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Characterization of Phospholipid Molecular Species and Peptide Molecules in Wheat Sprout Hydroalcoholic Extract

Paolo Lucci,[†] Deborah Pacetti,*[,‡] Isabella Calzuola,[§] Valeria Marsili,[§,⊥] Stefano Perni,[§,⊗] Flavio Giavarini,[∥] Natale Giuseppe Frega,[‡] and Gian Luigi Gianfranceschi[§]

[†]Department of Nutrition and Biochemistry, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotà, Distrito Capital, Colombia

[‡]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

[§]NPP Nutraceutical & Phytochemical Products, 3A Parco Tecnologico Agroalimentare dell'Umbria, Pantalla di Todi (PG), Italy

[⊥]Department of Cellular and Environmental Biology, Università degli Studi di Perugia, Via Pascoli, 06123 Perugia, Italy

[∥]Laboratory of Mass Spectrometry, Department of Pharmaceutical Sciences, Università di Milano, Via Balzaretti 9, 20133 Milano, Italy

ABSTRACT: The phospholipid molecular species and the main peptide molecules of wheat sprout hydroalcoholic extract have been fully characterized by normal-phase high performance liquid chromatography coupled online with positive electrospray ionization tandem mass spectrometry. The extract that resulted was rich in phospholipid molecular species formed by the combination of the two essential fatty acids (α-linoleic and α-linolenic). These species accounted for 51.7% of total phosphatidic acid, 47.3% of total phosphatidylethanolamine, 37.7% of total phosphatidylcholine, and 14.1% of total phosphatidylinositol. The last one showed the highest amounts of species containing palmitic acid, thus representing the most saturated phospholipid class. The extract was also shown to contain several peptide sequences with both potential antioxidant domains and interaction sites for phospholipids (i.e., H-Ala-Gly-Ser-Met-Met-Cys-NH_2, H-Tyr-Met-Thr-Val-Val-Ala-Cys-NH_2, etc.); this latter finding can have a highly positive impact on the poor peptides bioavailability. Because of the presence of essential fatty acids-rich phospholipids and bioactive peptides, wheat sprout hydroalcoholic extract can be considered a potential functional food ingredient.

KEYWORDS: *functional food, molecular species, peptide molecules, EFA-rich phospholipid, phospholipid domains, phospholipid−peptide complexes, LC-ESI-MS, hydroalcoholic extract, sprouts, wheat*

INTRODUCTION

Nowadays there is a global increasing interest among consumers in more natural and nutritional food products able to promote health and well-being. Within this context, seed sprouts have received considerable attention during the late twentieth century because of their very high health-maintaining nutrients, such as phytochemicals (glucosinolates and natural antioxidants), protein, vitamins, minerals, enzymes, and amino acids. Furthermore, the process of sprouting is a very simple and inexpensive procedure which can then be carry out to increase the nutritive value of different seeds such as legumes (bean, pea, lentil, soybean) and grains (rye, wheat, barley, oats), as well as some vegetables seeds such as alfalfa and radish.[1] In fact, the biochemical changes occurring during the germination (i.e., degradation of polysaccharides into oligo- and monosaccharides, hydrolysis of proteins into oligopeptides and free amino acids) significantly increase the biological value of protein, polyunsaturated fatty acid content, vitamin content, and efficiency of mineral utilization of sprouts when compared to the seeds.[2,3] On the other hand, a decrease of the antinutritive components such as trypsin inhibitors, tannins, and phytic acid can also be observed.[4] Thus, the germination process can undoubtedly represent an interesting tool to obtain functional ingredients to be used for the production of food supplements and functional foods with positive effects on human health. Over the past few years, extensive work has been focused on the "pharmacological" properties of sprouts and sprout extracts, and a large number of investigations (both in vivo and in vitro) have already assessed the potential health-promoting and protective effect of sprouts and their active components against various diseases, especially regarding cancer. For instance, Bonfili et al. (2009)[5] have recently shown that wheat sprout hydroalcoholic extract has the ability to induce apoptosis in human cancer cells, whereas Amici et al. (2008)[6] studied the effect of wheat sprout extracts on 20S proteasome functionality, revealing the capability of the hydroalcoholic extract to reduce the growth of the cancerous cells. Previously, Falcioni et al. (2002)[7] have also shown the presence in wheat sprouts of antioxidant compounds active in the protection of DNA against the oxidative stress induced by Fenton reaction (Fe^{2+}/H_2O_2).

Generally, the health-promoting effect of wheat sprout extracts is attributed to the high antioxidant content (i.e., polyphenols such as gallic acid, epigallocatechin-3-gallate, epigallocatechin, epicatechin, and catechin).[2,6,8] However, it could be also attributable to other components, such as phospholipid (PLs) and protein fractions. For instance,

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essential phospholipids (EPL), a purified extract from soybean whose main active ingredient is represented by 1,2-dilinoleoyl-phosphatidylcholine, have been successfully used in liver diseases of various origins.[9] Several authors considered PLs as synergists of phenolic antioxidants.[10−13] Furthermore, PLs have also been reported as anti- or pro-oxidants depending on the presence of metals and concentration and are known to increase the oxidative stability of fats.[14,15]

As far as peptides are concerned, they have also been reported as potent effectors able to control crucial metabolic pathways. For instance, the peptides isolated from the chromatin of both animal and vegetal tissues have been shown to exert potent inhibitory effects on the proliferation of several tumor cells in vitro (L1210, HL60, HeLa cells).[7] The fractions obtained from the gel filtration of chromatin peptides isolated from wheat sprouts have also been tested on the growth of HeLa cells; the results obtained revealed a sharp dose-dependent inhibition of HeLa cancer cell proliferation.[16] The evidence of peptides with antioxidant activity is also quite recent, but the number of papers demonstrating their strong antioxidant activity is quickly increasing. In this past decade, more than 800 papers have been reported in the international literature. Some examples are reported here: H-Phe-D-Arg-Phe-Lys-NH_2, cell-permeable, mitochondrial-targeted antioxidant peptides;[17] H-Tyr-Glu-Asp-Cys-Thr-Asp-Cys-Gly-Asn-OH, antioxidant peptides from *Cornububali* (water buffalo horn);[18] H-Phe-Ile-Lys-Lys-OH, antioxidant peptides from protease digest of prawn muscle;[19] H-Glu-Ser-Thr-Val-Pro-Glu-Arg-Thr-His-Pro-Ala-Cys-Pro-Asp-Phe-Asn-OH, antioxidant peptide from hoki (*Johniusbelengerii*) frame protein by gastrointestinal digestion;[20] H-Ser-Ser-Glu-Phe-Thr-Tyr-OH, antioxidative peptides in potato protein hydrolysate;[21] H-Ala-Cys-Phe-Leu-OH, antioxidant peptide from horse mackerel viscera protein.[22] However, a question associated with biologically active peptides is their poor bioavailability.[23,24] Interestingly, it has been reported that the complex with PLs can significantly increase this bioavailability.[25−28] In a previous work, we reported the recognition of peptide−phospholipid complexes in extracts of wheat sprouts and the discussion of their possible significance.[29]

Despite the widely testified biological activities of PLs and peptides, there are still no data available about the profile of the PLs and peptide fractions in wheat sprout extract. Therefore, the aim of the present work is to give a picture of the nutritional and biological potentiality of PLs and peptides bioactive substances of wheat sprout hydroalcoholic extract.

For this purpose, the PLs molecular species and the peptide molecules of wheat sprout extracts were characterized by means of high performance liquid chromatography (HPLC) coupled online with ion-trap mass spectrometry with electrospray ionization (ESI) source. Furthermore the presence of antioxidant domains and of domains suitable for the binding with phospholipids was recognized in the peptide sequences.

MATERIALS AND METHODS

Materials. HPLC-grade methanol, chloroform, acetonitrile, and water were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland). All other reagents were of analytical grade (purity greater than 99%). PLs standards (purity greater than 99%), including 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocoline (DPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS), L-α-phosphatidylinositol ammonium salt from bovine liver (PI), 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA), 1-oleoyl-*sn*-glycero-3-phosphocoline (OLPC), 1-palmitoyl-*sn*-glycero-3-phosphocoline (PLPC) were purchased from Sigma (St. Louis, MO).

Wheat Sprout Powder. Wheat (*Triticum aestivum*) seeds sprouted for 3−5 days on soft agar (0.8−1%) were dehydrated. Successively, the sprouts were mechanically separated from the seeds and grinded.

Preparation of the Hydroalcoholic Extract from Wheat Sprout Powder. Extract from wheat sprout powder was prepared according to a previously reported method.[8] Briefly, 20 g of powder were homogenized (by means of a Waring blender) with 400 mL of water/ethanol (70:30, v/v) and centrifuged at 10000*g* for 30 min at 4 °C. After overnight storage at −20 °C, the extract was again centrifuged at 10000*g* for 30 min at 4 °C. The ethanol was then removed by evaporation, and the aqueous residue was lyophilized.

Extraction of Total Lipids (TL). The lipid fraction from lyophilized hydroalcoholic wheat sprout extract was extracted using the method of Bligh and Dyer.[30]

Clean-up of Polar and Neutral Lipids. The polar lipid fraction was purified by means of solid-phase extraction (SPE) according to Pacetti et al. (2007).[31] An aliquot (25 mg) of the lipid fraction was dissolved in 200 μL of chloroform/methanol (2:1, v/v) and subjected to SPE using LC-Si tubes, 6-mL volume, 1 g of adsorbent (Supelclean, Supelco, Bellefonte, PA, USA). The column was washed with a sequential elution of 5 mL hexane/diethyl-ether (4:1, v/v), 5 mL hexane/diethyl ether (1:1, v/v), 5 mL of methanol, and 5 mL of chloroform/methanol/water (3:5:2, v/v/v). The neutral lipids were eluted with hexane and diethyl ether. The fractions of methanol and chloroform/methanol/water, containing polar lipids, such as PLs, were combined, dried, and used for analysis of PLs molecular species.

High Performance Liquid Chromatography (HPLC)−Tandem Mass Spectrometry (MS) Condition Analysis of PLs Molecular Species. The SPE fraction containing polar lipid was dissolved in chloroform−methanol (2:1, v/v), and it was injected into the HPLC system. HPLC/ESI/MS-MS analysis of PLs molecular species was carried out using a pump module (Jasco PU-980) and a ternary gradient module (Jasco LG-980-02, Tokyo, Japan). The column was a Polaris Si-A 3 μ 150 mm × 4.6 mm (Varian, Middelburg, The Netherlands) protected with a silica precolumn (4 mm × 3.0 mm i.d.) from Phenomenex (Torrance, CA, USA). The separation of phospholipids classes were obtained according to method reported by Pacetti et al. (2007).[31] The mobile phase was a gradient of solvent A [$CHCl_3$/MeOH/NH_4OH (30%) 80:19.5:0.5, v/v] and solvent B [$CHCl_3$/MeOH/H_2O/NH_4OH (30%) 60:34:5.5:0.5, v/v]. The gradient started at 100% of A, decreased to 0% in 10 min, then held for 15 min; and then reached back to 100% A in 5 min. The flow rate was 1.0 mL min^{-1}. The HPLC system was coupled online to an LCQ ion-trap mass spectrometer (Finnigan, San Jose, CA, USA) equipped with an electrospray ionization source (ESI). The HPLC effluent was split, and 0.4 mL min^{-1} entered the MS through a steel ionization needle set at 5.0 kV and a heated capillary set to 200 °C. The sheath gas flow was approximately 90 arbitrary units. The ion source and the ion optic parameters were optimized with respect to the positive molecular related ions of the phospholipids standards. Mass resolution was 0.1 Da. The molecular mass peaks from the HPLC effluent were detected using positive ion full-scan ESI−MS analysis. Tandem mass (MS^2) experiments were carried out with relative collision energy of 45%. The integration was performed with the Interactive Chemical Information System (ICIS) peak detection algorithm software provided by Finnigan, after correction for the contribution from the ^{13}C isotope effect.

Analysis of Peptide Molecules. Lyophilized hydroalcoholic wheat sprout extract was dissolved in 0.1% trifluoroacetic acid/acetonitrile (97:3, v/v) and fractionated by HPLC using a semi-preparative C18 column (150 mm × 10 mm, Phenomenex, Torrance, CA, USA). The column was equilibrated with 0.1% trifluoracetic acid/acetonitrile (97:3, v/v). After 6 min of isocratic elution, a gradient from 3 to 20% acetonitrile in 50 min was applied. Flow was set at 2.5 mL min^{-1}. The eluate of wheat sprout extract from reverse phase HPLC column was separated in 18 fractions from A to T (T_1−T_2).[29]

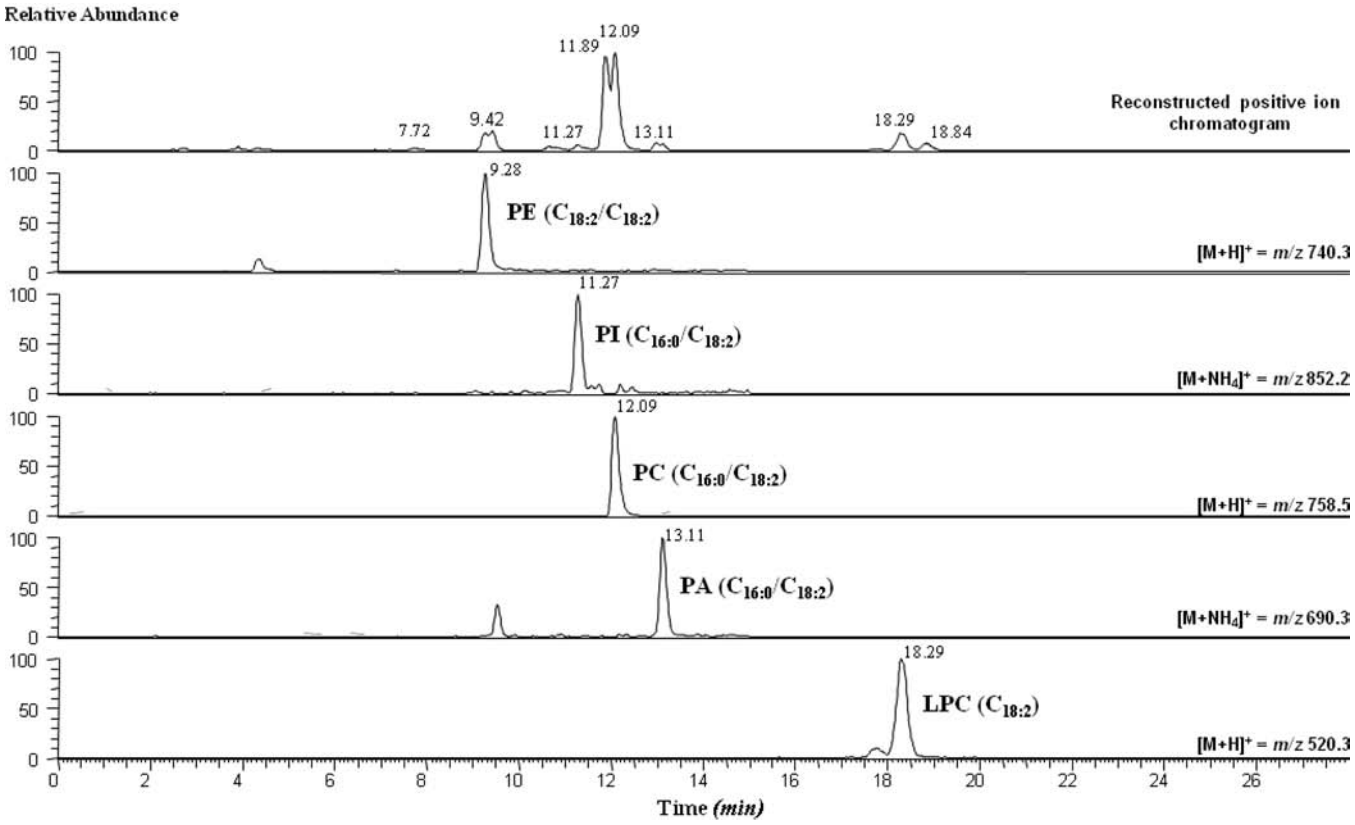

Figure 1. Positive HPLC-ESI/MS trace of PLs from wheat sprout hydroalcoholic extract with the MS operating in scan mode. PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; PA, phosphatidic acid; LPC, lysophosphatidylcholine. $C_{n:m}$ = fatty acid (n = carbon number; m = number of double bonds). The conditions of MS analysis are described in Materials and Methods.

Each fraction was then lyophilized, solubilized in methanol, and injected into ESI−MS using the flow injection analysis (FIA) mode. The ions shown by MS spectra (spectrum) of HPLC fractions were subjected to MS/MS analysis, and the results were scanned to cross over ions corresponding to peptide molecules. The MS parameters were capillary temperature 220 °C, capillary voltage 10 V, and spray voltage 4KV, collision energy from 17 to 40 keV.

Automatic Determination of Amino Acid Sequences Compatible with the Given Mass Spectral Data. For what concerns the study of peptide structure, the mass spectra analysis was performed with a recently reported automatic combinatorial method that carries out the computation of all amino acid sequences compatible with a given molecular ion.[32] The possible sequences of these compounds are automatically obtained by considering fragment ion masses that are potential breakdown products. This was obtained by developing a mathematical model of the problem and by searching for all possible sequences of given components satisfying certain constraints. The analysis does not rely on known protein database but on the computation of all amino acid sequences satisfying given mass spectral data. In some cases the information contained in the spectrum is sufficient to determine a unique sequence. Sometimes the spectrum does not contain enough information for an unequivocal determination of the sequence. In the latter case, all possible sequences that fit the spectrum are listed.

RESULTS AND DISCUSSION

Characterization of Phospholipid Molecular Species. The PL classes detected in the wheat sprout hydroalcoholic extract were phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidylinositol (PI), and lysophosphatidylcholine (LPC). The HPLC separation of PL classes is reported in Figure 1. The retention time for the different classes increased in the following order: PE, PI, PC, PA, LPC. All classes eluted within 18 min.

The molecular species of all the PLs were detected as their protonated molecular peak $[M + H]^+$, except PA and PI, which were detected as their adducts with ammonium $[M + NH_4]^+$. The positive ionization was preferred to the negative ion mode because the molecular species of PC and PE are better identified: the fragments originating from the loss of the fatty acid moieties are formed. The identification of the PL molecular species was confirmed by using standard PL solutions and comparing the mass spectra with those reported in the literature.[31,33−36] The relative abundance of individual molecular species within a PL class was calculated from the single ion current responses, since the peak intensity with ESI-MS among PL species of the same class is considered similar.[37,38]

As far as PL molecular species composition (Table 1) is concerned, the main molecular species of all the PL classes contained at least one molecule of α-linoleic acid ($C_{18:2\omega6}$). The preponderant molecular species of PE, PC, and PA were $C_{18:2}/C_{18:2}$ and $C_{16:0}/C_{18:2}$, followed by the combination of $C_{18:2}/C_{18:3}$. Differently, the main molecular species of PI were $C_{16:0}/C_{18:2}$ and $C_{16:0}/C_{18:3}$, followed by the combination of $C_{16:0}/C_{18:1}$. The LPC classes were principally formed by molecular species containing $C_{18:2}$.

The highest levels of PL molecular species formed by the combination of two essential fatty acids (EFAs), such as $C_{18:2\ \omega6}$ and $C_{18:3\ \omega3}$ (α-linolenic acid), were found in PA (51.7% of total PA), whereas the lowest in PI (14.1% of total PI). High levels of EFAs-PLs molecular species have been also observed

Table 1. Phospholipid Molecular Species Composition of Wheat Sprout Hydroalcoholic Extract[a]

PL molecular species		
ion (m/z)	fatty acids	relative abundance (% ± sd)
	PE ($[M + H]^+$)	
714.3	16:0/18:3−16:1/18:2	8.6 ± 0.8
716.3	16:0/18:2	27.9 ± 1.2
718.4	16:0/18:1	4.9 ± 0.7
736.3	18:3/18:3	4.6 ± 0.6
738.3	18:2/18:3	13.6 ± 1.6
740.3	18:2/18:2	29.1 ± 1.7
742.2	18:0/18:3	8.2 ± 0.4
744.3	18:1/18:1−18:0/18:2	1.8 ± 0.0
768,3	18:0/20:4	1.3 ± 0.2
	PI ($[M + NH_4]^+$)	
850.1	16:0/18:3	24.1 ± 1.1
852.2	16:0/18:2	45.2 ± 1.5
854.1	16:0/18:1	11.9 ± 1.7
874.2	18:2/18:3	4.9 ± 0.8
876.4	18:2/18:2−18:1/18:3	9.2 ± 0.9
878.3	18:1/18:2−18:0/18:3	5.6 ± 1.2
	PC ($[M + H]^+$)	
756.6	16:0/18:3	12.3 ± 1.2
758.5	16:0/18:2	26.6 ± 2.6
760.4	16:0/18:1	9.6 ± 1.2
762.3	16:0/18:0	0.8 ± 0.1
780.6	18:2/18:3	10.9 ± 1.2
782.5	18:2/18:2	23.8 ± 2.6
784.5	18:1/18:2	11.4 ± 0.9
786.6	18:0/18:2	4.1 ± 0.7
788.6	18:0/18:1	0.7 ± 0.2
	PA ($[M + NH_4]^+$)	
690.3	16:0/18:2	20.5 ± 1.5
692.2	16:0/18:1	5.9 ± 0.6
710.2	18:3/18:3	7.0 ± 0.6
712.2	18:3/18:2	19.8 ± 1.4
714.5	18:2/18:2	24.9 ± 0.3
716.2	18:1/18:2	11.4 ± 0.9
718.3	18:1/18:1−18:0/18:2	7.5 ± 0.6
720.5	18:0/18:1	3.1 ± 0.6
	LPC ($[M + H]^+$)	
494.2	16:1	0.9 ± 0.1
496.2	16:0	26.3 ± 1.8
518.3	18:3	18.2 ± 2.6
520.3	18:2	37.8 ± 2.4
522.2	18:1	14.1 ± 1.6
524.3	18:0	1.3 ± 0.5

[a]In each PL class, values are given as the average internal percentage ± standard deviation (n = 3); sd = standard deviation.

in PE (47.3% of total PE) and PC (37.7% of total PC). Furthermore, the molecular species $C_{18:3}/C_{18:3}$ was exclusively found in PE and in PA where it accounted for 4.6 ± 0.6% of total PE and for 7.0 ± 0.6% of total PA.

The highest amounts of the molecular species containing palmitic acid, such as $C_{16:0}/C_{18:3}$, $C_{16:0}/C_{18:2}$, and $C_{16:0}/C_{18:1}$, were revealed in PI, which represents the most saturated PL class. The molecular species $C_{16:0}/C_{18:3}$ accounted for about one-quarter of PI molecular species and for about 10% of PE and PC molecular species. On the contrary, it was not detected in PA.

Table 2. Main Ions $[M + H]^+$ Recognized in the Mass Spectrum of the Wheat Sprout Extract HPLC Fractions and Subjected to Tandem Mass Spectrometry (MS/MS)[a]

HPLC fractions	elution time (min)	acetonitrile (%)	ion $[M + H]^+$	abundance (%)	Δ abundance (%)
F	8.7	4.31	365.1	100	90
G	9.6	4.74	435.2	78	70
			663.5	100	54
H	11.25	5.17	428.6	100	75
I	12.38	5.60	437.2	100	90
			598.5	45	95
			882.8	22	72
L	15.93	7.11	663.4	100	95
M	2.80	10.34	446.2	95	76
			572.2	100	95
N	25.65	10.77	496.2	30	58
			528.1	100	88
			903.6	18	85
O	36.22	14.65	558.2	38	95
P	40.65	16.59	785.5	66	88
			821.7	64	86
Q	43.50	17.67	541.0	100	95
			671.4	33	95
R	44.55	18.10	702.5	38	32
S	50.25	20.25	603.4	100	82
T1	55.42	30.60	663.5	100	30
			815.6	65	45
T2	55.87	31.46	663.5	100	45

[a]For each ion the HPLC elution time, the related acetonitrile percentage, the abundance (%) in the mass spectrum, and the Δ abundance (%) (differences between the abundances before and after MS/MS fragmentation analysis), are reported.

Table 3. Peptide Sequences Predicted by MS/MS Spectrometry Analysis of Peptide Ions Recognized in HPLC Fraction of Wheat Sprout Extract[a]

ion $[M + H]^+$	considered ion fragments	HPLC fractions	sequence(s) consistent with MS/MS spectrum
365.1	184.9; 202.9; 220.4; 274.8; 305.1	F	Ac-Cys-Gly-Thr-ETH
428.6	309.2; 332.4; 351.1; 358.5; 370.2; 384.6	H	H-Pro-Pro-Gly-Gly-**Cys-NH_2**
598.5	216.1; 347.1; 407.2; 437.2; 509.2; 527.2	I	H-Ala-Gly-Ser-**Met-Met-Cys-NH_2**
785.5	395.6; 428.7; 455.9; 594.0; 604.9; 621.9; 664.8; 724.9	P	H-**Tyr**-Phe-Gly-Leu-Leu-Ala-**Cys-NH_2**
			H-**Tyr-Phe**-Leu-Val-Ala-Ala-**Cys-NH_2**
			N-**Tyr-Met**-Thr-Val-Val-Ala-**Cys-NH_2**
541.0	331.1; 348.0; 362.1; 393.0	Q	**Ac-Phe**-Cys-Ala-Gly-**Cys-NH_2**
			Ac-Cys-Met-Ser-Gly-**Cys-NH_2**
663.5	327.1; 463.3; 543.4; 603.9; 619.1	T2	Ac-Gly-Gln-Val-Leu-**Cys-Cys-NH_2**
			Ac-Asn-Val-Ala-Leu-**Cys-Cys-NH_2**
			H-Pro-Ala-Ala-Pro-**Cys-Cys-Cys-NH_2**

[a]Antioxidant aminoacids or domains are bolded while potential binding sites for phospholipids are underlined.

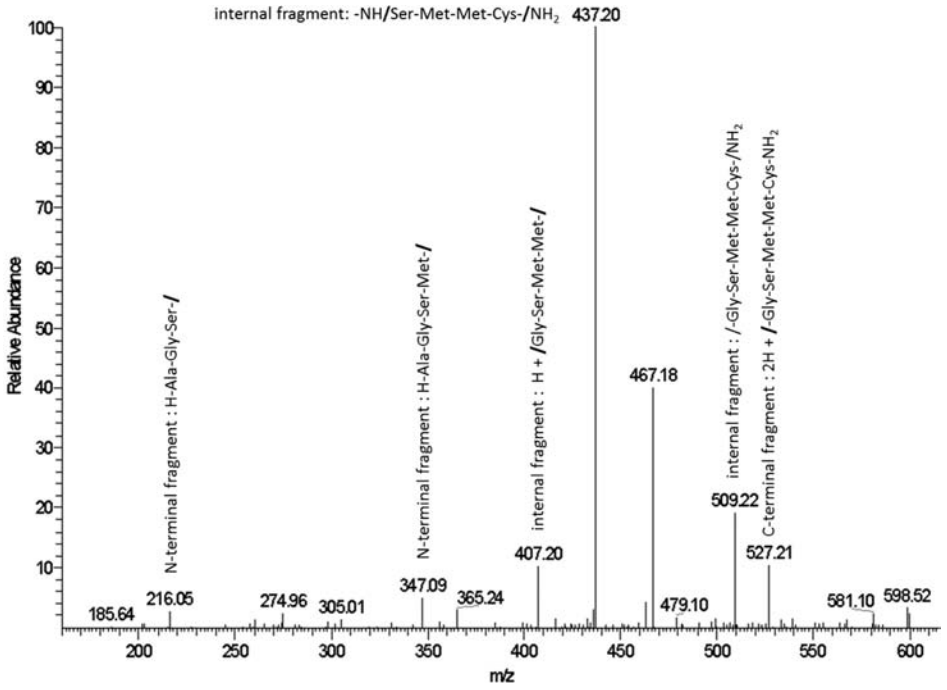

Figure 2. HPLC-ESI-MS/MS spectrum derived from fragmentation of the ion at m/z 598.5. The conditions of MS analysis are described in Materials and Methods.

These findings are very interesting, especially from a nutritional point of view. In fact, although PLs constitute a small fraction of total dietary fat, they can be used as important sources of EFAs and, from a large number of recent studies, it became evident that dietary PLs are highly effective in delivering their fatty acid residues for incorporation into the membranes of cells involved in different diseases. For instance, the therapeutic essential phospholipid PC $C_{18:2}/C_{18:2}$, a species usually purified from the semen of soybean, accounted for 23.8% of total PC of wheat sprout extract. Interestingly, the PC profile was found to be similar to that of purified soy PC,[39] thus revealing the potentiality of PL wheat sprout hydroalcoholic extract as a valuable alternative source of 1,2-dilinoleoyl-phosphatidylcholine, an active ingredient for the treatment of liver diseases. Furthermore, the analysis of the PL fraction of wheat sprout extract also revealed the presence of high percentages of EFAs-PL molecular species and thereby the possibility to successfully use wheat sprout extract as a natural PL source for the preparation of food supplements containing EFA-rich phospholipids. This finding is of particular note, especially considering that the supplementation of food products with phospholipids, especially polyunsaturated fatty acid (PUFA)-rich phospholipids, has recently emerged as promising way of increasing the assimilation and the health benefits of PUFA in the human body.[40]

Characterization of Peptide Fraction Extracted from Wheat Sprouts. The RP-HPLC-MS chromatogram of wheat sprout extract has been reported elsewhere.[29] From the MS/MS spectrometry analysis of the different HPLC fractions (Table 2), about 2000 potential sequences have been obtained. The ions that after MS/MS experiments show a variation of the relative abundance (Δ-abundance) lower than 30% were discarded because the collision energy of the spectrometer was set to be able to cleave peptide bonds, so a significant peptide fragmentation is expected.

The obtained sequences (F-T2) were then scanned to recognize single antioxidant amino acid or domains with potential antioxidant activity. At the same time, the presence of phospholipid binding domains was checked, following the vector machine prediction method.[41] The sequences determined by the peptide fragmentation spectra are reported in Table 3. As an example, the MS/MS spectrum of the m/z 598.5 ion together with the sequence-revealing fragment ions is reported in Figure 2. It is noteworthy that the C-terminal of almost all the reported sequences is constituted by amidated cysteine. Moreover the results show the presence of domains with two or three residues of cysteine and/or methionine some time together with aromatic amino acids, in accordance with previously reported antioxidant peptide sequences.[17−22]

Regarding the presence of phospholipid-binding domains, the main sequence feature of the peptides is being able to bind to PLs in the presence of a box of 2−4 hydrophobic amino acids, in agreement with the sequences of cell penetrating peptides.[41] This is confirmed by the N-terminal pentapeptide AcGALFL present in the P294 and P326 fusion peptides, which perform noncovalent interaction with phosholipids.[25] In addition, in the very short peptides we found another strong peculiarity such as the absence of a negative charge that prevents repulsive interaction with the phospholipids phosphoric group. In this context it is interesting that all the sequences obtained contain a C-terminal amidated cysteine which prevents the presence of C-terminal negatively charged OH. These observations are in agreement with the previously reported mass data concerning the presence in wheat sprout extracts of peptide−phospholipid complexes.[29]

The binding of peptides to phospholipids is of special interest because the complex with phospholipids, in agreement with the peculiarity of cell penetrating peptides, can strongly increase their bioavailability which represents an "Achilles heel" for their biological effectiveness. In fact, attempts at increasing the bioavailability of biologically active hydrophilic peptides are

a main research goal for many authors. In this context, designing and formulating a polypeptide drug have been a persistent challenge because of their unfavorable physicochemical properties, which include enzymatic degradation and poor membrane permeability.[42] For instance, porcine insulin has been used as a model drug due to its water solubility, ease of analysis, and ready availability. To improve oral delivery, insulin has been complexed with phospholipids by an anhydrous cosolvent lyophilization procedure. Results of an in vivo evaluation allow the conclusion that insulin-phosphatidylcholine complexes are able to markedly improve the intestinal absorption of insulin.[43] Another model of peptide−phospolipids complex designed to increase peptide bioavailability is represented by 5A apolipoprotein mimetic peptide. In human coronary artery endothelial cells (HCECs), apolipoprotein (apo) A-I mimetic peptide 5A complexed with 1-palmitoyl-2-linoleoyl-phosphatidylcholine (5A/PLPC), inhibited tumor necrosis factor-induced, intercellular adhesion molecule and vascular cell adhesion molecule expression, as well as the nuclear factor B signaling cascade and $[O_2]^-$.[44]

In this scenario, our results are very attractive since they provide evidence that several peptide sequences in wheat sprout extract with potential antioxidant activity that complexed with phospholipids can achieve good levels of activity also at the cellular level.

In conclusion, the present study supports the view that wheat sprout hydroalcoholic extract can be considered as a potential and promising functional food ingredient. In fact, it contained not only PLs characterized by a high content of essential fatty acids but also peptide sequences containing both potential antioxidant domains and interaction sites for phospholipids.

AUTHOR INFORMATION

Corresponding Author

*Phone: +39 071 2204307. Fax: +39 071 2204980. E-mail: d.pacetti@univpm.it.

Present Address

⊗(S.P.) Department of Physiology and Biophysics, University of Colorado, Anschutz Medical Campus, Aurora, CO 80045, USA.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

PL, phospholipid; EPL, essential phospholipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PA, phosphatidic acid; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; SPE, solid-phase extraction; EFA, essential fatty acid; PUFA, polyunsaturated fatty acid

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