre-digested amount of phenolic compounds and antioxidant capacity did not match the values obtained after digestion in the genotypes evaluated. This indicates that the health promoting properties of strawberries are modified after digestion, being necessary to assess the effect of digested extracts on cellular bioactivity. In this sense, the highest bioactivity (i.e. lower reactive oxygen species and cell death) was achieved in the cultivar with the highest bioavailable antioxidant capacity, suggesting the use of this parameter as a reliable indicator for selecting strawberry fruits with health promoting properties in breeding programs.
Suggested Reviewers:	Bruno Mezzetti b.mezzetti@univpm.it Dr Mezzetti is an expert in strawberry breeding for functional compounds such as antioxidants.
	Luca Mazzoni l.mazzoni@live.it
	Gordon McDougall gordon.mcdougall@hutton.ac.uk
Opposed Reviewers:	
Response to Reviewers:	

Highlights

- Strawberry polyphenols are one of the major antioxidant capacity dietary contributors
- Antioxidant capacity as an integrative parameter of antioxidant potential
- Digestion process affects the antioxidant bioavailability and their bioactivity
- *"AC-bioavailable"* as a new target for wholesome fruit in breeding program

Graphical Abstract v1

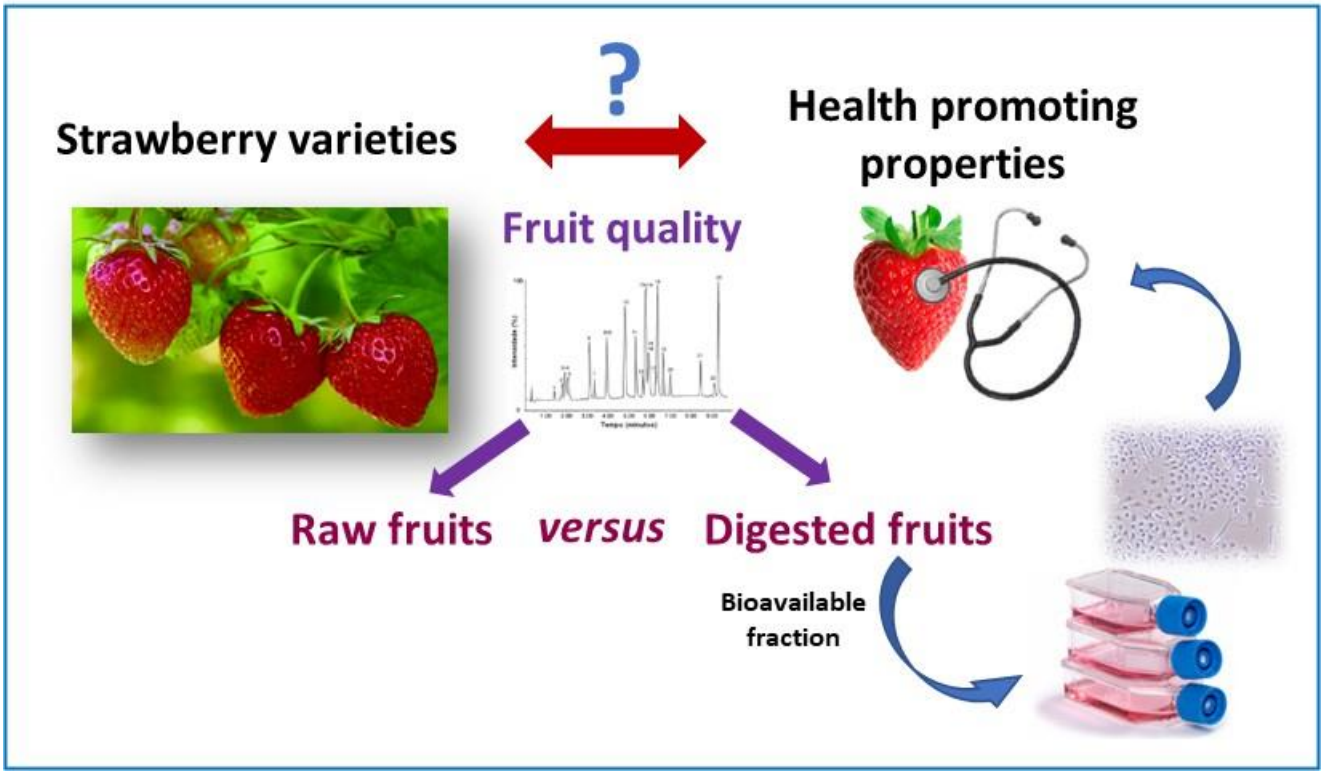

Figure 2

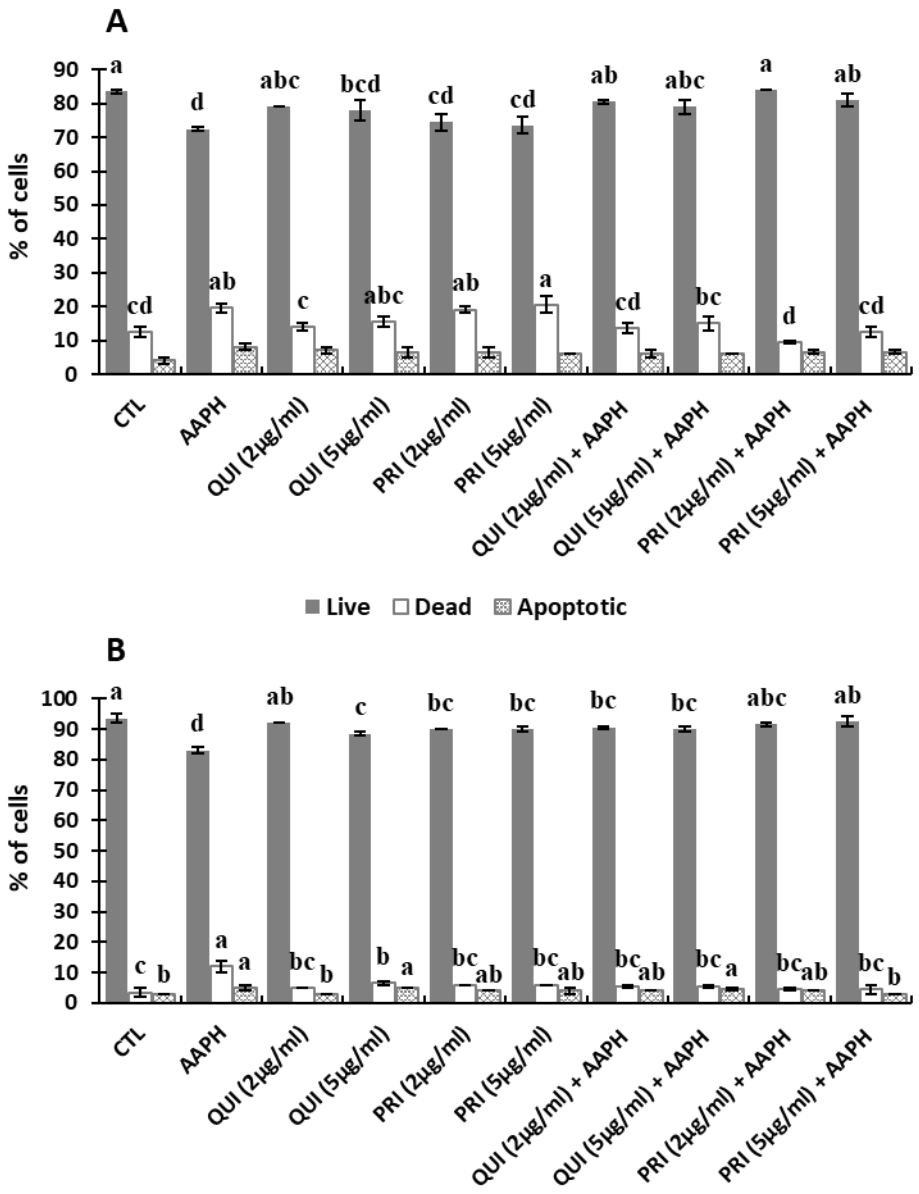

Figure 1

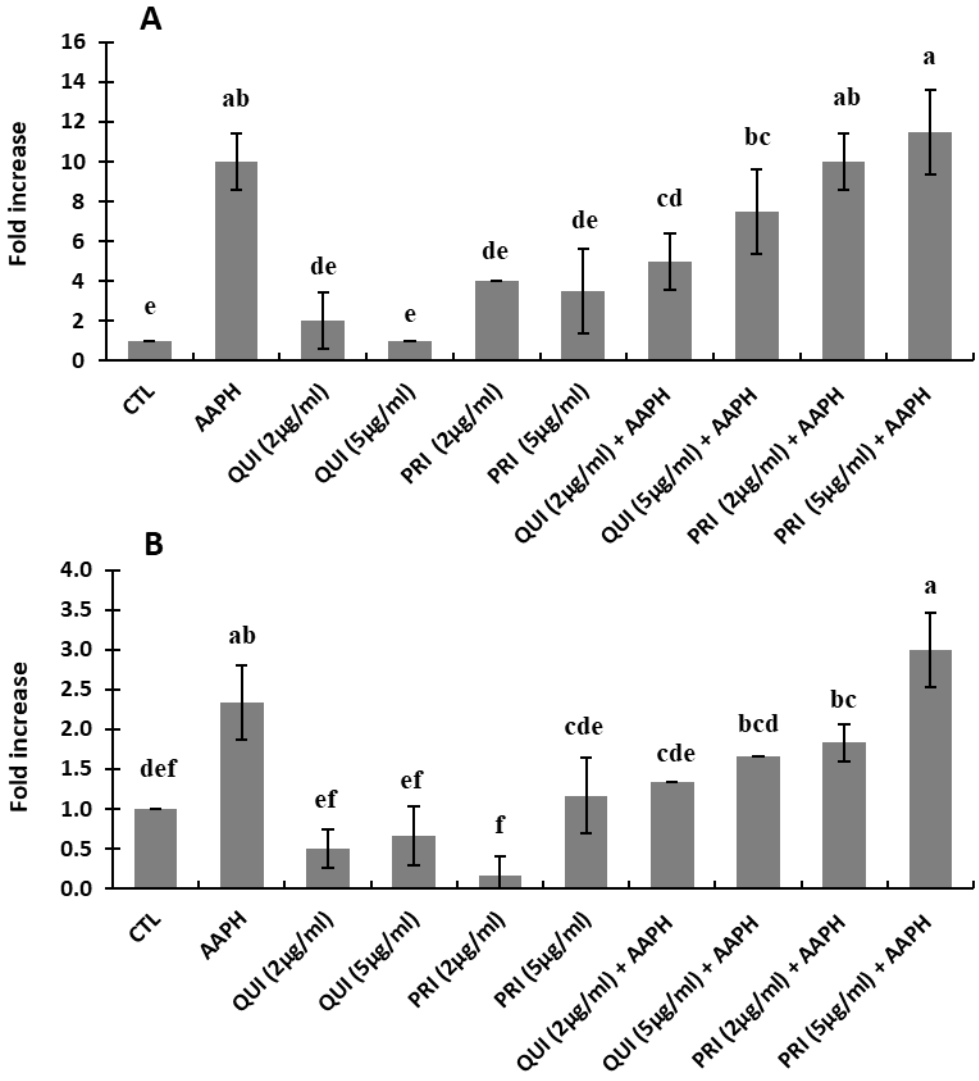

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and antioxidant capacity (AC) of raw fruits, as well as their ratios, from twelve commercial strawberry varieties. ~~Different letters indicate significant differences for each antioxidant group among varieties ($p<0.05$). Data are expressed as mean ± SE.~~

	TPC*	TFC*	TAC*	AC #	TFC/TPC ratio	TAC/TPC ratio
Candonga®	187.14 ± 8.34 de	83.06 ± 10.63 de	19.82 ± 0.87 bcd	42.13 ± 3.23 cde	0.44	0.11
'Charlene'	184.72 ± 5.39 de	90.89 ± 3.16 cde	13.96 ± 1.53 e	47.60 ± 3.66 abc	0.49	0.08
'Flaminia'	226.10 ± 10.46 a	133.46 ± 5.90 a	16.78 ± 1.57 de	48.49 ± 0.92 abc	0.59	0.07
'Flavia'	211.59 ± 12.23 abcde	86.36 ± 0.49 de	22.55 ± 1.07 abc	46.09 ± 3.80 bcd	0.41	0.11
'Fortuna'	181.49 ± 7.22 e	79.29 ± 1.14 e	24.92 ± 1.68 a	35.24 ± 0.39 ef	0.44	0.14
'Marisol'	188.48 ± 6.26 cde	78.81 ± 1.89 e	20.96 ± 0.51 abcd	50.85 ± 4.42 ab	0.42	0.11
'Marquis'	224.22 ± 7.38 ab	106.85 ± 4.50 bc	13.87 ± 1.23 e	40.80 ± 3.53 cde	0.48	0.06
'Melissa'	198.69 ± 9.77 bcde	74.38 ± 13.9 e	19.05 ± 1.28 cd	38.87 ± 0.94 def	0.37	0.10
'Primoris'	214.55 ± 9.41 abc	123.55 ± 4.80 ab	23.69 ± 1.10 ab	53.97 ± 1.11 a	0.58	0.11
'Rabida'	215.89 ± 13.32 ab	84.85 ± 3.15 de	13.78 ± 1.78 e	32.27 ± 2.46 f	0.39	0.06
'Rociera'	205.41 ± 8.08 abcde	98.82 ± 2.27 cd	19.69 ± 1.80 bcd	41.80 ± 1.72 cde	0.48	0.10
'Sabrina'	197.35 ± 10.74 bcde	74.94 ± 6.30 e	19.51 ± 2.21 bcd	41.14 ± 1.19 cde	0.38	0.10
Mean	202.97	92.94	19.05	43.27	0.46	0.10

Coefficient of Variation (CV)~~CV~~	7.68	20.70	19.94	14.70	15.80	24.96

Different letters indicate significant differences for each antioxidant group among varieties ($p<0.05$). Data are expressed as mean ± SE.

*mg standard/100 g Fresh Weight; #µmol Trolox Equivalent / g Fresh Weight

Table 2. Relative amount of antioxidant parameters (total phenolic content: TPC; total flavonoid content: TFC; total anthocyanin content: TAC; and antioxidant capacity: AC) in raw fruits of strawberry varieties, represented and categorized by quartiles ~~(Q1: first quartile; Q2: second quartile; Q3: third quartile; Q4: fourth quartile)~~.

	TPC	TFC	TAC	AC
'Primoris'	Q2	Q1	Q1	Q1
'Fortuna'	Q4	Q3	Q1	Q4
'Flaminia'	Q1	Q1	Q3	Q1
'Marquis'	Q1	Q1	Q4	Q3
'Charlene'	Q4	Q2	Q2	Q2

(Q1: first quartile; Q2: second quartile; Q3: third quartile; Q4: fourth quartile).

Table 3. Antioxidant composition in raw fruit and in bioavailable fractions (gastric and intestinal) of five strawberry varieties~~. Total potential bioavailability has been calculated from the absorbed fraction gastric + intestinal. For each variety and antioxidant group, the recovery of each compound in the bioavailable fractions respect to the raw fruits is shown in brackets. Different letters designate significant differences among varieties for each antioxidant group ($p < 0.05$). All analyses were performed in triplicate.~~

		'Primoris'	'Fortuna'	'Flaminia'	'Marquis'	'Charlene'
TPC*	**Raw fruit (RF)**	214.55 ± 9.41 a	181.49 ± 7.22 b	226.10 ± 10.46 a	224.22 ± 7.38 a	184.72 ± 5.39 b
	Gastric Absorbed (GA)	85.00 ± 1.30 a	71.37 ± 2.03 b	49.32 ± 3.14 c	43.36 ± 0.38 cd	37.71 ± 0.96 d
	Intestinal Absorbed (IA)	57.55 ± 1.75 b	67.28 ± 2.35 a	20.36 ± 0.93 d	23.25 ± 0.56 cd	26.54 ± 2.04 c
	Total bioavailability (GA+IA)	142.55 ± 13.02 a	138.65 ± 2.31 a	69.68 ± 3.76 b	66.61 ± 0.44 b	64.25 ± 1.27 b
	*(GA+IA)*100/RF*	(66.44%)	(76.40%)	(30.82%)	(29.71%)	(34.78%)
TFC*	**Raw fruit**	123.55 ± 4.80 a	79.29 ± 1.14 c	133.46 ± 5.90 a	106.85 ± 4.50 b	90.89 ± 3.16 c
	Gastric Absorbed	5.84 ± 0.09 a	3.10 ± 0.07 c	6.40 ± 0.28 a	6.52 ± 0.09 a	4.55 ± 0.35 b
	Intestinal Absorbed	5.66 ± 0.13 b	3.35 ± 0.10 c	8.29 ± 0.27 a	7.85 ± 0.26 a	4.87 ± 0.85 bc
	Total bioavailability	11.50 ± 0.22 b	6.44 ± 0.06 d	14.69 ± 0.75 a	14.37 ± 0.30 ab	9.43 ± 0.56 c
		(9.31%)	(8.13%)	(11.01%)	(13.45%)	(10.37%)
TAC*	**Raw fruit**	23.69 ± 1.10 a	24.92 ± 1.68 a	16.78 ± 1.57 b	13.87 ± 1.23 b	13.96 ± 1.53 b
	Gastric Absorbed	1.86 ± 0.05 c	4.02 ± 0.20 a	1.43 ± 0.02 c	1.80 ± 0.04 c	3.05 ± 0.19 b
	Intestinal Absorbed	1.04 ± 0.11 bc	1.20 ± 0.06 b	0.00 ± 0.00 c	2.14 ± 0.11 b	3.63 ± 0.37 a
	Total bioavailability	2.90 ± 0.12 bc	5.22 ± 0.42 a	1.43 ± 0.03 c	3.94 ± 0.08 ab	6.69 ± 0.76 a
		(12.24%)	(20.94%)	(8.54%)	(28.41%)	(47.90%)
AC #	**Raw fruit**	53.97 ± 1.11 a	35.24 ± 0.39 c	48.49 ± 0.92 a	40.80 ± 3.53 bc	47.60 ± 3.66 ab
	Gastric Absorbed	1.04 ± 0.02 c	0.36 ± 0.03 d	1.64 ± 0.07 b	2.47 ± 0.03 a	1.10 ± 0.05 c
	Intestinal Absorbed	2.90 ± 0.14 c	4.04 ± 0.19 b	5.54 ± 0.10 a	5.18 ± 0.20 a	3.10 ± 0.21 bc
	Total bioavailability	3.94 ± 0.13 b	4.40 ± 0.24 b	7.18 ± 0.09 a	7.66 ± 0.18 a	4.20 ± 0.19 b
		(7.30%)	(12.48%)	(14.80%)	(18.77%)	(8.82%)

*mg standard/100 g Fresh Weight; #µmol Trolox Equivalent / g Fresh Weight

Different letters designate significant differences among varieties for each antioxidant group ($p < 0.05$). All analyses were performed in triplicate.

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Table 4. Composition of phenolic acids and hydrolysable tannins, flavanols, flavonols and anthocyanins of two strawberry varieties ('Marquis' and 'Primoris') in raw fruits and digested fractions. ~~The~~ "T~~t~~otal ~~potential~~ bioavailability" has been calculated from the absorbed fraction gastric + intestinal. For each variety and individual compound, the recovery of each compound in the bioavailable fractions respect to the raw fruits is shown in brackets. ~~Different letters designate significant differences on the antioxidant composition among varieties for the same fraction (p< 0.05). All analyses were performed in triplicate. Data (mean ± SE) are expressed in mg/100 g fresh weight. LOQ: limit of quantification.~~

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		Raw Fruit		*Gastric bioavailability*		*Intestinal bioavailability*		*Total bioavailability*	
		'Marquis'	**'Primoris'**	**'Marquis'**	**'Primoris'**	**'Marquis'**	**'Primoris'**	**'Marquis'**	**'Primoris'**
Phenolic acids and hydrolysable tannins	Gallic acid	1.68 ± 0.02 a	0.95 ± 0.16 b	0.77 ± 0.07 a	0.86 ± 0.03 a	1.21 ± 0.01 b	2.05 ± 0.08 a	1.98 ± 0.07 (117.86%) b	2.90 ± 0.11 (306.32%) a
	Chlorogenic acid	4.19 ± 0.24 b	39.59 ± 1.77 a	1.07 ± 0.11 b	7.43 ± 0.36 a	0.35 ± 0.03 b	0.65 ± 0.16 a	1.42 ± 0.14 (33.89%) b	8.08 ± 0.49 (20.41%) a
	Caffeic acid	0.74 ± 0.02 b	1.03 ± 0.07 a	0.28 ± 0.00 b	0.35 ± 0.01 a	<LOQ b	0.33 ± 0.01 a	0.28 ± 0.00 (37.84%) b	0.68 ± 0.02 (66.02%) a
	p-Coumaric acid	0.50 ± 0.01 a	0.51 ± 0.01 a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Ellagic acid	20.62 ± 3.69 b	117.38 ± 4.30 a	1.76 ± 0.43 b	9.52 ± 0.27 a	<LOQ	<LOQ	1.76 ± 0.43 (8.53%) b	9.52 ± 0.27 (8.11%) a
	trans-Cinnamic acid	0.28 ± 0.00 b	0.42 ± 0.03 a	<LOQ b	0.13 ± 0.00 a	0.12 ± 0.00 b	0.17 ± 0.01 a	0.12 ± 0.00 (42.86%) b	0.30 ± 0.01 (71.42%) a
Flavanols	Procyanidin B1	16.48 ± 0.16 a	13.80 ± 0.79 b	0.07 ± 0.05 a	<LOQ a	<LOQ	<LOQ	0.07 ± 0.05 (0.42%) ns	<LOQ ns
	Catechin	16.18 ± 0.31 a	16.84 ± 0.27 a	1.02 ± 0.09 a	0.92 ± 0.10 a	2.19 ± 0.19 a	1.87 ± 0.27 a	3.21 ± 0.17 (19.84%) ns	2.79 ± 0.37 (16.56%) ns
	Procyanidin B2	7.12 ± 0.08 a	5.71 ± 0.16 b	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Epicatechin	<LOQ	<LOQ	0.94 ± 0.03 b	1.07 ± 0.03 a	<LOQ	<LOQ	0.94 ± 0.03 (100%) b	1.07 ± 0.03 (100%) a
Flavonols	Rutin	1.37 ± 0.07 a	1.57 ± 0.34 a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Myricetin	0.33 ± 0.09 a	0.44 ± 0.07 a	<LOQ	<LOQ	<LOQ b	0.57 ± 0.00 a	<LOQ b	0.57 ± 0.00 (129.55%) a
	Quercetin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ
	Kaempferol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ
Anthocyanins	Cya-3-glu	0.80 ± 0.03 b	1.75 ± 0.24 a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Pel-3-glu	14.65 ± 0.42 b	26.84 ± 1.58 a	1.28 ± 0.08 b	2.08 ± 0.11 a	0.33 ± 0.08 a	0.30 ± 0.04 a	1.61 ± 0.01 (10.99%) b	2.38 ± 0.08 (8.87%) a
	Pel-3-rut	1.18 ± 0.07 a	1.31 ± 0.03 a	0.17 ± 0.01 b	0.25 ± 0.01 a	0.01 ± 0.00 a	0.07 ± 0.00 a	0.18 ± 0.00 (15.25%) b	0.32 ± 0.01 (24.43%) a
	Peo-3-glu	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ

Different letters designate significant differences on the antioxidant composition among varieties for the same fraction (p< 0.05). All analyses were performed in triplicate. Data (mean ± SE) are expressed in mg/100 g fresh weight. LOQ: limit of quantification.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT authorship contribution statement

M.T.A., E.M.-F. and L.C. conceptualization, data collection, data evaluation, main writing, structuring of original draft, review, editing and supervision; C.S. conceptualization, data collection, data evaluation, review, editing and supervision; T.Y.F.-H., P.R.-R. and M.B. review, editing and supervision. All authors have read and agreed to the published version of the manuscript.

Antioxidant capacity in the bioavailable fraction as an indicator for selecting wholesomeness strawberry varieties in breeding programs.

Elsa Martínez-Ferri[1], Lucia Cervantes[1], Carmen Soria[1], Tamara Forbes-Hernández[2], Patricia Reboredo-Rodríguez[3], Maurizio Battino[4], María Teresa Ariza[1*]

[1] Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA), Consejería de Agricultura, Pesca y Desarrollo Rural, Junta de Andalucía, IFAPA de Churriana, Cortijo de la Cruz s/n, Churriana, 29140 Málaga, Spain; elsa.martinez@juntadeandalucia.es (E.M.-F.); lucia.cervantes@juntadeandalucia.es (L.C.); maria.soria@juntadeandalucia.es (C.S.); mariat.ariza@juntadeandalucia.es (M.T.A.).

[2] Department of Physiology; Institute of Nutrition and Food Technology José Mataix Verdú, Biomedical Research Center. University of Granada. Avda. del Conocimiento s.n. Armilla, 18100, Granada, Spain. tamara.forbe@gmail.com (T.Y.F.-H.);

[3] Food and Health Omics, Department of Analytical and Food Chemistry, Faculty of Science, University of Vigo, 32004-Ourense, Spain; preboredo@uvigo.es (P.R.-R.);

[4] Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche (DISCO)-Sez. Biochimica, Facoltà di Medicina, Università Politecnica delle Marche, 60131 Ancona, Italy; m.a.battino@univpm.it (M.B.).

* Correspondence: mariat.ariza@juntadeandalucia.es (M.T.A.). Tel.: +34-671532797

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Abstract

Strawberry phenolic compounds are responsible of many health benefits and are one of the major contributors to antioxidant capacity. In this study, different phenolic compounds as well as antioxidant capacity (TEAC) were determined spectrophotometrically and by HPLC on strawberry fruits ~~from~~ of different genotypes. In addition, ~~these~~ fruits of five selected genotypes were subjected to simulated *in vitro* digestion to determine their bioaccessibility and bioavailability. D~~d~~igested extracts from the two most contrasting genotypes were ~~used~~ further~~more~~ used to evaluate i*n vitro* bioactivity on HepG2 cells. The m~~M~~ain results showed that cultivar's raw fruits ~~differe~~differed in their ~~nt~~ amount of phenolic compounds ~~antioxidants~~ and antioxidant capacity ~~in raw fruits among cultivars~~. Besides, ~~that~~ ~~there was no correspondence~~ the pre-digested amount of ~~in either~~ phenolic compounds and ~~or~~ antioxidant capacity ~~between~~ did not match the values obtained ~~the raw fruits and these determinations before and~~ after digestion ~~digestion~~in the genotypes evaluated. This indicates that the health promoting properties of strawberries are modified after digestion, being necessary to assess the effect of digested extracts on cellular bioactivity. In this sense, the highest bioactivity (i.e. lower reactive oxygen species and cell death) was achieved in ~~Finally,~~ the ~~the~~ cultivar ~~The extracts~~ with the highest bioavailable antioxidant capacity, ~~showed the highest bioactivity values (i.e. lower reactive oxygen species and cell death ROS and cell death) indicating~~suggesting the use of ~~that~~ this parameter ~~e bioavailable antioxidant capacity could be considered~~ as a reliable ~~good~~ indicator for selecting strawberry ~~of~~ fruits with health promoting properties ~~which may be useful for~~in ~~strawberry~~ breeding programs.

Keywords: phenolic compounds, antioxidant capacity, *in vitro* digestion, healthy promoting properties, bioactivity, plant genetic resources.

1. Introduction

In recent decades there has been a significant change in the general attitude of consumers towards the relation between food and health. Concern about the health benefits of foods, in terms of positive effects and disease prevention, has increased. As a result, functional foods with nutraceutical compounds are progressively reaching the market, satisfying the growing consumer demand (Siró et al., 2008).

In this context, strawberries are among the most consumed fruits worldwide, not only for of their tasty flavour and nutritional value (Nile and Park, 2014), but also for of their functional quality. This quality is based on their natural content of phytochemicals (Nile and Park, 2014; Giampieri et al., 2012), mainly polyphenols that act as potent antioxidants with proven biological effects (Santhakumar, et al., 2018). The health benefits associated with the antioxidative properties of these phenolic compounds are based on the fact that they counteract the oxidative stress produced as a consequence of cellular metabolism, maintaining cellular redox homeostasis (within adequate levels). The imbalance between antioxidants and oxidative stress has been proposed as one of the main risk factors leading to various chronic diseases, such as cardiovascular diseases or cancer, among others (Willcox, et al., 2004).

Phenolic compounds are also a major contributor to the total antioxidant capacity (AC) of strawberry fruits (Ariza et al., 2016; Stoner and Seeram, 2011; Wang et al., 2018), which is defined as the ability of antioxidant compounds to protect a biological system against the potentially harmful effects of reactive oxygen and nitrogen species (ROS and RNS, respectively; Karadag et

al., 2009). In this sense, the AC is considered as an integrative parameter of their antioxidative properties and, therefore, AC could be suggested as a reliable index for the estimation of healthy effect on cells (bioactivity) as shown by Li et al. (2017) and Cervantes et al. (2020).

Strawberry varieties, resulting from breeding programs developed worldwide to obtain genotypes adapted to the agroclimatic conditions of specific areas, differ both in the amount and composition of antioxidants (Tulipani et al., 2008). However, they might have a similar impact on consumer health due to the different contribution of specific compounds to their AC (Wang, 2011). Furthermore, to properly assess the health-promoting effects of food matrices (i.e. fruits of strawberry varieties), it is also necessary to take into account the release and transformations of antioxidants along the gastrointestinal tract during intake and digestion (Granese et al., 2014), as the digestion process determines the release (bioaccessibility) and absorption (bioavailability; Ariza et al., 2016; 2018a) of phenolic compounds in the food matrices (Kosińska-Cagnazzo et al., 2015; Ariza et al., 2016; 2018a) and, therefore, their biological effects (bioactivity; Ariza et al., 2018b). Thereby, the amount and type of polyphenols in raw fruits do not necessarily coincide with those in digested extracts and, therefore, neither do their potential health effects.

In this regard, *in vitro* digestion models are powerful tools to simulate the physiological conditions that occur in human digestion (Cervantes et al., 2020), despite being more time consuming than the extraction of antioxidants from raw fruits (Bohn et al., 2017), and are currently used as a first approach to assess the extent to which fruit antioxidants are potentially released, transformed and/or metabolised from the food matrix and potentially absorbed (D'Archivio et al., 2010) after intake. Nevertheless, the absorption of these molecules does not in itself guarantee a beneficial action and, therefore, bioactivity assays are needed for determining their potential effect on human health.

Previous studies have revealed differences among strawberry varieties in the amount and type of antioxidants and AC determined in raw fruits (Nowicka et al., 2019; Ariza et al., 2015), and also in digested extracts (Cervantes et al., 2020; Ariza et al., 2018a; Thomas-Valdés et al., 2018; Ariza et

al., 2016). However, to date it is unknown to what extent higher AC in raw fruits (AC_{RF}) translates into greater AC in the bioavailable fraction (AC-bioavailable or AC_{BA}) in different strawberry varieties, and whether larger AC_{BA} translates into greater bioactivity (*i.e.*, differences in their bioprotective effects). If these relationships exist, it will allow the use of AC_{RF} and/or AC_{BA} as an accurate indicator of the health properties of fruits, suitable for selecting varieties in breeding programs aimed at obtaining wholesome fruits.

TWith this purpose, the objectivemail goal of the present work is to shed light on the relationship between the antioxidant and health promoting properties of strawberries. With this purpose, the objectives of the present work were: *i)* to determine evaluate the releasethe pattern of release (and absorption, if applicable) of antioxidants and the AC in both, raw fruits and in *in vitro* digested fractions, of different strawberry varieties,; and *ii) to define the major antioxidant group contributing to AC and to evaluate the relationship between AC_{BA} and AC_{RF} in different varieties; and i**ii)* to analyse the parameters that could be main responsible for a high bioactivity of high AC_{BA} of strawberry extracts in order to obtain a reliable index useful for to selecting wholesomeness varieties in breeding programs.

was to determine whether the digestion process affects the preservation of antioxidants and AC to establish which AC (raw or bioavailable fruit) could be analyzed to determine their bioactivity in order to obtain a reliable index useful for selecting healthiness varieties in breeding programs.

2. Materials and Methods

2.1. Plant material and experimental design

Twelve short-day strawberry (*Fragaria* x *ananassa* Duch.) cultivars, (Candonga®, 'Charlene', 'Flaminia', 'Flavia', 'Fortuna', 'Marisol', 'Marquis', 'Melissa', 'Primoris', 'Rabida', 'Rociera' and 'Sabrina') were grown under similar agronomical field conditions in a complete randomized block design with three replicate plots of 50 plants, spaced at 30 x 25 cm.

In order to determine polyphenol content and antioxidant capacity, fifty fresh and fully ripened strawberry fruits from each cultivar on each replicate plot were harvested in mid-April. Sampled fruits were immediately homogenized in a Multiquick MR 6500 blender from Braun (Kronberg, Germany), and puree samples were stored at -20 °C until analyses were conducted.

2.2. Preparation of antioxidant extracts from strawberry fruits

2.2.1. Raw fruits: hydromethanolic extraction

The antioxidant content and capacity in raw fruits of each cultivar were determined on hydromethanolic extracts obtained from ~1 g puree added to 10 mL of the extraction solution consisting of methanol/MilliQ water/HCl (37%) (80:19.9:0.1 v/v) and homogenized for 2 h at room temperature (Jiang and Joyce, 2003; Ariza et al., 2016; Forbes-Hernández et al., 2017). Extracts were filtered using hydrophilic cotton and centrifuged at 10000 rpm for 10 min at 4 ºC, and the supernatant was stored at -20 °C until spectrophotometric and HPLC analyses.

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2.2.2. In vitro *digestion: digested fractions*

Potential release (bioaccessibility) and absorption (bioavailability) of antioxidant compounds on selected cultivars were evaluated by an *in vitro* digestion process following the method proposed by Gil-Izquierdo et al. (2002), with some modifications (Ariza et al., 2018a). Thus, 10 g of strawberry puree were diluted with distilled water (100 mL) and stirred with pepsin (0.734 mg/g)

and HCl (reaching pH ~1.7-2) at 37 ºC for 2 h, simulating gastric conditions (gastric fraction). After gastric digestion, the pH of this mixture was raised to 7.8 with $NaHCO_3$ (7 M), and pancreatin and bile salts (9 mg/g and 56.25 mg/g, respectively) were added. The mixture was incubated at 37 °C for 2 h, simulating intestinal digestion (intestinal fraction).

Simultaneously on each sequential phase, a dialysis membrane (molecular weight cut-off 12000–14000 Da) was immersed into each fraction, containing ultrapure water, for gastric fraction; or the amount of $NaHCO_3$ necessary to titrate the mixture of gastric digestion fraction to pH 7.8, for the intestinal one.

After each phase, an aliquot of the solution outside and inside the membrane were collected, separately, and stored until analysis. The solution outside the dialysis membrane represents the potentially released or bioaccessible fraction, while the solution contained inside the membrane represents the potentially absorbed or bioavailable fraction, according to Carbonell-Capella et al. (2014) and Ariza et al. (2018a).

All collected aliquots were filtered by hydrophilic cotton and centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was stored at -20 °C until spectrophotometric and HPLC analyses. In order to avoid the interference of the *in vitro* digestion reactants on the spectrophotometric determinations, data were corrected using a blank digestion as described in Cervantes et al., (2019).

2.3. Determination of phenolic compounds

2.3.1. UV–Vis analysis

Phenolic compounds and AC of the hydromethanolic extracts (raw fruits) and all digested fractions from the study strawberry genotypes were determined in triplicate by UV–Vis spectrophotometry, using a Shimadzu PharmaSpec UV-1700 instrument (Kyoto, Japan). Thus, the total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), as

well as the antioxidant capacity (AC) evaluated by the Trolox Equivalent Antioxidant Capacity (TEAC) assay, were determined according to Ariza et al. (2018a). Results were expressed as milligrams of gallic acid equivalents (GAE), catechin equivalents (CAE), pelargonidin-3-glucoside equivalent (PE) per 100 grams of fresh weight (FW) and micromols of Trolox equivalents (TE) per gram of FW, respectively.

2.3.2. HPLC quantification

2.3.2.1. HPLC–DAD conditions

HPLC analysis of phenolic compounds was assessed on an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto-CA, USA) operated by a Windows based ChemStation software. The HPLC equipment consisted of a quaternary pump, degasser and auto sampler, and the system was used with a diode array detector (DAD). The column used was a Zorbax Eclipse Plus C-18 LC Column (3.00 mm x 150 mm, 3.5 µm) furnished with a guard column (4.6 mm x 12.5 mm, 5 µm), both from Agilent. All samples were filtered through a 0.45 µm filter (type Captiva Econofltr Nylon 25 mm, Agilent Technologies) prior to HPLC analysis.

2.3.2.2. Chemicals and reagents

The solvents used included ethanol, methanol, acetonitrile, acetic acid, formic acid and water, all purchased in HPLC-gradient grade from Sigma–Aldrich (St. Louis, MO, USA).

Stock standard solutions of individual compounds were prepared in pure methanol at a concentration of 500 mg/L and stored at -20 °C (Ariza et al., 2018a). Multi-compound working standard solutions were also prepared by dilution of the stock solutions in methanol and stored at -20 °C in amber coloured vials. Thus, different concentrations of working standard solutions were used to build up the calibration curve for each compound, so that the quantification of the analyte concentrations can be reliably detected (above the limit of quantification; LOQ).

189 *2.3.2.3. HPLC–DAD analyses*

190 The quantification of the individual compounds of a strawberry sample -or multi-compound
191 working standard solutions- was assayed by injecting a 20 µL aliquot into the column and eluted at
192 35 °C at a constant flow rate of 0.4 mL/min.

193 For the determination of phenolic acids and flavanols, the mobile phase consisted on water
194 containing 2–% acetic acid (v/v) and acetic acid/water/acetonitrile mixture (1:49:50, v/v/v;). The
195 gradient used has been described in Ariza et al. (2018a). The maximum characteristic wavelengths
196 for the analytes were 309 nm for *p*-coumaric acid; 280 nm for gallic acid, ellagic acid, *trans*-
197 cinnamic acid, procyanidin B1, procyanidin B2, (+)-catechin hydrate and epicatechin, and 320 nm
198 for chlorogenic and caffeic acid.

199 For the determination of flavonols and anthocyanins, the mobile phase consisted of
200 water/formic acid/acetonitrile (87:10:3, v/v/v) and water/formic acid/acetonitrile (40:10:50, v/v/v).
201 The gradient used has been described in Ariza et al. (2018a). The maximum characteristic
202 wavelengths for the analytes were 370 nm for rutin, myricetin, quercetin, naringenin and
203 kaempferol; and 520 nm for cyanidin-3-O-glucoside chloride, pelargonidin-3-O-glucoside chloride,
204 pelargonidin-3-O-rutinoside chloride, peonidin-3-O-glucoside chloride and malvidin.

205

206 *2.4. Bioactivity assay of the strawberry digested extracts*

207 The effect of bioavailable extracts on cells was assessed *in vitro* by analysing the intracellular
208 ROS production and apoptosis induction on a human hepatocyte carcinoma (HepG2) cells culture.

209 The time of application and dose used of each strawberry digested fraction as well as that of
210 the stressor agent (AAPH; 2,2'-Azobis (2-amidinopropane) dihydrochloride; CAS number: 2997-
211 92-4) were selected taking into account previous works which involved MTT viability assay,
212 ensuring a vitality greater than 90%, with similar and commercial strawberry cultivars grown in the
213 same region and conditions (Ariza et al., 2018b). Thus, for ROS and apoptosis assays, cells were

treated for 24 h with the gastric or intestinal strawberry bioavailable fraction at two chosen concentrations (2 and 5 µg/mL), in absence or presence of AAPH (2.5 mM), reported by Ariza et al. (2018b).

2.4.1. Culture of HepG2 cells

HepG2 cells were purchased from the American Type Culture Collection (ATCC® CL-173TM) and were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin until 80-90% of confluence when sub-cultured. Cells were maintained in an HeraCell CO_2 incubator at 37 °C with 5% CO_2.

2.4.2. Evaluation of intracellular ROS production and apoptosis

Assessment of intracellular ROS production was performed using the CellROX® Oxidative Stress Kit (Invitrogen TM, Life Technologies, Milan, Italy) according to the manufacturer's instructions. However, for the quantification of apoptosis, the Tali™ Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 (Invitrogen TM, Life Technologies, Monza, Italy) was used according to the manufacturer's instructions.

Briefly, cells were seeded in 6-well plates at a density of 1.5 x 10^5 cells/well and treated with the selected concentrations of the gastric or intestinal strawberry bioavailable fraction, in the presence or absence of AAPH. After treatment, cells were washed twice with phosphate buffered saline and detached by trypsinization with 0.5 mL of trypsin-EDTA solution for 2-5 min at 37 °C in 5-% CO_2 incubator. The trypsin was neutralized with 1.5 mL of complete medium and cells were collected prior to centrifugation at 1500 rpm for 10 min at 4 °C.

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For the evaluation of ROS production, the supernatant was discarded and pellet was re-suspended in 1 mL of complete medium, according to Ariza et al. (2018b). Results were expressed as the fold increase of the intracellular ROS content compared to the control.

For apoptosis evaluation, the supernatant was also discarded and, in this case, the pellet was resuspended in 100 µl of annexin binding buffer (ABB) and 5 µl of Annexin V Alexa Fluor® 488 was added according to Ariza et al. (2018b). Samples were analyzed with the Tali® Image-Based cytometer and the percentage of live, dead and apoptotic cells was determined on the basis of the respective fluorescence.

2.5. Statistical Analysis

Statistical analyses were performed using Statistix software 9.0 (Analytical Software, Tallahassee, FL, USA). Data were subjected to ANOVA and differences among means were assessed using Tukey honest significant difference (HSD) test. In order to estimate the variability of each polyphenol group within the strawberry varieties, the coefficient of variation ~~(CV)~~ was calculated as the standard deviation (SD)/mean ratio. Besides, quartiles were calculated for each one in order to categorize and classify varieties with contrasting profile and select varieties for assessing *in vitro* digestion analyses. In addition, correlations among the polyphenolic composition and AC parameters were assessed in raw fruits by Pearson's tests.

3. Results and discussion

3.1. Polyphenol composition and antioxidant capacity of strawberry raw fruits

Table 1 shows the total content of phenolic compounds (TPC), flavonoids (TFC), anthocyanins (TAC) and the antioxidant capacity (AC), of the twelve strawberry varieties. In raw fruits (*i.e.* undigested extract), the antioxidant content of the tested varieties ranged from 181.49 to 226.10 mg GAE/100 g FW for TPC, from 74.38 to 133.46 mg CAE/100 g FW for TFC, from 13.78 to 24.92 mg PE/100 g FW for TAC and from 32.27 to 53.97 µmol TE/g FW for AC. Although these values were in a similar range to those previously reported for many strawberry genotypes (Nowicka et al., 2019; Gündüz and Özdemir, 2014; Tulipani et al., 2008), some variability in the antioxidant composition and capacity was detected among the study varieties in agreement with previous works (Nowicka et al., 2019). These differences were not of the same magnitude in all polyphenol groups. In fact, the calculated coefficient of variation ~~CV~~ (7.68%, 20.71%, 19.48% and 14.69% for TPC, TFC, TAC and AC respectively) showed that TPC was the parameter with the least intra-varietal variability. These results suggest that genotype, to a greater or lesser extent, is an important factor when evaluating fruit quality in strawberry varieties, since the environmental factor was diminished due to the fact that all the varieties studied were cropped under the same conditions.

Comparing the different varieties, it can be observed that ‘Flaminia’, ‘Marquis’ and 'Primoris' showed the highest levels of TPC (226.10, 224.22 and 214.55 mg GAE/100 g FW, respectively) and TFC (133.46, 106.85 and 123.55 mg CAE/100 g FW), while ‘Fortuna’, ‘Charlene’ or Candonga® displayed the lowest levels of TPC (181.49, 184.72 and 187.14 mg GAE/100 g FW, respectively) and TFC (79.29, 90.89 and 83.06 mg CAE/100 g FW, respectively). However, ‘Fortuna’ stood out as the variety with the highest TAC (24.92 mg PE/100 g FW), in contrast to the lower content of ‘Marquis’, 'Charlene' and 'Rabida' (13.87, 13.96 and 13.78 mg CAE/100 g FW, respectively).

AC is an integrative parameter related to the healthy promoting properties of strawberries (Tulipani et al., 2008; Ariza et al., 2018a). The highest and lowest AC found in 'Primoris' and 'Rabida' (53.97 and 32.27 µmol TE/g FW, respectively), contrasted with the high TPC in both. These results are pointing out that the contribution of each polyphenol group to the AC may differ depending on the variety, suggesting that the AC does not predominantly come from polyphenols (Nowicka et al., 2019; Casadesús et al., 2020). This different contribution or lack of proportionality would explain the low correlation observed between TPC and AC, or TAC and AC (supplementary data~~data not shown~~). Only a slightly significant relationship between TFC and AC was found (R^2=0.40289; p<0.05) suggesting some involvement of flavonoids in AC. Thus, although some previous works have highlighted the relation between TPC and health promoting properties in many fruits through their antioxidant capacity (*i.e.* AC) (Ariza et al., 2016; Stoner and Seeram, 2011; Wang et al., 2018; Cervantes et al., 2020), our results are in agreement with Tulipani et al. (2008), showing no correspondence between the TPC and AC in raw fruits. Thus, the antioxidant potential of strawberries can be partly associated with the polyphenol composition (as seen in the correlation with TFC), but also other compounds such as vitamins, antioxidant enzymes, among others, could be involved as suggested Nowicka et al. (2019) and Casadesús et al. (2020).

The differences observed on the amounts of polyphenols in the study varieties did not translate into differences in the type of polyphenol, since the strawberry varieties maintained, to a greater extent, the ratio of each polyphenol group **(Table 1)**. Thus, the values of the TFC/TPC and TAC/TPC ratios were similar for all the varieties studied, exhibiting coefficients of variation~~CVs~~ not particularly elevated (15.80 and 24.96, respectively). This result is also indicating that, despite a variation in the composition of polyphenols among varieties, the proportion of phenolic compounds was nearly stable within the genotypes studied, probably because all the studied varieties come from a close pedigree, in contrast to Cervantes et al. (2020) where different berry species (i.e. strawberry, raspberry and blueberry) were compared.

303 The quantification and characterization of phenolic compounds in raw fruits have some
304 limitations when trying to determine the health potential of the fruit, as physiological processes
305 after intake may alter chemical integrity of antioxidant compounds, as well as their release from the
306 food matrix and their absorption (Ariza et al., 2018a; Cervantes et al., 2020). This situation
307 highlights the importance of taking into account the digestion process to determine the health
308 potential of fruits after consumption, for which *in vitro* digestion assays represent a reliable way to
309 mimic the physiological conditions.
310
311 *3.2. Polyphenol composition and antioxidant capacity after* in vitro *digestion*
312 Taking into account the differences among the study varieties in phenolic content and AC in
313 raw fruits, five out of the twelve initial strawberry varieties with contrasting antioxidant profile
314 (according to the relative amounts of each group of phenolic compounds, represented by quartile
315 position; **Table 2**) were selected for analyzing the potential release and absorption of antioxidants
316 after intake by simulating *in vitro* digestion process. These varieties were 'Primoris', 'Fortuna',
317 'Flaminia', 'Marquis' and 'Charlene'. The content of phenolic compounds in raw fruits and in the
318 bioavailable fractions (or potentially absorbed phenolic compounds) are summarized in **Table 3**.
319 Despite all study varieties belong to the same species with a conserved pedigree, the digestion
320 process affected the strawberry varieties differently, which is in agreement with previous studies
321 (Carbonell-Capella et al., 2014; Marhuenda et al., 2016). Thus, the highest values of TPC, TFC,
322 TAC and AC observed in 'Primoris' raw fruits did not translate into the highest TPC, TFC, TAC
323 and AC levels after the *in vitro* digestion **(Table 3)**. In contrast, 'Marquis', which showed
324 intermediate values of TPC, TFC, TAC and AC in raw fruits, displayed a higher total
325 bioavailability after digestion. This absence of consistency between raw fruits and digested fruits,
326 could be partially due to the reactants and conditions of the digestion process (different from those
327 of hydromethanolic extraction) but also to the intraspecific differences in antioxidant composition

328 (described in section 3.1), which could imply that transformations, degradations and release of
329 molecules from the food matrix might occur differently on each variety during the *in vitro*
330 digestion.

331 The *in vitro* digestion process includes two sequential phases simulating gastric and intestinal
332 digestion giving the potentially released (bioaccessible) and potentially absorbed (bioavailable)
333 fractions at each level.

334 Regarding to the bioaccessible fractions, phenolic composition and AC in the released fractions
335 of all the selected strawberry cultivars differed from raw fruit material (data not shown) in
336 agreement with previous studies in strawberry (Ariza et al., 2018a; Cervantes et al., 2019). All
337 varieties displayed a general trend to increased TPC values and decreased TFC, TAC and AC in the
338 bioaccessible fractions, revealing an uneven effect of the digestion process on the different
339 antioxidant compounds analyzed. A TPC increase in the bioaccessible fractions was also reported in
340 other strawberry varieties (Cervantes et al., 2020; Ariza et al., 2018), but is in contrast with other
341 food matrices like the tea extracts (Record and Lane, 2001), grapes (Granese et al., 2014),
342 pomegranate (Pérez-Vicente et al., 2002), blueberry (Cervantes et al., 2020). This result is
343 suggesting species differences in the interaction of phenolic compounds with the food matrix,
344 and/or differences on the transformation/releasing rate between high (i.e. anthocyanins) and low
345 molecular weight phenolic compounds, among others.

346 Regarding the potential absorption **(Table 3)**, 'Fortuna' displayed a higher proportion of TPC
347 bioavailable (76.40–% of TPC raw fruit was absorbed after digestion), in contrast to 29.71–%
348 showed by 'Marquis', while the reverse was true for the potentially absorbed TFC fraction, being
349 13.45–% and 8.13–% in 'Marquis' and 'Fortuna', respectively. This is indicating that the largest
350 proportion of TPC absorbed by 'Fortuna' is mostly of the non-flavonoid type. In contrast, although
351 raw fruits of 'Charlene' did not show high TAC values, this variety showed the highest potential
352 anthocyanin absorption (47.90% bioavailable TAC; **Table 3**), representing a 1.69-fold higher

bioavailable TAC than ‘Marquis’ (variety with similar values of TAC in raw fruit) and a 4.68-fold higher than ‘Flaminia’, the variety with the lower TAC bioavailable.

Regarding AC, as in raw fruits, no correspondence between the TPC and AC was found for the absorbed fractions (**Table 3**). Thus, ‘Primoris’ and ‘Fortuna’ showed the highest TPC bioavailable and the lowest AC-bioavailable (AC_{BA}) and the reverse was true for ‘Flaminia’ and ‘Marquis’.

It is noteworthy that although TPC, TFC and TAC, were equally or highly absorbed at the gastric level in all varieties, the AC was higher in the bioavailable intestinal fractions, suggesting that compounds absorbed at the intestinal level have higher AC compared to the bioavailable compounds detected in the gastric fractions (ranging from 2.09 to 11.22 times higher; **Table 3**).

Therefore, our results do not allow us to establish a sound relationship between the antioxidant capacity and the different groups of phenolic compounds analyzed neither in raw fruits nor after *in vitro* digestion of the fruits in the strawberry varieties. Likewise, it has not been possible to establish a direct relationship between TPC, TFC, TAC and AC before and after digestion, so the identification of some individual compounds responsible for the AC_{RF} and/or AC_{BA} on the selected varieties could be interesting for further selecting genotypes.

3.3. Comparative quantification of individual compounds and its bioavailability

In order to characterize the composition of individual phenolic compounds in both raw fruits and digested extracts **(Table 4)**, two varieties (‘Marquis’ and ‘Primoris’) were selected due to their contrasting pattern of AC before and after digestion.

In raw fruits, amounts of the different compounds analyzed were generally similar or statistically higher ($p<0.05$) in 'Primoris' than in 'Marquis', with the exception of the contents of gallic acid and procyanidin B1 and B2 (Table 4). Likewise, most of the phenolic compounds analyzed individually in both gastric and intestinal fractions, showed significantly higher values in 'Primoris' compared to 'Marquis', which contrasts with the higher AC_{BA} of 'Marquis' compared to

In this sense, although the health benefits of food extracts are not entirely dependent on AC, the properties related to protection against oxidative damage could be closely linked to AC_{BA}. With this in mind, 'Marquis' may have greater health benefits than 'Primoris', but bioactivity assays with bioavailable fractions are necessary before considering AC_{BA} as an indicator of the health properties of strawberry food extracts.

3.4. Bioactivity assessment of strawberry on HepG2 cells

Although previous studies have already indicated differences in the antiproliferative properties of different raw berry fruits (Boivin et al., 2007), very few have focused on the effects of digested samples on bioactivity (Ariza et al., 2018b; Cianciosi et al., 2020). Thus, in order to attribute a possible health effect to digested strawberry samples, their protective effects against oxidative damage in human hepatocellular carcinoma cells (HepG2) were evaluated. For this purpose, the effect of bioavailable extracts on the intracellular quantity of ROS and cell survival/apoptosis was tested in both control cells and cells subjected to a stressor (AAPH). The bioavailable fractions (gastric and intestinal) were considered the most suitable extracts, as they encompass the changes and transformations due to the digestion process that could reach the cells and therefore have a potential beneficial effect.

3.4.1. Evaluation of intracellular ROS production

Intracellular amount of ROS was quantified in untreated (control) and in AAPH-treated cells to determine basal ROS levels. As expected, intracellular ROS levels were increased in AAPH-treated cells compared to the control (**Figure 1 A and B**). However, when bioavailable extracts of "Marquis" or "Primoris" at two concentrations (2 and 5 µg/mL) were added to untreated cells, the amount of ROS observed was similar to that of control cells, indicating that the concentrations and composition of both extracts (gastric and intestinal) were adequate and harmless for the cells.

Cells pre-incubated with the bioavailable gastric and intestinal fractions of both varieties and subsequently treated with AAPH did not show a significant decrease in ROS values compared to AAPH-treated cells, with the exception of cells treated with bioavailable extracts of 'Marquis' (2 μg/mL + AAPH). This treatment significantly reduced ROS levels to values comparable to those of control cells (in the case of the intestinal fraction and slightly higher in the gastric fraction), being the most effective in counteracting oxidative damage caused by AAPH due to its antioxidant activity. It is striking that 'Marquis' extracts at higher concentration (5 μg/mL + AAPH) did not decrease ROS levels, possibly because this concentration was high enough to give rise to a pro-oxidation effect, as it has been observed in other assays when adding an antioxidant agent in excess (i.e. induction of apoptosis in tumor cells by tea catechins; Lambert and Elias, 2010). In fact, depending on their structure and the conditions, polyphenols can act as both antioxidants and prooxidants, the latter being directly proportional to the number of hydroxyl groups (i.e. their quantity; Lee and Lee, 2006). The higher ROS counteracting effects of 'Marquis' extracts compared to 'Primoris' extracts are consistent with their differences in the AC_{BA} (**Table 3**), although this cannot be attributed to any specific compound (**Table 4**).

3.4.2. Cell survival and apoptosis assay

Regarding the survival assay and apoptosis rate, cells treated with the stressor agent AAPH displayed lower percentages of live cells concomitantly with higher levels of dead cells than untreated cells. Except for the treatment with 'Marquis' at 2 μg/mL, the different concentrations of bioavailable strawberry extracts (gastric or intestinal) significantly decreased ($p < 0.05$) the percentage of live cells compared to control cells. With gastric fractions, survival percentages decreased to values similar to those of AAPH-treated cells, but with intestinal fractions, the percentages of live cells were slightly but significantly higher (**Figure 2 A and B**). This different effect between gastric and intestinal fractions could be due to their differences in the antioxidant

amount and composition, pointing out that concentrations used in the gastric fractions may have a cytotoxic effect (5 µg/mL~~l~~ 'Marquis' and 2 and 5 µg/mL 'Primoris').

When cells were treated with gastric extracts and AAPH, the percentage of live cells increased with respect to the AAPH-treated group, reaching values similar to those of control cells (**Figure 2 A**). Likewise, treatments with intestinal extracts and AAPH also counteracted the effect of AAPH by increasing the level of live cells, but only cells treated with 'Primoris' extracts reached values similar to untreated cells (**Figure 2 B)**.

Regarding to the apoptotic cells (one of the targets of cancer therapies as it is considered a clean die-off; Pfeffer and Singh, 2018), gastric and intestinal extracts of the study varieties did not result in significant differences in the percentage of apoptotic cells versus AAPH treatment, except for the intestinal extract of 'Primoris' 5µg/mL~~l~~+AAPH, which decreased the number of apoptotic cells.

Overall, the results described above show that 'Marquis' digested extracts were able to counteract to a greater extend the cell oxidative damage caused by a chemical agent, by decreasing intracellular accumulation of ROS and favoring cell survival. The various protective effects of different digested berry species have been widely demonstrated in literature (neuroprotective, Tavares et al., 2012; scavenging activity, Chen et al., 2016; anti-proliferative, Bermúdez-Soto et al., 2007), but the compounds responsible for such effects remain unclear. Some authors have attributed them to the anthocyanin group of compounds (Forbes-Hernández et al., 2016), or hydrolysable tannins (mainly ellagic acid; Chen et al., 2007); however, in 'Marquis' the amount of polyphenols (specifically anthocyanins and hydrolysable tannins; **Table 4**) was lower than in 'Primoris'. In this sense, the higher bioactivity observed in ~~of~~ 'Marquis' compared to 'Primoris' seems to be more related to their differences in the AC_{BA} rather than to their phenolic composition, since AC_{BA} was not correlated to TPC, TFC and TAC (Table 3), ~~since 'Primoris' showed higher bioavailable TPC, similar bioavailable TFC and TAC and lower AC_{BA} than 'Marquis'(Table 3)no significant correlation between AC_{BA} and bioavailable TPC and TFC was found,~~ reinforcing the use of AC-

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bioavailable as a feasible indicator of fruit wholesomeness. This can be very useful for selecting strawberry varieties with potential health benefits in different breeding programs. However, further molecular investigations are needed to better understand to what extent the ability to counteract oxidative stress *in vitro* depends exclusively on the AC-bioavailable of the extracts

4. Conclusions

The present study demonstrates that strawberry bioactivity is genotype-dependent and that the bioactive effects of strawberries depend on the antioxidant composition of the raw fruits, but also on the AC_{BA} resulting from the transformations of the fruits during the digestion process. In this sense, it is suggested the use of AC_{BA} as a bioactivity indicator for determining the potential health benefits of strawberries useful for breeding programs. Moreover, these results imply that other families of antioxidants, in addition to phenolic-type compounds, are also involved in strawberry bioactivity, with further efforts being necessary to identify the main contributors to the antioxidant capacity and bioactivity of each strawberry genotype in future works.

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Figure captions

Figure 1. Intracellular reactive oxygen species (ROS) accumulation in HepG2 cells determined by the Tali® Image-Based Cytometer. Cells were preincubated with the indicated fruit fractions (at 2 or 5µg/mL) and stressed or not with stressor agent (AAPH) for 24 h. **A:** Gastric fraction and **B**: Intestinal fraction. Values are the mean ± SE of three independent experiments (n = 3). Columns with different letters are significantly different ($p < 0.05$). CTL: Control cells without treatment; AAPH: cells incubated with AAPH; QUI: cells preincubated with bioavailable digestion extract from cv. Marquis; PRI: cells preincubated with bioavailable digestion extract from cv. Primoris; QUI + AAPH: cells preincubated with 'Marquis' extract and then stressed with AAPH; PRI + AAPH: cells preincubated with 'Primoris' extract and then stressed with AAPH.

Figure 2. Percentage of live, dead, and apoptotic HepG2 cells determined by the Tali® Image-Based Cytometer. Cells were preincubated with the indicated fruit fractions at two concentrations and stressed or not with stressor agent (AAPH) for 24 h. **A**: Gastric fraction and **B**: Intestinal fraction. Values are the mean ± SE of three independent experiments (n = 3). Columns belonging to the same data set with different letters are significantly different ($p < 0.05$). CTL: control cells without treatment; AAPH: cells incubated with AAPH; QUI: cells preincubated with bioavailable digestion extract from cv. Marquis; PRI: cells preincubated with bioavailable digestion extract from cv. Primoris; QUI + AAPH: cells preincubated with 'Marquis' extract and then stressed with AAPH; PRI + AAPH: cells preincubated with 'Primoris' extract and then stressed with AAPH.