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Yield, nutrient content and physico-chemical and organoleptic properties in green bean are affected by N:K ratios

E. M. Khah and I. S. Arvanitoyannis*
*University of Thessaly, School of Agricultural Sciences, Department of Agriculture Crop Production & Agricultural Environment, Fytoko Street 38446 Nea Ionia Magnesias , Volos, Hellas,Greece.*e-mail: parmenion@uth.gr*

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Abstract

There is insufficient information about whether cultivars of green bean can be separated on based sensory properties. This study was undertaken in order to determine if cultivars could be separated based on yield components and nutrient content with quality related (sensory and physical) properties. The effect of the ratio of N and K treatments on number and weight of green pods and fresh and dry weight of individual plants of the green bean cvs.'Montano' and 'Larma'. The physico-chemical parameter [moisture content, chlorophyll and carotenoid content, soluble solids, total acidity, pH, color parameters (L*, a* and b*), firmness, sugars, starch, dietary fiber, proteins, polyphenols, nitrate content, metal taste, vitamin C] were studied. Using a trained panel of ten persons, and untrained panel of 50 consumers, various sensory parameters were also studied. The physico-chemical parameters were correlated with the sensory properties, as well as with the yield. Although 'Montano' in general had higher yield than 'Larma', both cultivars irrigated only with water led to higher performance and increase in yield than the other three fertilizer regimes. Application of Principal Component Analysis resulted in classification of treatments (type and level of fertilizers) with regard to their sensory and physical properties. Overall impression was associated with sweetness, saltiness, color and odor.

Key words: Carotenoids, chemometrics, chlorophylls, cluster, color, fertilizers, firmness, green beans, PCA, regression, sensory.

Introduction

Vegetables are considered one of the most important items of the European diet, and are traditionally served alongside a protein (e.g. meat, fish) or a carbohydrate food (e.g. pasta, rice, potato). Vegetables provide textural and color variety to the meal and, more importantly, complementary nutrients such as dietary fiber, vitamins, minerals, folate[1] and flavonoids with beneficial cardiovascular activity[2]. Vegetables are one of the main sources of vitamin C, which has two active forms: ascorbic acid (AA) and its oxidized form dehydroascorbic acid (DHAA). Several methods have been developed for the analysis of this vitamin, including electrochemical, spectrophotometric, catalytic, spectrofluorometric and chromatographic methods. For liquid chromatography, reverse phase ion-exchange columns, and, more recently, octadecyl silane columns combined with electrochemical, fluorimetric or UV detection have provided good results for AA analysis[3]. Color is an important sensory property and may be used as a criterion of vegetable quality[4]. In plants, the major pigments are carotenoids, vitamin A precursors and possible inhibitors of cancer and ulcers [5,6] and chlorophylls (source of dietary magnesium[7]). The predominant carotenoids in green vegetables are lutein and β-carotene generally occurring at a ratio of 3:1 [8]. Nitrite and nitrate are intermediate states in the nitrogen cycle. Oxidation of nitrite to nitrate reduces the dissolved oxygen content of water. Fresh and processed vegetables have often been cited as the major sources of dietary nitrate intake, owing to their nitrate accumulation capacity. However, in recent years, there has been an increasing interest in the determination of nitrate levels in food products, essentially because of the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health due to formation of toxic and carcinogenic nitrosamine compounds. In most cases, the amount of available nitrate in soil (which may be related to the amount of commercial fertilizers applied) appears to be a major factor determining the nitrate concentration level in vegetables[9]. Application of increased fertilizer to greenhouse soil leads to soil contamination and lower production and assimilation of undesirable toxic elements in plant tissue [10,11]. Green bean (*Phaseolus vulgaris* L.) is one of the most popular vegetables in Europe and in the US. Green bean quality mainly depends on the cultivar and post-harvest practices [12,13,14]. Quality is one of the most defined and confusing terms in use in the food industry, and is strongly influenced by various factors such as geographic location, agricultural practices (e.g. irrigation, use of pesticides), cultivar employed and physiological maturity of the raw materials at the time of the harvest[15]. The chemical composition and quality of French bean pods and grains are influenced by soil fertility status[16,17], plant spacing [18,19], irrigation [20,21] maturity [22] and cultivar [23,24,25]. Crops respond according to the amount of water used and the appropriate fertilizer rates with better growth and improved yield, as well as high crop quality. Aguar et al.[26] found that although beans require less nitrogen than other vegetables, high rates of nitrogen improve vine growth and hasten fruit maturity. Ware and McCollum[27] found that high rates of potassium may be used to improve vegetable quality and yield, but excess nitrogen causes increased vegetative growth and greater sensitivity to mechanical damage at harvest. Talavera [28] found that nodulation responds positively to potassium application and the application of potassium in green beans is important for improvement of crop quality and physicochemical properties. The control, or modification, of texture has become a major objective in modern food technology[29]. At the fundamental level, research has been directed toward understanding which chemical and/or structural parameters of plant tissues contribute to texture and how they are affected by processing, which changes the pectic polymers of the cell wall and middle lamella[30]. Sensory attributes (appearance, texture, aroma, flavor and overall impression) are a function of the physico-chemical composition of vegetables. By determining which product sensory attributes are most important to consumers, one can focus research and development efforts on satisfying their expectations [31]. Correlation of food composition and sensory properties are important for predicting the sensory quality on the basis of physico-chemical measurements. This correlation is possible through the employment of chemometrics (multivariate data analysis aimed at facilitating the explanation and understanding of measuring various parameters) which, though introduced as a tool for use in chemical applications, has found a wider application for exploring the relationships between chemical and sensory data [32]. Although it is known that fertilizer rates can affect the yield of green beans it is not clear whether physico-chemical and sensory qualities are also affected. In order to produce improved varieties it is important to know if physico-chemical qualities are different in cultivars so that it might be possible to identify cultivars with desirable traits that can be exploited. It may be that there are combinations of traits that can be used to develop improved cultivars which can be identified by correlation analysis. The aim of this study was to determine how fertilizer levels affected the yield and physico-chemical and sensory properties of two cultivars of green beans ('Montano' and 'Larma') in an attempt to acquire an insight into the consumer acceptance, disclose correlations between sensory and instrumental data and separate the two cultivars on the basis of their attributes.

Materials and Methods

Plant production: Two cultivars ('Montano' and 'Larma') of green bean were grown in separate unheated glass greenhouses (10x18m) at the University of Thessaly's farm, Velestino, Volos, Greece, and supplied with various ratios of fertilizer consisting of NH_4NO_3 (110 g/L) and KNO_3 (130 g/L) which were diluted by 300 L water. Plants were irrigated twice per week with: only water as a control, 1N:1K 110:130 g/L, 1N:3K 110:390 g/L and 3N:1K 330:130 g/L. Seeds were sown into a commercial peat based compost in small pots (5x10x5 cm) on 14 September 2000. Pots were kept in a propagation greenhouse for 22 days until seedlings reached approximately 15 cm height and were then transplanted on soil level on 6 October 2000, and left until harvest on 20 November 2000. The soil of the greenhouses was previously prepared by plowing and then disking by small tractor. Prior to the initiation of the experiment, the soil used in greenhouses was randomly sampled in 0-30 cm depth (five samples were taken each approximately 500 g. from each greenhouse). Samples were sent to the laboratory of the National Agricultural Research Foundation, Athens, for analysis. The results showed that the soil was sandy clay with pH 7.55 and the values of organic matter, N and K were 3.0%, 140 mg/100 g and 0.56 meq/100 g, respectively. Irrigation water was analyzed and found to have a pH 7.55, electroconductivity = 578 mS/cm and 30 $mg \cdot L^{-1}$ NO_3^-. Air temperature and humidity (maximum and minimum) were recorded daily throughout the duration of the experiment. In each greenhouse, seedlings were sown in a split, completely randomized, design. Cultivar was the main treatment and fertilizers comprised the split with four blocks each in three replicates. Twenty plants were sown at 50 cm apart from each other in blocks. Four plants were evaluated and harvested for yield analysis and the other 16 plants, 8 plants from two sides were used for sensory testing. Irrigation and nutrients were provided via a drip system from individual 150 L barrel tanks. One tank was for control (water without fertilizer) and the other three contained one of the previously described fertilizer combinations. Plants were irrigated and fertilized twice per week. Pods were harvested when commercially mature and their number was recorded. The fresh weight of plant above ground was determined for four carefully uprooted plants. Plants and pods were dried in a ventilated oven at 85°C for 48 hours before dry weight determination.

Physico-chemical analysis:

Moisture content: Determined in a sample of 10 g according to AOAC method 964.22[33].

pH: Pieces of green bean pods (2 g 20 mL water) were pulped and measured with a pH meter (PHM 80 Portable, Denmark), and total acidity (expressed as malic acid) was determined by titration with 0.1 NaOH.

***Vitamin C*:** All analyses were carried out on vegetable tissue homogenized using pestle and mortar. Five grams of homogenate was mechanically stirred in 15 mL of a 4.5% (w/v) solution of metaphosphoric acid for 15 min. The mixture was filtered and the filtrate was diluted to 25 mL with HPLC grade water. A Hewlett-Packard HPLC apparatus was used equipped with a C_{18} column.

Soluble solids (water soluble, Brix number): A solution of pods (20% w/w at 20°C) was prepared and measured with a manual refractometer (A. Kruess, Optronic HR 901, Germany).

Color: Measurements were conducted with a Hunter colorimeter model D50 optical sensor (Hunter Associates Laboratory Inc., Reston, VA). Values for luminosity (L), greenness (a) and yellowness (b) were determined using a white tile as standard.

***Nitrate content*:** Measurements on 5 gr of pulped pods were carried out with a Reflectoquant (Merck, Germany) according to the following method: nitrate was reduced to nitrite by a reduction agent in the presence of an acidic buffer. This nitrite reacts with an aromatic amine to form a diazonium salt which in turn reacts with N-(1-naphthyl)-ethylene-diamine to form a red violet azo dye, the concentration of which is determined reflectometrically. Test strips were dipped into the freshly extracted juice (5 g of pulped pods were put in conical flasks with 50 mL of boiling water for 5 mins and then 50 more mL of cool water were added) and the strip color was allowed to develop for 60 s. The value of nitrate (NO_3^-) is calculated as follows: mg/L NO_3^- = (ml of water x machine reading) sample weight.

Firmness: Measurements on 15 samples of fresh bean pods (total 40 g) using an Instron Universal Testing Machine (model 4301, Bucks, UK) equipped with a Warner-Bratzler meat shear compression cell (catalogue no. 2830-013) were taken. Samples were placed in the cell with the length axis of the pods perpendicular to the opening of the shear cell. A 100 kg load and a blade speed of 60 nm/min were used for the measurements. The maximal shear force (top value) applied with the press to break through the beans was used to quantify the firmness of the beans.

Total starch: Content was determined after dispersion of the starch granules in 2M KOH (100 mg sample, 6 mL KOH) at room temperature (30 min, constant shaking) and hydrolysis of the solubilized starch with 80 mL amyloglycosidase (Boehringer-Mannheim, Germany). Glucose was quantified using the Peridochrom Oxidase/Peroxidase reagent (Boehringer-Mannheim, Germany). Total starch was calculated as glucose x 0.9 after correction of the free glucose content.

Dietary fiber (DF): DF content was analyzed by the enzymatic-gravimetric method described by Prosky et al. [34] and modified according to Manas et al. [35]. Samples (5 g) were treated with heat stable α-amylase (EC 3.2.1.1; A-3306), protease (P-3910) and amyloglucosidase (EC 3.2.1.3; A-9913) all from Sigma Chemical Co. (St. Louis, MO, USA) to remove digestible starch and protein. After centrifugation and washing with distilled water, soluble and insoluble dietary fiber content was determined according to the methods of Bravo et al.[36].

Total polyphenols (TPPH): Samples of 50 g were used and quantification was as gallic acid equivalents using the Folin-Ciocalteau's reagent [37].

Total nitrogen: Levels were determined in 3 g samples with the Kjeldahl method. Protein was calculated as N multiplied by 6.25; and fat was determined after extraction in a Soxhlet equipment.

Pigment analysis: Sliced fresh pods (5 g) were used for improving grinding with 1-2 g of anhydrous sodium carbonate to adjust the pH to 8-9 and to prevent chlorophyll conversion to pheophytin. Chilled acetone of HPLC grade was then added to the sample and the mixture was homogenized and centrifuged at 4000 *g*, for 10 min. The supernatant was then transferred to a separatory funnel and HPLC grade diethyl ether was added. The aqueous part was discarded, and the diethyl ether layer was dehydrated with sodium sulfate anhydride. This solution was filtered, transferred to a beaker and evaporated under a stream of nitrogen. The residue was dissolved and diluted to 4-5 mL of acetone. Triplicate samples 20 mL were injected for HPLC analysis.

Chromatographic conditions: Separation of pigments (sample size of 2 g) was performed on a C18 reverse phase column (HYPERSIL ODS, Hewlett Packard, USA). A combination of isocratic and gradient chromatography separated the oxygenated xanthophylls from chlorophylls and the hydrocarbon carotenoids. A gradient mixture of methanol - water (3:1) eluent A and ethyl acetate (eluent B) was used. The flow rate was 1mL/min and the runs were monitored at 430 nm. Chlorophyll and carotenoid pigments were identified according to their chromatographic behavior on HPLC and UV/visible absorption spectra, by comparing both their retention time and the absorption spectra to those of standard chlorophyll and carotenoid pigments. Values were compared to an external standard using commercially available pigment standards purchased from Aldrich-Sigma (Switzerland). Standard curves were constructed by plotting various concentrations of each pigment versus area measurement in HPLC chromatograms. Recoveries ranged between 80 and 90% for chlorophyll-a, chlorophyll-b, lutein, xanthophyll and β-carotene. Values are the mean value of at least three measurements.

Sensory evaluation: Quantitative descriptive analysis by a trained panel [38,39] and the acceptance test by consumers were part of the sensory evaluations of green beans. The descriptive panel consisted of ten panelists who had undergone training for more than six months by getting acquainted with various tastes and their intensity [40]. The intensity of all sensory impressions of each product sample was evaluated using an unstructured scale with the extremes of 0 ('not perceptible') and 10 ('strongly perceptible'). The evaluated sensory characteristics were: color, brightness, fibrousness, saltiness, bitterness, sweetness, grass taste, metal taste, moldy taste, tartness, acidity, stickiness, intensity, juiciness, odor and hardness. All sessions were held in the sensory laboratory located in the Department of Agriculture of the University of Thessaly (Volos, Greece). Subjects were seated in individual booths equipped with white fluorescent light. Samples (15 g for each combination of cultivar and treatment per person) were boiled with 50 mL of distilled water in a saucepan for 15 min. Samples were served warm (about 40^{0}C) and consisted of five green bean pods randomly labeled with a three digits number. Samples were evaluated by each panelist three times. Spring water and bread were provided for rinsing the palate between samples. In the consumer acceptance test, 50 consumers assessed the green beans for appearance, aftertaste, odor, tenderness, mouthfeel and overall impression using an unstructured scale with the extremes of 0 ('very unpleasant/bad') and 10 ('very pleasant/good'). The samples (50 g, 2 g for each combination of cultivar and treatment per person) were boiled with 500 mL of distilled water in a saucepan for 15 min. Samples were served warm (about 40^{0}C) and spring water and bread were provided for rinsing the palate between samples.

Statistical analysis*:* The yield components and composition data were analyzed for variance (ANOVA) using SPSS for Windows [41] and means separation with Duncan's multiple range test, as well as least significant difference (LSD) test at 5% level of probability.Principal Component Analysis (PCA), a popular multivariate analytical technique, is also known as the Hoteling transformation (image analysis), eigenvector analysis (physical sciences), correspondence analysis (French-speaking countries) [42] and is widely applied in industry, government and universities [43]. PCA is employed to reduce targets to the dimensionality of a data set in which there is a large number of intercorrelated variables while retaining as much as possible of the information present in the original data and introducing a minimum of *a priori* assumptions [44, 45]. Cluster analysis (CLA) groups variables or objects in terms of their similarities/properties are divided into hierarchical and non-hierarchical terms[44,46]. PCA and Cluster Analysis (CLA, dendrogram using average linkage between groups) were carried out with SPSS (Statistical Package for Social Sciences, Base 9.0, SPSS Inc., USA). Employment of PCA, Multiple Linear Regression Analysis (MLRA), Discriminant Analysis and CLA aimed at describing correlations between sensory and instrumental data.

Results and Discussion

Cultivation practices: The mean air temperature, and the mean humidity maximum and minimum, were 36.1^{0}C, 14.4^{0}C, 90.5% and 37.1%, respectively. Plant fresh and dry weights, and the number and dry weight of pods per plant were generally affected by cultivar, fertilization regime and their interactions (Table 1). The exception was for the number of pods per plant, which was not affected by the fertilizer regime. 'Montano' had heavier plants

Table 1. Effect of water and various fertilizer rates on the yield of four plants of two cultivars ('Montano" and 'Larma') of *Phaseolus vulgaris* L.

	Plant weight Fresh	Dry	No. pods Per plant	Pods dry weight (g) Per plant
Source				
Cultivar (C)	*	*	*	*
Fert. Regime (F)	*	*	NS	*
Interaction				
C x F	*	*	*	*
Cultivar				
'Montano'	36.6a*	16.7a	10.9a	11.2a
'Larma'	24.3a	9.5b	6.1b	4.8b
Fertilizer regime				
N0 : K0	34.2a	15.0a	10.0a	9.5a
N1 : K1	31.5a	13.0a	9.0a	8.6a
N1 : K3	34.3a	14.4a	8.3a	8.5a
N3 : K1	24.0b	10.1b	6.7a	5.4b
Interaction				
CV x Fert. Regime				
M 0 : 0	40.9[y]	18.7	11.3	12.7
1 : 1	41.1ns	16.7ns	11.0ns	11.7ns
1 : 3	40.1ns	18.1ns	11.7ns	12.2ns
3 : 1	28.4*	13.2*	9.7ns	8.1*
L 0 : 0	27.5	11.2	8.7	6.3
1 : 1	21.8ns	9.3ns	7.0ns	5.5ns
1 : 3	28.4ns	10.7ns	5.0ns	4.7ns
3 : 1	19.6*	7.0*	3.7*	2.6*

NS, * not significant or significant at P≤0.05, ANOVA.
[z] Values in a column followed by the same letter are not significantly different.
Means on each column were separated with Duncan S multiple range test.
[y] **NS**, * not significant or significant at P≤0.05, Least Squares Means analysis.

with more, and heavier, pods than did 'Larma'. The N3:K1 ratio produced the lightest plants with the lowest pod weight. For 'Montano', in the interaction, the N3:K1 ratio produced the lightest plants with the lowest pod weight. For 'Larma', in the interaction, the N3:K1 ratio had the lightest plants with the fewest number of pods and the lightest pod yield. Both 'Montano' and 'Larma' irrigated only with water exhibited results that were similar to most fertilizer treatments (Table 1). This could possibly be attributed to the soils in both greenhouses having high rates of N, K and P. Also, it was found that the irrigation water had considerable concentrations of N which provided the plants with extra N. Use of the N3:K1 fertilizer regime had a negative effect on yield. That the highest treatment of nitrogen led to low yields and quality agree with other researchers' findings that excessive application of N may result in reduction of growth and poor pod yield [26,27].

Physico-chemical properties: The percentage composition of the various nutritional components of green beans is found in Table 2. The average protein content of 'Montano' and 'Larma' (1.9%) is in agreement with that determined for French beans [47,48,49]. The

Table 2. Percentage composition of physico-chemical parameters* for two cultivars ('Montano' and 'Larma') of *Phaseolus vulgaris* L.

Cultivar	Protein %	Fat %	Moisture %	Sugars %	pH	Titratable acidity	Soluble solids (Brix)	Starch	Nitrate (mg/l)	Dietary fibre (%)	Total Nitrogen (%)	Poly-phenol (mg/l)
'Montano'												
0:0	2.00	0.15	94.32	4.5	5.8	0.15	6.21	38.0	343	1.4	4.0	0.60
1:1	1.85	0.13	93.04	4.6	6.0	0.14	6.82	37.2	270	1.8	3.9	0.55
1:3	1.89	0.21	92.10	4.4	6.0	0.16	7.81	36.4	300	1.2	3.8	0.42
3:1	1.80	0.16	92.75	4.4	5.9	0.11	7.51	38.5	314	1.9	4.2	0.49
'Larma'												
0:0	1.95	0.17	90.61	5.5	5.7	0.14	6.04	37.0	661	1.7	3.6	0.49
1:1	1.76	0.20	85.64	5.3	5.5	0.12	10.40	35.3	633	1.5	3.3	0.37
1:3	2.10	0.25	87.11	5.4	5.6	0.13	9.21	36.3	517	1.4	3.8	0.44
3:1	1.87	0.14	88.92	5.6	5.5	0.12	8.31	35.0	649	1.3	4.0	0.45

* The values reported stand for the average of at least three measurements.

Table 3. Vitamin C content* (mg/100 g) on wet basis and color parameters* for two cultivars ('Montano' and 'Larma') of *Phaseolus vulgaris* L.

Method	Total	Color parameters		
Titration	Vitamin C	L*	a*	b*
'Montano'				
0:0 (N/K)	12.53	40.7	-16.9	23.4
1:1	11.83	39.8	-16.9	24.3
1:3	10.57	41.1	-15.9	23.2
3:1	9.83	36.9	-16.4	22.7
'Larma'				
0:0 (N/K)	13.40	38.5	-18.0	25.9
1:1	12.47	39.9	-16.7	27.2
1:3	10.70	39.0	-18.2	26.7
3:1	11.17	32.8	-17.3	27.9

* The values reported stand for the average of at least three measurements.

average fat content was 0.16 and 0.19% for "Montano' and 'Larma', respectively, and is in agreement with previously reported values [47,49,50]. The water content determined for the two cultivars varied within a range 88-93% compared to 90% for green beans[50], but contrasts with very low 11.3% reported for green beans[51]. The water content is reported as a freshness index because high water content is closely related to field fresh green beans. Sugar contents were also in agreement with values reported elsewhere[36,51]. Dietary fiber (DF) contents are in agreement with previously reported values [47,48,49,50]. However, considerably higher DF values were determined for green beans by Kirk and Sawyer[51]. Total nitrogen content ranged 3.8-4.2 and 3.3-4.0% for 'Montano' and 'Larma', respectively. In fact, the higher nitrogen content values were reported for plants treated with extra nitrogen (3N:1K) and the control (0N:0K), whereas only in 'Larma' plants treated with 1N:1K the nitrogen content was slightly lower than the value 3.42% reported by Kirk and Sawyer[51]. Polyphenols occurred in concentrations of 0.52 and 0.44%. The carcinogenic potential of N-nitroso compounds such as amines and nitrates made the determination of the latter important. 'Larma' exhibited higher nitrate values (517-661 mg·g^{-1}) than did 'Montano' (270-343 mg/g). However, no correlation could be established between the N/K ratio and the nitrate content. Vitamin C content was found to be 11.2 and 11.9 mg/100 g for 'Montano' and 'Larma', respectively (Table 3). These values are in agreement with those determined for field fresh green beans [1,48,49] and are higher than market fresh, supermarket fresh, canned and boiled green beans which have average value of 7.12 mg/100 g,[1,49]. The vitamin C content was lower compared to those determined with spectrofluorimetry and titration [3,52] .The colorimetric parameters (L*, a* and b*) for 'Montano' and 'Larma' (Table 3) were in agreement with those determined for 'Strike' and 'Bina' green bean cultivars [15] . The pigment contents (Table 4) are in line with those reported by Mozsik et al.[6] . It is well-known that exposure of chlorophylls to thermal or solar energy results in degradation of chlorophylls to pheophytins. Another factor considerably influencing the degradation of chlorophylls was shown to be the acidic conditions [8] . The texture of French beans is usually measured with the Kramer shear press or with Warner-Bratzler shear compression (Table 5). The former gives values almost three times higher than the latter. An attempt was made to correlate instrumentally measured hardness with moisture content and sensory parameters with the aid of Multiple Linear Regression Analysis (MLRA). The parameters related to instrumental hardness were mainly moisture, bitterness, odor, hardness, acidity and only secondarily color and fibrousness.

Instrumental hardness = 68.860 + 0.384 Color -1.776 Fibrousness +7.521 Bitterness –17.452 Moisture +6.198 Acidity + 9.636 Smell -11.406 Hardness (1)

Sensory analysis showed that the overall impression of the consumer panel was in agreement with the results obtained by the trained panelists; that is high overall impression was closely related to color, brightness, juiciness, odor (trained panel) and appearance, mouthfeel, smell, tenderness and aftertaste (untrained panel) (Table 6). The results of the overall impression for the untrained panel were in the following order; 'Larma' (N:K/0:0) > 'Montano' (N:K/0:0) > 'Montano' (N:K/3:1) > 'Larma' (N:K/3:1) > 'Montano' (N:K/1:3) > 'Larma' (N:K/1:1) > 'Montano' (N:K/1:1) > 'Larma' (N:K/

Table 4. Changes in chlorophyll* and carotenoid* contents (mg/100 g) for two cultivars ('Montano' and 'Larma') of *Phaseolus vulgaris* L.

Cultivar	Chlorophyll -α	Lutein	Chlorophyll -β	Phaeophytin -α	β-carotene
'Montano'					
0:0 (N/K)	4.75	0.14	0.83	0.87	0.60
1:1	4.90	0.20	0.90	0.82	0.47
1:3	4.50	0.12	0.73	0.95	0.35
3:1	4.67	0.15	0.75	0.92	0.39
'Larma'					
0:0 (N/K)	4.37	0.27	0.99	0.81	0.32
1:1	4.32	0.20	0.88	0.74	0.29
1:3	4.44	0.30	1.05	0.85	0.34
3:1	4.33	0.24	0.93	0.76	0.31

* The values reported stand for the average of at least three measurements.

Table 5. Texture measurements* using the Warner Bratzler (shear compression) for two cultivars ('Montano' and 'Larma') of *Phaseolus.vulgaris* L.

Cultivar	Unit
	Max. Force (N)
'Montano'	
0:0 (N/K)	27.83
1:1	28.51
1:3	30.40
3:1	28.93
'Larma'	
0:0 (N/K)	29.52
1:1	34.53
1:3	32.86
3:1	32.21

* The values reported stand for the average of at least twelve measurements.

1:3) showing that green beans produced with no additional N or K were of better organoleptic properties. This may be due to the fact that greenhouse soil contained considerable fertilizer residues from previous crops. The worst performance was for the green beans produced with the 1N:1K ratio. It is interesting that cultivar is of minor importance at this instance *vis a vis* the fertilizer effect since both cultivars follow approximately the same general trend.

Principal Component Analysis (PCA): PCA shows that four eigenvectors are required for reaching a total variance of 82.2% (Table 7). PC1 vs PC2 (Figure 1) shows that 'Larma' (E1-3, F1-3, G1-3 and H1-3, for explanation of E, F, G and H see Table 6, numbers 1-3 stand for three replicates) is characterized mainly by the favorable sensory attributes of high overall impression, sweetness, saltiness, color and odor (group J). However, hardness and tartness are two unfavorable characteristics attributed to 'Larma'. 'Montano' (A1-3, B1-3, C1-3 and D1-3, for explanation of A, B, C and D see Table 6, numbers 1-3 stand for three replicates) is characterized by a lesser number of positive attributes such as a*, b* and brightness, whereas nitrate is included in the negative attributes (group M). The presence of nitrate is likely related to nitrogen in soils whereas the positive attributes for 'Larma' are likely due to colorimetric parameters, instrumentaly and by the panel. In PC1 vs PC3 (Figure 2), 'Larma' (E1-3, F1-3, G1-3 and H1-3) is characterized by positive attributes such as vitamin C, odor, saltiness, overall impression and intensity. Stickiness to the teeth and fibrousness are the only negative attributes (group L). 'Montano' is also characterized by positive attributes related primarily to colorimetric parameters (instrumental; a* and b*, sensory; brightness) and moisture related to freshness (group M). In PC2 vs PC3 (Figure 3) two clusters, L and M for 'Larma' and 'Montano', respectively, are distinguished. 'Larma' is characterized by the positive attributes overall impression, color, saltiness, intensity and moisture and stickiness and nitrate, negative attributes. In the case of 'Montano', the positive attributes (sweetness, brightness, a*) and the negative attributes (grass taste, tartness, hardness) are almost equally shared indicating that 'Montano' is inferior to 'Larma' in terms of desirable characteristics.

Cluster Analysis (CLA): Hierarchical Cluster Analysis was carried out using Pearson coefficient analysis resulting in a dendrogram construction both for physico-chemical and sensory parameters and for objects (plants under different fertilizer ratios) (Figures 4 and 5). The physico-chemical and sensory parameters were clearly grouped in three large clusters: J, K, M in Figure 4. Cluster J grouped

Table 6. Sensory properties of *Phaseolus vulgaris L.*, the mean values and standard deviation for the trained and untrained panels.

Cultivar	'Montano'				'Larma'			
Parameter	A(N:K 0:0)	B(N:K 1:1)	C (N:K 1:3)	D (N:K 3:1)	E(N:K 0:0)	F (N:K 1:1)	G(N:K 1:3)	H (N:K 3:1)
Descriptive analysis (trained panel)								
Color	7.35±0.23	6.04±0.32	7.11±0.61	8.22±0.51	8.15±0.42	6.15±0.42	5.78±0.84	7.45±0.84
Brighteness	8.37±0.14	6.52±0.17	7.39±0.56	7.85±0.39	8.85±0.32	6.85±0.32	4.93±0.76	7.93±0.76
Fibrousness	4.55±0.4	6.52±0.24	5.55±0.22	4.78±0.45	4.81±0.55	5.81±0.55	7.29±0.11	4.89±0.11
Saltiness	4.26±0.61	6.22±0.22	4.85±0.25	4.63±0.65	5.85±0.61	5.80±0.61	7.04±0.23	4.94±0.23
Tartness	4.74±0.61	6.11±0.29	5.63±0.71	4.92±0.71	4.67±0.24	5.67±0.24	6.52±0.17	5.52±0.17
Acrid	4.48±0.36	6.54±0.11	5.83±0.50	4.85±0.23	4.82±0.34	5.82±0.34	6.45±0.51	5.45±0.51
Sweetness	4.52±0.61	6.70±0.24	5.89±0.11	4.70±0.32	5.04±0.41	6.04±0.41	6.41±0.17	5.41±0.17
Grass taste	4.81±0.57	6.55±0.45	5.78±0.11	4.71±0.17	4.89±0.35	6.09±0.35	6.93±0.67	5.93±0.67
Metal taste	5.38±0.23	6.18±0.38	6.07±0.39	5.93±0.34	5.04±0.42	6.24±0.42	6.89±0.29	5.89±0.29
Mouldy taste	5.72±0.50	6.18±0.17	5.97±0.18	5.37±0.28	5.26±0.40	5.96±0.40	6.33±0.30	5.83±0.30
Acidity	4.56±0.20	5.41±0.49	5.00±0.40	4.81±0.61	5.22±0.39	5.22±0.39	5.63±0.42	4.93±0.42
Intensity	6.96±0.56	6.52±0.53	5.70±0.34	6.26±0.35	7.93±0.28	7.93±0.28	6.22±0.29	6.42±0.29
Stickiness	5.63±0.47	6.59±0.28	6.53±0.28	5.93±0.17	5.00±0.45	7.00±0.45	6.85±0.27	6.45±0.29
Juiciness	7.59±0.55	6.42±0.23	7.01±0.57	7.67±0.17	8.07±0.49	6.77±0.49	5.85±0.11	7.45±0.11
Odor	7.81±0.71	5.78±0.29	6.56±0.23	7.24±0.26	8.11±0.33	6.11±0.30	5.67±0.14	6.67±0.17
Bitterness	5.40±0.65	6.32±0.43	5.95±0.27	5.62±0.36	5.15±0.49	6.10±0.33	7.05±0.22	5.75±0.24
Hardness	6.00±0.44	6.37±0.32	6.85±0.17	6.66±0.59	6.55±0.42	6.55±0.42	6.63±0.56	6.33±0.56
Consumer Acceptance test (untrained panel)								
Appearance	8.07±0.59	6.15±0.59	7.05±0.59	7.57±0.59	8.37±0.59	6.47±0.59	6.03±0.59	7.19±0.59
Mouthfeel	8.00±0.38	6.00±0.38	6.72±0.38	7.60±0.38	8.11±0.38	6.11±0.38	5.72±0.38	7.00±0.38
Odor	7.79±0.41	6.21±0.41	6.78±0.41	7.37±0.41	7.55±0.41	6.51±0.41	6.88±0.41	7.14±0.41
Aftertaste	8.09±0.44	6.10±0.44	7.00±0.44	7.62±0.44	8.09±0.44	6.39±0.44	5.00±0.44	7.23±0.44
Tenderness	7.59±0.56	6.09±0.69	6.56±0.23	7.53±0.20	8.48±0.30	6.40±0.30	6.03±0.61	7.20±0.20
Overall impression	7.94±0.53	6.12±0.24	6.80±0.44	7.70±0.38	8.32±0.35	6.39±0.35	5.74±0.21	7.32±0.38

Table 7. Total variance explained as a result of implementation of PCAExtraction sums of squared loadings.

Component	Total	% of variance	Cumulative %
1	10.422	40.085	40.085
2	6.009	23.110	63.195
3	2.917	11.220	74.415
4	2.186	8.408	82.823
5	1.984	7.631	90.454
6	1.523	5.856	96.310

mainly the sensory properties showing that overall impression can be closely related to color, acceptability, aftertaste, intensity and hardness (as opposed to softness related to moisture or long storage). Juiciness and tenderness (related to freshness), saltiness (connected with taste) in conjunction with L* and smell are some more parameters also belonging to group J and indicative of the positive character of the properties expressed through this group. Cluster K grouped most of the negative, and undesirable to consumer, characteristics such as metal taste, grass taste, fibrousness, bitterness (as opposed to sweetness), instrumental hardness (as opposed to tenderness and freshness), and stickiness (adhesiveness to teeth). Cluster M comprises the instrumentally determined color parameters (a* and b*) and brightness, mouthfeel, moisture and nitrate content. As a whole, these parameters are positive expressions, except for nitrate, the concentration of which negatively affects the green bean profile. In the case of object grouping, two major clusters (M and L, for 'Montano' and 'Larma', respectively) emerged (Figure 5). This result shows that there is a very clear difference between the two cultivars which is further corroborated by the PCA results.

Multiple Linear Regression analysis (MLRA): Linear Regression Analysis is used to investigate the relationship between one dependent variable and one independent variable whereas in MLRA several variables are taken into account. The aim of MLRA is to construct a model predicting the overall impression. Instead of just an intercept and slope, the MLRA contains a constant (analogous to the intercept) and several coefficients (seven for sensory and six for sensory and instrumental attributes, see following equations (2) and (3) one for each of the independent significant variables ($P \leq 0.05$). The coefficients are known as partial regression coefficients.

Overall impression = -6.611 + 1.373 (metal taste) + 0.571 (fibrousness) - 0.464 (color) + 0.359(odor) + 0.317(acidity) + 0.223(hardness) + 0.045(bitterness) (2)

Overall impression = -6.557 + 1.024 (metal taste) + 0.845 (sweetness) + 0.652 (fibrousness) - 0.347(color) + 0.188(bitterness) + 0.030(vitaminC) (3)

Both equations have four attributes in common; metal taste, fibrousness, color and bitterness while the rest of the attributes differs. Metal taste and fibrousness and color are the three main parameters whereas bitterness appears to be of lesser importance. In equation 2 odor, acidity and hardness are also included in the major parameters while in equation 3 sweetness and vitamin C are the most important attributes. It is noteworthy that fibrousness was included in the suggested MLRA model which is in agreement with other publications that this attribute is of great importance [15].

Conclusions

The results of this study disclose that when the soil contains considerable fertilizer residues, it is unnecessary to apply fertilizer, especially N, when cultivation period is short-term. The values of organic matter, N and K contained in the soil were 3.0% and 140 mg/100 g and 0.56 meq /100 g, respectively. These amounts of nutrients were further enhanced with irrigation water containing NO_3^- (30 $mg \cdot L^{-1}$) and was apparently more than adequate to support plant development. High levels of N in relation to K (3:1) reduce growth and yield and act as an environmental contaminant. The application of multivariate techniques disclosed that separation of the green bean cultivars physical and organoleptic properties is feasible. Cluster analysis, in particular, proved to be quite effective towards this purpose because the genomic characteristics were shown to be more powerful than the treatments employed. Implementation of Principal Component Analysis provided a satisfactory classification of treatments (type and level of fertilizers) with regard to their properties. Discriminant analysis proved equally valuable because it produced equations confirming that overall consumer impression is closely related to color, odor, juiciness, tenderness and brightness which are characteristics associated with freshness.

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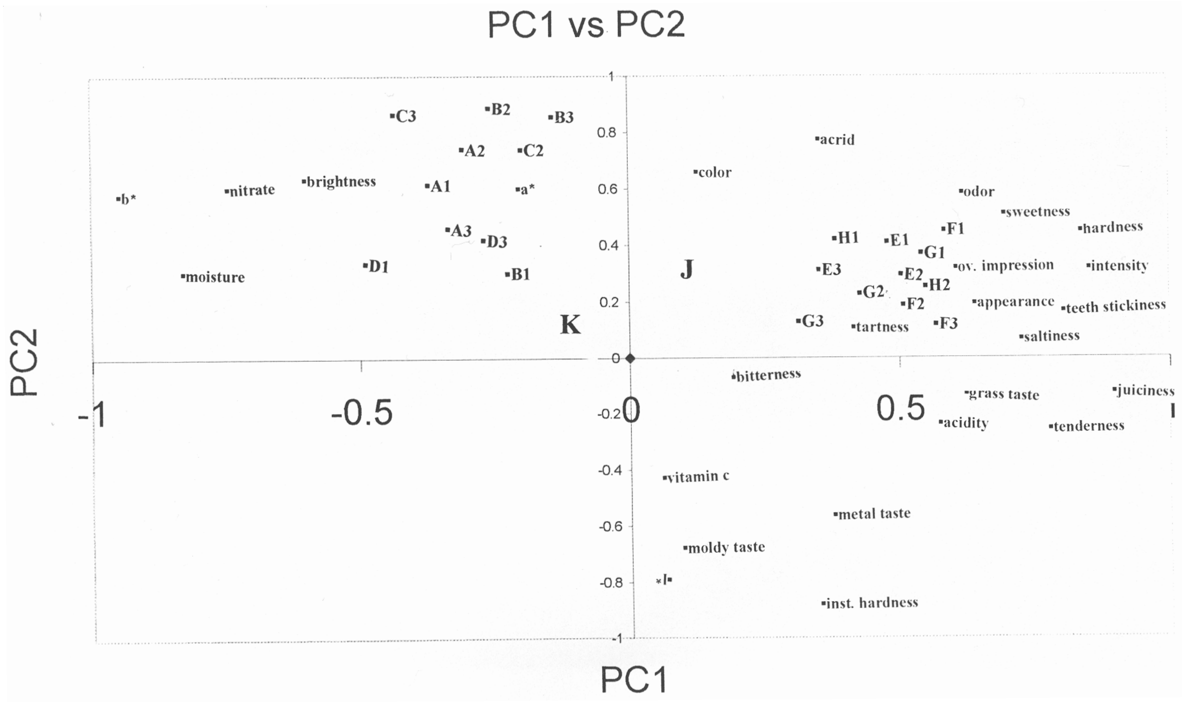

Figure 1. Principal Component Analysis of physico-chemical and sensory attributes of green bean cultivars along the axes PC1 vs PC2. M and L stand for two groups; 'Montano' and 'Larma', respectively.

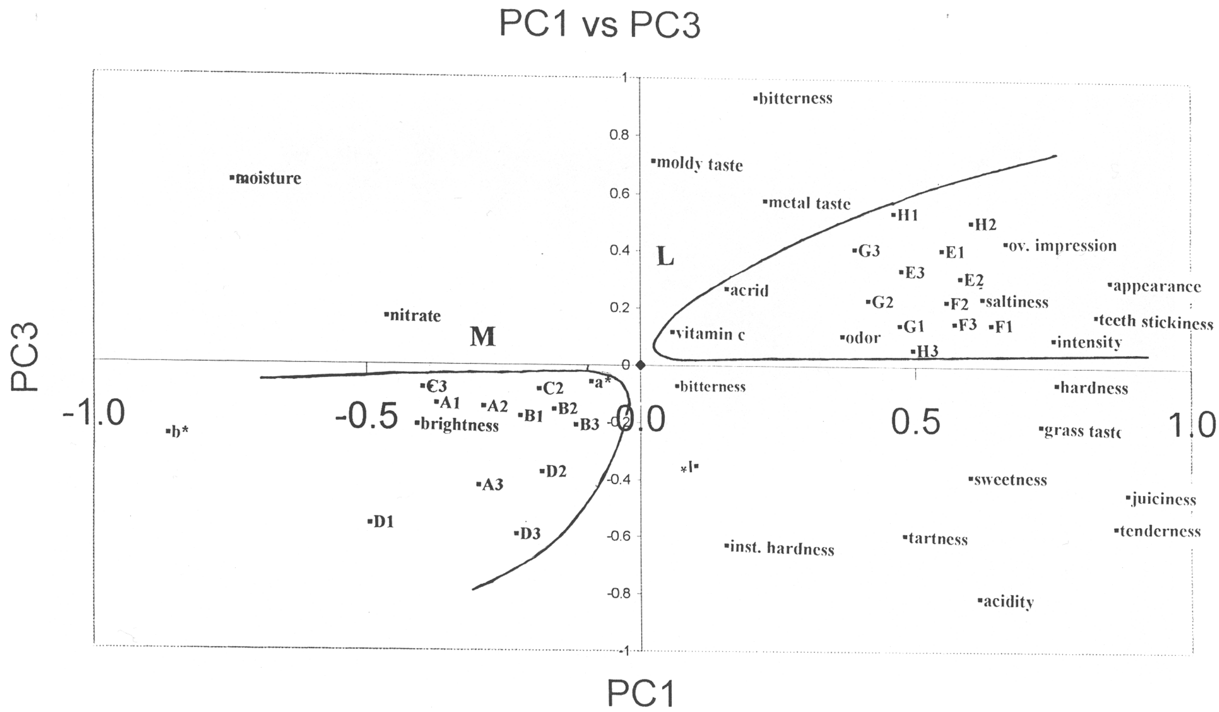

Figure 2. Principal Component Analysis of physico-chemical and sensory attributes of green bean cultivars along the axes PC1 vs PC3. M and L stand for two groups; 'Montano' and 'Larma', respectively.

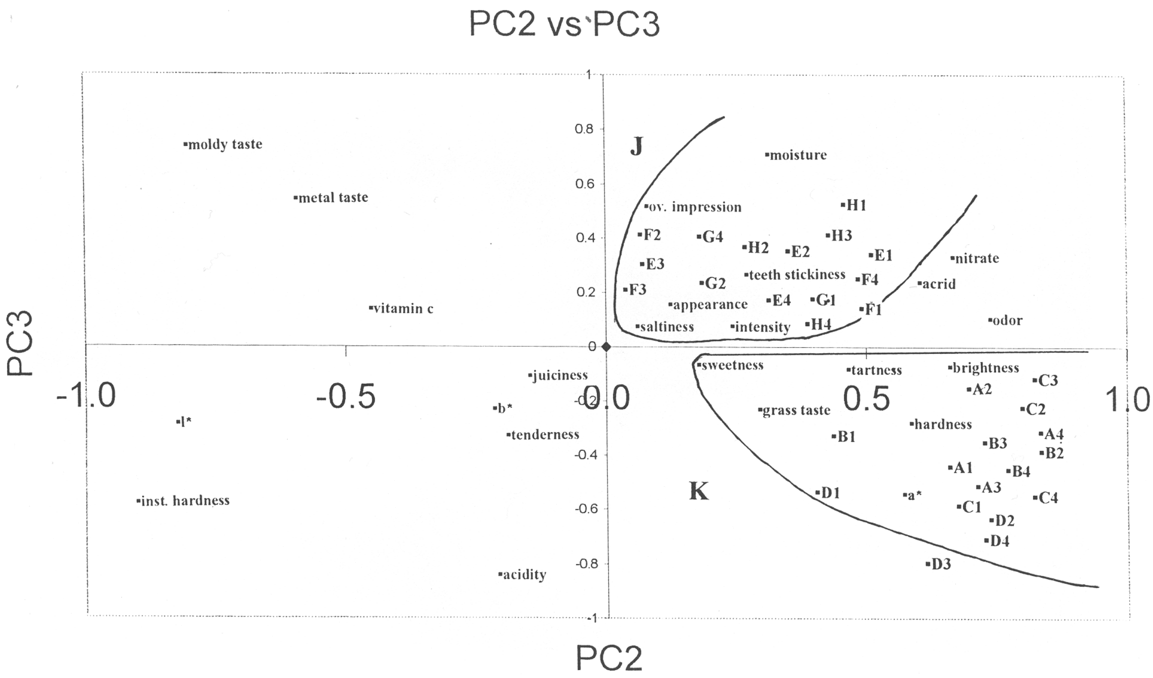

Figure 3. Principal Component Analysis of physico-chemical and sensory attributes of green bean cultivars along the axes PC2 vs PC3. M and L stand for two groups; 'Montano' and 'Larma', respectively.

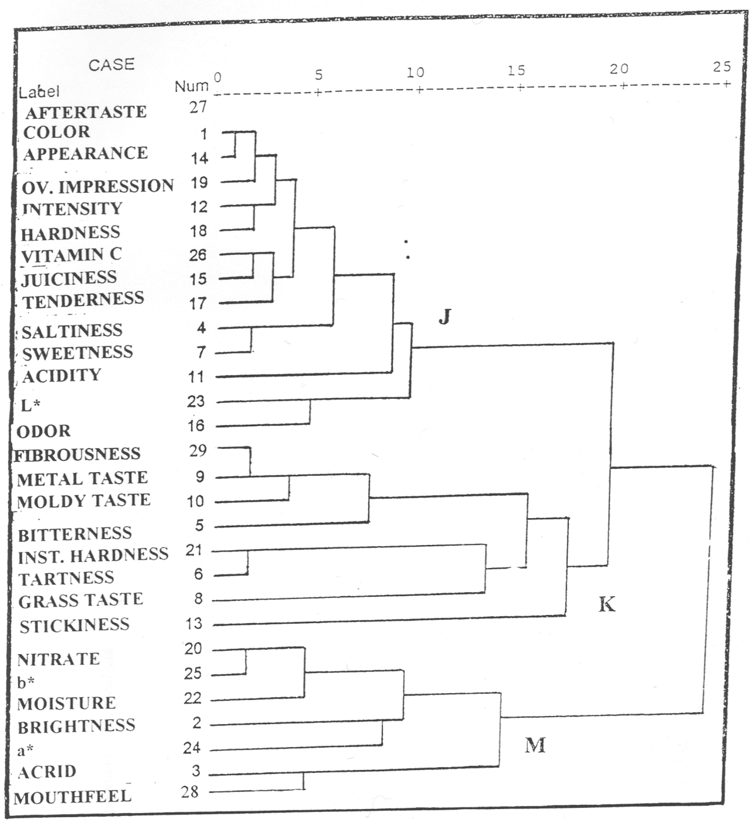

Figure 4. Tree diagram and classification according to Pearson coefficient. J, K and M stand for sensory and instrumental attributes.

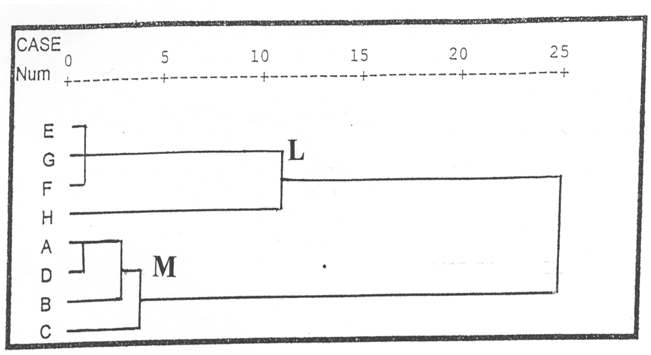

Figure 5. Tree diagram and classification of attributes according to Pearson coefficient. L and M stand for clusters grouping the two cultivars ('Larma' and 'Montano', see Table 6 with various fertilizer treatments).