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Determination of Soyasaponins I and βg in Raw and Cooked Legumes by Solid Phase Extraction (SPE) Coupled to Liquid Chromatography (LC)−Mass Spectrometry (MS) and Assessment of Their Bioaccessibility by an in Vitro Digestion Model

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ABSTRACT: Legumes contain a rich variety of phytochemicals as soyasaponins, triterpenoidal glycosides that possess multiple health-promoting properties, such as lowering of cholesterol. In this work, the quantification of soyasaponins I and βg in 60 raw and cooked legumes by using a solid phase extraction (SPE) coupled to a liquid chromatography (LC)−mass spectrometry (MS) method was carried out. Results showed that lentils are a good source of soyasaponins, with a content of soyasaponin I that ranged from 636 to 735 mg kg^{-1} and of soyasaponin βg from 672 to 1807 mg kg^{-1}. The cooking process produced a small loss of soyasaponins in water, that is, 4.8−8.7%, and partially converted soyasaponin βg into soyasaponin I. In addition, the bioaccessibility of soyasaponins I in lentils was studied; the values ranged from 8.9 ± 0.3 to 10.6 ± 1.1% in the duodenal compartment. On the basis of these results, soyasaponins could be effective in lowering exogenous cholesterol.

KEYWORDS: *legumes, soyasaponins, cholesterol lowering compounds, LC-MS, bioaccessibility*

INTRODUCTION

Legumes include lentils (*Lens culinaris* L.), beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lupins (*Lupinus* spp.), fava beans (*Vicia faba* or *Faba vulgaris*), soybeans (*Glycine max*), and others. Cultivated for thousands of years,[1] they have played an important role in the traditional diets of many regions throughout the world.[2,3] The nutritional profile of legumes shows that they have much to offer: high in protein, low in saturated fat, and high in complex carbohydrates and fiber, as well as being a good source of several micronutrients and phytochemicals.[1,4] Legumes, particularly lentils, are a primary dietary source of food saponins, which are bioactive compounds that have been demonstrated to possess multiple health-promoting properties, such as reduction of cholesterol levels, anticarcinogenic and antihepatotoxic properties, and antireplicative effects against HIV.[5,6] There is abundant evidence that dietary saponins (including soyasaponins) can lower plasma cholesterol values; this happens directly by inhibiting absorption of cholesterol from the small intestine or, indirectly by inhibiting the reabsorption of bile acids.[7,8] Reduced entry of cholesterol or bile acids into the enterohepatic circulation results in the stimulation of cholesterol synthesis mainly by the liver.[7] Additionally, Lee et al. reported that group B soyasaponins improve plasma cholesterol status by increasing the excretion of fecal bile acids and neutral sterols in hamsters. Larger production of group B soyasaponin metabolites was associated with improved plasma cholesterol status, suggesting that gut microbial variation in soyasaponin metabolism may influence the health effects of group B soyasaponins.[8]

Saponins chemically consist of a steroidic or triterpenic nucleus (aglycone) linked to a glycosidic chain and are considered to be a component of dietary fiber.[9] Soyasaponins are triterpenoidal glycosides, structurally divided into two groups, called A (bidesmosidic) and B (monodesmosidic).[6] Lentils mainly contain soyasaponin I (soyasaponin Bb) and soyasaponin βg (also called soyasaponin VI), both of which belong to the B group of soyasaponins.[6] Soyasaponin βg contains the group 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) at the C-22 position and may be the natural precursor of soyasaponin I.

Various analytical methods have been proposed for the quantification of soyasaponins, including liquid chromatography−mass spectrometry detection.[10] The use of solid phase extraction (SPE), which is a useful technique to concentrate and purify analytes of interest from a wide variety of matrices coupled to the high sensitivity of LC-MS, allows the quantification of all soyasaponins in legumes, including the minor components.[6,11−13] Legumes normally used in human nutrition need to be cooked to tenderize them and improve their flavor.[14] The effects of soaking and cooking on saponin composition and content are poorly understood; some work has demonstrated a relationship between chemical structure and biological activity, but further study is clearly needed.[15,16]

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Table 1. Soyasaponin Content (Dry Weight) in Different Raw Legume Samples[a]

no.	type of legume (trade name)	origin	soyasaponin I (mg kg^{-1})	soyasaponin βg (mg kg^{-1})	total (mg kg^{-1})
1	lentil	Canada	644	867	1511
2	hulled red lentil	Turkey	678	672	1350
3	lentil from Colfiorito	Italy	735	1807	2542
4	lentil from Castelluccio	Italy	689	1648	2337
5	lentil from Umbria	Italy	678	1103	1781
6	green lentil[c]	Italy	636	679	1315
7	bean borlotti	Canada	696	861	1557
8	bean Lamon	Italy	697	673	1370
9	bean cannellini	Argentina	695	889	1584
10	bean Corona	Poland	656	755	1411
11	green bean azuchi	Argentina	672	703	1375
12	red bean azuchi	Argentina	648	667	1315
13	bean Occhio	Peru	711	749	1460
14	black bean	Mexico	687	675	1362
15	bean Toscanelli	Italy[b]	650	714	1364
16	bean Saluggia	Italy	650	695	1345
17	bean Stregoni	Italy	704	914	1618
18	bean Rossoni	Argentina	661	694	1355
19	bean Bruni	Italy[b]	646	657	1303
20	bean Tondini	Canada	817	1209	2026
21	bean Stregoni	Italy	661	655	1316
22	bean Bunbunin	Italy	665	839	1504
23	bean Saluggia	Italy	675	704	1379
24	bean Billò Cuneo	Italy	661	907	1568
25	bean Purgatorio	Italy	699	1065	1764
26	bean cannellini	Italy	657	949	1606
27	bean vellutina	Italy	697	662	1359
28	bean borlotti	Italy	674	848	1522
29	bean cannellini	Italy	667	661	1328
30	bean borlotti	Italy	719	1058	1777
31	bean cannellini	Italy	739	930	1669
32	black bean	Italy	718	1026	1744
33	green bean azuchi	Italy	642	835	1477
34	fava bean broken	Egypt	661	717	1378
35	fava bean whole	Siria	646	700	1346
36	fava bean whole	Italy	642	716	1358
37	chickling	Italy	637	647	1284
38	chickling	Italy	737	661	1398
39	lupin	Italy	721	671	1392
40	soybean yellow	Canada	897	1595	2492
41	dry pea	Canada	702	1070	1772
42	green pea	Italy	907	1411	2318
43	chickpea	Mexico	688	866	1554
44	chickpea Puglia	Italy	721	1240	1961
45	chickpea Marches	Italy	761	1334	2095
46	chickpea	Italy	732	1412	2144
47	chickpea	Italy	714	933	1647
48	mixed legumes	Canada	670	820	1490
49	Tuscan soup	Turkey	678	797	1475
50	soup[d] beans/chickpeas	Mexico	719	949	1668
51	soup[d] Contadina	Canada	681	955	1636
52	soup[d] Colfiorito	Italy	721	932	1653
53	soup[d] Quattro Stagioni	Argentina	647	702	1349
54	soup[d] primavera	Poland	680	858	1538
55	soup[d] rustica	Argentina	670	781	1451
56	soup[d] Montagna	Argentina	727	1107	1834

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Table 1. continued

no.	type of legume (trade name)	origin	soyasaponin I (mg kg^{-1})	soyasaponin βg (mg kg^{-1})	total (mg kg^{-1})
57	soup[d] of lentils	Peru	651	666	1317
58	farro soup[d]	Italy	738	1038	1776
59	barley soup[d]	Italy	701	1046	1747
60	Tuscan soup[d]	Italy	636	668	1304

[a]Each sample was analyzed in triplicate. RSD% values in all cases were <9.3%. [b]In samples 15 and 19, the origin refers to the processing plant. [c]Samples 6, 30−33, 46, 47, and 58−60 are from organic cultivation. [d]The term "soup" indicates the kind of dish and is not related to legume type and name.

Additionally, to the best of our knowledge, only Ruiz et al.[4,17] have developed a method for the quantification of saponins by using LC and UV absorption in soaked and cooked chickpeas and lentils. A bioactive compound's bioaccessibility is defined in terms of the fraction of the compound in a food matrix that remains available for intestinal absorption after passing unmodified through the biochemical reactions of gastrointestinal digestion.[18] Studies on animals and humans have shown that oral bioaccessibility of some bioactive compounds in foods can be significantly modified depending on the food source. To our knowledge, no studies have been reported in the literature concerning the bioaccessibility of soyasaponins in food. The aim of this work was (a) to quantify soyasaponins I and βg in various raw and cooked legumes (lentils from Colfiorito, lentils from Umbria, chickpeas, chicklings, green peas, and Borlotti beans) by using a SPE-LC-MS method previously developed in our laboratory[6] and (b) to evaluate the bioaccessibility of soyasaponins I and βg in cooked lentils by using an in vitro digestion model. This study could be very useful for indicating the amount of soyasaponins that really reach the duodenal and intestinal tract, which might be beneficial for inhibiting cholesterol absorption.

MATERIALS AND METHODS

Chemicals and Materials. HPLC grade methanol, ethanol, and acetonitrile were purchased from Sigma-Aldrich (Milano, Italy), whereas HPLC grade acetic acid 99−100% was bought from J. T. Baker B.V. (Deventer, The Netherlands). Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulfate (Na_2SO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid, pepsin, pancreatin, bile salts, and phosphate buffer saline (PBS, pH 7.5) were purchased from Panreac Quimica SA (Barcelona, Spain). Deionized water (<8 MΩ cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All solvents and solutions were filtered through a 0.45 μm PTFE filter from Supelco (Bellefonte, PA, USA) before use. Strata C_{18}-E SPE tube cartridges (6 mL, 1 g) were purchased from Phenomenex (Bologna, Italy).

Standard Preparation. Pure standards of soyasaponind I and βg, used as reference compounds, were obtained from soybeans according to a previous isolation method developed in our laboratory.[6] Individual stock solutions were prepared by dissolving 5 mg of compound in 5 mL of methanol and storing this solution in glass-stoppered bottles at 4 °C. Standard working solutions, at various concentrations, were prepared when needed by appropriate methanol dilution of stock solution aliquots.

Preparation of Legume Samples. Legumes were kindly provided from a local company, Fertitecnica, of the Colfiorito (Perugia, Italy) community. For most samples (Table 1), origin was exactly known, whereas for some of them (samples 15 and 19), only packing location was available. Analyzed legumes include lentils (*Lens culinaris* L.), beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lupins (*Lupinus* spp.), fava beans (*Vicia faba* or *Faba vulgaris*), chicklings (*Lathyrus sativus*), and soybeans (*Glycine max*).

Soaking. Soaking was performed in distilled water. The proportion of seed to soaking medium was 1:10 w/v. The soaking time, 24 h at 25 °C, was chosen to obtain maximum seed weight and hydration. The soaking solution was filtered, and 50 g of the soaked seeds was weighed for cooking studies.

Cooking. Soaked seeds of legumes were weighed and boiled for 30 min (according to the package instructions) in distilled water (seed/water ratio 1:20 w/v) in an iron saucepan. The cooking liquids and seeds were separated by filtration, and then seeds were freeze-dried overnight before the same extraction procedure used for raw legumes was applied.

Sample Extraction. One gram of finely ground legume seeds was extracted, for 3 h under magnetic stirring, with 10 mL of 70% aqueous ethanol at room temperature. The mixture was then filtered under vacuum, and the solution was evaporated to 0.5 mL with a rotary evaporator at T < 30 °C under reduced pressure (60 mbar). The aqueous residue was purified on a Strata C_{18}-E cartridge (6 mL, 1 g). The cartridge was activated with 2 × 2 mL of methanol and conditioned with 2 × 2 mL of water, and then the aqueous residue was loaded onto the cartridge at a flow rate of <0.5 mL min^{-1}. When the sample was obtained from the soaking or cooking water, 10 mL of centrifuged water (4500 rpm for 10 min) was loaded onto the Strata C_{18}-E cartridge. The cartridge was then washed with 2 × 2 mL of water and thoroughly dried, and then elution was performed using 150 mL of methanol. The eluate was directly injected in LC-MS without further concentration.

Bacterial Strains and Growth Conditions. Thirteen commercial probiotic strains were obtained for the in vitro system that simulates the physiologic condition of the colonic intestinal compartment. In particular, *Lactobacillus animalis* CECT 4060T, *Lactobacillus casei* CECT 4180, *Lactobacillus casei rhamnosus* CECT 278T, *Lactobacillus plantarum* CECT 220, *Lactobacillus rhuminis* CECT 4061T, *Lactobacillus casei casei* CECT 277, *Bifidobacterium breve* CECT 4839T, *Bifidobacterium adolescentes* CECT 5781T, *Bifidobacterium bifidum* CECT 870T, *Corynebacterium vitaeruminis* CECT 537, *Streptococcus faecalis* CECT 407, *Eubacterium crispatus* CECT 4840, and *Saccharomyces cerevisiae* CECT 1324 were obtained at the Spanish Type Culture Collection (CECT, Valencia, Spain) in sterile 18% glycerol. For longer survival and higher quantitative retrieval of the cultures, they were stored at −80 °C. When needed, recovery of strains was undertaken by two consecutive subcultures in appropriate media prior to use.[19,20]

In Vitro Digestion Model. The procedure was adapted from the method outlined by Gil-Izquierdo et al.,[21] with slight modifications. The method consists of three sequential steps: an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion (Figure 1). The colonic conditions were simulated adding to the duodenal simulated fluid some bacteria representative of the gastrointestinal tract. For the saliva/pepsin/HCl digestion, 10 g of cooked lentils (lentil sample 1 of Table 1) was mixed with 6 mL of artificial saliva composed of KCl 89.6 g/L, KSCN 20 g/L, NaH_2PO_4 88.8 g/L, Na_2SO_4 57 g/L, NaCl 175.3 g/L, $NaHCO_3$ 84.7 g/L, urea 25 g/L, and 290 mg of α-amylase. The pH of this solution was corrected at 6.8 with 0.1 N NaOH. These mixtures composed by model solutions and by the artificial saliva were

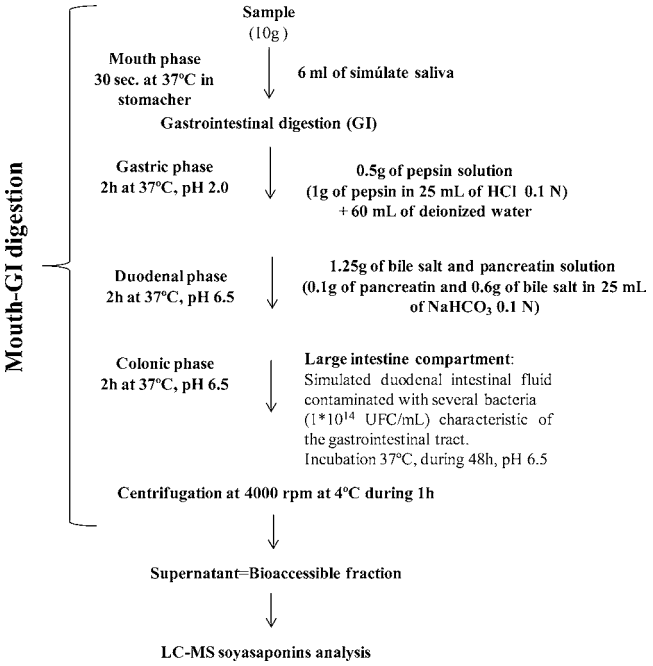

Figure 1. In vitro digestion model describing a four-step procedure simulating the digestive processes, considering the mouth, stomach, and small and large intestines. One milliliter of duodenal or colonic simulated fluids was taken and 20 μL injected in LC-MS.

placed in plastic bags containing 40 mL of water and homogenized by Stomacher IUL Instruments (Barcelona, Spain) for 30 s. To this mixture was added 0.5 g of pepsin (14800 U) dissolved in 25 mL of 0.1 N HCl. The pH of the mixture was corrected to 2 with 6 N HCl, and then the mixture was incubated in a 37 °C orbital shaker (250 rpm; Infors AG CH-4103, Bottmingen, Switzerland) for 2 h. After the gastric digestion, the pancreatic digestion was simulated. The pH was increased to 6.5 with $NaHCO_3$ (0.5 N), and then 5 mL of (1:1; v/v) pancreatin (8 mg/mL)/bile salts (50 mg/mL), dissolved in 20 mL of water, was added and incubated in a 37 °C orbital shaker (250 rpm) for 2 h. An aliquot of 1 mL of the duodenal simulated fluid was filtered on a 0.22 μm filter (Phenomenex, Bologna, Italy) and directly injected in the LC-MS for quantification of soyasaponin bioaccessibility. To simulate the colonic compartment, bacterial strains (previously described) were grown in a sterile plastic centrifuge tube overnight at 37 °C in MRS broth (Oxoid, Madrid, Spain) under aerobic conditions (5% CO_2/95% air).[19] Then, the tubes were centrifuged at 4000 rpm for 5 min at 23 °C, and the bacteria were resuspended in sterile PBS. A mixture of the bacterial suspensions (500 μL) at concentrations of 10^{14} CFU/mL (*Bifidobacteria* 10^{10} UFC/mL, *Lactobacillus* 10^{12} UFC/mL, *Bacterioides* 10^{11} UFC/mL, *Streptococcus* 10^{10} UFC/mL, *Saccharomyces* 10^{10} UFC/mL) was added to duodenal simulated intestinal fluid and incubated at 37 °C in 5% CO_2/95% air. After this step, 1 mL of the colon simulated fluid was filtered on a 0.22 μm filter and directly injected in the LC-MS for quantification of duodenal plus colonic soyasaponin bioaccessibility.

LC-MS Analyses. The separation of soyasaponins was achieved on a Gemini C_{18} (150 × 4.6 mm i.d., 5 μm) analytical column from Phenomenex (Chesire, UK). The mobile phase for LC-ESI-MS (single quadrupole) analyses was a mixture of (A) water with 0.25% acetic acid (v/v) and (B) methanol with 0.25% acetic acid (v/v), flowing at 1 mL min^{-1} in isocratic conditions: 20% A, 80% B.

LC-MS studies were performed using a Hewlett-Packard (Palo Alto, CA, USA) HP-1090 series II, made of an autosampler and a binary solvent pump, with a diode array detector (DAD) and a mass spectrometer detector (MSD) equipped with an ESI interface in negative ionization (NI) mode. The parameters of the ESI interface were as follows: vaporizer temperature, 325 °C; nebulizer gas (nitrogen) pressure, 60 psi; drying gas (nitrogen) flow rate, 13 mL min^{-1}; temperature, 350 °C; capillary voltage, 3500 V. Quantifications were performed by analysis in selected ion monitoring (SIM) mode of the ions m/z 941.5 $[M - H]^-$ for soyasaponin I in the first time window, 0−12 min, and m/z 1067.5 $[M - H]^-$ for soyasaponin βg in the second time window, 12−20 min.

Calibration curves of the analyzed soyasaponins were constructed by injecting 20 μL of mix standard solutions at six different concentrations, namely, 1.375, 13.75, 27.5, 55, 110, and 220 mg L^{-1} using the LC-MS technique. Three replicates for each concentration were performed during 5 days, and the relative standard deviations (RSDs) ranged from 1 to 2% for run-to-run precision and from 8 to 14% for day-to day precision. The calibration curves of the analyzed soyasaponins showed correlation coefficients equal to 0.998 (soyasaponin I) and 0.997 (soyasaponin βg). Limits of detection (LODs) and limits of quantification (LOQs) were estimated on the basis of 3:1 and 10:1 signal-to-noise ratios obtained with standards containing the compounds of interest at low concentration levels. LODs for soyasaponins I and βg were 0.02 and 0.2 mg L^{-1} (0.4−4 ng), respectively. LOQs for soyasaponins I and βg were 0.1 and 1 mg L^{-1} (2−20 ng), respectively. The recovery percentages of soyasaponins I and βg were investigated by spiking the different legumes with standard soyasaponin mixtures, reaching a final concentration level of 100 mg kg^{-1}, and by comparing the areas of spiked samples with those of nonfortified legume samples.

Mean recoveries of the two compounds ranged from 83 to 101%. The repeatability of the method, evaluated five times on each kind of legume, was expressed by RSD%, which was <14%.

Analysis of variance (ANOVA) was performed using the SPSS (v. 13.0) software package for Windows (SPSS Inc., Chicago, IL, USA). Values of $p < 0.05$ were considered to be statistically significant. Moreover, the repeatability interday was evaluated by one-way ANOVA using the Fisher test. The F test was used to evaluate the mean of the peak areas of each compounds. The analysis showed no significant differences between measurements relative to two marked compounds, obtaining F_{crit} of 3.13. The obtained results suggest that the proposed method is reliable.

RESULTS AND DISCUSSION

Quantifications of Soyasaponins I and βg in 60 Raw Legume Seeds. To quantify soyasaponins I and βg in legumes, a rapid and specific analytical procedure based on an SPE-HPLC-MS technique[6] was applied to the analysis of 60 commercial samples of raw legume seeds, from Italy, Canada, Turkey, Argentina, Poland, Peru, Mexico, Egypt, and Syria. The analytical data obtained in LC-MS from all analyzed samples are reported in Table 1. The quantification of soyasaponins was obtained by comparing the peak areas of compounds identified in the extracts of legume samples with those of pure standards. Sample 3, lentils from Colfiorito, Italy, displayed the highest content of total soyasaponins (2542 mg kg^{-1}) and a high level of soyasaponin βg (1807 mg kg^{-1}); also samples 4, 20, 40, 42, 45, and 46 showed total amounts >2000 mg kg^{-1} and corresponding high levels of soyasaponin βg. The highest quantity of soyasaponin I (907 mg kg^{-1}) was observed in green peas (sample 42) and the lowest (636 mg kg^{-1}) in green lentils (sample 6) and Tuscan soup (sample 60). In all cases, apart from hulled red lentils (sample 2), chicklings (sample 38), and lupins (sample 39), the content of soyasaponin βg was superior to that of soyasaponin I. Peas show a content of soyasaponin I that ranged from 702 to 907 mg kg^{-1} and of soyasaponin βg from 1070 to 1411 mg kg^{-1}, whetrsd chickpeas showed a content of soyasaponin I that ranged from 688 to 761 mg kg^{-1} and of soyasaponin βg from 866 to 1412 mg kg^{-1}. Apart from the soybean sample, which had a total soyasaponin content of 2492 mg kg^{-1}, these two kinds of legumes can be considered an important source of soyasaponins, with mean total soyasaponin

Table 2. Concentrations of Soyasaponins (SS) in Raw and Cooked Legumes and in Cooking and Soaking Water

		in seeds (mg kg^{-1}, dry wt)				in cooking solution (mg kg^{-1}, dry wt)				in soaking solution (mg kg^{-1}, dry wt)			
sample (trade name)	procedure	SS I	SS βg	total	RSD%[a]	SS I	SS βg	total	RSD%	SS I	SS βg	total	total saponin content
lentil from Colfiorito (3)	raw	735	1807	2542	0.14 (I) 0.46 (βg)	0	0	0		0	0	0	2542
	cooked	1074	1314	2388	0.13 (I) 0.39 (βg)	75	67	142	1.24 (I) 0.46 (βg)	0	0	0	2530
lentil from Umbria (5)	raw	678	1103	1781	0.44 (I) 3.91 (βg)	0	0	0		0	0	0	1781
	cooked	823	993	1816	1.77(I) 1.65 (βg)	68	66	134	0.21 (I) 0.11 (βg)	0	0	0	1950
chickpea (47)	raw	714	933	1647	0.67 (I) 2.50 (βg)	0	0	0		63	0	63	1647
	cooked	908	675	1583	2.77 (I) 0.17 (βg)	79	0	79	1.14 (I)	0	0	0	1662
chickling (37)	raw	637	647	1284	0.05 (I) 0.14 (βg)	0	0	0		0	0	0	1284
	cooked	642	635	1277	0.12 (I) 0.06 (βg)	64	0	64	0.32 (I)	0	0	0	1341
green pea (42)	raw	907	1411	2318	0.39 (I) 4.42 (βg)	0	0	0		0	0	0	2318
	cooked	701	695	1396	1.39 (I)	71	64	135	1.68 (I) 1.33 (βg)	0	0	0	1531
bean borlotti (7)	raw	696	861	1557	0.61 (I) 1.77 (βg)	0	0	0		0	0	0	1557
	cooked	757	671	1428	1.28 (I) 0.54 (βg)	73	63	136	1.11 (I) 0.32 (βg)	0	0	0	1564

[a]Each sample was analyzed in triplicate.

contents of 2045 and 1880 mg kg^{-1}, respectively. Lentils displayed a content of soyasaponin I that ranged from 636 to 735 mg kg^{-1} and of soyasaponin βg from 672 to 1807 mg kg^{-1}, whereas soups and mixtures of legumes showed a content of soyasaponin I that ranged from 636 to 738 mg kg^{-1} and of soyasaponin βg from 666 to 1046 mg kg^{-1}. Lentils and legume soups and mixtures displayed mean total soyasaponin contents of 1806 and 1557 mg kg^{-1}, respectively. Another legume that is a quite good source of soyasaponins, the bean, showed a content of soyasaponin I ranging from 642 to 817 mg kg^{-1} and of soyasaponin βg from 655 to 1209 mg kg^{-1}. It displayed a mean total soyasaponin content of 1498 mg kg^{-1}. Additionally, fava beans had a concentration of soyasaponin I that ranged from 642 to 661 mg kg^{-1} and a low concentration of soyasaponin βg that ranged from 700 to 717 mg kg^{-1} (the mean total soyasaponin content was 1361 mg kg^{-1}). In the two samples of chicklings analyzed, contents of soyasaponin I of 637 and 737 mg kg^{-1} and of soyasaponin βg of 647 and 661 mg kg^{-1} were found, respectively (mean total soyasaponin content was 1341 mg kg^{-1}). In lupins, concentrations of soyasaponin I of 721 mg kg^{-1} and of soyasaponin βg of 671 mg kg^{-1} were found in the only sample analyzed. Among legumes, taking into account the mean values, soybeans contain the highest level of soyasaponins, whereas lentils and peas show comparable levels of soyasaponins, higher than those in lupins. These data are in agreement with the literature.[17,22,23] Our data indicated that samples cultivated in Italy contain high amounts of group B soyasaponins compared to legumes cultivated abroad. This is clearly evident for lentils, chickpeas, and legume soups and mixtures and, to a lower extent, the same could be said for beans as well. This can indicate that many parameters may influence soyasaponin content in legumes, such as the different physical−chemical characteristics of the soil or tillage and the climatic conditions that are related to the territory geography. No significant differences in saponin content were observed between organic and normal legumes.

Cooking Studies on Legumes. Selected legumes, that is, two kinds of lentils, beans, chiklings, peas, and chickpeas, representative of the total set of the analyzed samples, were subjected to soaking and cooking processes for evaluating the changes in soyasaponin content. Data are reported in Table 2.

Both soyasaponins I and βg were detected in all cooked seeds analyzed. The concentrations of soyasaponin I in cooked legumes ranged from 642 to 1074 mg kg^{-1}; the lowest concentration was found in chicklings (sample 37) and the highest in lentils from Colfiorito (sample 3). The content of soyasaponin βg showed a similar trend, with the lowest level, 635 mg kg^{-1}, found in chicklings (sample 37) and the highest, 1314 mg kg^{-1}, found in lentils from Colfiorito (sample 3). When the data reported in Table 2 are taken into account, a conversion of soyasaponin βg into soyasaponin I in legume seeds during cooking is clearly evident. As an example, Figure 2

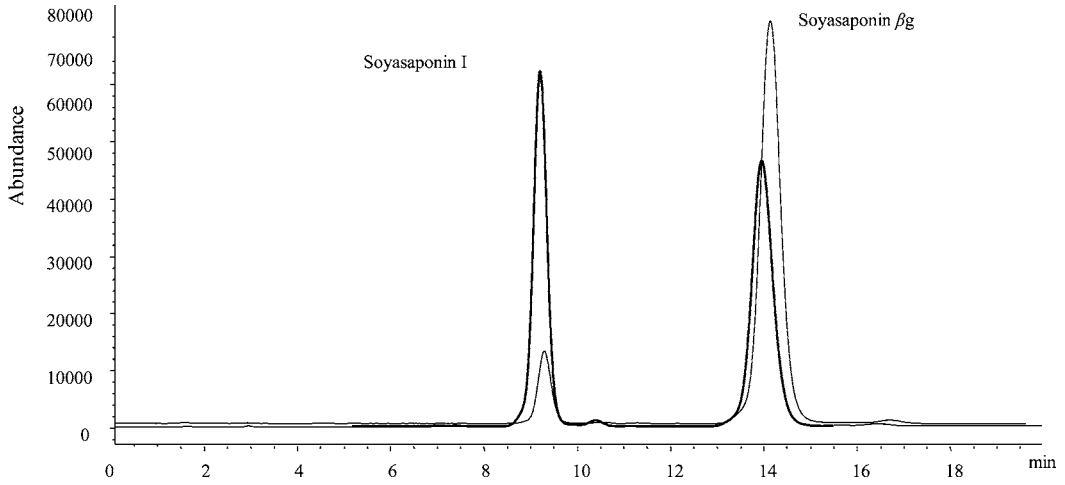

Figure 2. Overlapping of the LC-ESI-MS chromatograms of raw (gray) and cooked (black) lentil sample (no. 5) from Umbria.

Table 3. Bioaccessibility of Soyasaponin I Using the Simulated Gastrointestinal in Vitro Digestion (Referred to Dry Weight of Lentils)

	soyasaponin I (mg kg^{-1})				
cooking time (min)	cooked lentil	duodenal digestion	colon digestion	% duodenal bioaccessibility[a]	% colonic bioaccessibility[b]
30	640.52 ± 3.2	57.43 ± 1.4	0.54 ± 0.01	8.97 ± 0.3	0.084 ± 0.01
45	669.23 ± 2.5	65.32 ± 2.2	1.32 ± 0.4	9.76 ± 0.5	0.197 ± 0.02
60	684.00 ± 4.6	72.65 ± 1.3	1.87 ± 0.2	10.62 ± 1.1	0.274 ± 0.09

[a]Duodenal bioaccessibility is the percent ratio between the concentration of SS I in duodenal digestion and in cooked lentil. [b]Colonic bioaccessibility is the percent ratio between the concentration of SS I in colon digestion and in cooked lentil.

reports an overlapping of the LC-ESI-MS chromatograms of raw (gray) and cooked (black) lentil samples from Umbria (no. 5). This occurrence is the result of hydrolysis of the DDMP part of soyasaponin βg due to the high temperature reached during cooking, as confirmed by a previous study reported in the literature.[4] Considering the six cooked legume samples, in all cases, there was an increase of the concentration of soyasaponin I in cooked seeds compared to the raw seeds, and in four cooked samples (7, 37, 47, 42) this concentration was higher than that of soyasaponin βg. These conclusions apply to the four legumes analyzed, except for lentils from Colfiorito (3) and Umbria (5). In these samples, the concentration of soyasaponin I in cooked seeds was lower than that of soyasaponin βg, perhaps because the level of soyasaponin βg in raw legumes is particularly high and the conversion into soyasaponin I was not completed during the 30 min of the cooking experiment. The explanation of these losses of saponins is reported in the literature[4,16,24] and is confirmed in our work. It is suggested that, in addition to the conversion of soyasaponin βg into soyasaponin I, there is also a possible conversion of soyasaponin βg into soyasaponin Be.[4] This compound possesses soyasaponin E (sapogenol) as aglycone, and it presents the same sugar chain as soyasaponin I. Another suggestion is that soyasaponin I suffers from further degradation due to the heating process.[25] A discrete total saponin loss from raw to cooked legumes was evident only for green peas (about 34%), whereas in other samples it was minimal. It is possible that peas lose more total soyasaponin content in cooking than do lentils, chickpeas, beans, and chicklings because their seed structure was highly disrupted, releasing more saponins into the cooking solution and thus increasing the processes of further degradation. Soyasaponin I was also detected in all of the cooking liquids, whereas soyasaponin βg was not found only in the cooking liquids of chicklings (sample 37) and chickpeas (sample 47). Water used for cooking contained both soyasaponins in small concentrations, ranging from 64 to 79 mg kg^{-1} for soyasaponin I and from 63 to 67 mg kg^{-1} for soyasaponin βg. In all of these samples, soyasaponin I content was higher than that of soyasaponin βg (Table 2). The percentages of total soyasaponin contents leaked into the cooking solution correspond with those found by Ruiz et al.[4] The percentages of soyasaponins that leaked into the cooking solution were around 5.6 and 7.6% for lentils, 4.8% for chickpeas, 5.0% for chicklings, 5.8% for green peas, and 8.7% for beans. Instead, in soaking water, a small amount of soyasaponin I was found only for chickpeas (63 mg kg^{-1}), increasing for this kind of legume the percentage of soyasaponins lost into total water solution (cooking and soaking water) to 9.8%.

Study of Soyasaponin Bioaccessibility. For the first time, the duodenal and colonic bioaccessibility of soyasaponins I and βg in cooked lentils (lentil, sample 1, in Table 1) was investigated using an in vitro gastrointestinal digestion model that simulates the physiological human condition of the duodenal and colonic intestinal compartments. Three different cooking times of the analyzed lentils were tested. After the in vitro experiments, the simulated intestinal fluids were subjected to LC-MS analysis for the quantification of soyasaponins. The results of the bioaccessibility study are reported in Table 3. As can be observed, soyasaponin βg was found in neither the duodenal nor the colonic simulated compartments, which indicates that soyasaponin βg probably was easily converted into soyasaponin I during the digestion process.

With regard to soyasaponin I, it is interesting to observe that cooking times are strictly correlated with its bioaccessibility. In particular, the highest value of duodenal bioaccessibility, that is,

10.62 ± 1.1%, was found at 60 min of cooking time, whereas the lowest, 8.97 ± 0.3%, was at 30 min. A similar trend was observed for the colonic bioaccessibility with the highest value, 0.274 ± 0.09%, obtained at 60 min and the lowest, 0.084 ± 0.01%, at 30 min. This means that a more prolonged cooking time of lentils enhances the availability of the molecule to be active at the intestinal level. The data on duodenal bioaccessibility are very interesting if we consider that soyasaponins can act directly or indirectly as competitors for the absorption of exogenous cholesterol.[26] The colonic bioaccessibility data were from 39- to 111-fold lower than the data evidenced employing only duodenal digestion, probably due to the degradation of the soyasaponin I by the bacteria of the simulated gastrointestinal microflora. For this reason, as components of dietary fiber, soyasaponins should be considered a prebiotic component of food. Moreover, the conversion of soyasaponin βg in soyasaponin I, which was observed during the cooking in vitro digestion processes, confirms the metabolism proposed for soyasaponins that passes through the final formation of soyasapogenol B.[27]

In conclusion, for the first time, two cholesterol-lowering compounds, soyasaponins I and βg, have been quantified by the SPE-LC-MS method for 60 different samples of raw legumes. Soybeans contain the highest level of soyasaponins, but peas and chickpeas are also an excellent source of soyasaponins.

Among these samples, six cooked samples of legumes were analyzed using the same extraction procedure to compare the possible change in the concentration of soyasaponins I and βg in raw and cooked seeds, in water cooking solution, and in soaking water. The cooking experiment results showed clearly that soyasaponin βg converts into soyasaponin I and that a small amount of soyasaponins leaks into the cooking solution. Instead, soyasaponin content in soaking water was negligible. These data have established that total soyasaponin contents in raw and cooked legumes are similar and that, except for the pea sample, soyasaponin content in the legumes after cooking is complete.

In the bioaccessibility study, soyasaponin βg was not found in the colon or duodenum liquids, so we assume a transformation into soyasaponin I takes place. The soyasaponin I bioaccessibility values ranged from 8.9 ± 0.3 to 10.6 ± 1.1% in the duodenum compartment and from 0.08 ± 0.01 to 0.27 ± 0.09% in the colon compartment; soyasaponins at these levels, especially in the duodenum, could act directly or indirectly as competitors for the absorption of exogenous cholesterol.

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Notes

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