Original article

Variability of health and bioactive compounds in strawberry (*Fragaria x ananassa* Duch.) cultivars grown under an Indian temperate ecosystem

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Variability of health and bioactive compounds in strawberry (*Fragaria* × *ananassa* Duch.) cultivars grown under an Indian temperate ecosystem.

Abstract – Introduction. Strawberry is rich in health as well as bioactive compounds, and benefits resulting from the use of natural products rich in bioactive substances are receiving increased interest from the pharmaceutical, food and cosmetic industries. **Materials and methods**. Twenty-two cultivars of strawberry (*Fragaria* × *ananassa* Duch.) grown under a temperate ecosystem were screened for ascorbic acid, phenolic compounds, flavonoids, anthocyanins and antioxidant activities (DPPH and FRAP assays). The phenolic content was measured by Folin-Ciocalteu reagent using gallic acid as the standard. Antioxidant activity was determined in terms of DPPH and FRAP assays and expressed as ascorbic acid equivalent. Total anthocyanins and total flavonoid content were determined using a colorimetric method. Titratable acidity (citric acid) was determined by the titration method. The average data of two years was analyzed using SAS 9.2 software. **Results and discussion**. Significant differences in the health and bioactive compounds were detected among the cultivars. The range of ascorbic acid of the tested samples was (51.03 to 89.40) mg·100 g^{-1} fresh weight. Titratable acidity varied between 0.73% and 1.44%; however, total anthocyanins ranged between (28.24 and 43.32) mg cyanidin-3-glucoside Eq·100 g^{-1} fresh weight. Total phenols varied from (380.10 to 888.10) mg gallic acid Eq·100 g^{-1} and total flavonoids from (31.26 to 55.16) mg catechin Eq·100 g^{-1}. The total antioxidant activity ranged between (203.13 and 471.10) mg ascorbic acid Eq·100 g^{-1} fresh weight for DPPH, and between (326.06 and 701.13) mg ascorbic acid Eq·100 g^{-1} fresh weight for FRAP. Total phenols, DPPH and FRAP showed close association; however, PCA clearly categorized the selected cultivars into two broad groups. All of the diverse cultivars were clustered into two clusters which could be exploited for future qualitative breeding programs based on average cluster distance and can act as gene sources for making health foods. **Conclusion.** The importance of our findings would be significant for farmers, breeders, consumers and industries concerning food quality, disease prevention and healthcare.

India (Jammu and Kashmir) / *Fragaria ananassa* / temperate zones / fruits / antioxidants / ascorbic acid / phenolic content / anthocyanins

Variabilité de composés bioactifs et de santé chez des cultivars de fraisier (*Fragaria* × *ananassa* Duch.) cultivés dans un écosystème tempéré indien.

Résumé – Introduction. La fraise est riche en substances bioactives et de santé ; les avantages découlant de l'utilisation de produits naturels riches en substances bioactives intéressent de plus en plus les industries pharmaceutiques, alimentaires et cosmétiques. **Matériel et méthodes**. Vingt-deux cultivars de fraisier (*Fragaria* × *ananassa* Duch.) cultivés sous écosystème tempéré ont été étudiés vis-à-vis de leur teneur en acide ascorbique, composés phénoliques, flavonoïdes, anthocyanes, activités antioxydantes (dosages de DPPH et FRAP). La teneur en phénols a été mesurée par le réactif Folin-Ciocalteau en utilisant l'acide gallique comme standard. L'activité antioxydante a été déterminée en fonction de tests DPPH et FRAP et exprimée en équivalent d'acide ascorbique. Les anthocyanes totaux et la teneur totale en flavonoïdes ont été déterminés en utilisant une méthode colorimétrique. L'acidité titrable (acide citrique) a été déterminée par titrage. Les données moyennes de deux ans ont été analysées à l'aide du logiciel SAS 9,2. **Résultats et discussion**. Des différences significatives de teneurs en composés bioactifs et de santé ont été détectées au sein des cultivars étudiés. Dans les échantillons testés, l'acide ascorbique a varié de (51,03 à 89,40) mg·100 g^{-1} de matière fraîche (mf). L'acidité titrable a varié entre 0,73 % et 1,44 %, cependant la teneur en anthocyanes totaux a été de (28,24 à 43,32) mg Eq cyanidine-3-glucoside·100 g^{-1} mf. Les phénols totaux ont varié de (380,10 à 888,10) mg Eq acide gallique·100 g^{-1} mf et les flavonoïdes totaux, de (31,26 à 55,16) mg Eq catéchine·100 g^{-1} mf. L'activité antioxydante totale a varié entre (203,13 et 471,10) mg Eq acide ascorbique·100 g^{-1} mf pour DPPH, et entre (326,06 et 701,13) mg Eq acide ascorbique·100 g^{-1} mf pour FRAP. Les phénols totaux, DPPH et FRAP ont révélé une étroite association, alors que l'ACP des cultivars sélectionnés a clairement isolé deux grands groupes. Tous les cultivars se sont regroupés en deux clusters qui pourraient être exploités à l'avenir dans un programme d'amélioration qualitative basée sur la distance moyenne entre les clusters ; ils pourraient être utilisés comme source de gènes favorables à l'obtention d'aliments de santé. **Conclusion**. Nos résultats pourraient être importants pour les agriculteurs, les éleveurs, les consommateurs et l'industrie concernés par la qualité des aliments, la prévention des maladies et les soins de santé.

Inde (Jammu et Cachemire) / *Fragaria ananassa* / zone tempérée / fruits / antioxydant / acide ascorbique / teneur en phenols / anthocyane

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1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.), an important member of the family Rosaceae, is one of the most popular soft fruits in the world. Strawberry fruits have a very delicious taste and fresh aroma. The genus *Fragaria* consists of approximately 20 species, with a basic chromosome number of x = 7, that exists in four levels of ploidy [1]. The cultivated strawberry is an octaploid (*2n* = 8x = 56), stoloniferous perennial herb [2]. It has a wide range of climatic adaptation which includes Mediterranean, temperate, subtropical and taiga zones [3]. Strawberry is rich in health and bioactive compounds, and the benefits resulting from the use of natural products rich in bioactive substances are receiving increased interest from the pharmaceutical, food and cosmetic industries.

Several studies have shown that strawberries generally possess a high level of antioxidant activity, which is linked to the levels of phenolic and anthocyanin compounds in the fruit [4–7]. It was reported that strawberry juice exhibited a high level of antioxidant capacity against free radicals, including superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen [6, 7] and also confirmed that percent inhibition of each active oxygen species varied among the strawberry cultivars [5].

Standard quality characteristics for strawberry can be classified into visual or external quality traits and internal quality traits. As external quality traits, fruit size and homogeneity, color, and brightness seem to strongly influence consumer choices [8] and thus are important characteristics for retail. On the other hand, the inner quality traits include the chemical composition, influencing fruit taste. Strawberry taste has been shown to be mainly related to sugars, acids and aroma contents of the fruits [9, 10]. For these reasons the common standards to assess inner strawberry quality are the soluble solids contents (SSC) and titratable acidity (TA) of the fruit juice.

Furthermore, recent scientific evidence points out the importance of health-promoting compounds in strawberries in relation to their high level of antioxidants, including vitamin C and phenolic compounds, which lead to their wide consumption [5]. In fact, these compounds have protective effects against cancer, and cardiovascular and other chronic diseases [11, 12]. Therefore, knowledge about the existence and quantity of internal quality traits is becoming important for the consumer. Simultaneously assessing genetic divergence, in terms of health and bioactive compounds among the available plant genetic resources, is a vital tool for plant breeders for an efficient choice of parents for breeding programs. Genetically diverse parents are likely to contribute desirable segregants in future generations. Parents identified on the basis of divergence for any breeding program would be more promising [13]. Genetic divergence studies of strawberry germplasm on the basis of morpho-physico-chemical attributes have been assessed earlier by several authors [14, 15] but information on variability of health and bioactive compounds in strawberry genotypes is limited; the availability and informative value of strawberry germplasm are becoming more and more important for the future preservation and sustainable use of genetic resources. Since recognition and measurement of such diversity and its nature as well as magnitude are beneficial or even crucial to a breeding program, particularly for development of varieties rich in health compounds and antioxidants, our study had the purpose of characterizing the genetic diversity in strawberry cultivars, based on health and bioactive compounds, and classifying cultivars according to their similarity as well as dissimilarity for utilization and genetic enhancement under a temperate ecosystem of India.

2. Materials and methods

2.1. Experimental design

Our present study was carried out at the research farm of the Central Institute of Temperate Horticulture (CITH), Srinagar, Jammu and Kashmir (India). The experimental farm is located at 34°05' lat. N and 74°50' long. E, with an elevation of 1640 m

above the mean sea level. Twenty-two strawberry cultivars widely grown in Indian conditions were sourced from the National Bureau of Plant Genetic Resources, New Delhi, India, and used for our study. A complete randomized block design (CRBD) replicated three times and average data of two years were analyzed as per the method suggested by Gomez and Gomez [16]. Each experimental plot was 4.5 m^2 in size. The distance was 25 cm between strawberry transplants and 0.7 cm between rows. The planting dates were on 15th October, 2010 and 2011. The recommended package of practices was followed for better and healthy crops. Fruit was harvested at commercial ripeness in the first fortnight of May, 2011 and 2012, from randomly selected plants to represent the population of the plantation. The average maximum temperature 19.63 °C, minimum 6.52 °C, rainfall 650.72 mm and relative humidity 58.35%, evaporation 2.45 mm per day, and soil characteristics, *viz.* pH = 6.81, EC = 0.36 $dS \cdot m^{-1}$, were recorded during the growing seasons.

2.2. Strawberry samples

Randomly collected fruits of strawberry were brought to the lab at a temperature of (18 ± 2) °C and relative humidity of 95%. The fruits were squeezed manually under ice, filtered with a sieve and centrifuged for 5 min at 500 *g*. The ascorbic acid content of fruit was determined from fresh filtered juice while other analyses were performed on strawberries stored at (25 ± 2) °C.

2.3. Chemicals

Folin-Ciocalteu phenol reagent and all other chemicals were purchased from Sigma Aldrich (Milano, Italy).

2.4. Determination of total phenolic content

The phenolic contents were measured by Folin-Ciocalteu reagent [17, 18] using gallic acid as the standard. The juice (1 mL) was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted ten-fold with distilled water) and 4 mL sodium bicarbonate (7.5% w/v). The mixture was diluted to 100 mL with distilled water. The solution was kept in the dark at room temperature for 2 h and the absorbance was measured at 765 nm with a NanoDrop spectrophotometer (Model Thermo 8000, Thermo Fisher Scientific, USA). Total phenolic content was expressed as gallic acid equivalents (the concentration of gallic acid was established from a calibration curve) in $mg \cdot 100\ g^{-1}$ fresh weight.

2.5. 2, 2-diphenyl-1-picrylhydrazyl assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich Co. (USA). The effect of strawberry fruit extracts on DPPH radical was determined according to the method used by Yen and Chen [19] with some modifications. A 1-mL aliquot of strawberry juice was diluted 200 times and then 3 mL of ethanol (96%) and 1 mL of DPPH (0.012 g DPPH·100 mL) were added. The mixture was shaken and left at room temperature for 10 min; the absorbance was measured spectrophotometrically at 517 nm and reported as mg ascorbic acid equivalent (AAE)$\cdot 100\ g^{-1}$ fresh weight.

2.6. Ferric-reducing antioxidant power assay

The ferric-reducing antioxidant power (FRAP) was assessed according to Benzie and Strain [20]. Six milliliters of working FRAP reagent prepared daily was mixed with 20–100 µL of the extract. The absorbance was recorded at 593 nm after 30 min of incubation at 37 °C. Absorbance increase was calculated as FRAP values by comparing with standard curves created by vitamin C (0–15 µg), and reported as mg ascorbic acid equivalent (AAE)$\cdot 100\ g^{-1}$ fresh weight.

2.7. Titratable acidity

Titratable acidity was determined by using 10-g aliquots of strawberry fruits poured into 50 mL of distilled water and titrated with 0.1 N NaOH to an end-point of pH 8.1

where light pink color developed. Titratable acidity was expressed as percentage of citric acid and was calculated using the method given by the AOAC [21].

2.8. Determination of total flavonoid content

The total flavonoid content (FLC) was determined by a colorimetric method [22]. The alcoholic extract was diluted to a final volume of 5 mL with distilled water. After adding 300 µL $NaNO_2$ (5%) the mixture was left for 5 min. Then 300 µL $AlCl_3$ (10%) were added and, after 6 min, 2 mL NaOH (1 N) were also added. The solution was mixed well and the absorbance was measured against a prepared blank (water) at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents·100 g^{-1} fresh weight.

2.9. Determination of ascorbic acid

Ascorbic acid content of fresh fruit was determined spectrophotometrically by metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye as described by Robinson and Stotz [23] using a NanoDrop spectrophotometer (Model Thermo 8000) at a wavelength of 500 nm. Results were expressed as mg ascorbic acid·100 g^{-1} fresh weight.

2.10. Determination of total anthocyanins

Anthocyanins were determined according to Shin *et al.* [24] with some modifications. One gram of strawberry pulp was added to 10 mL of methanol containing HCl (0.5%, v/v), homogenized for 3 min and held at 4 °C for 1 h in the darkness. The slurry was centrifuged at 17,600 × *g* for 15 min at 4 °C. The absorbance of the supernatant was measured at 515 nm. Total anthocyanin content was calculated using the extinction coefficient (ε) equal to 3.6×10^6 $mol^{-1} \cdot m^{-1}$. Total anthocyanin content was expressed as mg cyanidin-3-glucoside equivalent·100 g^{-1} fresh weight.

2.11. Statistical analysis

Data were subjected to one-way analysis of variance for mean comparison, and intergenotype significant differences were calculated. Correlations were calculated on a cultivar mean basis, according to Pearson's test. Differences at $p < 0.01$ were considered to be statistically significant. Linear regression between antioxidant activities was performed by Minitab Statistical Software (Release 15, Minitab Inc., State College, PA, USA). The PCA produced eigenvectors and principal component scores that were used, respectively, to measure the relative discriminative power of the axes and their associated characters; a dendrogram was constructed using the Ward method. The distance is expressed as average cluster distance. *P-v*alues < 0.01 were regarded as significant using SAS 9.2 software [25].

3. Results and discussion

The results obtained from our present study describe the role of the genetic background of different cultivars in the bioactive compounds and antioxidant profiles of strawberries (*table I*). The ascorbic acid content varied between (51.03 and 89.40) mg·100 g^{-1} fw among the strawberry cultivars, and a 1.75-fold difference was registered among cultivars with the lowest (Brighton cv.) and highest contents (Missionary cv.). The minimum total titratable acidity content was recorded in Katrian Sweet (0.73 citric acid %), followed by Missionary (0.76 citric acid %), and maximum in Brighton (1.44 citric acid %) as compared with the other cultivars.

The strawberry is rich in polyphenolics and especially in anthocyanins, which demonstrates its rich antioxidant activity. Anthocyanin pigments are responsible for the characteristic red color of strawberry. A large variability was observed in anthocyanin content and significant differences were observed. The value varied from (28.24 mg cyanidin-3-glucoside Eq·100 g^{-1} fw (Douglas cv.) to 43.32 mg cyanidin-3-glucoside Eq·100 g^{-1} fw (Brighton cv.). Total phenolic

Table I.
Performance of 22 strawberry cultivars studied in relation to different health and bioactive compounds (New Delhi, India).

Cultivars	Ascorbic acid (mg·100 g^{-1} fw)	Titratable acidity (citric acid %)	Total anthocyanins (mg cyanidin-3-glucoside Eq·100 g^{-1} fw)	Total phenols (mg gallic acid Eq·100 g^{-1} fw)	Total flavonoids (mg catechinEq·100 g^{-1} fw)	DPPH	FRAP
						(mg of ascorbic acid Eq ·100 g^{-1} fw)	
Allstar	82.06	0.96	36.22	720.13	42.06	378.06	567.10
Athena	74.16	1.02	31.45	489.13	51.20	259.06	365.10
Banglora	82.13	0.84	39.16	401.06	39.53	235.10	333.10
Blackmore	59.10	1.22	37.82	598.16	38.20	325.13	425.06
Brighton	51.03	1.44	43.32	830.13	42.33	435.16	624.10
Camma Rosa	80.53	0.95	37.23	689.16	46.40	369.16	501.13
Chandler	68.10	1.22	41.31	738.06	45.46	425.16	637.06
Dilpasand	86.16	0.84	35.33	509.10	47.06	266.10	368.10
Douglas	83.06	1.03	28.24	380.10	37.46	203.13	326.06
EC-102642	76.16	1.02	30.46	547.10	50.13	304.06	378.13
EC-22355	69.10	0.96	35.34	468.10	47.33	250.13	349.10
Fiona	81.10	0.95	38.24	423.13	35.46	273.03	340.13
Heera	52.13	1.34	30.63	634.10	31.26	356.13	450.10
Howard	56.10	0.85	42.72	888.10	44.53	471.10	701.13
Katrian Sweet	59.16	0.73	33.11	456.06	55.13	247.10	348.06
Larson	74.06	0.91	38.34	637.10	41.33	358.10	457.10
Majestic	76.13	1.26	40.94	722.06	53.13	399.10	568.06
Missionary	89.40	0.76	40.31	656.13	39.26	365.06	459.13
Phenomenal	72.03	1.10	42.54	834.13	42.26	435.13	678.10
Red cross	75.10	0.86	30.22	631.16	39.26	347.20	447.20
Senga Sengana	85.10	1.11	31.25	706.13	38.40	369.13	522.13
Shasta	64.13	0.94	38.63	527.10	46.40	279.10	405.13
CD at 5%	3.62	0.10	2.55	33.70	4.80	0.34	32.10

fw: fresh weight.
DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power.

content varied from 380.10 mg gallic acid Eq·100 g^{-1} fw (Douglas cv.) to 888.10 mg gallic acid Eq·100 g^{-1} fw (Howard cv.). However, total flavonoids (mg catechin equivalents /100 g) ranged from 31.26 mg catechin Eq·100 g^{-1} fw (Heera cv.) to 55.13 mg catechin Eq·100 g^{-1} fw (Katrian Sweet cv.). Our findings are in accordance with earlier findings in strawberry [5].

The maximum value of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) was recorded in Howard cv. with (471.10 and 701.13) mg AAE·100 g^{-1} fw, respectively, followed by Phenomenal cv. with (435.13 and 678.10) mg AAE·100 g^{-1} fw, respectively; however, the minimum values were recorded in Douglas cv. (203.13 and 326.06) mg AAE·100 g^{-1} fw, respectively. The results exhibited the radical scavenging capacity of these cultivars and could be utilized for making health-promoting products as well as a parent for introgression of high nutraceutical traits in desired cultivars. These levels of DPPH and FRAP are in agreement with previously reported values for strawberries [26]. However, even when good experimental evidence exists, results need to be interpreted with caution in relation to human health benefits, as polyphenols may have limited bioavailability and may also be extensively metabolized. Bioavailability differs greatly between various polyphenols, and the most abundant polyphenols are not necessarily those that have the best bioavailability profile, because they either have a lower intrinsic activity or they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated.

Taking into account the descriptive statistical analysis for various traits (*table II*), the lowest values of standard deviation were recorded in the cases of the titratable acidity (0.18) and total anthocyanins (4.60), whereas the standard deviation values were highest for total phenols (145.95). The coefficient of variation value was lowest for the total anthocyanins (12.61%) followed by total flavonoids (13.75%), while the highest coefficient of variation was found for FRAP (25.38%) and DPPH (22.42%), respectively. Antioxidant and polyphenol content analysis based on different traits showed the high genetic divergence of the 22 strawberry cultivars, which could be exploited for future qualitative breeding programs and as a source for making health foods.

The correlation study (*table III*) which determines interrelationships among the bioactive compounds (ascorbic acid, titratable acidity, total polyphenol content, total flavonoids, total anthocyanins, and DPPH and FRAP values) shows that the total phenolic content, DPPH and FRAP exhibited a significant correlation with different antioxidant methods (*figures 1, 2*). This suggests that antioxidant activity is more correlated with total polyphenol content than with total anthocyanins. These results are in agreement with other studies [27–29]. The high correlation between the DPPH and FRAP and total polyphenol content can be attributed to the fact that both assays rely on the same reaction mechanism. There were no statistically significant correlations found between antioxidant activity (DPPH, FRAP) and total ascorbic acid, organic acids or total anthocyanins, which may be due to the presence of achenes, having high phenolic content but low anthocyanin concentrations [30]. The highest Pearson coefficient was obtained when it was compared between total phenols and DPPH antioxidant contents (0.983). High positive correlations between total phenols and total antioxidant activity (DPPH, FRAP) were also reported in blueberries [27–31] and in strawberry [5]. The high positive correlation among different pairs can be helpful in breeding for further improvement in the cultivars lacking in bioactive compounds.

Principal component analysis (PCA) was applied to identify the traits which were the main source of the variability and to explain the genetic diversity among populations. It is a multi-linear modeling method providing an interpretable overview of the main information in a multidimensional data table. Pearson's correlation matrix was used in the PCA analysis using seven qualitative variables. The results of the PCA showed that three of the seven principal component axes had eigenvalues greater than one and altogether accounted for 84.92% of the total variation (*table IV*). Two main principal components (PC) explained approximately

Table II.
Variability of ascorbic acid, antioxidant activity and phenolic content in 22 strawberry cultivars studied in relation to their different health and bioactive compounds (New Delhi, India).

Variable	Ascorbic acid (mg·100 g^{-1} fw)	Titratable acidity (citric acid %)	Total anthocyanins (mg cyanidin-3-glucoside Eq·100 g^{-1} fw)	Total phenols (mg gallic acid Eq·100 g^{-1} fw)	Total flavonoids (mg catechin Eq·100 g^{-1} fw)	DPPH	FRAP
						(mg ascorbic acid Eq·100 g^{-1} fw)	
Mean	72.54	1.014	36.49	612.97	43.34	334.11	465.92
Standard deviation	11.35	0.18	4.60	145.95	5.96	74.91	118.28
Range	51.03	0.73	28.24	380.10	31.26	203.13	326.06
	89.40	1.44	43.32	888.10	55.13	471.10	701.13
CV(%)	15.65	18.44	12.61	23.81	13.75	22.42	25.38

fw: fresh weight.
DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power.

Table III.
Pearson correlation coefficients among various traits studied for 22 strawberry cultivars in India (n = 22, Prob > |r| under H_0: Rh_0 = 0).

Characters	Ascorbic acid	Titratable acidity	Total anthocyanins	Total phenols	Total flavonoids	DPPH	FRAP
Ascorbic acid	1.00000	– 0.45818	– 0.20788	– 0.30845	– 0.04438	– 0.29885	– 0.29320
Titratable acidity		1.00000	0.13303	0.39301	– 0.20660	0.40437	0.39982
Total anthocyanins			1.00000	0.55216	0.08374	0.58993	0.61715
Total phenols				1.00000	– 0.02545	0.98344 **	0.96647 **
Total flavonoids					1.00000	– 0.07849	0.00019
DPPH						1.00000	0.95807 **
FRAP							1.00000

** significant at $p < 0.001$.
DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power.

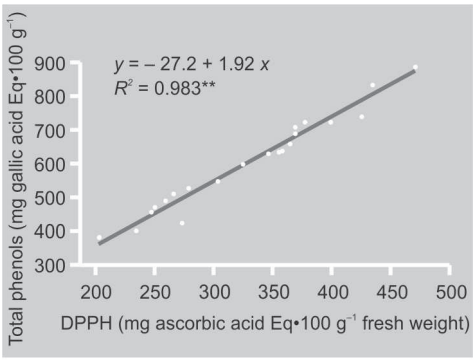

Figure 1.
Relationships between total phenolic and DPPH (2, 2-diphenyl-1-picrylhydrazyl) contents for 22 cultivars of strawberry.

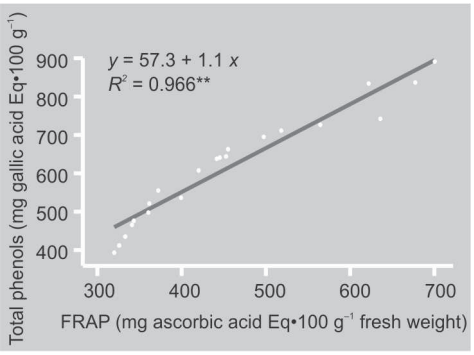

Figure 2.
Relationships between total phenolic and FRAP (Ferric-reducing antioxidant power) contents for 22 cultivars of strawberry.

70.55% of total data variability (PC1: 53.18% and PC2: 17.38%) (*figures 3, 4*). Variables related to antioxidant content, antioxidant activity, titratable acidity, total anthocyanins and total phenols were positively associated with PC1, with the exception of total ascorbic acid and total flavonoids, which were negatively correlated. Therefore, PC1 differentiates between two groups of strawberries *viz*. strawberries with a low antioxidant profile and strawberries with a high antioxidant profile. Lack of correlation between total flavonoids and antioxidant activity in strawberries was also found by Taruscio *et al*. [32] and Meyers *et al*. [33]. The antioxidant potential of flavonoids is dependent on the number and arrangement of hydroxyl groups across the structure, as well as the presence of electron-donating and electron-withdrawing substituents in the B-ring structure. A single hydroxy substituent, as observed in pelargonidin–3-glucoside, the main anthocyanin found in the strawberry, generates little antioxidant activity [34]. PC2 was associated with ascorbic acid and total flavonoids. This component identified cultivars with low total antioxidant activity.

The dendrogram of the 22 monitored strawberry cultivars was constructed on the basis of values of health and antioxidant compounds. The aim of determining this is that the set of cultivars can be divided into separate sub-clusters, which are internally homogenous, but mutually heterogeneous. All the cultivars were clustered into two major clusters based on average cluster distance (*figure 5*).

The first cluster, which included thirteen cultivars (59.09 % of the total cultivars studied), is formed by two sub-clusters I and II at a RMS distance 0.89; the sub-cluster I is further subdivided into I-1 (Howard cv.) and I-2 (Brighton and Phenomenal cvs.). It is particularly associated with total anthocyanin content. Sub-cluster II is quite heterozygous

Table IV.
Principal component analysis of 22 strawberry cultivars showing the principal component scores, eigenvalues and percentage of total variance accounted for the seven principal component (PC) axes.

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Ascorbic acid	–0.237	0.393	0.648	0.378	0.473	0.023	0.015
Titratable acidity	0.277	–0.600	–0.189	0.285	0.666	0.032	0.018
Total anthocyanins	0.352	0.335	0.008	–0.732	0.467	0.055	0.064
Total phenols	0.495	0.100	0.115	0.232	–0.215	0.246	0.754
Total flavonoids	–0.0253	0.581	–0.704	0.361	0.178	0.052	–0.0313
DPPH	0.498	0.082	0.156	0.158	–0.164	0.522	–0.628
FRAP	0.497	0.131	0.101	0.168	–0.087	–0.811	–0.172
Eigenvalue	3.722	1.2163	1.006	0.569	0.432	0.040	0.012
Proportion	0.5318	0.1738	0.1437	0.0814	0.0618	0.0058	0.0018
Cumulative	0.5318	0.7055	0.8492	0.9306	0.9924	0.9982	1

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power.

and divided into two clusters, *i.e.*, II-1 and II-2. The sub-cluster II-1 includes the cultivars Blackmore, Missionary, Heera, Larson and Red Cross, which have similarity in ascorbic acid content, total flavonols, DPPH and FRAP. However, cluster II-2 consists of Majestic, Allstar, Chandler, Senga Sangana and Camma Rossa, particularly characterized by total phenols and FRAP.

The second cluster includes nine cultivars (40.91% of the total cultivars studied); it is also divided into three sub-clusters. Douglas and Banglora formed a sub-cluster *viz.* II-1, which is characterized by ascorbic acid, flavonols and FRAP content. The cultivar Fiona formed the first monophyletic cluster and posses a medium value for all the antioxidant traits. Shasta and EC-102642 formed cluster II-2, having more similarity in ascorbic acid content, organic acid (citric acid), total anthocyanins and total flavonols. Sub-cluster II-3 consists of four cultivars, *i.e.*, Athena, Dilpasand, EC-22355 and Katrian Sweet, with similar antioxidant capacity.

Appliance of cluster analysis in this way is unique and very useful because it reveals groups of varieties with similar biochemical pathways. Parallel analysis based on homogenous groups points out similarities that may be useful while recommending new genotypes [35, 36], because it is possible to determine similarities and also divergence from the standardized and confirmed cultivars; in addition, it is possible to assume reactions of these new varieties to production and cultivation conditions. The diverse genotypes from different clusters could be utilized in strawberry improvement programs for introducing health-promoting traits. Cultivars with a wide intercluster distance can be used for improving desired

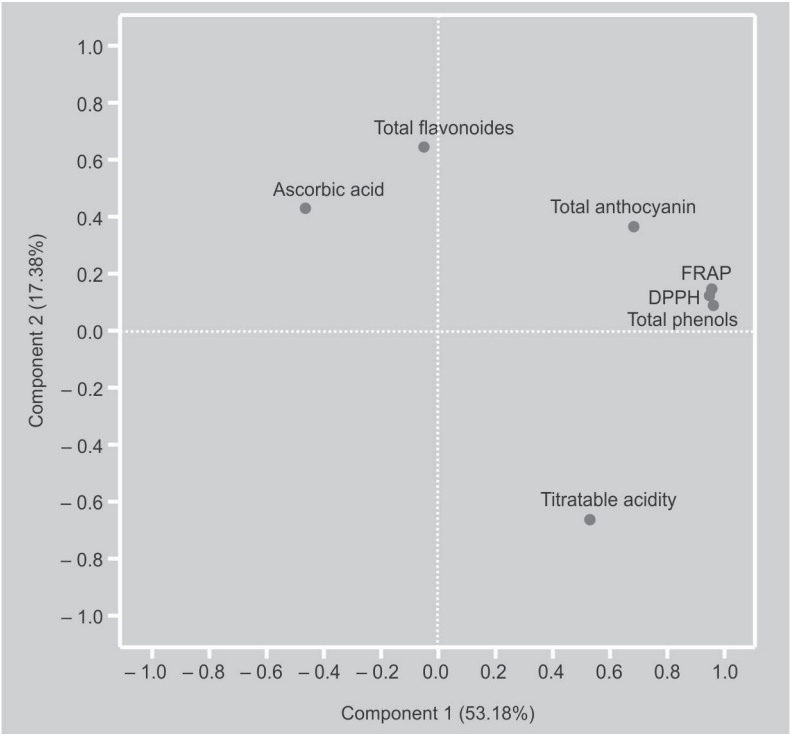

Figure 3.
Configuration of the seven health and bioactive compound traits from 22 strawberry cultivars under principal component axis 1 and axis 2 of a PCA (DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing antioxidant power).

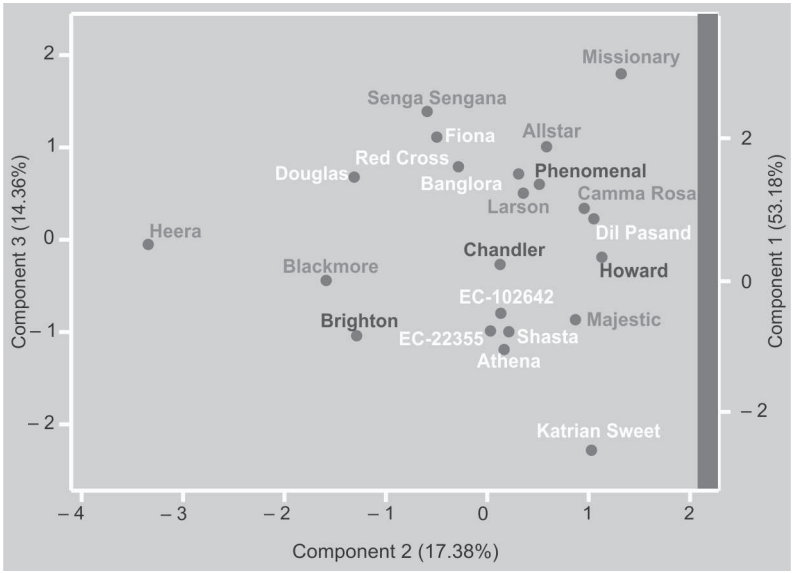

Figure 4.
Relationships among 22 strawberry cultivars shown by a 2D scatter for the first three principal components of a PCA based on health and bioactive compound traits.

traits through hybridization to obtain cultivars rich in health and bioactive compounds.

4. Conclusion and future prospects

The results obtained in our study can be used for better selection of varieties, formulating breeding and evaluation strategies and confirming the importance of the genetic background of cultivars for the availability of specific compounds in strawberry fruits. Nowadays, these aspects are considered to be highly valuable for the commercial valorization of new varieties, but mostly for the selection of genotypes with high fruit nutritional quality combined with yield efficiency. The choice of cultivar turns out to be the most important factor to increase health- and taste-promoting compounds in strawberry fruits. In the future, increasing

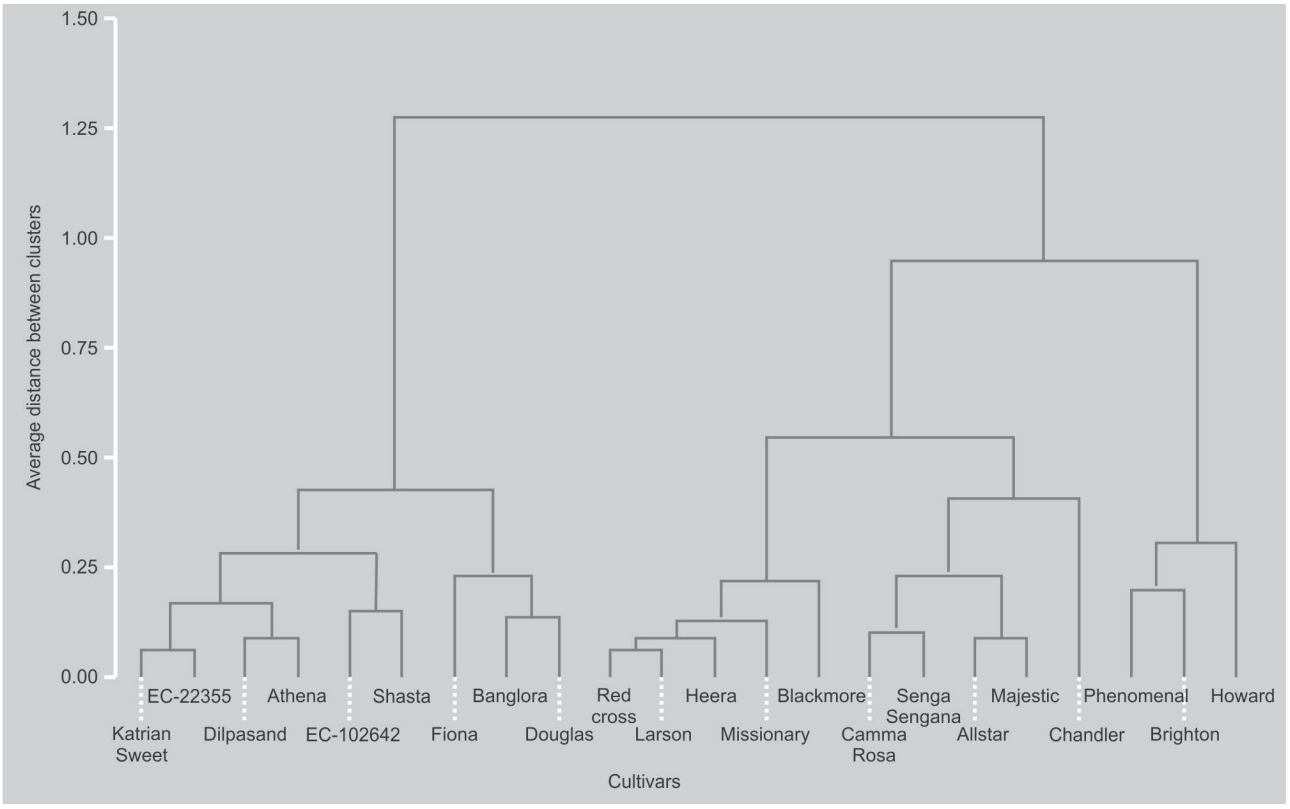

Figure 5.
Dendrogram depicting genetic relationships among 22 strawberry cultivars based on health and bioactive traits produced by Ward analysis (scale: average distance).

taste- and health-related components in strawberry fruits could be achieved by breeding new cultivars or by acting on plant biosynthetic pathways so that bioactive-rich fruits could be used for direct consumption or as extracts to increase the nutritional value of different foods and diets.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Variabilidad de compuestos bioactivos y de salud en los cultivares de de la fresa (*Fragaria* × *ananassa* Duch.) en un ecosistema templado de la India.

Resumen – **Introducción**. La fresa es rica en sustancias bioactivas y saludables; las ventajas derivadas de la utilización de productos naturales ricos en sustancias bioactivas interesan cada vez más a la industria farmacéutica, alimenticia y cosmética. **Material y métodos**. Se estudiaron veintidós cultivares de fresa (*Fragaria* × *ananassa* Duch.) en un ecosistema templado según su contenido en ácido ascórbico, compuestos fenólicos, flavonoides, antocianinas, actividades antioxidantes (dosis de DPPH y FRAP). El contenido en fenoles se midió con el reactivo Folin-Ciocalteau usando ácido gálico como control. La actividad antioxidante se determinó con los ensayos DPPH y FRAP y se expresó con el equivalente de ácido ascórbico. El contenido en flavonoides y las antocianinas totales se determinaron con un método colorimétrico. La acidez valorable (ácido cítrico) se determinó mediante valoración. Las medias de los datos correspondientes a dos años se analizaron con la ayuda del programa SAS 9.2. **Resultados y discusión**. Se detectaron diferencias significativas en el contenido de compuestos bioactivos y de salud en los cultivares estudiados. En las muestras de estudio, el ácido ascórbico varió (51,03 a 89,40) mg·100 g^{-1} de materia fresca (mf). La acidez valorable varió entre el 0,73% y el 1,44%, mientras que el contenido en antocianinas totales fue de (28.24 a 43.32) mg Eq cianidina-3-glucosida·100 g^{-1} mf. Los fenoles totales variaron (380,10 a 888,10) mg Eq ácido gálico·100 g^{-1} mf y los flavonoides totales (31,26 a 55,16) mg Eq catequina·100 g^{-1} mf. La actividad antoxidante total varió (203,13 y 471,10) mg Eq ácido ascórbico·100 g^{-1} mf en el caso del DPPH, y (326,06 a 701,13) mg Eq ácido ascórbico·100 g^{-1} mf en el caso del FRAP. Los fenoles totales, el y DPPH el FRAP mostraron estar estrechamente ligados, mientras que el ACP de los cultivares seleccionados los divide claramente en dos grandes grupos. Todos los cultivares se agruparon en dos clusters que podrían explotarse en un futuro con un programa de mejora cualitativa basado en la distancia media entre dichos clusters; podrían utilizarse como fuente de genes favorables para la obtención de alimentos saludables. **Conclusión**. Nuestros resultados podrían ser relevantes para agricultores, ganaderos, consumidores e industrias que se preocupen por la calidad de los alimentos, la prevención de enfermedades y el cuidado de la salud.

India (Jammu y Kashmir) / *Fragaria ananassa* / zona templada / frutas / antioxidantes / acido ascórbico / contenido fenólico / antocianinas