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Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes

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Abstract

Methanol extracts of seeds from 23 accessions of 3 *Phaseolus vulgaris* ecotypes ("Sarconi", "Lamon", "Zolfino del Pratomagno"), grown in different Italian regions (Basilicata, Veneto, Tuscany) were analyzed for their flavonoid content. Flavonoid glycosides were found in the seed coat from ten accessions of the "Zolfino" ecotype and in one accession of the "Sarconi" ecotype. From highest to lowest concentration these compounds were kaempferol 3-*O*-glucoside (compound **2**), kaempferol 3-*O*-xylosylglucoside (compound **1**) and a not completely identified kaempferol monoglucoside (compound **3**). Total flavonol content varied from 0.19 to 0.84 g/kg of seed fresh weight. A great variability in the total flavonol content, being between 18% and 50%, and in the relative abundance of different kaempferol derivatives was observed for the same genotypes sampled in the original locations in the 2001–2003 period. Fluctuation in flavonol content suggests that further researches are necessary for an exhaustive comprehension of physiological mechanisms influencing the expression of these phenolic compounds. Obtained results evidenced that some Italian bean ecotypes may be an important source of functional compounds as kaempferol glycosides.

Keywords: *Phaseolus vulgaris*; Italian bean ecotypes; Flavonols; Kaempferol; Nutraceutical compounds

1. Introduction

Common bean had been recognized since a long time for its protein content, but in general there have been relatively little research and discussion on its nutraceutical value (Aragao et al., 1996; Bengtsson, 1991; Bollini, Carnovale, & Campion, 1999; Sharma, 1993). This crop is widely cultivated in Italy and nevertheless the relevant decreasing in production in all Europe, Italy is within European Union a leader in bean production (Laghetti, Xhuveli, Perrino, Olita, & Hammer, 1998). Trade necessities favored bean commercial cultivars, confining local ecotypes in very small areas characterized by traditional cropping practices (Limongelli, Laghetti, Perrino, & Piergiovanni, 1996). As regards Italian bean ecotypes, very few of them possess a high seed or pod quality negatively influencing the relative commercial value (Piergiovanni, Cerbino, & Gatta, 2000). These ecotypes are cropped in few farms precisely located in mountainous or hilly areas: they are known by local names and some of them are labeled by Indication of Geographical Provenance (IGP). Some examples are "Fagiolo di Lamon", "Fagiolo di Sarconi", "Fagiolo Zolfino del Pratomagno". "Lamon" bean comes from the mountainous area of Lamon and Sovramonte (Veneto) and only four populations are known: Spagnolet, Spagnolon, Calonega and Canalin (Piergiovanni, Cerbino, & Brandi, 2000). "Sarconi" bean is a locally grown variation of the *Cannellino* and *Borlotto* bean ecotypes. The special environmental conditions and the plentiful supply of water of western

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Basilicata combine to give the beans grown in Sarconi a quality quite distinct from those cultivated in other zones. Whether fresh or dried, the Sarconi beans are much appreciated for their thin coat, short cooking time and good taste (Piergiovanni et al., 2000). Finally, "Zolfino del Pratomagno" bean is a dwarf bean, with a characteristic pale yellow color. It is cropped in Pratomagno area, a hilly region in Tuscany which encompasses seven villages. Production is characterized by a low yield, limited storage capacity and poor response to technical inputs. These factors brought this bean on the verge of extinction in the 1970s. This ecotype has benefited from EU agri-environments funds aimed at increasing cultivation of threatened species (Turchi, 2000). All cited Italian ecotypes are cropped with traditional methods preventing crop from pathogens attack and permitting to obtain seeds without any chemical treatment.

Flavonoids, which are found in virtually all plants, encompass a wide range of structural classes and biological functions (Kuhnau, 1976): they include several chemical classes such as flavanones, flavones, flavonols, anthocyanins, biflavonoids, isoflavones, coumestans, isoflavans, pterocarpans (Seigler, 1998). Plant flavonoid content can be influenced by different factors, such as light, temperature, mineral nutrition, pathogens, mechanical damage, plant growth regulators (Jaakola et al., 2002). They could act as antioxidants, enzyme inhibitors, pigments for light absorbance, visual attractants for pollination, light screen, inhibitors of plant growth, chemical signals in nodulation gene induction and phytoalexins (Beier & Nigg, 1992; Paolacci et al., 2001). In common bean a conspicuous number of flavonoids were isolated and identified (Feenstra, 1960; Kucera, Leubner-Metzger, & Wellmann, 2003). Researchers accept the fact that the pigments responsible for seed coat color in *Phaseolus vulgaris* are flavonoids (Beninger, Hosfield, & Nair, 1998). Many of the flavonoids pigments that give rise to seed coat color in beans may also impart positive health benefits as antioxidants (Amic, Davidovic-Amic, Beslo, & Trinajstic, 2003; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog, Hollman, Katan, & Kromhout, 1992). In particular several glycosidic forms of quercetin and kaempferol have been identified in seeds of *P. vulgaris* (Beninger et al., 1998; Beninger & Hosfield, 1999; Clifford, 1996; Hertog et al., 1993; Hertog, Hollman, & Katan, 1992; Hodges & Forney, 2000; Lioi, 1989; Romani et al., 2004; Scalbert & Williamson, 2000; Vinson, Hao, Su, & Zubik, 1998; Wells, 2004). In particular Romani et al. (2004) quantified the content of flavonols in some landraces from Pratomagno area.

Since there is still little knowledge about the flavonoid content of Italian bean ecotypes, we undertook the present investigation to identify flavonoid compounds in 23 bean ecotypes from three different Italian hilly areas (Sarconi, Pratomagno, Lamon) and to compare them with flavonoid content of four commercial bean cultivars. The specific objectives were to determine the storage localization in the seed (coat, embryo, endosperm) and to evaluate the trend of the flavonoid content in a three-year lasting period (2001–2003).

2. Materials and methods

2.1. Plant materials

Phaseolus vulgaris L. seeds of three Italian ecotypes (Sarconi, Lamon, Zolfino del Pratomagno) were directly sampled from local farmers or producer associations. Ten, three and ten populations were collected for Sarconi, Lamon and Zolfino del Pratomagno ecotypes, respectively (Table 1). The Italian bean

Table 1
List of analyzed Italian ecotypes and cultivars of *P. vulgaris* L.

Ecotype/cultivar	Code	Original location	Local name
Sarconi	S13	Sarconi (PZ, Italy)	Riso bianco
	S14a	Sarconi (PZ, Italy)	Tuvagliedda bianca
	S14b	Sarconi (PZ, Italy)	Tuvagliedda rossa
	S15	Sarconi (PZ, Italy)	Nasieddu
	S17	Sarconi (PZ, Italy)	Munachedda
	S18	Sarconi (PZ, Italy)	Ciuoto
	S19	Sarconi (PZ, Italy)	Cannellino bianco
	S20	Sarconi (PZ, Italy)	Verdolino nano
	S21	Sarconi (PZ, Italy)	Tabacchino nano
	S22	Grumento Nova (PZ, Italy)	San Michele o' Ciuoto
Lamon	L1	Lamon (BL, Italy)	Spagnolon
	L2	Lamon (BL, Italy)	Spagnolet
	L3	Lamon (BL, Italy)	Calonega
Zolfino del Pratomagno	Z1	Loro Ciuffenna (AR, Italy)	–
	Z2	Loro Ciuffenna (AR, Italy)	–
	Z3	Loro Ciuffenna (AR, Italy)	–
	Z4	Loro Ciuffenna (AR, Italy)	–
	Z5	Reggello (AR, Italy)	–
	Z6	Terranova Bracciolini (AR, Italy)	–
	Z7	Terranova Bracciolini (AR, Italy)	
	Z8	Terranova Bracciolini (AR, Italy)	–
	Z9	Terranova Bracciolini (AR, Italy)	–
	Z10	Terranova Bracciolini (AR, Italy)	–
Contender	C	–	–
Tendergreen	T	–	–
Sanilac	S	–	–
Lingua di fuoco	LDF	–	–

ecotypes were sampled for three years (2001–2003) from the same farmers or producer associations. In addition, four commercial bean cultivars (Sanilac, Tendergreen, Contender, Lingua di fuoco) were analyzed. Seeds of "Zolfino del Pratomagno" ecotypes and "Sanilac" cultivar were characterized by a uniform, light yellow seed coat pigmentation, while bean seeds of all the other samples showed different seed coat pigmentation.

2.2. Standards and chemicals

Authentic standard of flavonols (kaempferol, kaempferol 3-*O*-xylosylglucoside, kaempferol 3-*O*-glucoside) were purchased by Indofine Co., Hillsborough, USA. The chemical structures of the three flavonols is reported in Fig. 1. Methanol, hydrochloric acid, acetic acid and all solvents used for HPLC were of analytical or HPLC grade from Carlo Erba, Milan, Italy.

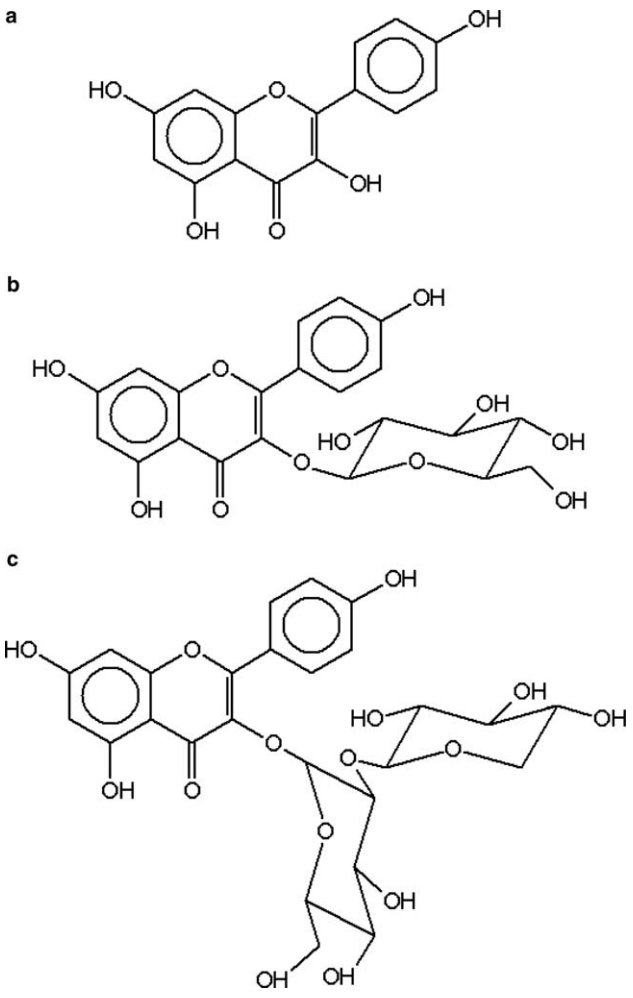

Fig. 1. Chemical structure of kaempferol (a), kaempferol 3-*O*-glucoside (b) and kaempferol 3-*O*-xylosylglucoside (c).

2.3. Standard solutions, calibration curves and calculation of flavonol content

Flavonoids stock solutions were prepared by dissolving the crystalline standards first in 1000 ppm stock solutions. Subsequently, stock solutions were diluted to 5 ppm with 80% methanol (v/v). Calibration curves were obtained for each standard with high linearity ($r > 0.996$) by plotting the standard concentrations as a function of the peak area obtained from HPLC analysis with 40 μl injections. For this purpose, the stock solutions of the standards were diluted with 80% methanol to five different concentrations ranging from 1 to 20 mg l^{-1}. Each concentration was analyzed by triplicate injections.

2.4. Extraction of flavonols

As regards whole seed extraction, 500 mg of ground dry seeds were dispersed in a mixture of 2 ml of acetonitrile and 0.5 ml of 0.1 N HCl by stirring for 3 h (Barnes, Kirk, & Coward, 1994; Wang & Murphy, 1994). After 3 h, the mixtures were centrifuged at 15,000 rpm for 10 min at 10 °C. Clear supernatant was filtered through a 0.45 μm nylon filter (Millipore Co., USA) and dried by a vacuum pump. Samples were reconstituted with 2.5 ml of 80% methanol.

For the differential analysis of seed coat, endosperm and embryo, 100 g (fresh weight) of beans were washed and then placed in enough distilled water to soak the beans. After 10 min of soaking the seed coats were separated from cotyledons and embryos. Seed coats, endosperms and embryos were freeze-dried. The water exudates from the soaked bean were also freeze-dried and stored. Seed coats, endosperms, embryos and water exudates were lyophilized, ground to fine powder and flavonols were extracted as previously reported.

Since the extraction method has been mainly employed for the analysis of isoflavones, it was validated for the purpose of the present research. Amounts of external standards (kaempferol 3-*O*-xylosylglucoside, kaempferol 3-*O*-glucoside and kaempferol at the concentration of 10, 50 and 100 ppm) in a methanol solution were added to 500 mg samples (ground dry seed of Z1 accession). The samples were mixed and left at room temperature until the methanol had evaporated. The HPLC analysis method is described below. The average overall recovery in the 5–100 ppm range was 98.3 ± 1.8%, 98.6 ± 1.5% and 95.3 ± 0.3% for kaempferol 3-*O*-xylosylglucosid, kaempferol 3-*O*-glucoside and kaempferol, respectively (Table 2). For kaempferol 3-*O*-xylosylglucoside and kaempferol 3-*O*-glucoside the mean recovery was slightly higher in the 100 ppm fortified samples, but the differences with respect to the other fortifications were not significant. Since compound **3**

Table 2
Mean recovery (MR, %) and coefficient of variation (CV, %) of kaempferol compounds (compound **1** = kaempferol 3-*O*-xylosylglucoside; compound **2** = kaempferol 3-*O*-glucoside; compound **4** = kaempferol) in six replicates of Zolfino (Z1) dry ground seed fortified with standard concentrations (5, 50, 100 ppm)

Compound	5 ppm		50 ppm		100 ppm	
	MR (%)	CV (%)	MR (%)	CV (%)	MR (%)	CV (%)
1	96.2	3.2	98.9	2.8	99.8	2.8
2	97.1	3.3	98.6	2.9	100.2	2.5
4	95.3	3.2	95.1	3.1	95.6	3.2

was not clearly identified, the relative recovery was deduced by comparing the present extraction method with the extraction procedure (70% ethanol) proposed by Romani et al. (2004) for the analysis of bean flavonols. Six grams of intact seeds (Z1 accession) were ground and the resulting flour was divided in 12 aliquots (500 mg each). Six aliquots were extracted with 70% ethanol (Romani et al., 2004), while the remaining six aliquots were extracted with acetonitrile (Barnes et al., 1994; Wang & Murphy, 1994). On the basis of the comparison between the two extraction procedures, the mean recovery of compound **3** was 97.3 ± 2.2%. The mean recovery values, experimentally determined, were used to correct the flavonol content in the bean accessions sampled in the original location in the period 2001–2003.

2.5. Acid hydrolysis

Bean extract solutions (whole seed, seed coat, endosperm, embryo) (2 ml) was mixed with concentrated HCl (37%, 0.5 ml) and heated in a sealed vials at 80–85 °C for 2 h. The acid hydrolysis procedures was shown to completely convert glycosides to aglycones by using authentic flavonols standards (data not shown). Subsequently, flavonols were extracted with ethylacetate, evaporated in vacuum and suspended in methanol 80% (2 ml). The solutions were re-filtered prior to HPLC injection.

2.6. Preparative HPLC

The seed coats of 50 g of beans were removed, extracted and hydrolyzed as previously described. The purification of compound **4** (Fig. 2) for NMR analysis was achieved on a Waters Xterra MS reverse-phase C18 column (10 × 150 mm, 5 μm) using a Beckman System Gold 126 multisolvent pump, photodiode array detector Beckman 168 and a Spark Holland autosampler. The solvent system was (A) H_2O/CH_3COOH (99.9:0.1) and (B) $ACN/H_2O/CH_3COOH$ (80:19.9:0.1). Separations were done in isocratic mode with a flow rate of 2 ml/min. The detection was at 260 nm. Each injection volume was 1.5 ml. If the peak collected was not pure, it was re-chromatographed under the above conditions and the compound **4** collected pure after one or more purification runs. All chromatograms were analyzed using System Gold software (9.0).

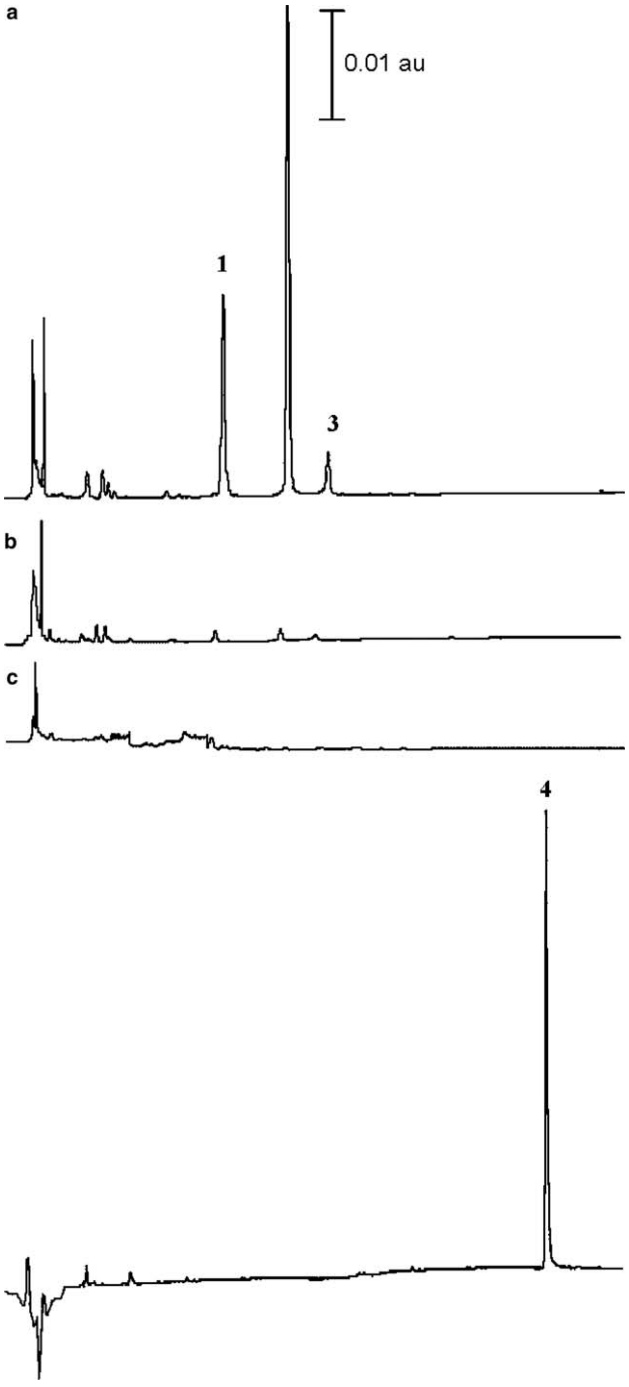

Fig. 2. HPLC–DAD ($\lambda = 260$ nm) profiles of methanol extracts from accession Z2 (Zolfino bean ecotype) collected in the 2001. (a) seed coat extract, (b) endosperm extract, (c) embryo extract, (d) acid hydrolysis of seed coat extract. Compound **1** = kaempferol 3-*O*-xylosylglucoside, compound **2** = kaempferol 3-*O*-glucoside, compound **3** = unidentified kaempferol monoglucoside, compound **4** = kaempferol.

2.7. Analytical techniques and equipment

HPLC–DAD analysis. Analyses of flavonols were carried out using a Beckman liquid chromatograph consisting of a Gold 126 multisolvent pump, photodiode array detector Beckman 168 and a Spark Holland autosampler. For the separations a Waters XTerra MS reverse-phase C18 column (2.1 × 150 mm, 5 μm) operating at 25 °C was employed. The eluent was composed of (A) H_2O/CH_3COOH (99.9:0.1) and (B) $ACN/H_2O/CH_3COOH$ (80:19.9:0.1). A two-step linear solvent gradient system was used starting from 20% to 40% of solution B for a 30-min period at a flow rate of 0.2 ml/min. The percentage of solution B reached 30% from 5 to 9 min, then 40% from 10 to 20 min. The injection volume was 10 μl. UV–Vis spectra were recorded in the 200–500 nm range and the chromatograms were recorded at 260 nm.

HPLC–MS analysis. HPLC–MS analyses were carried out with a Finnigan Surveyor LC System linked to a TSQ Quantum-Finnigan mass spectrometer with an electrospray interface (Finnigan). The separations were performed using a Waters XTerra MS reverse-phase C18 column (2.1 × 150 mm, 5 μm) operating under the same chromatographic conditions previously reported. Spectra were recorded in negative (fragmentor 80) and positive (fragmentor 120) ion mode. The mass spectrometer operating conditions were: capillary temperature 300 °C; nebulizer pressure 45 psi, spray voltage 3200 V, mass range of 50–1000 amu, quadrupole temperature 40 °C.

NMR analysis. NMR analysis of the purified flavonol **4** and of the standard kaempferol were carried out at 600 MHz for ^1H spectra (Varian Inova 600 MHz spectrometer, equipped with an indirect detection probe). A standard sample of authentic kaempferol was prepared by dissolving 1.0 mg of the compound in 0.7 ml of CD_3OD. An analytical sample of purified flavonol **4** was prepared from 1.0 mg of compound dissolved in 0.7 ml of CD_3OD. Direct comparison between the proton spectra of the two samples confirmed the structural identification of compound **4** as kaempferol (Fig. 3).

2.8. Identification and quantification of flavonoids

Identification of flavonoids was based on the comparison of their retention times, diode array scans and mass spectra with those of authentic standards. The compound **4** was purified and the chemical structure determined by NMR analysis. Quantification of individual flavonoids was based on HPLC analyses using a five-point regression curve ($r^2 > 0.97$). For each collection site in each year five different seed samples were analyzed. Analytical results were expressed as means with their SDs. Data were elaborated using the SAS Institute statistical package. Analysis of variance was carried out using a general linear model procedure with Student–Newman–Keuls comparison of means to test for significant differences between means.

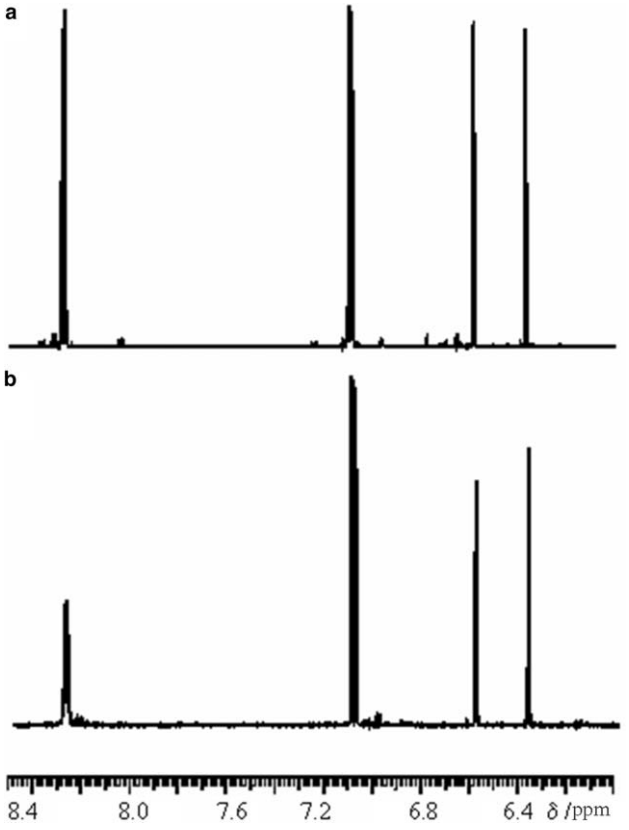

Fig. 3. 1H NMR spectrum of purified compound **4** as compared to spectrum of kaempferol standard. (a) 1H NMR spectrum of kaempferol standard; (b) 1H NMR spectrum of compound **4**.

3. Results and discussion

HPLC–DAD analysis of bean samples collected in the 2001 evidenced three flavonols, hereinafter named compounds **1**–**3**, in one Sarconi accession (S20) and in all the analyzed Zolfino populations (Z1–Z10). No flavonol was observed in all the other samples (remaining Sarconi and Lamon accessions, bean cultivars). For example, the chromatographic profile of Z1 populations at 260 nm, evidencing the qualitative composition of flavonols, is reported in Fig. 2. The three compounds were characterized by similar absorption spectra, with absorption peaks at 266 and 347 nm (data not shown). As evidenced by differential analysis of various seed parts (embryo, endosperm, seed coat), the three compounds are primarily stored in the seed tegument (Fig. 2). In fact only traces of the three flavonols were found in the endosperm (less than 0.1% of the total content) and no compound was found in the embryo. This result is in agreement with the literature (Beninger

et al., 1998; Beninger & Hosfield, 1999). In general, in the *Phaseolus* genus flavonols and other phenolic compounds are usually stored in the seed tegument for the following main reason: these compounds are characterized by anti-pathogen and anti-feeding activities and their tegument localization assures the best protection of the seed from external attacks (pathogens, insects). The acid hydrolysis of seed coat extracts evidenced that compounds **1**–**3** are conjugated forms of the same aglycone (hereinafter called compound **4**) (Fig. 4). The DAD spectrum of compound **4** was characterized by three absorption peaks at 207, 266 and 347 nm (data not shown). According to spectroscopic data reported in the literature (Lin et al., 2000), the compound **4** was tentatively identified as kaempferol. The identification was confirmed by the retention time of authentic kaempferol standard (data not shown), mass and NMR spectra. On the basis of mass spectra recorded in positive and negative ion mode, the molecular weight of compound **4** was 286 (Fig. 4). In addition, MS/MS experiments carried out in both positive (+MS2 precursor *m*/*z* 287; +MS3 precursors *m*/*z* 241, *m*/*z* 213; *m*/*z* 164) and negative (−MS2 precursor *m*/*z* 285; −MS3 precursors *m*/*z* 256, *m*/*z* 239, *m*/*z* 228) polarity showed for the compound **4** identical fragmentation pattern to that of authentic kaempferol standard (data not shown). Finally, 1H NMR spectra of compound **4** match exactly the spectra obtained from the standard sample. In the case of the proton spectra of compound **4** (Fig. 3(a)) it has to be noted that the signal at 8.28 ppm is broader with respect to the same signal in the standard sample (Fig. 3(b)). This signal (2 hydrogens) belongs to the *para*-substituted phenolic ring, and the broadening effect is probably due to a very little coupling constant with the phenolic hydroxyl group.

Compounds **1**–**3** are different glucosidic forms of kaempferol. Negative ion mass spectrum of compound **1** had a peak at *m*/*z* 579, corresponding to the quasi-molecular ion of kaempferol 3-*O*-xylosylglucoside $[M + H]^+$ (Fig. 4). Positive ion mass spectrum of com-

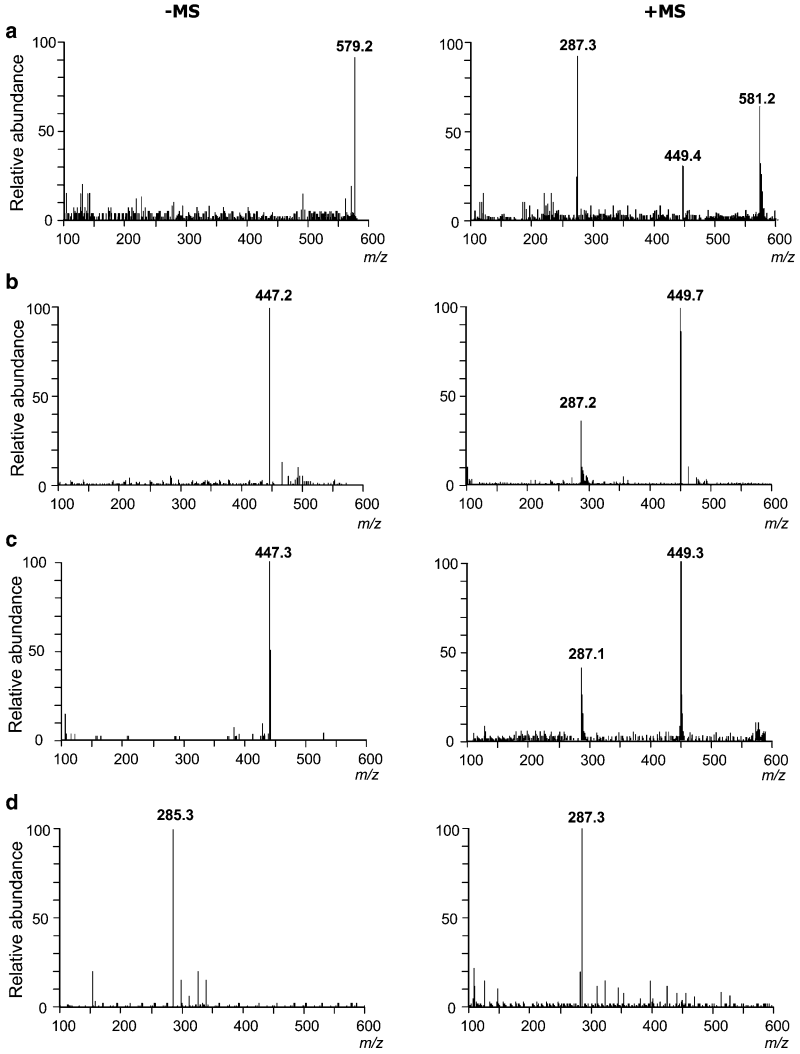

Fig. 4. Negative and positive ion mass spectra of compound **1** (a), **2** (b), **3** (c) and **4** (d) obtained by HPLC–MS analysis.

pound **1** showed three signals at m/z 581, 449 and 287, corresponding, respectively, to the quasi-molecular ion of kaempferol 3-*O*-xylosylglucoside $[M - H]^-$, the fragment after the loss of a xyloside moiety $[M - 132 - H]^-$ and the kaempferol aglycon $[M - 294 - H]^-$. Negative ion mass spectrum of compound **2** had a peak at m/z 447, corresponding to the quasi-molecular ion of kaempferol 3-*O*-glucoside $[M + H]^+$ (Fig. 4). Positive ion mass spectrum of compound **2** showed two signals at m/z 449 and 287, corresponding, respectively, to the quasi-molecular ion of kaempferol 3-*O*-glucoside $[M - H]^-$and to kaempferol aglycon $[M - 162 - H]^+$. The identification of compounds **1** and **2** was confirmed by the injection of authentic kaempferol 3-*O*-xylosylglucoside and kaempferol 3-*O*-glucoside standards and by comparison of their retention times (data not shown). Kaempferol 3-*O*-glucoside and kaempferol 3-*O*-xylosylglucoside were previously isolated and identified by several authors (Beninger et al., 1998; Beninger & Hosfield, 1999; Feenstra, 1960) in different *Phaseolus* genotypes characterized by yellow seed coats. Recently, the presence of these two compounds was also confirmed in Zolfino landraces (Romani et al., 2004). Negative and positive mass spectra of compound **3** were nearly identical to those of compound **2** (Fig. 4). Despite of the identical mass spectra, compounds **2** and **3** are characterized by different retention times. This evidence suggests that compound **3** is a monoglucoside form of kaempferol different from kaempferol 3-*O*-glucoside. Moreover in Romani et al. (2004) a compound, extracted from Zolfino landraces and characterized by chromatographic features similar to those of the compound **3** of the present paper, was tentatively identified as kaempferol 3-acetylglucoside. However, the molecular weight of kaempferol 3-acetylglucoside (490 Da) was not compatible with mass spectra observed for compound **3** (Fig. 4). Studies are in progress in order to obtain a clear identification of the compound **3**.

The quantitative data on the content of flavonols in the investigated *P. vulgaris* ecotypes during the period 2001–2003 are reported in Table 3. As previously reported, flavonols (kaempferol and related conjugated forms) were found in all the Zolfino accessions and in one Sarconi accession. To our knowledge, this is the first report testifying the presence of kaempferol and related conjugated forms in a Sarconi genotype. The total content of flavonols ranged from 0.19 to 0.84 g/kg and was similar with that reported by Romani et al. (2004). In addition, the mean values observed were in general agreement with those found for other yellow and green French beans (Manteca-type and Prim-type from Chile) (Beninger et al., 1998). According to Beninger and Hosfield (1999), kaempferol 3-*O*-glucoside (compound **2**) was the main flavonol found in all samples with a percentage ranging between 47% and 68% of total flavonol content. The second most abundant flavonol was kaempferol 3-*O*-xylosylglucoside (compound **1**) with a percentage ranging between 16% and 33% of total flavonol content. The analyses of the same genotype sampled in the original location in different years evidenced a great variability in the total flavonol content and in the relative abundance of different kaempferol derivatives. The coefficients of variation for the mean total content of flavonols observed in the period 2001–2003

Table 3
Content (g/kg seed) of kaempferol (compound **4**) and relative conjugates (compound **1** = kaempferol 3-*O*-xylosylglucoside; compound **2** = kaempferol 3-*O*-glucoside; compound **3** = unidentified kaempferol monoglucoside) in Zolfino accessions (Z1–Z10) and in one Sarconi accession (S20)

Year	Compound	Sarconi (S20)	Zolfino		
			Loro Ciuffenna (Z1–Z4)	Reggello (Z5)	Terranova (Z6–Z10)
2001	**1**	0.16 ± 0.03	0.11 ± 0.09	0.07 ± 0.01	0.09 ± 0.02
	2	0.30 ± 0.12	0.21 ± 0.06	0.23 ± 0.06	0.21 ± 0.07
	3	0.03 ± 0.01	0.05 ± 0.03	0.04 ± 0.02	0.03 ± 0.01
	4	–	–	–	–
Total		0.50 ± 0.08	0.38 ± 0.11	0.35 ± 0.11	0.34 ± 0.09
2002	**1**	0.13 ± 0.04	0.08 ± 0.02	0.13 ± 0.07	0.03 ± 0.01
	2	0.37 ± 0.07	0.34 ± 0.09	0.52 ± 0.29	0.09 ± 0.02
	3	0.04 ± 0.01	0.06 ± 0.03	0.16 ± 0.10	0.03 ± 0.01
	4	–	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02
Total		0.54 ± 0.11	0.51 ± 0.12	0.84 ± 0.45	0.19 ± 0.07
2003	**1**	0.17 ± 0.06	0.11 ± 0.07	0.15 ± 0.04	0.16 ± 0.02
	2	0.38 ± 0.05	0.20 ± 0.04	0.44 ± 0.10	0.37 ± 0.12
	3	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.03 ± 0.01
	4	–	–	–	–
Total		0.62 ± 0.13	0.38 ± 0.08	0.65 ± 0.18	0.56 ± 0.09

were ±18%, ±20%, ±40%, ±50% for the Sarconi ecotype, for the Zolfino ecotypes sampled at "Loro Ciuffenna", "Reggello" and "Terranova", respectively. In addition, in the 2001 and 2003 no kaempferol in the aglycon form was observed in the Zolfino ecotypes, while in 2002 the percentage of this compound ranged between 4% and 21% of total flavonol content. In the 2001 and 2003 the mean percentage of kaempferol 3-*O*-xylosylglucoside in Sarconi and Zolfino accessions was 28% of total flavonol content, while in 2002 the mean percentage of this kaempferol derivative was significantly lower (18%) than that observed in the other years. A great variability of flavonoid content was also observed in soybean seed. Experimental studies carried out on the same soybean genotype showed year by year a relevant fluctuation (±50%) of seed isoflavone content as a function of growth environmental conditions and adopted cropping techniques (Elridge & Kwolek, 1983; Tsukamoto et al., 1995; Wang & Murphy, 1994). In particular, Carrao-Panizzi, Del Pino Beleia, Kitamura, and Oliveira (1999) and Tsukamoto et al. (1995) demonstrated that in soybean the seed isoflavone content increased when seed development occurred at moderate temperatures. However, an unique hypothesis to justify such a phenomenon was not proposed. Probably isoflavones, as other phenolic compounds (flavonols) are phytoalexins, substances involved in plant defense mechanisms against pathogens (Hahlbrock & Scheel, 1989; Nicholson & Hammerschmidt, 1992; Van Etten & Pueppke, 1976). In particular, phytoalexins are involved in mechanisms of systemic acquired resistance (SAR). Many experimental papers showed that in the first phases of infection a relevant biosynthetic activity of different phytoalexins in plant tissues takes place (Kuc, 1995). Today, common opinion is that, also several abiotic stresses act as elicitors, inducing defense mechanisms in plants. Some molecular studies showed that the transcription of enzymes involved in the synthesis of these compounds is spatially and temporarily coordinated and that enzyme transcripts are induced by either compatible and no compatible interactions (Meier, Shaw, & Slusarenko, 1993). The observed fluctuation of flavonol content in the investigated bean ecotypes suggests that further researches are necessary for an exhaustive comprehension of physiological mechanisms influencing the expression of these phenolic compounds. Such an understanding will represent the starting point for the identification and development of proper cropper techniques able to maximize the yield of flavonols in bean genotypes. Several studies have extensively reported the beneficial effects of flavonoids intake for human health. As reported from several researchers fruits and vegetables are important sources of these compounds. Moreover, it has been determined that beans (*P. vulgaris* L.) are sources too of these compounds, especially kaempferol. In general, flavonols are antioxidant and chelating molecules with several beneficial effects on human health (Heim, Tagliaferro, & Bobilya, 2002). In particular, kaempferol and related conjugated forms are reported to exert anti-cancer activity (Di Carlo, Mascolo, Izzo, & Papasso, 1999). The undertaken study about evaluation of flavonoid contents in Italian bean ecotypes has enlighten the possible role of these compounds in a further valorization of these ecotypes to consumers from a nutraceutical point of view. At the present soybean is considered among legume crops the most relevant source of flavonoids (isoflavones) with beneficial health effects. However, soybean is not a traditional food crop in Italy and other Mediterranean countries. In addition soybean was one of the first crop genetically modified for agronomic purposes. Most European consumers do not accept the principle of substantial equivalence between GM- and GM-free foodstuffs. Soybean-derived products are influenced by this spread attitude of European consumers and this crop can hardly be accepted as a source of nutraceutical compounds. As a consequence, a scientific effort on Mediterranean area legumes crops, as GM-free sources of flavonoids is particularly interesting. In particular, the determination of flavonoid content as a function of the bean genotype and the applied agronomic practices could be usefully exploited both for breeding programs aiming to increase the yield of these compounds and for orienting the consumer choice towards common bean as a source of nutraceutical compounds with antioxidant and chemo-protective activity.

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