SUPPLEMENTARY MATERIAL

Functional properties and fatty acids profile of different beans varieties.

Vincenzo Lo Turco, Angela Giorgia Potortì*, Rossana Rando, Pietro Ravenda, Giacomo Dugo, Giuseppa Di Bella.

Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute (S.A.S.T.A.S.), Università di Messina, Viale Ferdinando Stagno d'Alcontres, 31 – 98166 Messina, Italy.

*Corresponding author: Angela Giorgia Potortì, Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute (S.A.S.T.A.S.), Università di Messina (Italy), Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy. Phone: +39 090 676 5497 fax: +39 090 676 5436; e-mail address: agpotorti@unime.it.

Abstract

Dried seeds of four varieties of *Phaseolus vulgaris*, three of *Vigna unguiculata* ssp. *unguiculata* and two of *Vigna angularis* grown and marketed in Italy, Mexico, India, Japan, Ghana and Ivory Coast were analyzed for fatty acids content. In oils from seeds of *Phaseolus vulgaris* the main fatty acids were linolenic (34.7-41.5%) and linoleic (30.7-40.3%), followed by palmitic (10.7-16.8%). The first three aforementioned fatty acids in the lipid fraction of *Vigna unguiculata* varieties were 28.4%, 28.7% and 26.2%, respectively; while in *Vigna angularis* varieties main fatty acids were linoleic (36.4-39.1%) and palmitic (26.9-33.3%), followed by linolenic (17.9-22.2%). Statistical analyses indicate that botanical species play a role in bean fatty acids distribution, while the same was not verified for geographical origin. Furthermore, the atherogenic index (IA) and the thrombogenic index (IT) were investigated for health and nutritional information. The results showed that these wide spread legumes have functional features to human health.

Keywords: *Phaseolus vulgaris*; *Vigna unguiculata* ssp. *unguiculata*; *Vigna angularis*; Fatty acids; Health lipid indices; Multivariate statistics.

Experimental

Samples

The analyses were carried out on 33 samples of dried edible beans from 9 varieties (3 or 6 for each one). The list of investigated sample typologies is given in Table S1, which also shows the country of origin. Dried beans were stored in screw-top jars at room temperature. Aliquots were ground to powder by mechanical mill just before analysis.

Lipids extraction of

The lipids extraction was carried out by ISO 659 official method. 100 g of powdered samples were extracted with n-hexane (Merck, Darmstadt, Germany) in Soxhlet apparatus for 8 h. The solvent was partly removed in a rotary vacuum evaporator (Buchi, Donau Lab., Switzerland), the residue was transferred in pre-weight glass vessel and the rest of the solvent was removed under nitrogen stream (99.9990% purity) to a constant weight in order to determine the lipids content (ISO 659 2009).

Lipids analysis

The fatty acid composition of lipid extract was determined by gas chromatography after transmethylation of the respective sample with 2% H₂SO₄ in methanol (Aldrich Chemical, Chicago, IL, USA) at 80°C (ISO 5509 2000). The separation and quantification of fatty acid methyl esters were conducted with a Dani Master GC 1000 equipped with a FID detector (Dani Instrument, Milan, Italy) and a capillary column Supelco SLB-IL100 (60 m x 0.25 mm, film 0.20

μm). The injector temperature was 220°C and the splitless injection mode was used. The injection volume was 1 μl with a split ratio of 1:100 for 1 min and the oven temperature was from 130°C to 200°C (10 min hold) at a rate of 3°C/min. The carrier gas was helium (99.9995% purity) at a constant flow rate of 30 cm/sec with a detector temperature of 240°C. All analytes were identified by comparison to the retention time of reference standards (Aldrich Chemical, Chicago, IL, USA). Results were expressed as a percentage of the total fatty acids. All determinations were run in triplicate.

Statistical analysis

All statistical calculations were made by SPSS 13.0 software package for Windows (SPSS Inc., Chicago, IL, USA). The studies of significant differences were carried out by Kruskal-Wallis test. Principal Component Analysis (PCA) was used to try to discriminate among samples from different botanical and geographical origins.

Statistical analysis were carried out on one starting multivariate matrix that was constituted by 33 cases (bean samples under analysis) and 11 variables (concentrations of 11 different fatty acids determined in analyzed samples: C14:0, C16:0, C16:1n9, C18:0, C18:1n9, C18:2n6, C18:3n3, C20:0, C20:1n9, C22:0 and C22:2n6).

The data were subdivided into three groups according to the specie of origin and into six groups according to the geographical origin. Both Principal Components Analysis were carried out on variables before normalized.

Lipid quality analysis

The atherogenic (IA) and thrombogenic (IT) indices were investigated in order to give information on health and nutritional values of beans lipid fraction .

The IA was calculated by the following equation:

$$IA = [(4 \times C14:0) + C16:0 + C18:0] / [\sum MUFA + \sum PUFA_{n6} + \sum PUFA_{n3}]$$

this index is indicative of the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro and macro coronary diseases) (Garaffo et al. 2011).

The IT was calculated by the following equation:

$$IT = (C14:0 + C16:0 + C18:0) / \left(0.5 \times MUFA + 0.5 \times PUFA_{n6} + 3 \times PUFA_{n3} + \frac{PUFA_{n3}}{PUFA_{n6}} \right)$$

this index is indicative of the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFA, PUFA_{n6} and PUFA_{n3}) (Garaffo et al. 2011).

Table S1 – Details on the different beans analyzed.

Sample	Sample labeling	Species	Country of origin
Small Pinto Bean	SP	<i>Phaseolus vulgaris</i>	Italy
Black Eyed Green Bean	BEG	<i>Vigna unguiculata</i> ssp <i>unguiculata</i>	Italy
Black Turtle Bean	BT	<i>Phaseolus vulgaris</i>	Mexico
Red Kidney Bean	RK	<i>Phaseolus vulgaris</i>	India
Red Adzuki Bean	RA	<i>Vigna angularis</i>	Japan
Small Red Bean	SR _G	<i>Vigna angularis</i>	Ghana
Red Flat Bean	RF	<i>Phaseolus vulgaris</i>	Ghana
Black Eyed White Flat Bean	BEWF _G	<i>Vigna unguiculata</i> ssp <i>unguiculata</i>	Ghana
Small Red Bean	SR _{IC}	<i>Vigna angularis</i>	Ivory Coast
Black Eyed White Bean	BEW	<i>Vigna unguiculata</i> ssp <i>unguiculata</i>	Ivory Coast
Black Eyed White Flat Bean	BEWF _{IC}	<i>Vigna unguiculata</i> ssp <i>unguiculata</i>	Ivory Coast

Table S2 – Oil Yield (%) and fatty acid composition (% of total FA) of different species of beans.

	<i>Phaseolus vulgaris</i>				<i>Vigna unguiculata</i> ssp. <i>unguiculata</i>				<i>Vigna angularis</i>		
	SP	BT	RK	RF	BEG	BEWF _G	BEW	BEWF _{IC}	RA	SR _G	SR _{IC}
Oil yield	1.47 ± 0.06	1.41 ± 0.12	1.66 ± 0.08	1.87 ± 0.21	1.89 ± 0.09	1.86 ± 0.09	1.09 ± 0.16	1.36 ± 0.11	1.36 ± 0.08	1.32 ± 0.09	1.13 ± 0.12
C14:0	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C16:0	16.8 ± 0.7	10.7 ± 0.5	13.1 ± 0.9	12.7 ± 0.7	21.8 ± 0.4	28.2 ± 0.4	28.1 ± 0.8	26.8 ± 0.7	26.9 ± 1.3	32.0 ± 0.7	33.3 ± 1.0
C16:1n9	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
C18:0	1.2 ± 0.5	2.2 ± 0.1	2.3 ± 0.2	1.6 ± 0.5	6.0 ± 0.4	4.4 ± 0.4	3.3 ± 0.5	4.4 ± 0.8	3.0 ± 0.8	2.7 ± 0.1	2.7 ± 0.1
C18:1 n9	8.2 ± 0.3	11.0 ± 0.7	6.8 ± 0.3	6.6 ± 0.7	10.3 ± 0.6	7.3 ± 0.4	6.5 ± 0.5	7.7 ± 1.2	5.2 ± 1.1	4.2 ± 0.3	4.2 ± 0.3
C18:2 n6	30.7 ± 1.1	31.5 ± 1.0	32.3 ± 0.8	40.3 ± 1.1	24.1 ± 1.6	31.5 ± 0.5	30.0 ± 0.8	29.2 ± 1.0	38.8 ± 1.5	36.4 ± 1.8	39.1 ± 1.2
C18:3 n3	39.7 ± 1.1	40.6 ± 1.5	41.5 ± 0.7	34.7 ± 1.4	33.7 ± 0.4	24.5 ± 0.7	28.2 ± 0.5	27.2 ± 0.9	22.2 ± 1.6	20.9 ± 0.3	17.9 ± 0.3
C20:0	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.3 ± 0.2
C20:1 n9	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C22:0	0.6 ± 0.2	0.7 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.8 ± 0.4	2.0 ± 0.5	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
C22:2 n6	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Unknown (sum)	1.6 ± 0.3	1.3 ± 0.7	0.8 ± 0.4	1.9 ± 0.8	1.6 ± 0.3	1.8 ± 0.4	0.8 ± 0.1	1.0 ± 0.3	1.5 ± 0.6	2.3 ± 0.6	1.7 ± 0.2
Σ SFA	19.3 ± 0.5	14.5 ± 0.6	17.8 ± 0.9	15.7 ± 0.6	29.5 ± 0.8	34.3 ± 0.9	33.8 ± 1.1	34.0 ± 0.4	31.3 ± 1.7	35.5 ± 0.7	36.6 ± 0.8
Σ MUFA	8.5 ± 0.4	11.7 ± 0.8	7.4 ± 0.2	7.1 ± 0.8	10.9 ± 0.8	7.8 ± 0.3	6.9 ± 0.5	8.3 ± 1.4	5.8 ± 1.0	4.6 ± 0.3	4.6 ± 0.3
Σ PUFA	70.6 ± 0.1	72.5 ± 1.6	74.0 ± 0.5	75.3 ± 0.8	58.1 ± 1.8	56.1 ± 0.9	58.5 ± 0.7	56.7 ± 1.9	61.4 ± 3.0	57.5 ± 1.6	57.1 ± 1.2

Figure S1 - 2D Scatterplot for the 39 honey samples categorized by geographical origin (A) obtained using 11 variables and (B) using 6 variables. Insert: loading plot for PC1 and PC2.