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Genetic Diversity Analysis of Some Ethiopian Specialty Coffee (Coffea arabica L.) Accessions for Cup Quality Attributing Traits

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Abstract
knowledge of nature and extent of genetic variation and diversity available in the germplam or breeding materials helps breeder for planning sound breeding program. Hence, the present investigation was undertaken to evaluate 47 coffee germplam accessions, which were collected from Gomma wereda of Jimma zone and two standard check varieties. The experiment was conducted in simple lattice design with two replications during 2011/12 cropping season. Data on eight organoleptic traits were recorded and the analysis revealed that all organoleptic quality traits showed significant variation among the accessions at ($P<0.05$). High phenotypic and genotypic coefficients of variation was observed for bitterness and astringency. Organoleptic traits such as flavor, overall standard, acidity and aromatic quality had high heritability. Bitterness and astringency showed moderate heritability coupled with high genetic advance. Similarly, flavor had high heritability and moderate genetic advance as percent of mean. Hence, bitterness, astringency and flavor can be improved through selection. Cluster analysis based on organoleptic traits grouped the accessions into three groups which make them also moderately divergent. Besides, inter-cluster distances were significantly different and crossing coffee accession from these divergent clusters will result in heterosis and recombinant in segregating generation. The principal component analysis showed the variation in first principal component, 63.7%, had been attributed to overall standard, flavor, acidity and aromatic quality. Hence, these traits should be given importance during hybridization and selection in the segregating population. In general , the present study indicated the presence of variability for organoleptic traits among the accessions. Therefore, the observed variability should be exploited in order to improve the quality of Gomma wereda coffee. However, since high quality variation between accessions is not a guarantee for a high genetic variation, biochemical studies need to be considered as complementary to organoleptic studies.
Keywords: Genetic variability, principal component, organoleptic traits, genetic diversity, heritability

1. INTRDUCTION

Coffee (*Coffea arabica* L.*)* is originated in Ethiopia and there is a great genetic diversity in the country. Ethiopia is currently producing an estimated 9.8 million bags that would rank the country as the third largest coffee producer in the world after Brazil and Vietnam, beating out Columbia (ICO, 2012). Despite the availability of coffee genetic diversity in the country, coffee genetic resources (CGR) are under serious threats of extinction, mainly due to deforestation, replacement of traditionally grown landrace by improved varieties, environmental degradation and change in land use (Gole and Teketay, 2001).

Thus, it is pertinent and need of the day to collect and conserve coffee accessions from different coffee growing regions of the country so as to reduce the loss of coffee genetic resources and improve the productivity of the crop by developing coffee varieties which are high yielder, disease resistant and best quality. In this regard, JARC has been conserving more than 6000 accession under the two major collection programs. Diversity of Arabica coffee for coffee cup quality was observed among crosses and hybrids (Van der Vossen, 1985). Moreover, Selvakumar and Sreenivasan (1989) observed coffee cup quality variation ranging from good to excellent among 54 Arabica coffee accessions collected from Keffa, Ethiopia. Abeyot *et al.* (2011) and Olika *et al.* (2011) have also reported the presence of diversity for organloeptic traits in Ethiopian coffee collections. Nevertheless, despite tremendous specialty coffee genetic resources that the country is endowed with, specialty coffee germplasm accessions are not yet systematically characterized and detailed information on the extent of genetic diversity is not yet available. Thus, the purpose of this study was to see the magnitude of genetic diversity of ex situ conserved specialty coffee germplasm using organoleptic traits.

2. MATERIALS AND METHODS

The experiment was conducted at Agaro Station of the Jimma Agricultural Research Center. The center is located 45 km far from Jimma and 397 km from Addis Ababa. Agaro is located at 7''50'35- 7'' 51' 00'' N latitude and 36''35'30''E longitude and at an altitude of 1650 meters above sea level. The mean annual rainfall of the area is 1616 mm with an average maximum and minimum air temperatures of 28.4 ^{0}C and 12.4^{0}C, respectively. (Zebene and Wondwosen, 2008).

Forty nine coffee (C. *arabica* L.*)* germplasm accessions, which have been collected from the *Gomma* woreda of Jimma Zone, were used for this study (Table 1). The study was conducted during 2011/12 cropping

season. The experiment was laid out in a 7x7 simple lattice design with two replications and with seven genotypes per each incomplete block. Each plot was comprised of four coffee trees. Spacing between trees and plots was two meter and spacing between replications was 3 meter. All the improved agronomic practices were applied uniformly according to the recommendations (Endale *et al.,* 2008).

During peak harvesting time, only healthy and red-ripe berries were harvested from each accession selectively by hand and processed according to the wet processing method. For this purpose, 3-6 kilograms were collected from each coffee accession. The whole processing steps were done according to the research recommendation (Behailu *et al*., 2008). Fully ripened and healthy berries were separated from foreign material and unripe green cherries. Parchments were separated from the skin and pulp by using hand pulper. Immediately after pulping the parchments were sorted from the pulp and dipped into water to separate the floaters. The moist parchments were fermented in fermentation box for 48 hours till the first washing was made. The samples were then stored in fermentation tank for additional 24 hours. After fermentation when the slippery mucilage removed, the fermented coffee was washed by soaking with clean water and dried. During drying, the moisture content of the beans was measured by moisture tester to maintain the moisture level at 10- 12 % for all samples uniformly. The dried parchment were separately labeled and packed. Finally, the parchment was removed and 300-600g clean green beans were prepared from each sample for quality evaluation. A total of 98 samples were prepared and from 49 coffee accessions. To attain homogeneous bean size and healthy beans for organoleptic quality analysis, samples were screened on a mesh sieve 15(5.95mm). Then, samples on screen 15 and above were used for organoleptic quality analysis. Brew was prepared and cup tasting was carried out after a beverage cooled to a drinkable temperature. Two cups per sample were prepared for tasting. Cup tasting was made by a group of experienced and well trained coffee tasters. Aroma (aromatic quality and intensity), acidity, astringency, bitterness, body, flavor and overall standard of the brew were scored using scale ranging from zero to five (Table 2). The mean of the assessment result given by panelists was used for statistical analysis.

The variability of each organoleptic trait was estimated by simple measures such as mean, range, standard deviation, phenotypic and genotypic variances, and coefficients of variation. The phenotypic and genotypic coefficients of variation were computed based on the formula suggested by Burton and de Vane (1953) as follows:

Phenotypic variance $(\sigma^2 p) = \sigma^2 g + \sigma^2 e$

Where: $\sigma^2 g$ = genotypic variance

$\sigma^2 e$ = environmental variance

Genotypic variance $(\sigma^2 g) = (MSt - MSe)/r$

MSt = mean square due to genotypes

MSe = environmental variance (error mean squares)

r = the number of replication

Environmental variance ($\sigma^2 e$) =error mean squares

Phenotypic Coefficient of Variation, $(PCV) = \frac{\sqrt{\sigma 2p}}{\bar{x}} X100$

Genotypic Coefficient of Variation, $(GCV) = \frac{\sqrt{\sigma 2g}}{\bar{x}} X100$

$\bar{x}$ = *mean of the character*

Broad sense heritability values were estimated using the formula given by of Falconer (1989)as follows:

$$(H^2 b) = (\sigma^2 g / \sigma^2 ph) \times 100$$

Where: $H^2 b$ = heritability in the broad sense

$\sigma^2 p$ = phenotypic variance

$\sigma^2 g$ = genotypic variance

Expected genetic advance for each character at 5% selection intensity was computed using the methodology described by Johnson *et al*. (1955).

$$GA = k * \sigma p * H^2$$

Where: GA = the expected genetic advance under selection

σp = the phenotypic standard deviation;

H^2 = heritability in broad sense and k is selection Intensity.

Genetic advance as percent of mean was calculated to compare the extent of predicted advance of different traits under selection using following formulae of Johnson *et al*. (1955).

$$GAM = \frac{GA}{\bar{x}} X100$$

Where: GAM= genetic advance as percent of population mean
GA = the expected genetic advance under selection

$\bar{x}$ = mean of the character
In this study, eight organoleptic characters were used for clustering the accessions into homogeneous groups. Organoleptic data were subjected separately to cluster analysis so as to determine the variability among the accessions. For cluster analysis covariance matrix was used. Hierarchical clustering was employed using the similarity coefficients among the 49 coffee accessions. Clustering was performed using the proc cluster procedure of SAS version 9.2 (SAS, 2008) by employing the method of average linkage clustering strategy of the observation. The numbers of clusters were determined by following the approach suggested by Copper and Miligan (1988) by looking into three stastics namely Pseudo F, Pseudo t^2 and cubic clustering criteria.

Genetic divergence between clusters was determined using the generalized Mahalanobis's D^2 statistics (Mahalanobis, 1936) using the equation: $D^2p = ((Xi - Xj)S - 1(Xi - Xj)$.
Where: D^2_p= the distance between any two groups i and j;
X_i and X_j = the p mean vectors of accessions $_i$ and $_j$, respectively.
S^{-1} = the inverse of the pooled covariance matrix.
The D^2 values obtained for pairs of clusters were tested for significance at 5% and 1 % level of significance against the tabulated values of p degrees of freedom, where p is the number of variables considered (Singh and Chaudhary, 1987).

Principal component analysis was performed by employing Minitab statistical software using covaraince matrix (Minitab, 2007).

3. RESULTS AND DISCUSSION

3.1. Genotypic and phenotypic coefficients of variation

High PCV and GCV were recorded for bitterness and astringency with PCV values of 144.09 % and 124.72 % and GCV values of 93.01 % and 78.8 %, respectively. Moderate PCV and GCV were recorded for flavor with the respective values of 11.52% and 10.16%. However, low levels were recorded for aromatic intensity and body with PCV values of 6.78% and 7.84% and GCV values of 3.89% and 5.31%, respectively. Moderate PCV and low GCV were recorded for overall standard, aromatic quality and acidity with respective values of 11.35%, 10.77% and 10.12% for PCV and 9.84%, 8.21%, and 8.21% for GCV (Table 3).

The narrow gap between PCV and GCV for flavor, overall standard, acidity and aromatic quality in the present study indicates that environment had little influence in the expression of the traits. Thus, selection of genotypes based on phenotypic appearance of these traits would be effective in improving coffee quality. Conversely, relatively wide difference between PCV and GCV values for astringency, bitterness, aromatic intensity and body, indicating environment influenced the expression of the traits.

The magnitude of phenotypic coefficient of variation in coffee quality attributes has been reported by several investigators. Getu (2009) has reported high PCV value for bitterness; medium values for aromatic intensity, aromatic quality, astringency, flavor and overall standard and low value for acidity. He has also reported medium GCV value for overall standard and low values for all other quality attributes. Similarly, Abeyot *et al*.(2011) has also reported high PCV values for all organoleptic quality traits, except for bitterness and body, which were in medium and low ranges, respectively. The same author has also reported high GCV for acidity, astringency and flavor; medium values for aromatic quality, aromatic intensity, bitterness and overall standard and low GCV value for body. Interestingly, the current finding is quite similar with work of Olika *et al.*(2011) who reported high PCV values for astringency and bitterness; medium values for aromatic quality, acidity, flavor and overall standard and low values for aromatic intensity and body. The same authors also reported low GCV values for all organoleptic quality traits.

3.2. Heritability and genetic advance

Flavor (77.78%), overall standard (75.18%), acidity (65.81%) and aromatic quality (58.04%) had high heritability; the rest of the traits such as body (45.95%), bitterness (41.67%), Astringency (40%) and aromatic intensity (32.79%)had moderate heritability estimates (Table 3).

Generally, medium and high heritability estimates for the characters indicate that these characters can be easily improved through selection, as there would be close correspondence between genotypic and phenotypic expressions due to relative small contribution of the environment to phenotype. Van der Vossen (1985) observed

fairly high heritability for the overall standard of cup quality and indicated the possibility of good selection progress for this character with the assistance of experienced coffee tasters. The present finding is in agreement with the finding of Getu (2009), who reported high heritability for overall standard and aromatic quality. However, the low heritability reported for bitterness and astringency by the same author is contrary to the present finding. The contradictory result might have happened due to differences in test materials and the environment. Nevertheless, the present finding partly agrees with the finding of Abeyot *et al*. (2011), who reported that all quality attributes, except body, had high broad sense heritability. Olika *et al*.(2011) also reported high heritability for aromatic quality, medium heritably for flavor and overall standard and low heritability for acidity, aromatic intensity, astringency, body and bitterness. In the present study, high genetic advance as percent of mean at 5% selection intensity was recorded for bitterness (123.69%) and astringency (102.77%); moderate for flavor (18.45%), overall standard (17.58), acidity (13.72) and aromatic quality (12.88%) and low for body (7.42) and aromatic intensity (4.58%) (Table 3).

This study showed that bitterness and astringency had high genotypic coefficients of variation, moderate heritability and high genetic advance as percent of mean. Similarly, flavor had high heritability, medium genotypic coefficient of variation and considerable genetic advance. These three traits could, therefore, be improved more easily than the rest of the characters, as selection based on those traits with high and moderate genetic advance as percent of mean will result in the improvement of the performance of the accessions for the traits. On the other hand, although, body and aromatic intensity had moderate heritability, the low genotypic variability and genetic advance as percent of mean restricted their potential for improvement through selection. Similarly, overall standard, acidity and aromatic quality had high heritability and moderate genetic advance, thus, improvement of these traits through selection is impossible as they had low genotypic variability and hence, heterosis breeding would be recommended to improve them. Abeyot *et al*. (2011) has reported the genetic advance at 5% selection intensity to be within the range of 11.18% for body and 336.71% for bitterness. The author also reported that, of all good coffee quality attributes, only flavor and aromatic intensity appeared to combine relatively high value of heritability and genetic advance as percent of mean.

3.3. Cluster analysis

The Clustering patterns of 49 coffee germplasm accessions based on eight organoleptic characters are presented in Table 4. These accessions were grouped into three clusters. The first cluster comprises of 42 accessions (85.70%) and the second cluster consists of five accessions (10.20%), while the third cluster comprises of only two (4.10%) best quality accessions, which have been collected from Omo-Boko and Omo-Gobo farmers'association of Gomma wereda. The clustering pattern of accession revealed the existence of moderate genetic diversity in coffee accession for organoleptic quality traits studied and accessions were not grouped according to their area of collection. To this end, accessions collected from all kebeles were found to be grouped in the first cluster. The observed diversity for these traits is important in the effort exerted to increase the genetic base of winy flavored Arabic coffee varieties for future coffee breeding program. Yigzaw (2005) reported that cluster analysis based on coffee quality traits grouped 42 coffee accessions into two main clusters. According to this author, genotypes were not clustered according to area of collection. Olika *et al.*(2011**)** also reported that the cluster analysis grouped 49 Limu coffee germplasm accessions into three clusters based on eight organoleptic traits.

3.4. Cluster mean characterization

The mean organoleptic quality attributes of clusters for eight organoleptic quality attributes in 49 coffee germplasm accessions is given in Table 5. Accessions in cluster I are characterized predominantly by medium value for all organoleptic attributes (aromatic intensity, aromatic quality, acidity, astringency bitterness, body, flavor and overall standard) (Table 5). Accessions in cluster II are characterized by their poor organoleptic quality attributes, viz. maximum bitterness which is undesirable for good quality coffee, low aromatic intensity, aromatic quality, acidity, astringency, body, flavor and overall standard (Table 5). Conversely, cluster III comprises only two genotypes which are characterized by high aromatic intensity, aromatic quality, acidity, astringency, body, flavor and overall standard. Besides, accessions in this cluster are known to have low value for bitterness (Table 5). Generally, accessions in cluster III are found to be best for all organoleptic quality attributes.

3.5 . Genetic divergence

The pair wise generalized square distances (D^2) between the three clusters are presented in Table 6. The genetic diversity prevalent in the germplam accessions was assessed by adopting Mahalanobis (1936) concept of generalized distance. Characters selected in this study for multivariate analysis include organoleptic quality traits. The distances between all the three clusters were significant ($P < 0.05$). The maximum inter cluster distance was between cluster II and III (122.09) followed by I and III (50.80). The minimum is being between I and II (20.03)

(Table 6). By and large, this finding exhibited that the germplasm accessions included in this study are moderately divergent. Therefore, crossing of parents selected from cluster I & III and Cluster II & III produce desirable recombinants in views of the genetic diversity. Arunachalam *et al*. (1984) reported that genotypes belonging to clusters separated by high estimated statistical distances could be used for the hybridization program to obtain a wide spectrum of variations and good manifestations of heterosis in the F_1

3.6 . Principal component analysis

Principal component (PC) analysis grouped the eight organoleptic characters in eight components, which accounted for 100% of the variability existing among the tested coffee germplasm accessions. The first two principal components accounted for 75.8 % of the entire variability apparent among the accessions (Table 7). The first principal component which explained 63.7 % of the variability among the accessions was attributed to variations in overall standard, flavor, acidity, aromatic quality. The second principal component explained 12.1% of the variation among the tested materials was mainly due to aromatic intensity ,aromatic quality. and astringency. In the present study, the first principal component was more related to good quality attributes of coffee quality (flavor, overall standard, acidity and aromatic quality). Hence, these quality traits were played a vital role in classifying the accessions into different groups and should be considered while selecting diverse parents for breeding programs .

4. Summary and Conclusion

In conclusion, the present study exhibited the presence of genetic diversity for several organoleptic traits among coffee germplasm accessions. The existence of genetic diversity is potential resource for improvement of the crop through selection and hybridization. Therefore, the observed variability should be exploited in order to improve the quality of this valuable crop. However, the diversity observed for organoleptic traits should also be confirmed using biochemical constituents of coffee beans.

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Table 1. Geographical origin of the study coffee (*Coffea ararica L.)* germplasm accessions at Agaro

Acc. no	Specific collection site	Wereda	Acc.no	Specific collection site	Wereda
L01/05	Chadero Suse	Gomma	L27/05	Bako Kuju	Gomma
L02/05	Chadero Suse	Gomma	L28/05	Bako Kuju	Gomma
L03/05	Chadero Suse	Gomma	L29/05	Bako Kuju	Gomma
L04/05	Chadero Suse	Gomma	L30/05	Bako Kuju	Gomma
L05/05	Gabena Abo	Gomma	L31/05	Bako Kuju	Gomma
L06/05	Gabena Abo	Gomma	L32/05	Debi Kechamo	Gomma
L07/05	Gabena Abo	Gomma	L33/05	Debi Kechamo	Gomma
L08/05	Gabena Abo	Gomma	L34/05	Debi Kechamo	Gomma
L09/05	Gabena Abo	Gomma	L35/05	Debi Kechamo	Gomma
L10/05	Gabena Abo	Gomma	L36/05	Debi Kechamo	Gomma
L11/05	Gabena Abo	Gomma	L37/05	Limu Sapa	Gomma
L12/05	Gabena Abo	Gomma	L38/05	Limu Sapa	Gomma
L13/05	Omo-Boko	Gomma	L39/05	Limu Sapa	Gomma
L14/05	Omo-Boko	Gomma	L40/05	Limu Sapa	Gomma
L15/05	Omo-Boko	Gomma	L41/05	Limu Sapa	Gomma
L16/05	Omo-Boko	Gomma	L42/05	Omo Gobo	Gomma
L17/05	Omo-Boko	Gomma	L43/05	Omo Gobo	Gomma
L18/05	Omo-Boko	Gomma	L44/05	Omo Gobo	Gomma
L19/05	Omo-Boko	Gomma	L45/05	Omo Gobo	Gomma
L20/05	Goja Kemisse	Gomma	L46/05	Omo Gobo	Gomma
L21/05	Goja Kemisse	Gomma	L47/05	Omo Gobo	Gomma
L23/05	Goja Kemisse	Gomma	L48/05	Omo Gobo	Gomma
L24/05	Goja Kemisse	Gomma	744	-	Standard check
L25/05	Goja Kemisse	Gomma	Dessu	-	Standard check
L26/05	Goja Kemisse	Gomma			

Table 2. Cup quality parameter and their descriptive value

Character	Scale	Description of each scale					
		0	1	2	3	4	5
Aromatic intensity	0-5	Nill	Very light	Light	Medium	Strong	Very strong
Aromatic quality	0-5	Nill	Very light	light	Medium	Strong	Very strong
Acidity	0-5	Nill	Very light	light	Medium	Strong	Very strong
Astringency	0-5	Nill	Very light	light	Medium	Strong	Very strong
Bitterness	0-5	Nill	Very light	light	Medium	Strong	Very strong
Body	0-5	Nill	Very light	light	Medium	Strong	Very strong
Flavor	0-5	Nill	Very light	light	Medium	Strong	Very strong
Overall standard	0-5	UA	Bad	Regular	Good	Very good	Excellent

UA= unacceptable

Table 3. Estimates of range, mean, phenotypic variance, genotypic variance, phenotypic (PCV) and genotypic coefficient of variation (GCV), broad sense heritability (H^2), genetic advance and expected genetic advance (GAM) as percent of mean for eight organoleptic quality attributes at Agaro (2011/12)

Trait	Range	Mean	σ^2p	σ^2g	PCV (%)	GCV (%)	H^2 (%)	GA	GAM (%)
Aromatic intensity	3.17-4.085	3.64	0.061	0.020	6.78	3.89	32.79	0.17	4.58
Aromatic quality	2.75-4.415	3.51	0.143	0.083	10.77	8.21	58.04	0.45	12.88
Acidity	2.50-4.335	3.38	0.117	0.077	10.12	8.21	65.81	0.46	13.72
Astringency	0-0.835	0.15	0.035	0.014	124.72	78.88	40	0.15	102.77
Bitterness	0-1.415	0.17	0.06	0.025	144.09	93.01	41.67	0.21	123.69
Body	2.83-4.25	3.47	0.074	0.034	7.84	5.31	45.95	0.26	7.42
Flavor	2.33-4.415	3.19	0.135	0.105	11.52	10.16	77.78	0.59	18.45
Overall standard	2.50-4.415	3.26	0.137	0.103	11.35	9.84	75.18	0.57	17.58

σ^2g =Genotypic variance, σ^2p =phenotypic variance, GCV= Genotypic coefficient of variation, PCV= phenotypic coefficient of variation, H^2 = Heritability in broad sense, GA= expected genetic advance, GAM= Genetic advance as percent of mean.

Table 4. The distribution of germplasm accessions into three clusters based on analysis for 49 coffee germplasm accessions tested at Agaro (2011/12).

Cluster no	No acce.	%	Accession
Cluster I	42	85.70	L21/2005, L29/2005, L20/2005, L04/2005, L34/2005, L27/2005, L38/2005, L06/2005, L15/2005, L35/2005, L01/2005, L39/2005, L42/2005, L18/2005, L14/2005, L03/2005, L12/2005, L31/2005, L02/2005, L33/2005, L05/2005, L43/2005, L30/2005, L08/2005, L32/2005, L36/2005, L46/2005, L47/2005, L13/2005, L09/2005, L26/2005, L41/2005, L28/2005, L24/2005, L40/2005, L37/2005, L07/2005, L10/2005, L44/2005, L19/2005, L23/2005 and L11/2005,
Cluster II	5	10.20	L25/2005, L16/2005, 744**, L48/2005 and F 59**
Cluster III	2	4.10	L17/2005 and L45/2005

** represents check varieties

Table 5. Mean values of eight organoleptic traits for three clusters of 49 coffee germplasm accessions tested at Agaro (2011/12)

Traits	Clusters		
	Cluster I	Cluster II	Cluster III
Aromatic intensity	3.65	3.57*	3.75**
Aromatic quality	3.53	3.03*	4.25**
Acidity	3.42	2.83*	4.04**
Astringency	0.16	0.099*	0.17**
Bitterness	0.11	0.617**	0,00*
Body	3.47	3.25*	4.00**
Flavor	3.22	2.57*	4.21**
Over all standard	3.29	2.62*	4.25**

** and * represent higher and lower cluster mean values, respectively

Table 6. Average inter cluster divergence (D^2) values obtained based on organoleptic quality attributes for 49 coffee germplasm accessions tested at Agaro (2011/12)

Cluster I	Cluster II	Cluster III
Cluster I	20.03*	50.80**
Cluster II		122.09**

**= Highly Significant at P=0.01(χ 2) = 20.09, *= Significant at P=0.05(χ 2)= 13.36

Table 7. Eigenvector and eigenvalues of the first two principal components for eight organoleptic characters of 49 Arabica coffee germplasm accessions

Traits	**Eigenvectors**	
	PCI	PCII
Aromatic intensity	0.102	**-0.431**
Aromatic quality	**0.411**	**-0.636**
Acidity	**0.451**	0.083
Astringency	0.017	**-0.495**
Bitterness	-0.185	-0.157
Body	0.218	0.254
Flavor	**0.512**	0.198
Over all standard	**0.522**	0.172
Eigenvalue	0.419	0.079
Proportion	0.637	0.121
Cumulative	0.637	0.758

PC= principal component

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