



(Poly)phenolic characterization of three food supplements containing 36 different fruits, vegetables and berries

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ABSTRACT

There is continuously accruing evidence to suggest the health benefits of consuming fruits and vegetables. Food supplements may represent an effective and acceptable method to deliver a way of providing bioactive compounds to consumers. The aim of this work was to characterize the (poly)phenolic composition of three plant-based food supplements designed to integrate and increase the daily intake of dietary phenolics. The supplements are blends of berries, fruits, or vegetables made from a total of 36 different edible food plants. The best conditions for phenolic extraction were assessed and the total phenolic content of each supplement was estimated (it ranged from 50 to 176 mg/g powder). The analysis of the three supplements by uHPLC–MSⁿ allowed to tentatively identify in all 119 (poly)phenolic compounds belonging to different classes, namely ellagitannins, gallotannins, dihydrochalcones, flavan-3-ols including proanthocyanidins, flavanones, flavones, flavonols, anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids, phenylethanoids, and lignans. The contribution of these food supplements to the daily intake of (poly)phenolic compounds and, in turn, the potential contribution of such intake to health were discussed.

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1. Introduction

High consumption of plant based foods is recommended in dietary guidelines worldwide [1]. Their high daily intake promotes human health, playing an important role in prevention of chronic diseases [2]. The consumption of 5 portions or at least 400 g of fruits and vegetables daily is the ideal target for many national nutrition societies and public policies [1,3]. The amount of daily plant based food has been associated with a reduction in all-cause mortality [4], a reduction in hypertension, coronary heart disease and stroke risk [2], and a decrease in overall cancer risk [5], with specific effects on colorectal [6], esophageal and gastric cancer [7]. In addition, the consumption of fruits and vegetables has been

proven to reduce the risk of certain eye diseases, dementia, osteoporosis, and metabolic diseases like type II diabetes [8,9].

The nutritional relevance of fruits, vegetables and their derived products seems to be linked to a range of micronutrients and non-nutrient bioactive compounds, including phytochemicals such as (poly)phenolic compounds and carotenoids, vitamins (mainly vitamin C, folate, and provitamin A), minerals as potassium, calcium, and magnesium, and dietary fibre [10,11]. Among them, (poly)phenolic compounds are maybe the most investigated components in plant foods. They are principally represented by phenolic acids, flavonoids, and tannins [11] and their dietary intake has been related to beneficial health effects, including reduction of inflammation, hypertension, and risk of cardiovascular diseases, neurodegenerative diseases and cancer [12,13]. This fact turns likely this wide class of plant compounds into the best candidates to justify the benefits associated with plant-based diets.

Consumers are becoming more aware of the importance of consuming a healthy diet due to health promotion and are inclined to buy products rich in bioactive compounds [14]. Among other reasons, lifestyle changes and consumer health demands have boosted the market of plant-based nutraceuticals and food supplements in recent years. These products represent new ways

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of providing bioactive compounds to consumers, becoming a relevant strategy to ideally guarantee the health benefits attributed to plant foodstuffs [15]. Despite the fact that most of the food supplements present on the market to date are merely faint reproductions of the phytochemical richness spread in plant foods, new technologies, new approaches and, hence, new products with very relevant nutritional characteristics are becoming available. The aim of the present study was to characterize the (poly) phenolic profile of three different plant-based food supplements designed to integrate and increase the daily intake of dietary phenolics. They are made from a total of 36 different vegetal matrices and have shown to exert wide biological effects in diverse health conditions [16–20].

2. Materials and methods

2.1. Samples and chemicals

Samples, named Juice PLUS+[®] Vineyard (a berry blend, JBB), Juice PLUS+[®] Fruit Blend (JFB) and Juice PLUS+[®] Vegetable Blend (JVB), were kindly supplied by Juice PLUS+[®] company. The powder samples differed for their composition: JBB contained 850 mg of dried powder blend of juice and pulp from grapes and berries (45.7%) including Concord grape, blueberry, cranberry, blackberry, bilberry, raspberry, redcurrant, blackcurrant, elderberry, in varying proportions, besides green tea, ginger root, grape seed, artichoke leaf powder, cocoa powder, pomegranate powder; JFB contained 850 mg of dried powder blend of juice and pulp (52%) of apple, orange, pineapple, cranberry, peach, acerola cherry, papaya, in varying proportions, besides beet root powder, date fibre and prune fibre; and JVB contained 750 mg of dried powder blend of juice and pulp (60%) of carrot, parsley, beet, kale, broccoli, cabbage, tomato, and spinach, in varying proportions, as well as sugar beet fibre, garlic powder, oat bran fibre and rice bran. Moreover, each powder was enriched with vitamins (vitamin C, vitamin E and folic acid) and carotenoids (β -carotene). All chemicals and solvents were of analytical grade. All solvents and reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Ultrapure water from MilliQ system (Millipore, Bedford, MA, USA) was used throughout the experiment.

2.2. Sample extraction

The dried powder contained in each gelatine capsule of JBB, JFB and JVB was separately extracted using four different methods in order to select the most effective one.

2.2.1. Aqueous extraction method

An aliquot of 0.5 g of powder was extracted with 5 mL of acidified water (0.1% formic acid). The samples were left 5 min in a sonic bath, vortexed vigorously for 2 min and then centrifuged for 10 min at 2000 \times g. The supernatant aqueous extract was collected and kept at -80°C until analysis.

2.2.2. Warm aqueous extraction method

According to the method previously reported [21], 0.5 g of powder was extracted with 5 mL of acidified water (0.1% formic acid). The samples were kept for 30 min in a sonic bath, then heated at 70°C for 1 h in a Dubnoff bath at 20 strokes/min. At the end of the extraction procedure, the samples were centrifuged for 10 min at 2000 \times g, the supernatant was removed and kept at -80°C until analysis.

2.2.4. Methanolic extraction method

An aliquot of 0.5 g of powder was extracted with 5 mL of methanol/acidified water (0.1% formic acid) (50:50 v/v). The

samples were left 5 min in a sonic bath, vortexed vigorously for 2 min and then centrifuged for 10 min at 2000 \times g. The supernatant was collected and kept at -80°C until analysis.

2.2.5. Sonic bath methanolic extraction method

According to the method previously reported [21], 0.5 g of powder were extracted with 5 mL of methanol/acidified water (0.1% formic acid) (50:50 v/v). The samples underwent sonic bath extraction for 15 min, followed immediately by 15 min of vortex mixing. This procedure was repeated twice. The extracts were then centrifuged for 10 min at 2000 \times g and the collected supernatants were stored at -80°C prior analysis.

2.3. Folin–Ciocalteu assay

Both aqueous and methanolic extracts were analysed by the Folin–Ciocalteu assay [22] to determine the total phenolic content. Values were expressed as (+)-catechin equivalents. The results were used to determine the best extraction method.

2.4. Identification of phenolic compound by uHPLC–MSⁿ analysis

According to the Folin–Ciocalteu assay results, the aqueous extract was analysed by ultra-high performance liquid chromatography coupled with mass spectrometry, to characterize their phenolic profile. An Accela ultra-high performance liquid chromatography (uHPLC) 1250 apparatus equipped with a linear ion trap mass spectrometer (LIT-MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-ESI (H-ESI-II) probe (Thermo Fisher Scientific Inc.) was used. Separations were carried out by means of a BlueOrchid C18 (50 \times 2 mm) column, 1.8 μm particle size (Knauer, Berlin, Germany). To detect different phenolic classes, three different analytical methods were used [23]. Anthocyanins were detected in positive ionization mode, with mobile phase, pumped at a flow-rate of 0.3 mL/min, consisting of a mixture of acidified acetonitrile (0.1% formic acid) (solvent A) and 0.1% aqueous formic acid (solvent B). Following 2 min of 5% solvent A in B, the proportion of A was increased linearly to 30% over a period of 10 min, followed by 3 min of 80% solvent A and then 5 min at the start conditions to re-equilibrate the column. The H-ESI-II interface was set to a capillary temperature of 275°C and the source heater temperature was 300°C . The sheath gas (N_2) flow rate was set at 40 (arbitrary units) and the auxiliary gas (N_2) flow rate at 5. During anthocyanin analysis, the source voltage was 4.5 kV, and the capillary voltage and tube lens voltage were 20 and 95 V, respectively. Initially, a preliminary analysis of 5 μL of aqueous samples was carried out using full-scan, data-dependent MS³, scanning from a mass to charge (m/z) of 100–1000 using a collision induced dissociation (CID) equal to 35 (arbitrary units) to obtain fragmentation. After this first step, further specific MS² analyses were carried out to unambiguously identify the compounds revealed in the first step, by monitoring specific m/z transitions. Molecules were fragmented using pure helium (99.99%), with a CID setting of 15 for the production of the molecular ion, and 35 for subsequent fragmentations. Identification was performed by comparison with literature [23,24].

For all other phenolic classes, spectrometric analyses were performed in negative ionization mode using two different tune methods, whereas the chromatographic gradient was maintained. The mobile phase, pumped at a flow rate of 0.3 mL/min, consisted of 5% of solvent A for 3 min, changing then linearly from 5 to 40% solvent A in B over 9 min. After 4 min of 80% solvent A, the column was re-equilibrated for 5 min at the start conditions.

For the first method, H-ESI-II interface was set to a capillary temperature of 275°C and the source heater temperature was 200°C . The sheath gas (N_2) flow rate was set at 40 (arbitrary units)

and the auxiliary gas (N₂) flow rate at 5. The source voltage was 4 kV, the capillary voltage was –42 V and tube lens voltage was –118 V. For the second method, the H-ESI-II interface was set to a capillary temperature of 275 °C and the source heater temperature was 250 °C. The sheath gas (N₂) flow rate was set at 60 (arbitrary units) and the auxiliary gas (N₂) flow rate at 15. The source voltage was 4 kV, the capillary voltage was –49 V and tube lens voltage was –153 V. As for the anthocyanins, a preliminary analysis of phenolic compounds in the aqueous samples was carried out using full-scan, data-dependent MS³, scanning from an *m/z* ratio of 100–1500. After this first step, specific experiments reaching up to 3 consecutive fragmentations were carried out to unambiguously identify the compounds revealed by the initial analysis. Molecules were fragmented using pure helium (99.99%), at a CID setting of 30 for the production of the molecular ion and for the subsequent fragmentation. All compounds were identified by comparing with mass spectral data reported in literature.

3. Results and discussion

Total phenolic content of the aqueous and methanolic extracts of the plant-based food supplements was measured (Fig. 1). This assay allowed not only to determine the richest powder in term of phenolic substances, but also to discriminate the best extraction procedure. The aqueous extraction method at room temperature resulted in the highest rate of phenolic compound extraction and was selected for the uHPLC–MSⁿ analyses. Furthermore, as Fig. 1 depicts, JBB showed the highest total phenolic content (176 mg/g for the aqueous extraction), followed by JFB (128 mg/g for the aqueous extraction) and JVB (50 mg/g for the aqueous extraction). The analysed products, in particular JBB and JFB, exhibited an extremely high total phenolic content when compared to common food items [25]. They were only comparable to cloves. In contrast, JVB showed a total phenolic content similar to those of many dried spices such as dried spearmint, dried sweet basil, and Ceylan cinnamon [25]. Although the powders included non-phenolic constituents like vitamins, which have been reported to react with the Folin–Ciocalteu assay and could lead to overestimation [25], the high phenolic content of these plant-based food supplements may account for its usefulness to increase the daily intake of dietary phenolics.

The phytochemical fingerprint of the food supplements was assessed using a new procedure for non-targeted screening of

(poly)phenolic compounds by uHPLC–MSⁿ consisting in three different MS operating conditions [23]. The compounds were tentatively identified based on the interpretation of their mass spectral behaviour obtained from MS² and MS³ experiments and by comparison with literature [21,23,26–41]. The characterization of the complete (poly)phenolic profiles of JBB, JVB, and JFB are reported in Tables 1–4.

A total of 119 compounds were tentatively identified. JBB showed the richest profile, with 75 identified compounds belonging to different phenolic classes, namely ellagitannins, gallotannins, dihydrochalcones, flavan-3-ols, flavanones, flavones, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, phenylethanoids (Table 1), and anthocyanins (Table 4). The comprehensive evaluation of JVB allowed the tentative identification of a total of 28 compounds (Table 2), with a relevant predominance of flavonols, whereas 25 compounds belonging to different phenolic classes (Table 3), including anthocyanins (Table 4), were identified in JFB.

It is important to consider the large variety of (poly)phenols within the analysed capsules. JBB (Tables 1 and 4), produced from 15 fruits, contained a large number of ellagitannins, characteristic of berries and pomegranate [15], as well as anthocyanins and proanthocyanidins, which can be found in both berries and red grapes [15,33]. Together with ubiquitous flavonols like quercetin derivatives [28], JFB (Tables 3 and 4), composed of 10 different fruits, contained flavones such as luteolin, apigenin, diosmetin, and chrysoeriol C-linkage derivatives and flavanones such as hesperetin and naringenin O-derivatives, main polyphenolic compounds of oranges [42]. Considering the lower consumption of vegetables in contrast to fruits [43], the phytochemical profile of JVB (Table 2), composed of 12 vegetables, is also a point worth mentioning. The intake of JVB would supply flavones as luteolin, apigenin, diosmetin, and chrysoeriol derivatives, which are common in parsley [40], spinacetin and patuletin derivatives, typical for spinach [39], and flavonols such as kaempferol and quercetin linked to sugar moieties and phenolic acids, which are commonly found in broccoli [27]. In addition, two glucosinolates, nitrogen-sulphur compounds present in *Brassica* plants [44], were tentatively identified in JVB. Overall, the daily consumption of these three food supplements made from a total of 36 vegetal species – cranberry is present in both JBB and JFB – would broaden the array of phenolic compounds ingested, maximizing the putative health effects of these compounds [13] on the basis of a regular consumption.

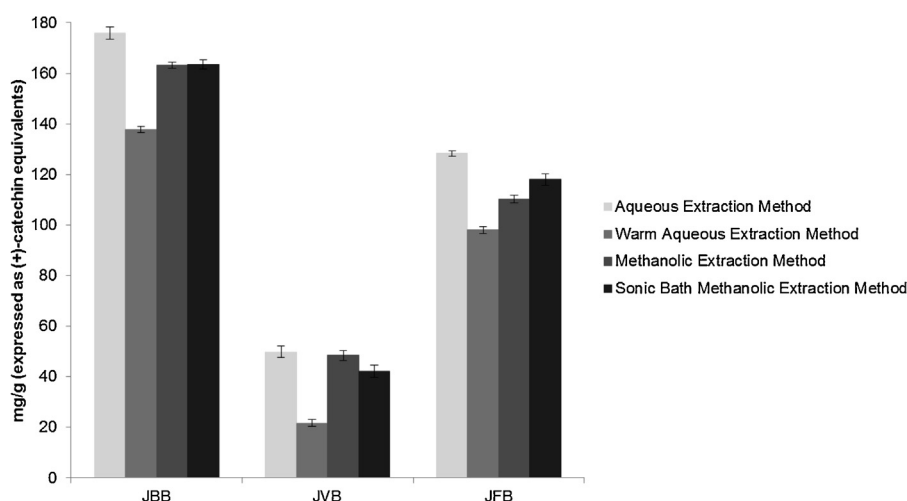


Fig. 1. Total phenolic content of juice PLUS+[®] berry blend (JBB), Juice PLUS+[®] vegetable blend (JVB) and juice PLUS+[®] fruit blend (JFB). Values are expressed as mean ± SD (*n* = 3).

Table 1
Mass spectral characteristics of (poly)phenolic compounds identified in Juice PLUS+[®] berry blend (JBB). The ions in parentheses are fragmented in MSⁿ experiments; HHDP: hexahydroxydiphenyl; (epi)GC: (epi)gallicocatechin.

Compound	RT	[M-H] ⁻ (m/z)	MS ² ions (m/z)	MS ³ ions (m/z)	MS ⁴ ions (m/z)
Ellagitannins					
Galloyl-HHDP-hexoside	1.20	633	301, 249, 275, 463	(301) 301, 257, 229	
Galloyl-HHDP-hexoside	1.96	633	301, 275, 249	(301) 301, 257, 229	
Galloyl-HHDP-hexoside	4.40	633	301, 421, 275, 615	(301) 301, 257, 229	
Galloyl-HHDP-hexoside	6.55	633	301, 302, 463	(301) 301, 257, 229, 185	
Punicalin	1.62	781	601, 721, 602, 575	(601) 299, 271, 601, 300, 301	
Bis-HHDP-hexoside (pedunculagin I isomer)	1.72	783	481, 721, 765, 507, 275, 437, 299, 301, 341, 329	(481) 437, 419, 299, 329, 463	(765) 721, 419, 463, 703, 341, 747, 329
Bis-HHDP-hexoside (pedunculagin I isomer)	2.50	783	301, 481, 275	(301) 301, 257, 229	
Bis-HHDP-hexoside (pedunculagin I isomer)	5.41	783	301, 481, 275, 302	(301) 301, 257, 229	
Digalloyl-HHDP-hexoside (pedunculagin II)	6.87	785	483, 301, 633, 419, 249, 275, 615, 313, 331, 741	(483) 331, 313, 169, 193, 223	
Punicalagin isomer	2.70	1083	1065, 601, 807, 1021, 721, 959, 1003, 635, 1047, 575		
Punicalagin	5.39	1083	781, 601, 575, 549, 721, 1065, 763, 745	(781) 601, 721, 575	
Punicalagin	6.27	1083	781, 601, 575, 721, 549, 763, 719	(781) 601, 721, 575	
Gallotannins					
Digalloylglucose	6.39	483	271, 331, 313, 272	(271) 211, 169	
Trigalloylglucose	7.74	635	465, 483, 466, 313, 484, 271	(465) 313, 295, 169	
Dihydrochalcones					
Phloretin	9.43	273	167	(167) 123, 125	
Flavan-3-ols					
(+)-Catechin	6.16	289	245, 205, 179, 231, 203, 247	(245) 203, 227, 187, 161, 188, 217, 175, 230	
(-)-Epicatechin	7.02	289	245, 205, 179, 247, 231, 203	(245) 203, 227, 188, 187, 161, 217, 230, 175	
(+)-Gallocatechin	2.80	305	179, 221, 219, 261, 165, 137, 247, 125, 287, 161	(179) 164, 151, 135, 137	
(+)-Epigallocatechin	6.06	305	179, 221, 219, 261, 165, 137, 125, 247, 287, 137	(179) 164, 151, 135, 137	
(-)-Epicatechin-3-O-gallate	8.37	441	289, 331, 167, 271, 397, 423	(289) 245, 205, 179, 231, 247	
(+)-Catechin-3-O-gallate	8.50	441	289, 331, 167, 271, 397, 423	(289) 245, 205, 179, 231, 247	
(-)-Epigallocatechin 3-O-gallate	7.20	457	169, 331, 305, 287, 269	(169) 125	
(+)-Gallocatechin 3-O-gallate	7.60	457			
(Epi)gallocatechin-O-hexoside	2.42	467	305	(305) 179, 221, 219, 261, 165, 137, 247, 125, 287, 161	
(Epi)gallocatechin-3-O-methylgallate	8.09	471	305, 183	(305) 179, 221, 219, 261, 165, 137, 247, 125, 287, 161	
Procyanidin dimer B-type	6.81	577	425, 451, 407, 559, 289, 287, 299	(425) 407, 273, 381	
Prodelfphinidin dimer B-type [1 unit of (epi)GC]	4.52	593	575, 441, 305, 423, 467, 585, 425, 287		
Prodelfphinidin dimer B-type [1 unit of (epi)GC]	5.7	593	425, 467, 289, 407, 303, 423, 441		
Prodelfphinidin dimer B-type [1 unit of (epi)GC]	5.98	593	575, 423, 467, 441, 305, 425, 287		
Prodelfphinidin dimer B-type [2 units of (epi)GC]	2.01	609	441, 305, 423, 483, 565, 444		
Prodelfphinidin dimer B-type [2 units of (epi)GC]	5.18	609	441, 423, 305, 591, 483, 471	(441) 423, 273	
(Epi)gallocatechin-derivative	5.51	611	305, 485	(305) 179, 221, 219, 261, 165, 137, 247, 125, 287, 161	
Prodelfphinidin dimer B-type gallate [1 unit (epi)GC]	6.76	745	575, 593, 423, 619, 727, 467, 405, 457, 449	(575) 423, 449, 557, 287	(593) 575, 423, 467, 405, 305
Prodelfphinidin dimer B-type gallate [1 unit (epi)GC]	6.90	745	593, 575, 423, 619, 727, 457, 467, 557, 305, 577	(593) 575, 467, 423, 305, 549, 217, 397	
Prodelfphinidin dimer B-type gallate [2 units (epi)GC]	6.35	761	593, 609, 423, 575, 591, 635, 483, 465, 743, 457	(593) 575, 423, 405, 441	(609) 591, 483, 305, 303, 565, 177, 439
Prodelfphinidin dimer B-type gallate [2 units (epi)GC]	6.26	761	609, 423, 593, 591, 635, 575, 743	(609) 591, 305, 483, 303, 591, 453, 177	
Procyanidin trimer B-type	7.49	865	695, 577, 739, 713, 575, 425, 847, 407, 587, 451	(695) 543, 405, 525, 451, 677, 243, 289, 363, 407, 299	(739) 449, 577, 407, 287, 587, 435, 695, 451, 588, 425
Flavone					
Apigenin-C-pentoside-C-hexoside	7.83	563	443, 473, 503, 545, 383, 444, 353, 474, 504, 455	(443) 353, 383, 425, 354	(473) 353, 383, 413
Apigenin-C-hexoside-O-hexoside	8.18	593	413, 293, 473	(413) 293	
Apigenin derivative	9.75	605	269, 473, 563, 315, 545	(269) 269, 225, 197, 199	
Flavonols					
Kaempferol	11.58	285	285, 241, 257, 187		
Myricetin	9.28	317	179, 151		
Quercetin-O-pentoside	8.96	433	301	(301) 179, 151, 273	
Kaempferol-3-O-galactoside	8.88	447	285, 284, 327, 255, 283, 301, 151		

Table 1 (Continued)

Compound	RT	[M-H] ⁻ (m/z)	MS ² ions (m/z)	MS ³ ions (m/z)	MS ⁴ ions (m/z)
Kaempferol-3-O-glucoside	9.04	447	285, 284, 327, 255, 283, 301, 151	(285) 257, 285, 267, 256, 229, 241, 163, 151 (285) 257, 285, 267, 256, 229, 241, 163, 151	
Quercetin-3-O-galactoside	8.51	463	301, 300, 302	(301) 179, 151, 273, 229, 257	
Quercetin-3-O-glucoside	8.6	463	301, 300	(301) 179, 151, 257, 273, 193	
Myricetin-3-O-galactoside	7.9	479	316, 317, 179	(316) 271, 179, 270, 287, 151, 288	(317) 179, 151, 192, 193
Myricetin-3-O-glucoside	7.96	479	317, 316, 318, 461, 179	(317) 179, 151, 192, 272, 288	
Syringetin-O-hexoside	9.12	507	344, 345, 387	(344) 316, 301, 330, 273	
Quercetin derivative	10.89	567	301, 300, 445	(301) 179, 151, 272	
Kaempferol-3-O-rutinoside	8.89	593	285	(285) 257, 267, 229, 241, 197, 199, 213	
Quercetin-3-O-rutinoside	8.48	609	301, 300, 457, 302, 321	(301) 179, 151, 273, 193, 257, 272, 283	
Kaempferol rhamnoside-hexoside-rhamnoside	8.59	739	285	(285) 257, 151, 285, 213, 267, 197, 241, 229, 223, 163	
Kaempferol rhamnoside-hexoside-rhamnoside	8.77	739	285, 257	(285) 257, 229, 151, 267, 285, 197, 241	
Kaempferol 3-O-galactosylrutinoside	8.45	755	285	(285) 285, 151, 257, 241, 229, 199, 267, 197, 213, 239	
Kaempferol 3-O-glucosylrutinoside	8.68	755	285, 593, 286	(285) 257, 285, 151, 241, 163, 229, 240, 267	
Quercetin 3-O-galactosylrutinoside	8.13	771	301, 343, 609, 302	(301) 179, 151, 273, 257, 239, 107, 192, 211	
Quercetin 3-O-glucosylrutinoside	8.27	771	301, 609, 343, 302, 271	(301) 179, 151, 257, 192, 273, 228, 301	
Kaempferol-rutinoside-acetylhexoside	9.09	797	755, 285, 737, 756, 738	(755) 285, 593	(285) 267, 229, 151, 241, 257
Kaempferol-rutinosyl-dirhamnoside	10.8	885	739, 431	(739) 285, 593, 575, 453	
Kaempferol-rutinoside-hexoside-rhamnoside	9.45	901	755, 285, 615, 828, 513	(755) 285, 609	
Flavanone					
Hesperidin (hesperetin-7-O-rutinoside)	9.16	609	301	(301) 286, 242, 283, 257, 258, 125, 199, 227, 215	
Hydroxybenzoic acids					
Vanillic acid	3.13	167	123, 152, 108		
Gallic acid	1.84	169	125		
5-Galloylquinic acid (Theogallin)	1.88	343	169, 191		
Hydroxycinnamic acids					
Coumaroyl quinic acid	5.75	337	163, 173, 119, 191	(163) 119	
4-Coumaroyl quinic acid	7.02	337	173, 163, 191,	(173) 93, 111, 155, 109, 71, 99, 83	
Phenylethanoids					
Hydroxytyrosol	3.25	153	123		
Hydroxytyrosol-O-hexoside	2.14	315	153		

Despite the importance attributed to (poly)phenolic compounds in the framework of human health, robust information on their dietary intake is still lacking. This may be justified by some limiting factors usually associated with food components databases, like intrinsic variability among fruit and vegetable cultivars, years, and growing conditions or modifications in the phenolic levels due to food processing [45]. However, in spite of the obvious socio-demographic, lifestyle, and anthropometric differences [46], the mean intakes of phenolic compounds reported in literature are not so different from each other. Scalbert and Williamson reported an average polyphenol daily intake of ~1000 mg/d, among which fruit and beverages, in particular fruit juice, wine, tea, coffee, chocolate, and beer, and, to a lesser extent, vegetables, dry legumes, and cereals, were considered the main sources [47]. Finnish adults introduce approximately 863 mg of phenolics per day [48]. Among all phenolic classes consumed in Finland, phenolic acids represented the predominant group (75% of total phenolic intake), derived principally from coffee and cereals. Proanthocyanidins and anthocyanins accounted for the 14% and 6% of total phenolic intake, respectively, and their contribution was mainly due to berries, berry products, and fruit intake. Finally, a minor percentage of flavonoids (5%) such as flavonols, flavanones, and flavones, was related to apple, citrus fruit, and tea consumption [48]. A higher (poly)phenolic intake was originally reported for the Spanish population in 2007, ranging

between 2590 and 3016 mg/d [49], whereas the more recent PREDIMED study reduced this estimation to 820 mg/d, with 443 mg/d of flavonoids and 304 mg/d of phenolic acids, with hydroxycinnamic acids as the highest phenolic subclass consumed (276 mg/d) [43]. In this case, fruits were the main source of polyphenols (44%) and vegetables provided more than 12% of the total phenolic intake, representing the third source of phenolic acids. Coffee represented an important source of phenolic compounds providing 55% of total phenolic acids. Alcoholic beverages, cereals, olive oil, cocoa products, nuts and seeds, and legumes contributed for less than the 10% of the total polyphenol intake [43]. In France, the daily polyphenol consumption was estimated to be equal to 1193 mg/d [50], primarily due to non-alcoholic beverages (mainly coffee and tea) and fruits, followed by alcoholic beverages (mainly wine), cocoa products, vegetables, cereals, seeds and oils. Fruit was the main source of flavonoids (35%), while non-alcoholic beverages were the most important sources of phenolic acids (nearly 80%) [50]. Therefore, considering the reported mean values of phenolic intake among different countries, the daily consumption of 6 gelatine capsules –2 of each product (which corresponds to ~600 mg of phenolic compounds), which has been used as a dose in the most recent intervention studies applying these supplements [19], would contribute to notably enhance the daily average (poly)phenolic ingestion.

Table 2
Mass spectral characteristics of phytochemical compounds identified in Juice PLUS+[®] vegetable blend (JVB). The ions in parentheses are fragmented in MSⁿ experiments.

Compound	RT	[M-H] ⁻ (m/z)	MS ² ions (m/z)	MS ³ ions (m/z)	MS ⁴ ions (m/z)
Dihydrochalcones					
Phloretin-2'-O-(2''-O-xylosyl)glucoside	8.94	567	273	(273) 167, 125	
Flavone					
Apigenin-7-apiosylglucoside (apiin)	9.05	563	269, 431, 270, 443	(269) 269, 225, 149, 183	
Diosmetin-O-apiosylglucoside	9.17	593	299, 284, 461, 285, 341, 473	(299) 284	
Chrysoeriol-O-apiosylglucoside	9.27	593	299, 284, 300	(299) 284	
Diosmetin-7-O-(2-apiosyl-6-acetyl)glucoside	9.79	635	299, 593, 284, 503, 575, 285, 473, 341	(299) 284	
Chrysoeriol-7-O-(2-apiosyl-6-acetyl)glucoside	9.86	635	299, 284, 593, 341, 575	(299) 284	
Flavonols					
Kaempferol-3,7-di-O-glucoside	6.27	609	447, 285, 489	(447) 285, 284, 327	
Isorhamnetin-3,7-di-O-glucoside	7.28	639	477, 519, 315	(477) 314, 315, 357, 299, 286, 329	(315) 300, 285, 286, 271
Patuletin-3-O-gentiobioside (patuletin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside)	8.06	655	331, 316, 330, 373	(331) 316, 209	
Spinacetin-3-O-gentiobioside (spinacetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside)	8.63	669	345, 330, 287	(345) 330	
Kaempferol-3-O-sophoroside-7-O-glucoside	6.41	771	609	(609)	
Patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside (patuletin-3-O-β-D-glucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside)	7.81	787	331, 655, 330, 637, 505, 772, 315, 303, 625, 316	(331) 316	(655) 373, 316, 331, 330, 358, 374, 385
Quercetin-3-sophoroside-7-glucoside	6.15	787	625	(625) 300, 445, 301, 505, 463, 271, 343, 299, 179, 355	
Spinacetin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside (spinacetin-3-O-β-D-glucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside)	8.24	801	345, 651, 669, 344, 330, 387, 769, 302, 329, 786	(345) 330	
1,2'-Disinapoyl-2-feruloylgentiobioside	10.18	929	705, 511, 723	(705) 529, 499, 481, 511, 259, 427, 467, 469, 247, 367	
Kaempferol-3-O-(caffeoyl)sophoroside-7-glucoside	6.79	933	771	(771) 609	(609) 285 ^a ,429
Patuletin-3-O-β-D-(2''-β-coumaroylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside)	8.56	933	787	(787) 331, 655, 330, 637, 505, 373, 315, 316, 625, 772	
Kaempferol-3-O-(feruloyl)-sophoroside-7-O-glucoside	7.24	947	785	(785) 609, 623, 591, 299	(609) 285 ^a ,429
1,2,2'-Trisinapoylgentiobioside	10.01	959	735, 529, 511	(735) 529, 511, 497, 717, 223, 457, 247, 427	
Acylated kaempferol 3-sophoroside-7-glucoside (tentative kaempferol 3-hydroxyferuloylsophoroside-7-glucoside)	6.66	963	801	(801) 609, 623	
Patuletin-3-O-β-D-(2''-feruloylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside)	8.68	963	787, 801, 769	(787) 655, 637, 330, 331, 316, 315, 373, 505, 772	
Kaempferol-3-O-(sinapoyl)-sophoroside-7-O-glucoside	7.10	977	815	(815) 609, 623, 591	
Kaempferol-3-O-sinapoyltrigluconide-7-O-glucoside	7.04	1139	815, 977, 609	(977) 771,785,754	(815) 623,609,591,515
Hydroxycinnamic acids					
Coumaric acid	6.21	163	119	119	
5-Caffeoylquinic acid	6.32	353	191	(191) 127, 173, 111, 109, 171, 155	(173) 111,155,93,71
Lignans					
Pinoselinol-O-diglucoside	7.73	681	519, 315, 477, 561, 357	(519) 314, 315, 357, 459, 285, 271, 299, 329, 204	(357) 329, 342, 193, 271
Glucosinolate					
Glucobrassicin	4.27	447	259, 291, 269, 275, 367, 205, 169, 195, 227, 224	(259) 139, 97	
Neoglucobrassicin	7.47	477	446, 447	(446) 283, 259, 416, 224, 383	

^a MS⁵ fragments (257, 241, 151, 179, 213) served to confirm the presence of kaempferol.

During recent years, many phytochemicals have been diffused as food supplements among health-conscious consumers to achieve intake levels otherwise unattainable with standard diets. Pharmaceutical forms (pills, powders, capsules, etc.) as source of putative bioactive compounds have been largely diffused and phenolic compounds have become very relevant contributors to nutraceutical products. About this issue, certain scepticism should be maintained for two paramount reasons. First, the compliance between the labelled content and the amount of bioactives actually present in products is not always guaranteed. For instance, among 40 food supplements containing anthocyanins, only the 50% were

well labelled in terms of standardized content, while the content was negligible for the 12% of them [51]. Similar data have been reported for isoflavone-containing dietary supplements [52] and, concerning resveratrol, a recent study demonstrated that only 5 of 14 analysed brands had near label values, compliant with Good Manufacturing Practices requirements, and two samples were below the limit of detection [53]. The second reason deals with the bioavailability, metabolism, tissue distribution, dose/response and toxicity of these bioactive compounds in form of nutraceuticals, which have not been well established. A lot of the published scientific evidence derives from animal and *in vitro* assays, whereas

Table 3

Mass spectral characteristics of (poly)phenolic compounds identified in Juice PLUS+[®] fruit blend (JFB). The ions in parentheses are fragmented in MSⁿ experiments; HHDP: hexahydroxydiphenic acid.

Compound	RT	[M-H] ⁻	MS ² ions (m/z)	MS ³ ions (m/z)	MS ⁴ ions (m/z)
Dihydrochalcones					
Phlorizin (phloretin-2'-O-glucoside)	9.36	435	273	(273) 167, 125	
Ellagitannins					
Galloyl-HHDP-hexoside	6.50	633	301, 249, 275	(301) 301, 257, 213, 229, 185	
Flavan-3-ols					
Procyanidin trimer B-type	7.43	865	695, 577, 739, 713, 575, 425, 847, 407, 587, 451	(695) 543, 405, 525, 451, 677, 243, 289, 363, 407, 299	(739) 449, 577, 407, 287, 587, 435, 695, 451, 588, 425
Flavones					
Diosmetin-6-C-glucoside	8.59	461	341, 371	(341) 298, 313, 326	
Apigenin-6,8-C-diglucoside	7.25	593	473, 503, 353, 575, 383, 474	(473) 353, 383	
Luteolin-6,8-C-diglucoside	6.90	609	489, 519, 591, 399	(489) 369, 399, 471	
Luteolin-C-dihexoside	8.24	609	429, 285, 284, 447, 257, 255, 489	(429) 339, 309, 313, 327, 297, 257, 393, 411, 371, 381	
Diosmetin-6,8-C-diglucoside	7.47	623	503, 383, 533, 504, 413, 605	(503) 383, 413, 485	
Chrysoeriol-6,8-C-diglucoside	7.62	623	503, 383, 533, 504	(503) 383, 413, 485	
Flavonols					
Quercetin-3-O-arabinoside	8.71	433	301, 300	(301) 151, 179, 257, 273, 229, 193, 107	
Quercetin-3-O-xyloside	8.88	433	301, 300	(301) 179, 151, 257, 273, 229, 193, 107	
Quercetin 3-O-rhamnoside	9.00	447	301, 300	(301) 179, 151, 272, 283, 257, 193	
Quercetin-3-O-hexoside	8.45	463	301, 300	(301) 179, 151, 273, 257	
Myricetin 3-O-hexoside	7.84	479	316, 317	(316) 271, 270, 287, 179, 288, 151	
Quercetin-3-O-rutinoside	8.37	609	301, 300	(301) 179, 151, 257	
Isorhamnetin-O-rutinoside	8.91	623	315, 300, 316	(315) 300, 287	
Isorhamnetin-O-rutinoside (tentative)	9.70	623	315		
Flavanones					
Hesperidin-O-hexoside	8.12	463	301	(301) 286, 242, 283, 257, 125, 258, 199, 233	
Naringenin-7-O-rutinoside (narirutin)	8.79	579	271	(271) 151, 177	
Isosakuranetin-7-O-rutinoside (didymin)	10.35	593	285	(285) 270, 243, 164, 241, 285, 151, 217, 175, 226	
Hesperetin-7-O-rutinoside (hesperidin)	9.09	609	301, 286	(301) 286, 283, 242, 257, 125, 258, 199, 215	
Hesperetin-O-glucosyl-rutinoside	8.10	771	463, 301, 609	(463) 301	(301) 286, 125, 257, 199, 283,
Hesperetin derivative	8.70	915	463, 771, 505, 301, 607, 813, 609, 545	(463) 301	(301) 286, 283, 125, 257, 242
Hydroxycinnamic acids					
5-Caffeoylquinic acid	6.31	353	191	(191) 127, 173, 111, 109, 171, 155	

Table 4

Mass spectral characteristics of anthocyanins identified in Juice PLUS+[®] berry blend (JBB) and Juice PLUS+[®] fruit blend (JFB).

Compound	RT	M ⁺ (m/z)	MS ² ions (m/z)	FB	BB
Cyanidin-3-O-glucoside	7.2	449	287	+	+
Peonidin-O-hexoside	7.8	463	301	-	+
Delphinidin-O-hexoside	6.7	465	303	-	+
Petunidin-O-hexoside	7.44	479	317	-	+
Malvidin-O-hexoside	8.1	493	331	-	+

human trials are scarce and inconclusive. Furthermore, the association with the biological effects of these phenolic-containing nutraceuticals needs to be elucidated [54].

4. Conclusions

The phytochemical analysis of these three plant-based food supplements revealed that their content in (poly)phenolic compounds is substantial. The variety of plant ingredients declared on label is clearly confirmed by the presence of such a huge amount of different and sometimes very specific phenolic structures. Moreover, the preparation of these capsules appears to preserve

these components from degradation, keeping them robustly in the forms they are present in plants.

Further studies dealing with the bioavailability of these phytochemicals should be performed to confirm the presence of these compounds or their metabolites in circulation. This paper represents a key step in characterization of polyphenol intake through supplementation enabling the investigation of the physiological effects of these food supplements and their potential health benefits.

Conflicts of interest

The authors declare no conflict of interest.

Laypersons' summary

The scientific evidence of the health benefits linked to fruits and vegetables consumption is growing, and food supplements may represent an effective method to provide additional bioactive F&V compounds to consumers. Among these bioactives, polyphenols have been widely studied and it looks like they might be responsible for a great share of the preventative effects of plant food based diets. The aim of this

work was to characterize the polyphenolic composition of three plant-based food supplements designed to integrate and increase the daily intake of dietary phenolic compounds. The supplements are blends of berries, fruits, or vegetables made from a total of 36 different edible food plants. Using cut-edge analytical instruments, we have identified 119 (poly)phenolic compounds in the supplements, showing a richness and a variety that had never been reported before for this type of products. The contribution of these food supplements to the daily intake of (poly)phenolic compounds and, in turn, the potential contribution of such intake to health are discussed.

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