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Variability of Nutritional, Antioxidant, and Textural Traits of a Collection of Snap Beans of Different Colors

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Abstract: A set of 54 snap bean lines grown under organic farming was characterized for different traits: pod color, pod cross-section shape, pod section width (PSW), protein content (PC), and sugar content (S). After cooking, the lines were analyzed for firmness and color parameters (CIE-L*, a*, b*). The snap bean lines were grouped based on pod cross-section shape and pod color into eight groups, and significant differences were observed among lines and groups for all the traits investigated. In particular, the yellow pods were harder than the other snap beans and less sweet due to the negative correlation between firmness and sugar content. Fourteen selected lines with contrasting firmness and belonging to different color groups were investigated for their phenolic composition and antioxidant activity (TEAC) before and after domestic cooking. A general decrease was observed only for vanillic acid, quercetin, and apigenin-8-C-glucoside. Quercetin-3-O-rutinoside and kaempferol-3-O-rutinoside were detected in all samples with the highest values in the SBP042 line in both raw and cooked samples. Antioxidant activity decreased with cooking (average of 39%), but purple line SBP053 showed the lowest and no significant loss (3.1%). The results reported in this study could be useful to design specific varieties for different markets and purposes.

Keywords: *Phaseolus vulgaris* L.; snap beans; nutritional quality; antioxidant activity; texture; cooking

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1. Introduction

Snap beans (syn. French beans, green beans) are common beans (*Phaseolus vulgaris* L.) grown for their edible immature pods. The common bean is considered the most important food legume for direct human consumption in Europe and worldwide [1,2], with production of up to 27.7 and 1.31 million tons in 2021 worldwide for dry and snap beans, respectively (items: "beans, dry" and "string beans") [3]. Differently from dry common beans, the fresh pods of snap beans are consumed preferentially as fresh vegetables. They are rich in water and represent a source of primary nutrients, such as protein, minerals, vitamins, dietary fiber, and soluble sugars [4]. The use of snap beans is highly recommended in low carbohydrate diets, and their low glycemic index makes their consumption suitable also for diabetics [4,5]. In addition, snap beans are also an important source of phytonutrients as polyphenols that play a significant role in the human diet and well-being [6]. Several studies on common beans have pointed out the relationship between color and phytochemicals, molecules that play an important role in human health because they possess antioxidant activity [7], which has anti-diabetic [8], anti-obesity [9], anti-inflammatory [10], anti-mutagenic [11], and anti-carcinogenic properties [12]. Domesticated common bean germplasm is characterized by two major eco-geographically and genetically distinct gene pools, the Andean and the Mesoamerican, where domestication occurred independently

(see [2] as a review). Subsequently, domesticated materials from both gene pools were widespread worldwide, where the lack of spatial isolation between the Mesoamerican and Andean gene pools led to greater possibilities for hybridization and introgression between Mesoamerican and Andean genotypes [13]. Landraces with edible fresh pods are rarely found in North, Central, and South American collections [14,15]. Snap beans are suitable for green pod production due to the low fiber content in the pod walls and sutures [16]. The exact origins of snap beans are still unclear; however, snap bean cultivars likely arise because of selective pressures on pod characteristics exerted on dry materials consumed as mature seeds [17]. Common beans exhibit high levels of morphological diversity in their pods [18]; that was also observed in the pod morphology variation of a large snap bean panel (SBP) collected in Europe from which was established a core collection with the maximum diversity of pod phenotypes [19].

Textural properties are also relevant among the different traits that drive consumer choices, such as the absence of fibers, flavor, and excellent nutritional characteristics [20]. During post-harvest, biochemical changes occur in green beans mainly due to mechanical damage, such as wilting, chlorophyll pigment degradation, and increased fiber content or additionally due to treatment severity and treatment duration in processing [21,22]. All these factors reduce their economic and nutritional value due to loss of texture [23], making this trait worth evaluation. From a sensory point of view, this property is generally defined as the overall sensation that the food leaves in the mouth after consumption and includes all properties that can be evaluated by touch [24,25]. In green beans, the texture is the result of the interaction between firmness and crunchiness but even the absence of parchment layers and string-less defines the texture that can be evaluated through instrumental or sensory analysis [26].

Vegetable color also represents an important sensory property and can be used as a criterion of vegetable quality [27]. After harvest, beans show a higher respiration rate, and this process occurs even when kept at low temperatures; furthermore, in the cut green bean, the respiratory rate is higher than in the whole bean, and this results in physiological changes that reduce quality, such as wilting and enzymatic browning [28].

Contrary to fruits and some vegetables, pods of snap beans are principally consumed after cooking; during the thermal process, their properties (e.g., biological, chemical, physical, etc.) undergo several modifications including changes in texture or turgor, smell, color, and from a nutritional point of view, on the concentration and bioavailability of bioactive compounds as polyphenols [29]. The cooking process can affect positively or negatively these metabolites causing their thermal degradation or increasing their total content by enhancing their availability for extraction, inactivating the polyphenol oxidase, or releasing fiber-bound polyphenols into free polyphenols [29,30]. However, the result depends upon differences in process conditions and morphological and nutritional characteristics of vegetable species [31,32]. However, few studies are available concerning the variation among snap bean traits having different morphological characteristics and belonging to different gene pools. Therefore, in this study, we characterized a core collection of snap bean genetic resources to (i) establish the nutritional and the instrumental texture of snap beans of different pod colors; (ii) define the relationship between nutritional profile and texture; and (iii) establish the cooking effect on the antioxidant properties and phenolic composition of pods of selected snap beans lines.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

The plant materials used in this study consist of 54 snap bean lines developed within the Horizon 2020 project BRESOV—Breeding for Resilient, Efficient and Sustainable Organic Vegetable Production [33]. These lines were selected from the BRESOV Snap Bean Panel (SBP), which consists of 311 snap bean lines collected in Europe including landraces and elite cultivars previously described [19]. The selection was based on a preliminary evaluation of agronomic and phenotypic data recorded for the entire SBP during three field

trials carried out in 2019 in three localities, Italy, Spain, and Romania (data not shown). The SBP lines purified by several cycles of selfing under insect-free conditions are homozygous. Table 1 reports the list of the 54 SBP lines characterized in the present study, along with passport information. Within the activities planned for the BRESOV project, genotyping-by-sequencing of SBP lines provided the data to carry out a population structure analysis and to classify the lines as Andean or Mesoamerican when the percentage of membership (q_i) to the respective genetic group was higher than or equal to 70%; lines showing a q_i ranging between 30% and 70% were considered as admixed between the two gene pools (personal communication, Roberto Papa) (Table 1). Eleven SBP lines were in common with the Core-SBP developed by García-Fernández et al. [19] based on 14 quantitative pod dimension traits along with three qualitative traits: pod color, seed coat color, and growth habit.

The fresh pods investigated in this study came from a field trial conducted in Spain in the 2021 season under an organic farming system, using a randomized design with three plots per line. A plot included 8–10 plants per line distributed in 1 m. The seeds were germinated in trays containing peat and then transplanted to ensure the homogeneity of the crop. The field crops were grown on loam soil (pH = 7.4 and 2.43% organic matter) and mulched with plastic to control weeds. Organic farming management practices were followed and adopted to ensure adequate plant growth and development during the period of 7 May 2021 to 16 September 2021 at Villaviciosa, Spain (43°2901 N, 5°2611 W; elevation 6.5 m).

After harvesting, about eight fresh pods were packed in aluminum envelopes, collected in liquid nitrogen, and stored at −80 °C until the analysis.

2.2. Phenotyping of Fresh Snap Beans

The set of 54 SBP lines was visually classified based on the fresh pod color (yellow, green, mottle green, purple) and pod cross-section shape (flat, elliptical, round). In addition, the pod section width (PSW), crude protein content (PC), and total sugar content (S) were measured for each snap bean line and replicates. In particular, the PSW of six individual pods was manually measured in the cross-section perpendicular to the suture filament using a micrometer (Beta Utensili S.p.A., Milan, Italy).

The PC was determined by the Official Method of Analyses, Dumas's method, 990.03 [34], and the protein percentage was calculated from total nitrogen using factor 6.25 [35].

The total sugar content was measured on the juice obtained from the fresh pods using a refractometer and expressed as Brix degree (°Brix).

2.3. Phenotyping of Cooked Snap Beans

2.3.1. Cooking of Snap Bean

For each biological replicate of the 54 SBP lines, a pool of six pods was cooked in a traditional domestic mode (250 mL of boiling water at 100 °C) for ten minutes. Afterwards, the pods were cooled in cold water (r.t.) for one minute and successively dried on blotting paper. All treatments (cooking and refreshing) were carried out using distilled water to avoid the interference of ions on the firmness.

Table 1. List of the 54 snap bean lines, along with passport information and classification according to pod phenotype (pod color and cross-section shape).

Line Code	DOI [1]	Gene Pool [2]	Biological Status	Growth Habit	Fresh Pod Color	Cross-Section Shape	Group	Total Number of Lines
SBP010	10.18730/SBC1E	AND	cultivar	indeterminate	green	flat	I	10
SBP016	10.18730/SC0XG	AND	cultivar	determinate	green	flat	I	
SBP039	10.18730/SC1J0	AND	landrace	determinate	green	flat	I	
SBP040	10.18730/SC1K1	AND	landrace	determinate	green	flat	I	
SBP070	10.18730/H7QGQ	MESO	landrace	determinate	green	flat	I	
SBP082	10.18730/H7PJY	AND	cultivar	indeterminate	green	flat	I	
SBP108	10.18730/H825Z	AND	landrace	determinate	green	flat	I	
SBP227	10.18730/SC4MR	AND	cultivar	determinate	green	flat	I	
SBP280	10.18730/SC61*	AND	breeding line	determinate	green	flat	I	
SBP283	10.18730/SC62~	AND	landrace	determinate	green	flat	I	
SBP022	10.18730/SC13P	AND	cultivar	determinate	green	round	II	16
SBP035	10.18730/SC1E~	MESO	landrace	indeterminate	green	round	II	
SBP046	10.18730/SC1S7	AND	cultivar	determinate	green	round	II	
SBP061	10.18730/SC24J	MIX	cultivar	determinate	green	round	II	
SBP064	10.18730/SC27N	AND	cultivar	indeterminate	green	round	II	
SBP113	10.18730/H82H6	AND	landrace	determinate	green	round	II	
SBP116	10.18730/H847Q	MESO	landrace	indeterminate	green	round	II	
SBP120	10.18730/SC2K~	MESO	landrace	indeterminate	green	round	II	
SBP137	10.18730/SC34D	MIX	cultivar	determinate	green	round	II	
SBP157	10.18730/SC543	AND	cultivar	determinate	green	round	II	
SBP246	10.18730/SC5DC	AND	cultivar	determinate	green	round	II	
SBP257	10.18730/SC5TS	AND	cultivar	determinate	green	round	II	
SBP271	10.18730/SBB9V	AND	cultivar	determinate	green	round	II	
SBP288	10.18730/SC6E8	AND	cultivar	determinate	green	round	II	
SBP301	10.18730/SC6F9	MIX	cultivar	determinate	green	round	II	
SBP302	10.18730/SC1F$	MIX	cultivar	determinate	green	round	II	
SBP036	10.18730/H81C6	MESO	landrace	indeterminate	green	elliptical	III	8
SBP073	10.18730/H7VG3	AND	landrace	determinate	green	elliptical	III	
SBP085	10.18730/SC415	AND	landrace	determinate	green	elliptical	III	
SBP194	10.18730/SC426	MIX	cultivar	determinate	green	elliptical	III	
SBP195	10.18730/SC4Z=	MIX	cultivar	determinate	green	elliptical	III	
SBP240	10.18730/SBC4H	MIX	cultivar	determinate	green	elliptical	III	
SBP287	10.18730/SBBV8	MESO	cultivar	determinate	green	elliptical	III	
SBP289	10.18730/SC1G=	AND	cultivar	determinate	green	elliptical	III	

Table 1. *Cont.*

Line Code	DOI [1]	Gene Pool [2]	Biological Status	Growth Habit	Fresh Pod Color	Cross-Section Shape	Group	Total Number of Lines
SBP037	10.18730/SC1HU	AND	landrace	indeterminate	yellow	flat	IV	
SBP038	10.18730/H7XV4	AND	landrace	indeterminate	yellow	flat	IV	
SBP086	10.18730/H7YEQ	AND	landrace	determinate	yellow	flat	IV	
SBP090	10.18730/H7YY2	n.a.	n.a.	determinate	yellow	flat	IV	
SBP094	10.18730/H80CB	AND	landrace	determinate	yellow	flat	IV	
SBP098	10.18730/H82G5	AND	landrace	determinate	yellow	flat	IV	
SBP112	10.18730/H82M9	AND	landrace	determinate	yellow	flat	IV	13
SBP114	10.18730/H82NA	AND	cultivar	determinate	yellow	flat	IV	
SBP115	10.18730/H83Q7	AND	landrace	determinate	yellow	flat	IV	
SBP117	10.18730/H84EY	n.a.	landrace	indeterminate	yellow	flat	IV	
SBP121	10.18730/H851C	AND	landrace	indeterminate	yellow	flat	IV	
SBP125	10.18730/SC5SR	AND	landrace	indeterminate	yellow	flat	IV	
SBP270	10.18730/SC1V9	AND	cultivar	determinate	yellow	flat	IV	
SBP049	10.18730/SC22G	AND	cultivar	determinate	yellow	round	V	
SBP059	10.18730/SC5MK	MIX	cultivar	determinate	yellow	round	V	3
SBP265	10.18730/SC1XB	AND	cultivar	determinate	yellow	round	V	
SBP053	10.18730/SC1N3	MESO	cultivar	indeterminate	purple	flat	VI	1
SBP042	10.18730/SC3BM	MIX	cultivar	determinate	purple	round	VII	1
SBP166	10.18730/SC50U	AND	cultivar	determinate	mottled green	round	VIII	2
SBP242	10.18730/SBC1E	AND	cultivar	determinate	mottled green	round	VIII	

[1] DOI, Digital Object Identifier, https://ssl.fao.org/glis/, accessed on 1 December 2022. [2] Gene pool assignment was based on molecular AND = Andean gene pool; MESO = Mesoamerican gene pool; MIX = Admixed lines.

2.3.2. Texture Determination

Pod texture was measured by a TA. XT plus texture analyzer, equipped with a 25 kg load cell (Stable Micro System, Godalming, UK), according to a modification of the method by Pevicharova et al. [20] with a Warner-Bratzler blade. Firmness was quantified as the maximum force (N) to cut the pods in half between two immature seeds on the side without suture. The instrument was set up with a test speed of 1 mm s^{-1} and a travel distance of 30 mm.

2.3.3. Color Measurement

The color was registered using a Minolta CR200 colorimeter (Minolta Corp., Ramsey, NY, USA). Before use, the instrument was calibrated with a ceramic reference, illuminant C. CIE-L*a*b* coordinates were obtained using D65 illuminant and 10° observer as the reference system. Registered parameters were L* (brightness: L* = 0 [black], L* = 100 [white]), a* (−a* = greenness, +a* = redness), b* (−b* = blueness, +b* = yellowness). The measures were repeated twice on each pod. In addition, the Brownness (B) was determined according to Equation (1):

$$B = (100 - L^*) \quad (1)$$

2.4. Extraction and Analysis of Phenolic Compounds

For each of the eight snap bean groups, the two lines showing contrasting firmness were selected except for groups VI and VII, represented by unique lines (SBP053 and SBP042, respectively), and used for the determination of phenolic compounds and antioxidant activity. The analyses were conducted for each of the selected 14 lines and biological replicates in both raw and cooked materials. Before the extraction, both fresh and cooked samples of the selected lines were freeze-dried and milled using a planetary mill with an agate jar and balls (Pulverisette 7 Planetary Micro 200 Mill, Classic Line; Fritsch GmbH Milling and Sizing, Idar-Oberstein, Germany) to obtain a fine powder.

Phenolic compounds were extracted according to Laparra et al. [36] with minor modifications. The samples (60 mg) of ground powder, were extracted using 2 mL methanol acidified with 1% HCl (80:20), for 30 min in an ultrasonic bath. The mixtures were centrifuged at 2000× *g* for 15 min, and the supernatants were collected into clean tubes and stored at −20 °C until further analysis.

Each extract was used for the antioxidant activity determination, expressed as Trolox equivalent antioxidant capacity (TEAC), using the ABTS radical scavenging assay, and for the phenolic compounds determination by HPLC analysis.

2.5. Trolox Equivalent Antioxidant Capacity (TEAC)

Trolox equivalent antioxidant capacity (TEAC) was determined according to the procedure of Re et al. [37] with some modifications. ABTS (2,2-azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]) was dissolved in water to a 7 mM concentration. ABTS radical cation was produced, allowing the reaction of the ABTS stock solution with potassium persulfate (2.45 mM, final concentration) for 12–16 h in the dark and at room temperature before use. ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. TEAC values of the extracts were calculated, using a Trolox standard curve, based on the percentage inhibition of absorbance at 734 nm and expressed in µmol of Trolox equivalents (TE)/g sample on a dry matter basis.

2.6. Phenolic Content

The methanolic extracts were used for the phenolic compounds determination according to Kim et al. [38] with some modifications. The extracts were analyzed on a 1200 HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector, and metabolites separation was achieved using a reverse phase C18 column (InfinityLAB Poroshell 120 RC-C18, 100 × 2.1 mm; particle size = 2.7 µm) (Agilent Technologies, Santa Clara, CA, USA). The column temperature was maintained at 35 °C, and

the injection volume was 2 µL. The mobile phase consisted of (A) water with phosphoric acid 10^{-3} M and (B) acetonitrile at a flow rate of 0.5 mL/min, using the following linear gradient program: 5% B for 2.0 min, from 5% to 30% B for 10 min, from 30% B to 55% B for 1.0 min, from 55% to 70% for 2 min, isocratic at 70% for 1.0 min, linear gradient from 70% to 5% B for 6 min. The wavelengths used for peak detection were 280 and 320 nm; compounds were identified by their characteristic UV/vis spectra and comparison of the retention times with those of pure standards (Figure S1). The quantification of each phenolic acid and flavonoid was based on the corresponding calibration curves (0–50 mg/L). Total phenolic acids (TPAs) were the sum of individual metabolites and expressed as µg g^{-1} d.w. The solvents are HPLC grade; solvents and standard compounds were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.7. Statistic Analysis

Analysis of variance was performed for each trait; means comparisons were carried out by the "Least Squares" method and separated by a post hoc Tukey's test or Student t-test and statistically significant differences were determined at the significance level of $\alpha = 0.05$ ($p \leq 0.05$) for the evaluation of genotype, group or cooking effect on the set of materials used and on the selected 14 SBP lines.

To obtain a general comprehensive characterization of the entire set of materials (54 lines × 3 replicates), the traits were subjected to principal component analysis (PCA) based on correlations. Pearson correlation was carried out between antioxidant activity and quercetin-3-O-glucoside, kaempferol-3-O-glucoside, and TPAs. The statistical treatments of the data were performed using the JMP software (SAS Institute Inc., Cary, NC, USA, version 8).

3. Results and Discussion

3.1. Characterization of the Entire Set of SBP

The 54 SBP lines set analyzed in the present study includes lines belonging to both Andean and Mesoamerican gene pools, developed as single seed descent from original gene bank accessions that are landraces or cultivars and characterized by different growth habits, determinate and indeterminate (Table 1). In particular, 67%, 13%, and 16% of the lines were Andean, Mesoamerican, and admixed lines, respectively; this classification was not possible for two lines for which genetic data were not available (Figure S2a). Concerning the biological status, almost all the lines were developed from landraces and cultivars, with one line being a breeding line, and one not classified for biological status (Figure S2b). Finally, 41 and 13 lines showed a determinate and indeterminate growth habit, respectively (Figure S2c).

The entire set of materials was characterized for relevant morphological and nutritional traits: fresh pod color, pod cross-section shape, pod section width (PSW), protein content (PC) and total sugar content (S) for fresh pods, firmness, and color after cooking (Table 2). The lines were grouped based on the fresh pod color and pod cross-section shape into eight groups: I: Green/Flat; II; Green/Round; III: Green/Elliptical; IV: Yellow/Flat; V: Yellow/Round; VI: Purple/Flat; VII: Purple/Round; VIII: Mottled Green/Round (Figure 1 and Table 1); images of fresh pods for all the characterized lines are available [19,39].

Table 2. Mean, interval of variation (Min, Max), and standard error (SE) for the traits recorded in the set of 54 snap bean lines.

Traits [1]	Description of Trait	Unit	Mean	Min	Max	SE
Pod Section Width (PSW)	Width of the section taken perpendicular to the suture of 6 randomly pods	mm	7.13	5.22	9.02	0.10
Total Sugar Content (S)	Total sugar content determined from the extracted juice	°Brix	5.04	4.27	6.40	0.08
Protein Content (PC)	N × 6.25	%	21.59	16.82	26.60	0.31
Firmness	Rupture Force	N	13.15	5.87	23.76	0.60
CIE-L*	Brightness (L* = 0 [black], L* = 100 [white])		43.46	31.95	56.27	1.02
CIE-a*	(−a* = greenness, +a* = redness)		−7.63	−9.82	−4.36	0.15
CIE-b*	(−b* = blueness, +b* = yellowness)		17.90	9.96	25.80	0.46
B	Brownness (100 − CIE-L*)		56.57	43.73	68.05	1.03

[1] PSW, S and PC measured on fresh pods; firmness, CIE-L*, CIE-a*, CIE-b* and B measured on cooked pods.

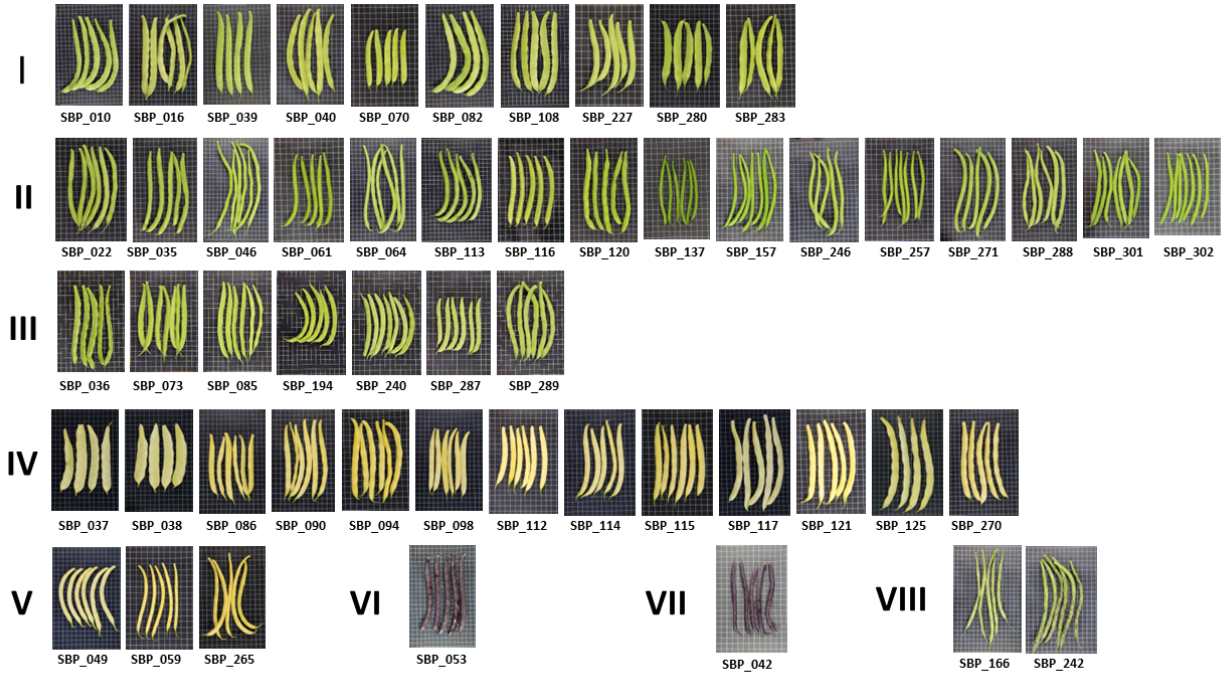

Figure 1. Classification of the lines of core-SBP into eight groups based on pod color and pod shape. I: Green/Flat; II; Green/Round; III: Green/Elliptical; IV: Yellow/Flat; V: Yellow/Round; VI: Purple/Flat; VII: Purple/Round; VIII: Mottled Green/Round.

The results showed a wide range of variation for the traits measured and significant differences among the lines (Tables 2 and S1). For instance, PSW ranged from 5.22 mm (observed in SBP137) to 9.02 mm (observed in SBP289). Regarding the two main nutritional traits, protein content and sugars content, the relative ranges were between 16.82% (SBP064) and 26.59% (SBP073) and between 4.27 °Brix (SBP036) and 6.40 °Brix (SBP098), respectively. The broad range of variability of protein content observed among the snap bean lines agreed with other studies [40–42]. Likewise, according to the literature, as well as in this study, significant differences were detected in the sugar content among the lines (Table S1). In particular, in previous works with a panelist test, it was demonstrated that *P. vulgaris* varieties, classified as sweet, are preferred, and thus the sweetness can be used as a characteristic to differentiate between cultivars [43,44]. Regardless of the tissue type, sugar concentration is cultivar-dependent and highly variable [27], and significant

differences between green bean genotypes were reported both in the content of sugars as well as in the patterns of sugar accumulation [45,46].

The SBP analyzed showed wide variation also for the traits investigated after cooking. Firmness, considered very similar to the masticatory action, is one of the principal texture properties in piece-form vegetables, resulting important for the consumer's choice. The broad variability of this parameter, ranging from 5.87 N (SBP036) to 23.76 N (SBP016), was confirmed by the genotype effect of the one-way analysis of variance (Table S1) according to a previous study where the higher contribution to the variability of this trait was attributable to the genotype effect [20].

A broad variability was reported also for the color parameters. L* ranged between 31.95 (SBP301) and 56.27 (SBP038), and the opposite trend was reported for B (43.73 and 68.05 for SBP038 and SBP301, respectively). In a previous study, an interval of 36.5–50.3 for the luminosity parameter was reported in *P. vulgaris* L. green beans [47]. Furthermore, significant differences were observed between *P. vulgaris* L. landraces for L* values ranging between 50.80 and 57.80 [48]. Consistently, the lines with higher L* investigated in this study are landraces (Table 1 and Table S1).

The a* and b* resulted in significant differences between the lines with the ranges from −4.36 to −9.82 (SBP117 and SBP283) and from 9.96 to 25.80 (SBP082 and SBP302), respectively.

The pairwise correlation between the traits of the entire data set showed a significant weak negative correlation ($r = -0.18$ $p = 0.026$) between firmness and sugar content suggesting that the lines with a strong firmness are less sweet, and this result could be a key driver for agro-industrial application. Firmness did not correlate significantly with PC and PSW but is positively correlated with L* and b* values and negatively correlated with the Brownness (Table S2), suggesting that the hardness lines tend to remain more brilliant after cooking, probably due also to a lower content of sugars which are highly susceptible to the browning process. In a recent study, it was reported that in native beans, the grain color was not associated with the protein or the total sugar content [40]. Our results confirm this trend for the sugar content of snap beans, whereas a weak positive correlation was reported between the protein content and L* (Table S2). Moreover, the data set was subjected to principal component analysis (PCA), and the first two components account for about 51% of the total variability. The score plot (Figure 2) showed a clear separation among the lines due to the color parameters correlated to PC1 (L* and b* in a positive manner, a* and B in a negative mode, respectively) and firmness but with less strength, whereas the sugars and the protein content were positively and negatively correlated to PC2. Then, the yellow pods were harder to respect than the other snap beans.

Table 3 shows the results of the comparison between the groups. Significant differences were observed for all the traits except for PSW. The highest PC was observed in group VII (purple, round pods), whereas the lowest was in the green groups (I, II, and III), respectively. The highest sugar content was observed in the purple/flat (Group VI), whereas the lowest contents were reported for group V (yellow/round) and VIII (mottled green/round). The green groups I (flat) and II (round) showed the highest and lowest firmness, respectively. Concerning the color parameters, the yellow groups (IV and V) showed the highest values of L* and b* and consequently the lowest brownness (B), whereas an opposite trend was registered for the green and purple groups. Finally, for the a*, very similar values were observed but as expected, the lowest one was for group I (green/flat).

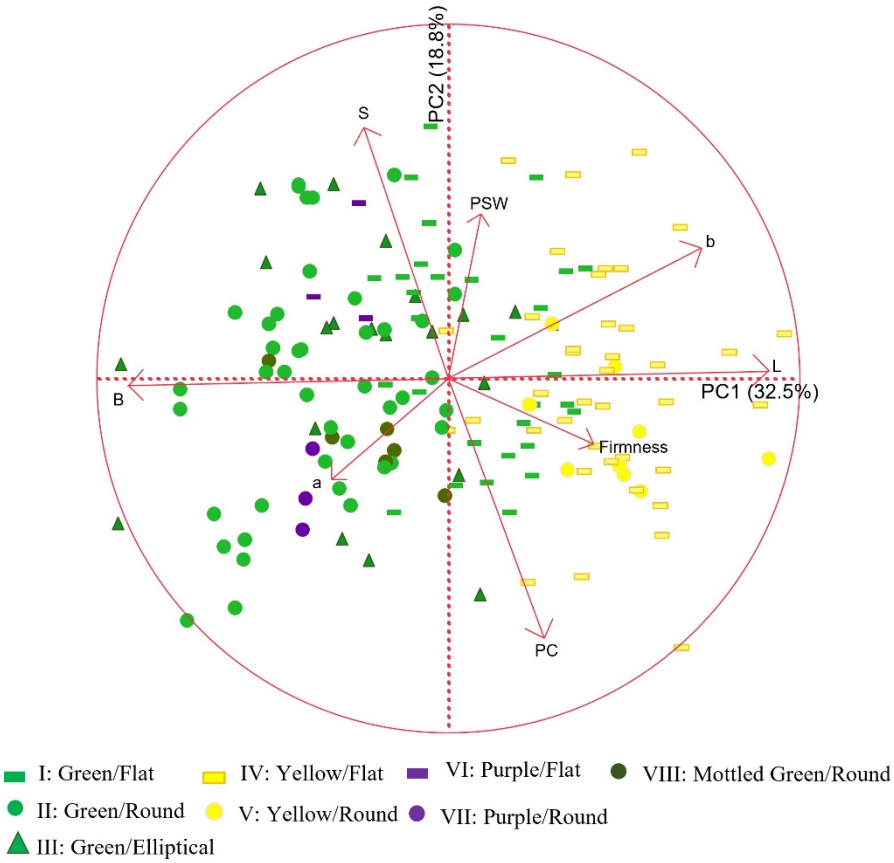

Figure 2. Principal component analysis (PCA) score plot of the entire set of SBP. Loadings are indicated by arrows, and lines are highlighted with different colors and shapes based on the different groups.

Table 3. Mean values for the traits recorded in the eight pod phenotypic groups established for the set of 54 SBP lines.

Group Code	PSW	PC	Sugar	Firmness	L*	a*	b*	B (100-L*)
I	6.98	20.67 c [1]	5.16 b	19.45 a	42.25 b	−8.84 b	20.13 a	57.75 c
II	7.07	20.97 c	5.12 b	10.30 c	36.97 d	−7.30 a	15.47 bc	63.02 a
III	7.29	21.31 c	5.16 b	11.20 bc	38.76 d	−7.85 a	16.94 b	61.24 b
IV	7.30	21.99 abc	4.94 bc	13.58 b	54.34 a	−7.12 a	20.06 a	45.66 d
V	7.09	23.92 ab	4.63 c	12.29 bc	53.33 a	−7.85 ab	21.04 a	46.66 d
VI	6.42	20.10 bc	6.37 a	10.75 bc	39.55 bcd	−7.64 ab	17.77 abc	60.45 abc
VII	6.99	25.13 a	5.17 bc	11.32 bc	37.33 cd	−6.34 a	12.20 c	62.66 ab
VIII	7.41	21.52 abc	4.52 c	12.11 bc	39.30 cd	−6.99 a	15.29 bc	60.70 ab
p	n.s.	0.0241	0.0005	<0.0001	<0.0001	0.0007	<0.0001	<0.0001

[1] Values in the same column followed by different letters are significantly different (Tukey test; $p < 0.05$).

3.2. Characterization of the Selected Snap Bean Lines

For both raw and cooked pod samples of the 14 selected snap bean lines, the phenolic composition and antioxidant activity have been evaluated.

Figure 3 shows the cooking effect on the mean values of the phenolic profile for both raw and cooked samples. In total, ten metabolites have been identified, only two of which are phenolic acids (vanillic acid and 3,4-dihydrobenzoic acid), whereas the other compounds belong to the flavonoid's family (quercetin-3-O-rutinoside [rutin], kaempferol-3-O-rutinoside [nicotiflorin], catechin, epicatechin, quercetin-3-O-glucoside [isoquercetin], apigenin-8-C-glucoside [vitexin], quercetin, and quercetin-3-O-rhamnoside [quercitrin]). As

shown, the phenolic profile is mainly represented by flavonoids rather than phenolic acids in raw as well as cooked samples. Flavonoids accounted for about 90% and 99% of total phenolics in raw and cooked samples, respectively. The main compound was quercetin-3-O-rutinoside, followed by kaempferol-3-O-rutinoside and quercetin-3-O-glucoside. Our results agree with those reported by Pereira Lima et al. [49], who investigated the cooking effect on the content of bioactive compounds for snap beans grown in organic and conventional farming systems highlighting as the raw organic snap beans showed a higher content of flavonoids and antioxidant activity compared to the conventional ones which, on the contrary, showed higher chlorophyll and phenolic compounds.

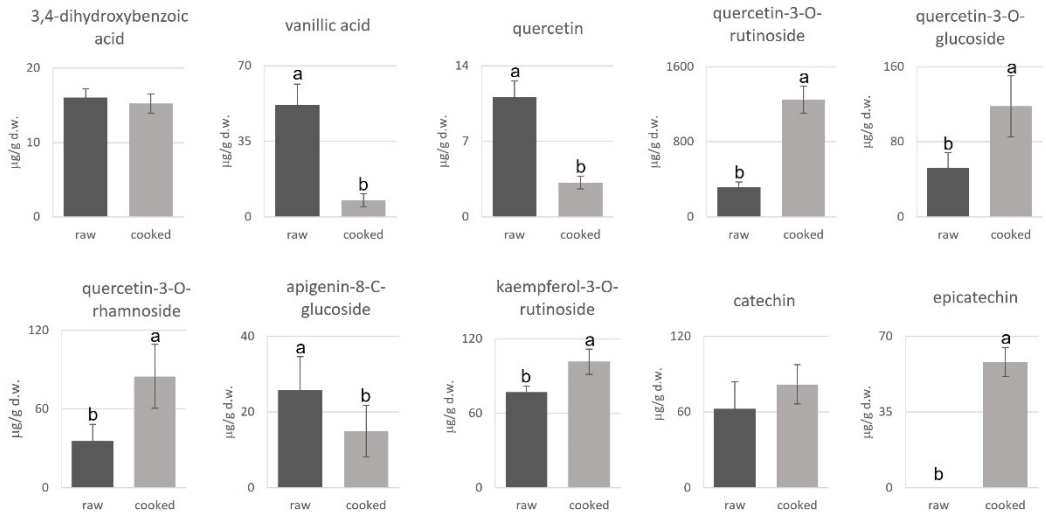

Figure 3. Cooking effect on the average content of each phenolic compound in raw and cooked samples. Different letters mean significant differences ($p \leq 0.05$). No letters mean no significant differences.

As shown in Figure 3, the cooking significantly affects the content of all the metabolites detected except for 3,4-dihydroxybenzoic acid, whereas catechin was detected only in cooked samples with a mean content of 58,2 µg/g. A significant decrease from raw to cooked samples was reported for vanillic acid, quercetin, and vitexin with a decrease of 85%, 42%, and 71%, respectively. According to Natella et al. [50], the decrease in phenolic compounds after cooking may be caused by the leaching phenomenon, which is the function of the time, temperature, and water volume of cooking. Similar results have been reported in other vegetables also with a short cooking time [51,52]. However, not all the metabolites showed a decrease due to the cooking, but a significant increase was detected for five of them, with a range of percentage from 1.31-fold to 3.92-fold and the following ranking: catechin < kaempferol-3-O-rutinoside < quercetin-3-O-glucoside < quercetin-3O-rhamnoside < quercetin-3-O-rutinoside. Benner et al. [53] reported that the boiling treatment improves the extraction rate of anthocyanins and other phenolic compounds, whereas other reports showed as domestic cooking methods can increase the polyphenol content in vegetables, including flavonoids, because extractable parts of these molecules could be improved by plant tissue disruption when subjected to high temperatures [31,52,54]. In the present study, the increase in flavonoid content induced by the cooking treatment could be due to a combination of different factors related to the cell structure as a consequence of the organic growing conditions. In organic crops, the cell differences were principally related to thickening and lignification rendering the matrix less susceptible to physical damage [55], and accordingly, a low-softening matrix might favor lower leaching and/or degradation (as occurs in the cooking process). Therefore, the increased amounts of some metabolites observed could be related to this phenomenon.

However, not all the metabolites were detected in each selected line investigated: the only two metabolites present in both raw and cooked samples were quercetin-3-O-

rutinoside and kaempferol-3-O-rutinoside (Figure 4a,b). In general, SBP042 showed the highest contents of both metabolites in raw as well as in cooked samples. After cooking, quercetin-3-O-rutinoside increased significantly for all the lines with a range from 1.8-fold for SBP108 to 28-fold for SBP037. Quercetin-3-O-rutinoside was heat-stable for several cooking methods and durations, even if in asparagus a leach was reported in boiling water [56]. For kaempferol-3-O-rutinoside, the variation was not significant for the lines SBP016, SBP265, and SBP053 (Figure 4b), and the range was between 0.1-fold for SBP036 and 5.7-fold for SBP242.

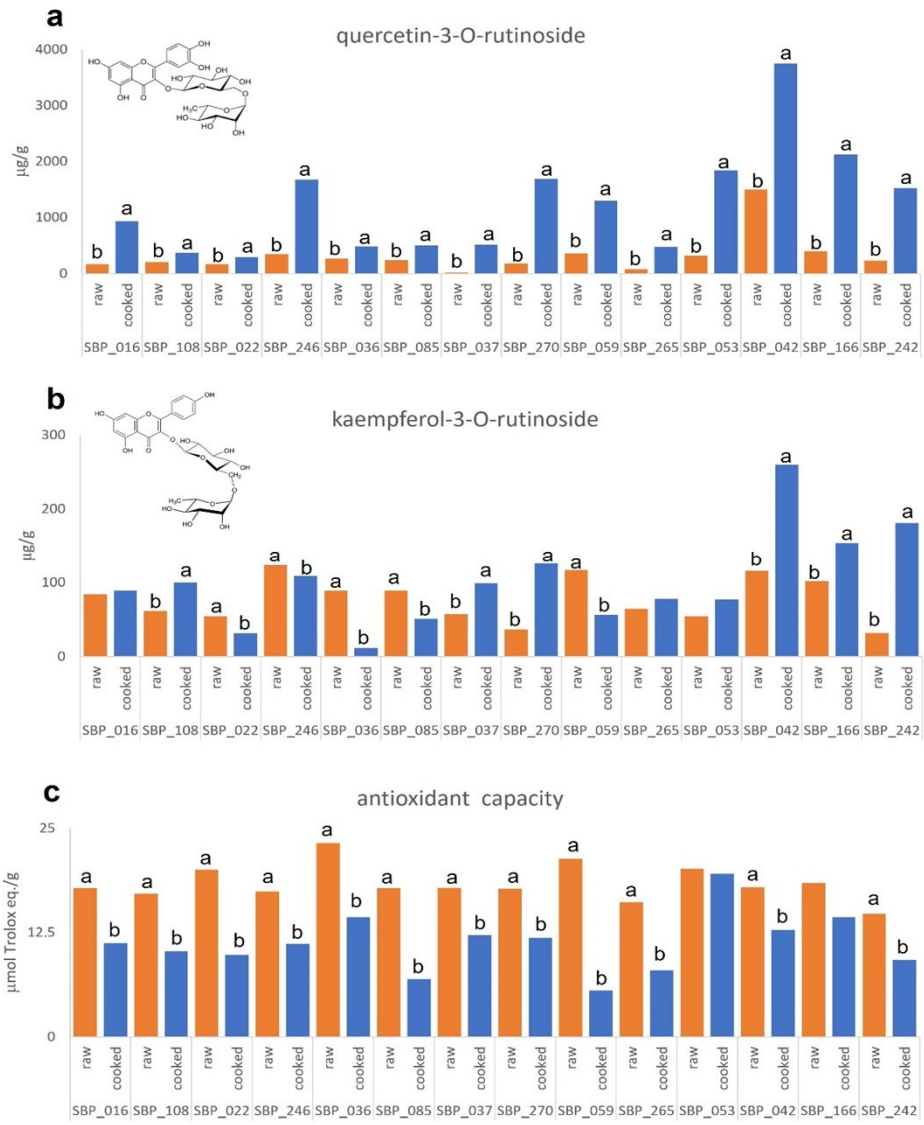

Figure 4. Mean content for the quercetin-3-O-rutinoside (**a**), kaempferol-3-O-rutinoside (**b**), and antioxidant activity (TEAC) (**c**) in the raw and cooked samples of each selected line investigated. Different letters mean significant differences ($p \leq 0.05$). No letters mean no significant differences.

Concerning the antioxidant properties, the cooking process leads to a significant decrease in TEAC of about 39% (18.43 and 11.24 µmol Trolox eq/g in raw and cooked samples, respectively). A similar loss of antioxidant capacity was already observed for several vegetables including green beans after boiling [50,51,57]. Besides the general decrease, among the selected lines, different behaviors were observed regarding the antioxidant capacity (Figure 4c). Here, among the green and yellow pods, higher antioxidant activity was observed for the SBP036 (raw and cooked samples) which belong to the Mesoamerican gene pool respecting other snap beans that are Andean. We can argue that the origin could

be ascribable for this different behavior, but additional investigations need to address this hypothesis.

The cooking effect on the antioxidant activity of three green bean cultivars of different colors (green, yellow, and purple) was investigated [58]. The authors referred to a significant decrement of the antioxidant capacity after treatment over 30% using the ABTS assay except for the purple type whose value did not show any significant change after cooking. In this study, we observed a similar trend, and among purple lines, the SBP053 showed the lowest, not significant, variation (−3.1%). On the contrary, the green and yellow lines registered the major decrements with SBP059 showing the highest significant loss (−73.9%) (Figure 4c). As well documented in the literature, the decrease in the antioxidant capacity in vegetables during cooking was attributable to the loss of antioxidants and, in particular, to the drastic reduction in the vitamin C and phenolic contents of the raw materials [59–61]. Here, considering the increase in some flavonoid-glucosides contents and the decrease in the TEAC observed after cooking, we can guess that in cooked snap beans, these metabolites do not contribute greatly to the antioxidant activity, and the higher contribution could be attributable to other compounds such as free phenols and vitamins losses by leaching or thermal degradation. Moreover, previous studies compared the antioxidant activity of quercetin and its derivatives which antioxidant power reported in the following order: quercetin > quercitrin > isoquercitrin > rutin [62,63]. In addition, in green beans, the antioxidant capacity of the kaempferol-glucosides turned out much lower than the corresponding quercetin species but like that of the kaempferol aglycone [64]. Regarding the correlation between the total phenolic compounds and antioxidant activity, we found a moderately significant correlation only in the cooked samples (r = 0.40, p= 0.001). In addition, for the correlation between the two metabolites detected in all samples and the antioxidant activity, the r value was significant but low only for quercetin-3-O-glucoside in cooked samples (r = 0.31, p = 0.045). However, the consumption of cooked snaps rich in rutin could contribute to improving health due to the indirect antioxidant power linked to its aglycone which can develop during digestion [64].

4. Conclusions

From our knowledge, the set of snap bean lines used in this study constitutes a large panel of natural variation for different traits: nutritional, textural, and antioxidant properties. In particular, the total sugar content and the firmness can be used as reciprocal features to differentiate between cultivars for the consumer's request. In addition, as highlighted in this study, domestic cooking affects the antioxidant activity and the metabolite composition of snap beans as reported in previous studies, but the magnitude of the influence is different among the lines investigated based on their morphological and genotypic characteristics. All these results may be useful in breeding programs to design specific lines with different characteristics for different markets and purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030311/s1, Table S1. Mean values for the traits recorded in the different lines of the entire set of snap beans. Table S2. Correlation coefficients between traits from the lines of the entire set of snap beans. Figure S1. HPLC Chromatogram, for each phenolic compound, indicated the retention time (RT) and the wavelength of detection. Figure S2. Percentage of the snap bean lines based on the gene pool (a), biological status (b), and growth habit (c).

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