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RESEARCH ARTICLE

Platelet hyperactivity: a comparison of water-soluble, bioactive compound levels in commercial tomato products and water-soluble tomato concentrate, a supplement with an approved EFSA antiplatelet health effect

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ABSTRACT

The aim of this study was to evaluate and compare the concentration of water-soluble bioactive compounds in tomato products (polyphenols profile, water-soluble vitamins and nucleophilic substances) with the concentration of the same bioactive molecules existing in a water-soluble patented tomato extract, water-soluble tomato extract (WSTC), commercially available as FruitFlow®. This patented tomato extract has been recognised by EFSA (European Food Safety Authority) in a specific Health Claim declaration as having an "Antiplatelet health effect". More than 100 commercial tomato samples, coming from 18 different processing tomato companies worldwide, were analysed and compared with the FruitFlow® supplement. According to the multivariate statistical analyses applied to the data matrix, it is possible to conclude that the commercial tomato products measured (pastes, purees, others) show a significantly higher concentration of water-soluble bioactive molecules (nucleosides/nucleotides and polyphenols) responsible for an anti-platelet aggregation effect than the FruitFlow® dietary supplement.

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Processing tomato products; water-soluble tomato bioactive molecules; blood antiplatelet effect; LC-HRMS high-resolution mass spectrometry analysis; polyphenols; nucleosides; nucleotides

Introduction

Consumer interest in improving well-being through clean, natural, sustainable foods and beverages has never been greater than it is currently.

With the increase of the average human life (from less than 50 to over 75 years in the last half-century), chronic diseases or "well-being society diseases" (heart disease, cancer, mental disorders, respiratory and digestive diseases) have consistently increased globally. Consequently, so have the costs that national governments face for public health systems (OECD et al. 2017).

Several scientific studies (Tresserra-Rimbau et al. 2014; Zamora-Ros et al. 2016; Agarwal et al. 2019; Mitchell et al. 2020) have shown that for every euro invested in disease prevention, three euros are saved in disease treatment. Moreover, given that more and more scientific evidences are determining the very close correlation between eating habits and health. It is clear, for instance, that the Mediterranean diet, characterised by a very well-balanced consumption of fruits, legumes, vegetables, olive oil, fish products, etc., precipitates safe and lasting beneficial effects and higher life expectancy, confirmed by statistical evidences (Tektonidis et al. 2016; Salas-Salvadó et al. 2018).

In parallel, the agro-industrial system is facing some tough challenges linked to global social evolution, such as an ever-increasing urban population, a decrease in the consumption of home processed food, constant evolution in cookery towards middle-class tendencies, diets for growing an ageing society, an awareness of the health benefits of bioactive molecule-rich foods (functional foods) among others (Rao et al. 2018).

Moreover, international nutritional guidelines (FAO, EUFIC, etc.) (EUFIC 2023; FAO 2023) simultaneously recommend eating more fruits and vegetables, fibres, vitamins, potassium, etc. and eating less foods rich in calories, saturated fats, and sodium.

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The undeniable success of tomato and its derivatives, its increasingly extensive use in kitchens all over the world, also thanks to its stabilisation and industrial transformation, the continuous development of technologies respectful of its nutritional and nutraceutical–functional characteristics are the result of research and experimentation that were introduced primarily thanks to the great technological applications brought about by scientific research (Rao et al. 2018).

Tomato fruit, a major component of the "Mediterranean diet", is often linked with a healthier lifestyle (Corpet and Gerber 1997; Grosso et al. 2017; Rao et al. 2018). Owing to the presence of a set of lipophilic and hydrophilic biomolecules, tomato products are a natural source of bioactive compounds such as carotenoids (lycopene, β-carotene, lutein, phytoene, etc.), vitamins C and A, and phenolic molecules such as flavonoids and phenolic acids and are also important sources of potassium, folates, fibres, unsaturated fatty acids and enzymes (Ali et al. 2021). The clinical and health scientific literature increasingly proves that a direct correlation exists between the consumption of foods rich in bioactive substances and positive effects on human health (Del Rio et al. 2010). In this context, vegetables in general, and more specifically tomato-based products, have always been considered foods containing multiple health-protecting bioactive molecules (Corpet and Gerber 1997; Grosso et al. 2017; Rao et al. 2018).

For these important nutritional characteristics, tomato products are considered very important worldwide in terms of public health and well-being. With increasing interest and awareness of the health benefits of natural tomato bioactive compounds (mainly lycopene), the tomato industry has drawn more and more attention to the nutritional quality and healthy benefits of processed tomato-based foods (Ali et al. 2021). In recent years, several studies have shown the importance of the natural synergistic effect shown by all the tomato's bioactive compounds: from new product applications (high pigment varieties, lycopene ingredients, new functional tomato recipes, lycopene-enriched products, etc.), new sustainable process technologies, green extraction of by-products, etc (Arab and Steck 2000; Sesso et al. 2004; Costa-Rodrigues et al. 2018).

Cardiovascular diseases (CVDs) are considered important health problems for the population worldwide, being highly influenced by food consumption and unhealthy lifestyle (Yamamoto et al. 2003; Virani et al. 2020). The cardio-protective benefits of the Mediterranean diet have been extensively studied and further associated with the consumption of tomato products, being one of its most relevant constituents, due to the potential to reduce the level of specific associated risk factors (Stampfer et al. 2000; Mackay and Mensah 2004; Odai et al. 2019; Rattanavipanon et al. 2021). Studies have been conducted to associate the antiplatelet aggregation properties for different nutrients in tomato, starting with their main carotenoid constituent, lycopene, also responsible for other very important health benefits. However, despite the fact that the consumption of tomato-based products or lycopene-enriched alternatives has been reported to have a cardio-protective effect, other studies suggest that there is not a strong correlation between singular lycopene and CVD risk factors (Sesso et al. 2004), in addition to the fact that the mechanism of action of this molecule is not completely elucidated (Arab and Steck 2000; Hak et al. 2003; Costa-Rodrigues et al. 2018). These results open a door to the evaluation of other compounds existing in the fruit, which might be responsible for desired outcome effects, mainly thanks to a synergistic complex effect and bioavailability (hydro-soluble and fat-soluble molecules).

FruitFlow® is a patented lycopene-free, water-soluble tomato concentrate extract (WSTC) owned by the DSM Company that has been evaluated by an EFSA NDA panel for its potential to reduce platelet aggregation, providing scientific evidence for two different products: WSTC I (completely water-soluble syrup) and WSTC II (a low-sugar derivative) in *in-vitro* and *ex-vivo* studies. In 2009, an EFSA NDA panel approved the specific claim: "Helps to maintain normal platelet aggregation, which contributes to healthy blood flow" under the category "emerging science" of the European Food Safety Authority (EFSA) (EFSA 2009; O'Kennedy et al. 2017).

DSM's FruitFlow® is a dietary supplement made from bioactive compounds naturally found in the jelly around tomato seeds. Tomatoes are crushed into a paste, and then the skin and seeds are removed. The compounds are then extracted from the clear juice and concentrated to create FruitFlow®. The primary function of FruitFlow® is to smooth blood platelets, protecting against hyperactivity and unwanted blood clots, ultimately aiding healthy blood flow.

Consequently, with FruitFlow® being an extract directly obtained from processing tomato (tomato pastes), with this work, we want to answer a simple question about how the antiplatelet compounds presented in tomato-based products currently available in the market can be compared with their nutraceutical alternative used as a dietary supplement.

The aim of this study is therefore to verify and compare the concentration of water-soluble bioactive compounds naturally present in different tomato-based

products coming from worldwide factories, with the actual concentration of those compounds in FruitFlow®, based on the recommended daily intake made by EFSA.

Materials and methods

Materials

Tomato products

More than a hundred samples (107 in triplicate) of tomato-based products, coming from different producers globally, were divided into three different categories: *pastes*, *purees* and *others* (including tomato pulps, juices, peeled and sauces). A total of 18 companies from 13 countries worldwide joined this study, which represents the largest study conducted with the widest scientific collaboration of subjects related to the tomato industry sector ever organised.

This study gave us access to many types of tomato pastes and other tomato products (purees, crushed, pulps, whole peeled, sauces, etc.) coming from worldwide sources that accurately represent the main commercial tomato products present in the world market.

The total tomato pastes analysed was 63, the total purees analysed was 10, the total amount of analysed products included in the category "others" was 34 (Table 1).

Chemicals

Nucleosides/nucleotides: guanosine, uridine, adenosine, cytidine, adenosine-5-monophosphate and adenosine-3-monophosphate were obtained from Sigma-Aldrich. Stock solutions of Nucleosides/nucleotides were prepared in water at 1000 mg $L^{-1}$and were then stored at −20 °C. Just before use, standard solutions were prepared from the stock solutions by dilution to the desired concentration with water.

Flavonoids: quercetin, rutin, hyperoside, quercitrin, quercetin-3-glucoside, kaempferol, kaempferol-3-glucoside, kaempferol-7-glucoside, kaempferol-3-rutinoside, kaempferol-7-neohesperidoside, naringenin, naringenin-7-glucoside and naringin were purchased from Extrasynthese (Lyon, France). Phenolic acids and derivatives: trans-cinnamic acid, caffeic acid, ferulic acid, sinapic acid, p-coumaric acid, o-coumaric acid, m-coumaric acid, chlorogenic, neochlorogenic acid were obtained from Extrasynthese (Lyon, France). 2-Hydroxybenzoic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, syringic acid, 2,5-dihydroxybenzoic acid and 3,4- dihydroxybenzoic acid were obtained from Sigma-Aldrich. Stock solutions of Phenolic acids and Flavonoids were prepared in methanol at 100 mg $L^{-1}$and were then stored at −20 °C. Just before use, working solutions were prepared from the stock solutions by dilution to the desired concentration with a solution of water:methanol, 50:50, except for working solutions of the aglycones quercetin, kaempferol and naringenin, which were prepared from the stock solutions by dilution to the desired concentration with methanol. UHPLC-MS grade solvent, water, formic acid, and methanol were obtained from Scharlau (Barcelona, Spain).

Methods

Determination of nucleosides and nucleotides content:

The nucleosides/nucleotides determination was carried out by means of HPLC-DAD.

Sample preparation

From 0.5 to 5 g samples were diluted to 250 ml with water, depending on tomato base-product type. Each sample was homogenised using Ultra-Turrax (T25 basic IKA®, IKA-Werke, Staufen, Germany),filtered with paper filter (Chemifarm, Parma, Italy) and then with syringe drive nylon filter unit 0.2 µm (Millex-GN Millipore; Bedford, UNITED STATES). Analyses were carried out in triplicate.

Apparatus and analysis conditions

A liquid chromatographic system consisting of Alliance 2695 Sep. Module, Alliance column heater, 2996 photodiode array detector (Waters) was used. Chromatographic separation was performed by a 250×4.6 mm, 5 µm C18 Hypersil Gold column (Thermo Scientific) kept at 30 °C. An isocratic elution with 1.0 ml/min flow was applied. Eluent A was water/0.1% formic acid and eluent B was methanol: 90% eluent A-10% eluent B. Data acquisition and chromatograms integration as well as management of chromatographic system were performed using Empower 2.0 software (Waters). Quantification of nucleosides/nucleotides was performed using the external standard method with calibration graphs, as a function of concentration based on peak area, detected at wavelength corresponding to their maximum absorbance. Acquisition wavelengths: 280 nm for cytidine and 260 nm for others compounds. The linearity and calibration experiments were performed in the range 0.1–10 mg/kg for nucleosides and 2–200 mg/kg for nucleotides.

The nucleosides/nucleotides identified were confirmed by means of UHPLC-HRMS/MS. A Q-Exactive™ quadrupole-Orbitrap mass spectrometer equipped

Table 1. Tomato products evaluated discriminated by origin and category.

Category	Consecutive	Origin
Puree	1	Spain and Portugal
	2	Greece
	3	Italy
	4	Italy
	5	Italy
	6	Italy
	7	Greece
	8	Portugal
	9	Italy
	10	Italy
Paste	1	United States
	2	United States
	3	United States
Paste	4	United States
	5	United States
	6	Portugal
	7	Portugal
	8	Portugal
	9	Portugal
	10	Portugal
	11	Portugal
	12	United States
	13	United States
	14	United States
	15	United States
	16	United States
	17	United States
	18	United States
	19	United States
	20	Ukraine
	21	Ukraine
	22	Ukraine
	23	Greece
	24	Greece
	25	Australia
	26	Australia
	27	Australia
	28	Australia
	29	Australia
	30	Australia
	31	Australia
	32	Australia
	33	Australia
	34	Italy
	35	China
	36	China
	37	United States
	38	United States
	39	United States
	40	Portugal
	41	Portugal
	42	Portugal
	43	Portugal
Paste	44	United States
	45	United States
	46	United States
	47	United States
	48	United States
	49	United States
	50	Argentina
	51	Japan
	52	Japan
	53	Japan
	54	Greece
	55	Portugal
	56	Italy
	57	Spain
	58	Spain
	59	Italy
	60	Italy
	61	Italy
	62	Italy
	63	Italy

(*Continued*)

Table 1. Continued.

Category	Consecutive	Origin
Others*	1	United States
	2	United States
	3	United States
	4	Spain and Portugal
	5	United States
	6	United States
	7	Spain
	8	Greece
	9	Greece
	10	Greece
	11	Greece
	12	Greece
	13	Australia
	14	Australia
	15	Australia
	16	Italy
	17	Italy
	18	Italy
	19	Italy
	20	United States
	21	United States
	22	United States
	23	Argentina
Others*	24	Greece
	25	Greece
	26	Japan
	27	Italy
	28	Italy
	29	Italy
	30	Italy
	31	Italy
	32	Netherlands
	33	Netherlands
	34	Netherlands

*Other products include pulp, juice, whole peeled tomatoes and sauces.
The dietary supplement FruitFlow® was considered as a sample. Five different batches were purchased and the average sample obtained from mixing them was analysed.

with heated electrospray ionisation (ESI) source was used to obtain MS data.

UHPLC-HRMS/MS analysis

Liquid chromatographic analysis was performed by a Dionex Ultimate 3000 RS UHPLC system fitted with Q-Exactive™ quadrupole-Orbitrap mass spectrometer equipped with heated ESI source. Chromatographic separation was performed by a 100×2.1 mm, 1.9 µm Hypersil Gold C18 column (Thermo Scientific) kept at 35 °C. An isocratic elution with 0.3 ml/min flow was applied. Eluent A was water/0.1% formic acid and eluent B was methanol: 90% eluent A-10% eluent B. Ten microliters of sample was injected for analysis. Mass spectra were acquired in positive ion mode through full MS and higher energy collisional dissociation (HCD) data-dependent MS/MS analysis. The mass range was from m/z 150 to 500. The resolving power was set at 70 000 for the full MS scans and 17 500 for HCD MS/MS scans. The normalised collision energy (NCE) was set at 30%. The mass spectrometer operated as follows: spray voltage, 3.60 kV; sheath gas flow rate, 40 arbitrary units; auxiliary gas flow rate, 10 arbitrary units; capillary temperature, 270 °C; and auxiliary gas heater temperature, 300 °C. The instrument was externally calibrated by the Pierce® LTQ VELOS ESI-positive ion calibration solution (product number 88323, Thermo Scientific, Rockford, UNITED STATES). Data acquisition was performed using XCALIBUR 2.1 software (Thermo Scientific, Rockford, UNITED STATES). Full mass spectral data were used for the identification of analytics through comparison of high-resolution m/z values, retention time and data-dependent MS/MS spectra with data collected from analysis performed on commercially available standard compounds (Table 2).

FruitFlow® supplement

Determination of phenolic acids, flavonoids and derivatives content:

The polyphenols determination was carried out by means of UHPLC-HRMS/MS.

Table 2. *Target compounds* MF, molecular formula of the compound; Rt (min), retention time, in minutes; $[M-H]^-$, observed accurate mass of precursor ion; MS^2 main fragment, main fragment in the negative ion ESI-HCD MS/MS spectrum (NCE = 30%) of precursor ion.

Analytes	MF	Rt (min)	$[M+H]^+$ (*m/z*)	MS^2 main fragments (*m/z*)
Cytidine	$C_9H_{13}N_3O_5$	1.01	244.0928	112.0507
Uridine	$C_9H_{12}N_2O_6$	1.20	245.0768	113.0347
Guanosine	$C_{10}H_{13}N_5O_5$	1.40	284.0989	152.0564
Adenosine	$C_{10}H_{13}N_5O_4$	1.33	268.1040	136.0617
Adenosine-3-monophosphate	$C_{10}H_{14}N_5O_7P$	1.05	348.0708	136.0616
Adenosine-5-monophosphate	$C_{10}H_{14}N_5O_7P$	0.97	348.0708	136.0616

Sample preparation

From 1 to 20 g sample was diluted to 100 ml with a solution of water:methanol, 50:50, depending on tomato base-product type and single polyphenol analysed. Only for quercetin, kaempferol and naringenin determination and quantification sample was diluted with methanol, due to solubilty. Each sample was homogenised using Ultra-Turrax (T25 basic IKA®, IKA-Werke, Staufen, Germany),filtered with paper filter (Chemifarm, Parma, Italy) and then with syringe drive nylon filter unit 0.2 µm (Millex-GN Millipore; Bedford, UNITED STATES). Analyses were carried out in triplicate.

UHPLC-HRMS/MS analysis

Liquid chromatographic analysis was performed by a Dionex Ultimate 3000 RS UHPLC system fitted with Q-Exactive™ quadrupole-Orbitrap mass spectrometer equipped with heated ESI source. Chromatographic separation was performed by a 100 × 2.1 mm, 1.7 µm ACE Excel C18-PFP column kept at 35 °C. Mobile phase flow was maintained as 0.3 ml/min. Eluent A was water/0.1% formic acid and eluent B was acetonitrile/0.1% formic acid. The separation gradients were 0 min, 1%B; 10 min, 95%B; 13.5 min, 1%B; and 16 min, 1%B. Ten microlitres of sample was injected for analysis. Mass spectra were acquired in negative ion mode through full MS and higher energy collisional dissociation (HCD) data-dependent MS/MS analysis. The mass range was from m/z 100 to 950. The resolving power was set at 70 000 for the full MS scans and 17 500 for HCD MS/MS scans. The NCE was set at 30%. The mass spectrometer operated as follows: spray voltage, 3.00 kV; sheath gas flow rate, 40 arbitrary units; auxiliary gas flow rate, 10 arbitrary units; capillary temperature, 250 °C; and auxiliary gas heater temperature, 300 °C. The instrument was externally calibrated by the Pierce® ESI negative ion calibration solution (product number 88324, Thermo Scientific, Rockford, UNITED STATES).

Data acquisition was performed using XCALIBUR 2.1 software (Thermo Scientific, Rockford, UNITED STATES). Full mass spectral data were used for identification and quantification of analytics through comparison of high-resolution m/z values, retention time and data-dependent MS/MS spectra with data collected from analysis performed on commercially available standard compounds (Table 3).

When standards were not available, as in the case of caffeic acid, ferulic acid, p-coumaric acid derivatives and flavonol derivatives, the identifications were carried out through the comparison of HCD MS/MS spectra shown with data in the literature and in the Reference Library of mzCloud-Advanced mass spectral database.

Then they were quantified with respect to the corresponding hydroxycinnamic acid (caffeic, ferulic and p-coumaric acids) or to the corresponding aglycone for flavonoids.

The linearity and calibration experiments were performed in the range 0.01–5 mg/kg for flavonoids and in the range 0.01–4 mg/kg for phenolic acids and derivatives, except for 2-hydroxybenzoic acid, chlorogenic acid and neochlorogenic acid in the range 0.5–10 mg/kg.

All quantitative data were calculated as mg/kg of fresh product.

Statistical analysis

The statistical analysis was performed using SPSS® program SPSS® Inc. – Codes SIEJ9LL + SIELFLL Statistics Categories (Statistical Package for the Social Science). (Version 26.0; IBM Corp., Armonk, NY, USA) Analysis of variance (ANOVA) and one sample t-test were used as tools to identify differences in WSTBM (Water Soluble Tomato Bioactive Molecules) in between different tomato products coming from worldwide divided into homogeneous categories and FruitFlow®. The results are presented as mean and standard deviation for category, and the significance level is 0.05.

Results and discussion

The concentration of water-soluble bioactive compounds in the different categories is presented in Table 4. Nucleotides and nucleosides are the main constituents presented in all tomato-based products and the food supplement, followed by phenolic acids

Table 3. *Target compounds* MF, molecular formula of the compound; Rt (min), retention time, in minutes; $[M-H]^-$, observed accurate mass of precursor ion; MS^2 main fragment, main fragment in the negative ion ESI-HCD MS/MS spectrum (NCE = 30%) of precursor ion.

Analytes	MF	Rt (min)	$[M-H]^-$ (*m/z*)	MS^2 main fragments (*m/z*)
Flavonoids				
Quercetin	$C_{15}H_{10}O_7$	6.76	301.0343	301.0354/151.0027
Rutin (quercetin-3-rutinoside)	$C_{27}H_{30}O_{16}$	5.12	609.1450	300.0277
Hyperoside (quercetin-3-galactoside)	$C_{21}H_{20}O_{12}$	5.27	463.0871	300.0276
Quercetin-3-glucoside	$C_{21}H_{20}O_{12}$	5.30	463.0871	300.0276
Quercitrin (quercetin-3-rhamnoside)	$C_{21}H_{20}O_{11}$	5.58	447.0922	301.0341
Kaempferol	$C_{15}H_{10}O_6$	7.21	285.0404	285.0405/151.0027
Kaempferol-3-glucoside	$C_{21}H_{20}O_{11}$	5.51	447.0937	284.0327
Kaempferol-7-glucoside	$C_{21}H_{20}O_{11}$	5.67	447.0937	284.0327
Kaempferol-3-rutinoside	$C_{27}H_{30}O_{15}$	5.32	593.1512	285.0398
Kaempferol-7-neohesperidoside	$C_{27}H_{30}O_{15}$	5.49	593.1508	285.0398
Naringenin	$C_{15}H_{12}O_5$	7.09	271.0615	151.0027
Naringenin-7-glucoside	$C_{21}H_{22}O_{10}$	5.66	433.1141	271.0615-151.0037
Naringin	$C_{27}H_{32}O_{14}$	5.48	579.1719	271.0614
Benzoic acids and derivatives				
trans-cinnamic acid	$C_9H_8O_2$	6.87	147.0453	147.0452-61.9880-102.8488
Sinapic acid	$C_{11}H_{12}O_5$	5.61	223.0601	208.0378
Caffeic acid	$C_9H_8O_4$	4.84	179.0350	135.0452
p-Coumaric acid	$C_9H_8O_3$	5.36	163.0401	119.0502-163.0400
o-Coumaric acid	$C_9H_8O_3$	5.96	163.0401	119.0502-163.0400
m-Coumaric acid	$C_9H_8O_3$	5.67	163.0401	119.0502-163.0400
Ferulic acid	$C_{10}H_{10}O_4$		193.0506	134.0373
Chlorogenic acid (5-caffeoylquinic acid)	$C_{16}H_{18}O_9$	4.47	353.0880	191.05555
Neochlorogenic acid (5-caffeoylquinic acid)	$C_{16}H_{18}O_9$	4.11	353.0880	191.0555-179.0349-135.0452
2-hydroxybenzoic acid (salicylic acid)	$C_7H_6O_3$	6.35	137.0240	93.0345-137.0243
3-hydroxybenzoic acid	$C_7H_6O_3$	4.98	137.0240	93.0345-137.0243
4-hydroxybenzoic acid	$C_7H_6O_3$	4.62	137.0240	93.0345-137.0243
Syringic acid		5.00	197.0444	182.0220-197.0456-103.9766
3,4-dihydroxybenzoic acid (protocatechuic acid)	$C_7H_6O_4$	4.12	153.0182	109.0295-153.0193
2,5-dihydroxybenzoic acid (gentisic acid)	$C_7H_6O_4$	4.77	153.0182	109.0295-153.0193

and flavonoids. A similar study made by O'Kennedy et al. in 2017, where different commercially available tomato products were compared with FruitFlow®, showed different results compared to those obtained in this study: while nucleosides were the main category of bioactive molecules, flavonoids were the second most abundant biomolecules. Regarding the concentration, much higher results were found. However, the analytical techniques were completely different. Additionally, the variability within categories was smaller compared to the present study, but the number of products compared was also smaller in their study (10 products per category) (O'Kennedy et al. 2017). For the tomato-based products, the concentration increased with the processing steps. In this case, the paste presented the highest concentration, compared to the puree and other products (including whole tomatoes, juice, and pulp, among others). These results are in agreement with Bugianesi et al. who in 2004 demonstrated an increase in the levels of naringenin and chlorogenic acid in plasma, after the consumption of heat-treated tomatoes, compared with unprocessed fruit (Bugianesi et al. 2004). This could be explained considering the mechanical and thermal

Table 4. Quantities of bioactive compounds identified in tomato-based products and compared to FruitFlow® mg/kg of fresh product.

	Nuleosides/Nucleotides		Phenolic Acids and derivatives		Flavonoids and derivatives	
Product	Mean	Range	Mean	Range	Mean	Range
FruitFlow®	1880.8	1870.0 – 1891.6	292.7	288.8 – 296.6	78.4	73.9 – 82.9
Paste	2518.7	1352.1 – 3510.2	390.9	149.5 – 643.5	235.2	50.9 – 556.5
Puree	1184.4	581.4 – 2218.8	186.7	73.3 – 345.9	134.9	36.9 – 365.3
Others	1053.0	502.6 – 1797.6	143.0	45.5 – 530.0	74.4	17.1 – 388.4

conditions which tomatoes undergo to produce sauces and pastes that promote a release of those bioactive compounds, increasing their bioavailability (Kamiloglu et al. 2013; Martínez-Huélamo et al. 2015), in addition to the fact that pastes compared to other tomato-based products present a lower concentration of water, making the water-soluble fraction more concentrated.

Nucleotides and nucleosides

The concentration of Nucleotides/nucleosides for the evaluated products (tomato paste, tomato puree and other tomato-based products) was compared with the concentration of those available in FruitFlow®, finding a significant difference at a 0.05 level between samples. In more detail, the concentration of total nucleotides/nucleosides in tomato paste was significantly higher compared to FruitFlow®, with adenosine-5-monophosphate being the most abundant nucleotide and adenosine, uridine and guanosine the main nucleosides (Figure 1). Interestingly, guanosine was not detected in the FruitFlow® sample. Concentrations in tomato puree and other tomato products were significantly lower in comparison with the supplement. All the samples presented a similar distribution of bioactive molecules.

Nevertheless, after a comparison between the recalculated values considering the portion size recommended by the USDA and the dose suggested for FruitFlow® that supports the EFSA claim, (3 capsules per day), all tomato-based products were significantly higher than FruitFlow®, for all nucleotides and nucleosides (Figure 2).

Fuentes et al. in 2014 had demonstrated the protective effect on antiplatelet activation and *in vivo* thrombus formation of adenosine-5-monophosphate (AMP), showing that the increase in the concentration of AMP is correlated with the response in the selected markers evaluated during the study. These results provide an understanding of the AMP action mechanism (Fuentes et al. 2014). More recent studies have also demonstrated the effect of uridine triphosphate thio analogues on the inhibition of an adenosine diphosphate receptor responsible for platelet aggregation (Gündüz et al. 2017) as well as the association between cardiovascular risk factors and the guanosine phosphate pathway (Ying et al. 2019), showing their effect as cardio-protective agents.

Phenolic acids and derivatives

Chlorogenic, neochlorogenic and salicylic acids are the main representatives in all the different products evaluated. However, the distribution of these compounds differs between products: Tomato-based products presented chlorogenic acid as the main representative of phenolic acids, followed by salicylic and neochlorogenic acid. In contrast, FruitFlow® presented a high concentration of salicylic acid followed by smaller amounts of chlorogenic and neochlorogenic acids. A similar trend compared to the composition of nucleosides and nucleotides was found for phenolic acids: tomato paste presented a significantly higher concentration compared to FruitFlow®, despite the fact that tomato puree and other tomato-based products were significantly lower (Figure 3).

Considering the portion size (Figure 4), all tomato-based products presented a significantly higher concentration in all the phenolic acids and derivatives found compared to FruitFlow®. The effect of chlorogenic acid has been tested in different

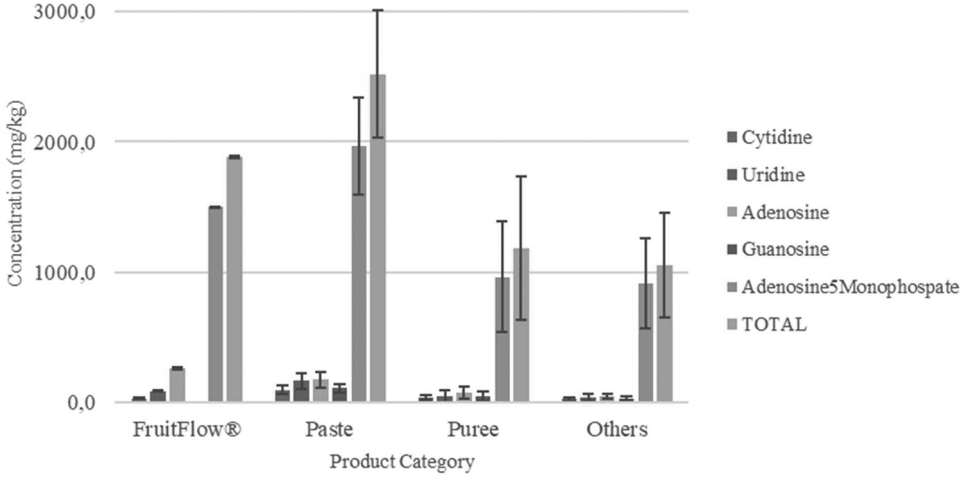

Figure 1. Nucleosides/nucleotides distribution for tomato-based products compared to FruitFlow®.

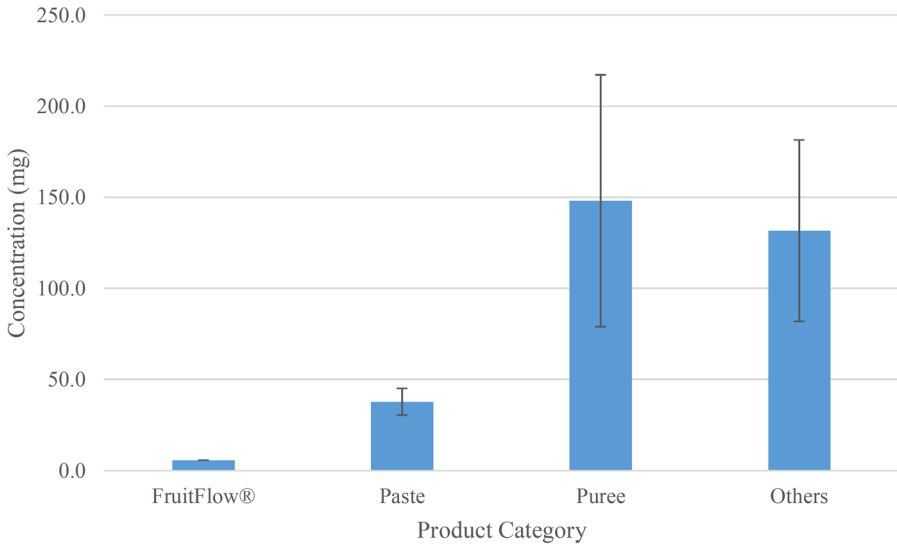

Figure 2. Nucleosides/nucleotides per portion size.

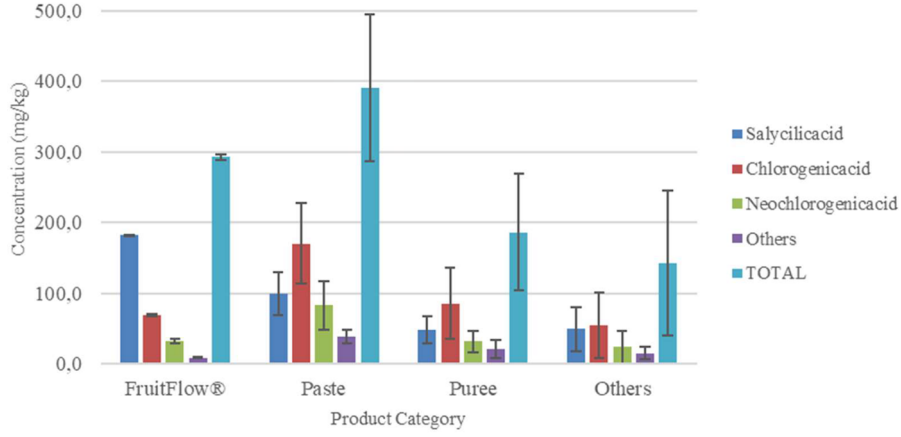

Figure 3. Phenolic acids and derivatives distribution for tomato-based products compared to FruitFlow®.

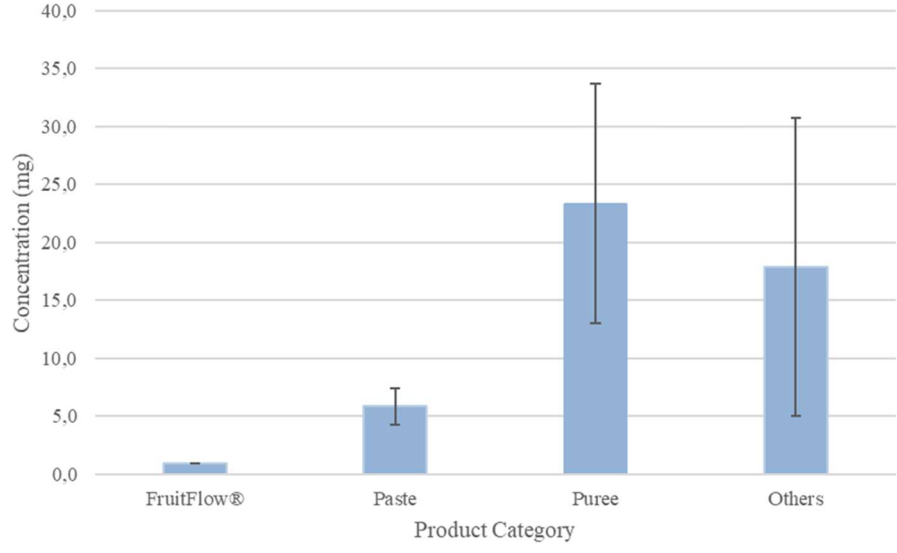

Figure 4. Phenolic acids and derivatives per portion size.

studies (Li et al. 2020). Amin et al. (2023), finding a linear positive correlation between platelet aggregation inhibition and chlorogenic acid concentration, extracted from W. tinctoria in an *in vitro* assay. Moreover, Fuentes et al. in 2014 elucidated the antithrombotic and antiplatelet mechanism of chlorogenic acid, associating it with a specific pathway (Fuentes et al. 2014).

Flavonoids and derivatives

The distribution of flavonoids followed a different pattern compared to phenolic acids and nucleosides. All tomato-based products presented a significantly higher mean value of flavonoids compared to FruitFlow® (Figure 5); compared to the individual flavonoids distribution, despite the tomato-based products and FruitFlow® presenting a different distribution, both presented Rutin as the main constituent, accounting for about 50% of total flavonoids (data not shown).

Consequently, in a comparison based on recommended daily intake, similar results were found (Figure 6). Significant differences were found between FruitFlow® and tomato-based products, with all presenting higher values compared to the food supplement. Different authors have studied the role of flavonoids in platelet aggregation (including the beneficial effects on lipid metabolism, their capability to potentiate the endothelial and vascular function and to reduce cell adhesion (Guerrero et al. 2005; Kim and Yun-Choi 2008; Faggio et al. 2017). More specifically, the effect of rutin as an antiplatelet aggregation ingredient, elucidating its action mechanism in human platelets (Sheu et al. 2004; Dar and Tabassum 2012).

The biomolecules identified in each cluster and recalculated according to the recommended serving size are presented in Table 5. It is clear that in all cases, tomato-based products provide at least a similar but generally a much higher quantity of bioactive molecules than those provided by the FruitFlow® supplement. The differences are more evident for those molecules presented previously as the major component in each category. These values represent the mean value per category, meaning they are subjected to the high variation presented between samples, due to the differences in origin that translates as a difference in biomolecule content related to the intrinsic variation of vegetable products but also the solids content allowed in different locations, making samples more or less concentrated.

4. Conclusions

Epidemiological findings confirm the beneficial effects on human health of different antioxidants contained in tomato fruits, whose amount and bioavailability can be modified by mechanical and heat treatments as well as the addition of ingredients such as oil or salt, characterising the industrial transformation into the final products (Martínez-Huélamo et al. 2015). It was found that the content of antioxidants is significantly affected by the genotype, their content being mostly concentrated inside the skin, followed by pulp, and it is important to understand how these compounds vary during and after the processing phases (George et al. 2004).

According to the obtained results in this sample study, it is possible to conclude that the tomato-based products considered (Pastes, Purees, Others), show a

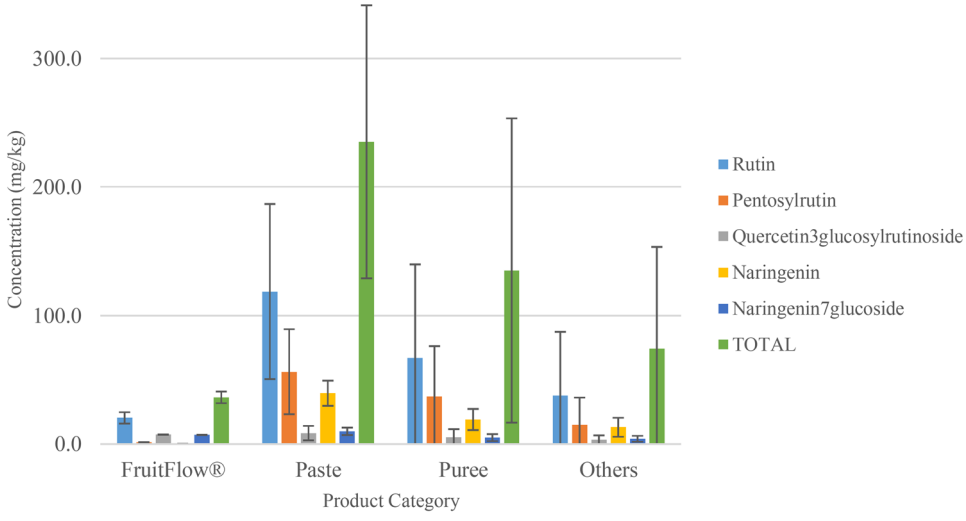

Figure 5. Flavonoids and derivatives distribution for tomato-based products compared to FruitFlow®.

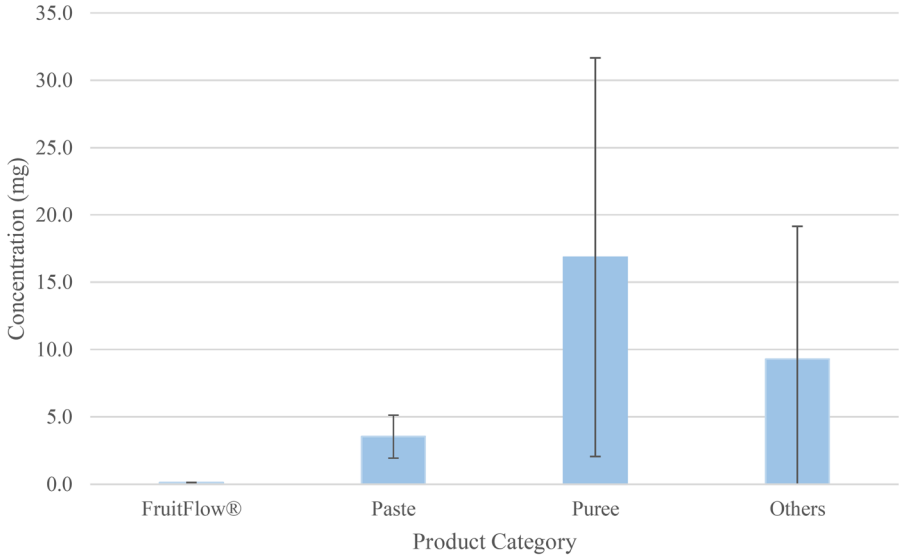

Figure 6. Flavonoids and derivatives per portion size.

Table 5. Bioactive compounds identified in tomato-based products and compared to FruitFlow® per serving size (mg).

	FruitFlow®	Paste	Puree	Others
	3 Capsules (3g)	1 tbsp. (15g)	1/2 cup (125g)	1/2 cup (125g)
Cytidine	0.1	1.49	5.05	3.24
Uridine	0.27	2.52	6.20	4.41
Adenosine	0.78	2.65	9.68	6.01
Guanosine	<0.01	1.66	8.77	3.87
Adenosine-5-monophosphate	4.49	29.46	120.64	114.08
Salicylic acid	0.515	1.49	6.00	6.23
Protocatechuic acid	<0.01	0.01	0.06	0.04
Caffeic acid	0.0015	0.03	0.07	0.07
Caffeic acid hexoses	0.0072	0.25	1.04	0.77
Chlorogenic acid	0.22	2.55	10.67	6.84
Neochlorogenic acid	0.097	1.24	3.99	2.94
p-coumaric acid	<0.01	0.01	0.03	0.02
p-coumaric acid hexoses	0.018	0.11	0.62	0.37
p-coumaroylquinic acids	<0.01	0.02	0.07	0.04
Ferulic acid hexoses	<0.01	0.16	0.80	0.54
Quercetin	<0.01	0.01	0.04	0.06
Rutin	0.06	1.78	8.39	4.73
Pentosyl rutin	0.004	0.84	4.62	1.87
Quercetin-3-glucosyl-rutinoside	0.022	0.13	0.66	0.42
kaempferol-3-rutinoside	<0.01	0.02	0.12	0.06
Naringenin	<0.01	0.59	2.39	1.64
Naringenin-7-glucoside	0.02	0.15	0.64	0.51

significantly higher concentration of water-soluble bioactive molecules (nucleosides/nucleotides and polyphenols), responsible for an anti-platelet aggregation effect, than the same compounds assessed in FruitFlow® capsules, considering the recommended portion size suggested by the USDA.

The same bioactive components evaluated in WSTC/FruitFlow® are also present (in higher concentration) in tomato products, with the same potential beneficial health effect. However, we should consider that beyond these water-soluble bioactives there are many other important bioactive compounds in processed tomato products (carotenoids, vitamin E, Potassium, etc.) that are not present in the FruitFlow® supplements, which therefore cannot exert these very important synergistic health effects.

However, the situation is more complex. Not all the constituents in the fruit may synergistically interact with the bioactive compounds responsible for antiplatelet aggregation; some could have an antagonistic effect, potentially reducing the expected health benefits. Additionally, there are challenges in standardising food products, considering both the inherent variation from different crops and the difficulties in consistently controlling the composition of specific bioactive molecules during industrial processing.

With this work, we were able to demonstrate a direct correlation between WSTC and tomato derivatives that

showed in a single standard serving of the tomato products examined a sufficient concentration of bioactive compounds needed to produce the claimed health effect on platelet aggregation recognised by EFSA.

This study was conceptually built for the entire tomato supply chain (from seed producers to packaging producers, even to large-scale retail trade), the data produced are able to scientifically demonstrate, once more, the positive correlation between tomato consumption and the improvement of human health, and indeed the inevitability of increasing its conscious consumption per capita globally.

The Mediterranean Diet has always been recognised as healthy, and the consumption of tomato products has been always part of it. We want to demonstrate that although some human clinical trials have been undertaken with tomato products, there is a great need for a well-designed human intervention study that takes into consideration the capacity of the bioactive native molecules present in tomato products and their synergistic effect in human health.

Thanks to these results, the next step of the ongoing research will be to organise specific human trials to prove the anti-platelet health benefits of tomato products and their efficacy in the battle against CVD and emerging health threats.

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Author contributions

L.S., D.S. and J.H. designed and conceived the research. E.C., C.S., R.V., F.D.S, M.T.R. provided the experimental data on bioactive substances, E.C. and C.S. performed the statistical analyses, L.S., A.M.B., D.S. and J.H. edited the manuscript.

Conflicts of interest

We declare that there are no conflicts of interest.

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