Exploring the nutritional and nutraceutical significance of legumes: A comparative analysis across Mashreq countries

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Abstract

This study investigates the nutritional and nutraceutical profiles of four legume species - bean, faba bean, chickpea, and lentil - collected from Lebanon, Syria, Jordan, Palestine, and Egypt. Chemical analyses assessed antioxidant, nutritional, and nutraceutical properties, followed by Principal Component Analysis and Hierarchical Cluster Analysis to examine similarities and differences among legume varieties and regions. Key findings include Lebanese beans with distinct tocopherol (vitamin E), polyphenols, flavonoids, and TEAC, along with high protein, lysine, and methionine. Syrian faba beans showed superior vitamin E, TEAC, protein, lysine, and methionine levels, while a Palestinian variety excelled in polyphenols and flavonoids. Syrian chickpeas were rich in vitamin E, flavonoids, protein, and methionine, and a Palestinian variety stood out for histidine and lysine. Lentils from Lebanon, Syria, and Palestine had notable vitamin E and methionine contents. These findings highlight the role of genetic, environmental, and geographical factors in legume quality, emphasizing their potential in addressing malnutrition and promoting sustainable food systems.

Keywords: *Nutritional profile, Nutraceutical characteristics, Legumes, Chemometric analysis, Food security, Mediterranean diet, Mashreq Countries*

1. Introduction

The Mashreq region, which includes Lebanon, Syria, Jordan, Egypt and Palestine, faces significant economic political and security challenges stemming from conflicts, poverty, unemployment, and displacement. These difficulties have led to malnutrition, food insecurity, and limited access to balanced diets, especially affecting vulnerable groups such as refugees, internally displaced persons, women, and children (Capone *et al.*, 2021). One of the most pressing nutritional issues is ensuring enough high-quality proteins, which remains a primary focus of nutritional interventions in the region. Access to high-quality animal protein sources remains

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challenging for low-income communities due to limited economic resources. However, partially replacing these protein sources with legumes can offer numerous benefits for both individuals and the community as a whole. Legumes boast high nutritional value, being rich in protein, fiber, vitamins, and minerals, making them a valuable alternative to meat. They provide essential nutrients crucial for growth, development, and overall health, which may be deficient in the diets of low-income individuals. Moreover, legumes are cost-effective, sustainably produced with a lower environmental footprint, and offer health benefits by reducing the risk of non-communicable diseases (NCDs).

Legumes constitute a staple source of nutrition in the traditional Mediterranean Diet (MD) (Sikalidis *et al.*, 2021). The main legumes consumed in the Mediterranean region include faba beans (*Vicia faba* L.), chickpeas (*Cicer arietinum*), lupins (*Lupinus albus*), and lentils (*Lens culinaris*) (Godos *et al.*, 2024). These versatile crops are renowned for being nutrient-dense and possessing health-promoting properties (Amoah *et al.*, 2023). They are rich in protein, complex carbohydrates, fibers, vitamins, minerals (Montejano-Ramírez and Valencia-Cantero, 2024), low in fat, cholesterol-free, and have a low glycemic index (Amoah *et al.*, 2023; Vijayakumar and Haridas, 2021). It is important to note that, with the exception of soybeans, legumes should be combined with grains to form a complete diet, rich of all essential amino acids (Lisciani *et al.*, 2024). Beyond their nutritional value, legumes have nutraceutical properties, as they contain numerous bioactive compounds, including polyphenols (flavonoids and non-flavonoids) and phytosterols (Ganesan and Xu, 2017).

Various studies have highlighted the positive health outcomes associated with legume consumption, including decreased risk of type 2 diabetes mellitus, cardiovascular diseases, and certain cancer types (Yanni *et al.*, 2023). Moreover, legumes are recognized for their anti-inflammatory, antihypertensive, and antioxidant properties (Naureen *et al.*, 2022). In addition to these health benefits, legumes have a low environmental footprint and can be grown in a variety of climates and soil types, making them a sustainable option for farmers (Yanni *et al.*, 2023). Thus, a protein-rich diet based on legumes is a feasible, cheaper, and sustainable alternative to animal-based diets and helps prevent malnutrition in developing countries (Lisciani *et al.*, 2024; Montejano-Ramírez and Valencia-Cantero, 2024). Despite that, the global legume intake, being around 21 g/person/day (Semba *et al.*, 2021), is substantially lower than the recommendation of ~100g of cooked legumes by EAT-Lancet and MD (Hughes *et al.*, 2022).

Research has shown that phenolic content could vary between different varieties of legume species due to genetic factors, climatic conditions, storage, and variation in color of coats between cultivars (Carbas *et al.*, 2020; Yang *et al.*, 2018), as well as degree of maturity (Marathe *et al.*, 2011). Moreover, the geographic location where legumes are grown influences their nutraceutical and nutritional content (Johnson *et al.*, 2021; Shea *et al.*, 2024) due to environmental factors such as soil composition, annual rainfall, altitude, and humidity (Yegrem, 2021). As such, within legume species to have the highest nutritional and antioxidant profiles is crucial for maximizing the benefits deriving from their consumption.

The multivariate method includes Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Partial Least Squares Regression (PLS) are useful tools in science for classifications (Al Bakain *et al.*, 2020, 2021; Belharar and Chakor, 2023). These tools were performed in analytical studies for many purposes; to identify the most relevant compounds in distinguishing legumes varieties, to find the variation in chemical profiles as a result of growing legumes in different batches and with variations in growth locations, to confirm whether the cultivars in the cluster analysis would also be grouped together, to reveal the compounds that were responsible for grouping cultivars between clusters and to predict the geographical origin of the sample using linear discriminant analysis.

The Mediet project has been launched in 2022 by the International Center of Advanced Mediterranean Agronomic Studies of Bari (CIHEAM-Bari) and funded by the Italian Ministry of Foreign Affair and International Cooperation (MAECI), aims to address Sustainable Development Goal (SDG) number 2, Zero Hunger, by promoting legume production and consumption. The project focuses on the role of legumes in improving food security, nutrition, and sustainable agricultural practices, particularly in Mediterranean regions. This paper presents the initial findings of the project, relate to the nutritional and nutraceutical analysis of four legume species, which serve as a critical component of the project's broader objective. Using advanced multivariate statistical methods, results were analyzed to identify and select legume samples that exhibit promising nutritional and nutraceutical properties. The obtained results are addressed to improve consumer's awareness about the main nutritional and nutraceuticals benefits achievable by the legume consumption. The findings hold potential implications for public health and agricultural policy, as they can inform strategies to promote sustainable diets and enhance food security through the increased consumption of selected legumes. The final goals are to improve dietary diversity, reduce malnutrition, and ultimately foster better health outcomes in both local and global contexts.

2. Materials and methods

2.1. *Legumes samples*

Forty-seven dry seeds represent local cultivars and selected varieties of four legumes: bean, faba bean, chickpea, and lentil were collected during 2022 and 2023 from various locations in Lebanon, Syria, Jordan, Palestine, and Egypt. These regions are known for their long-standing tradition of legume cultivation and their integration of legumes into crop rotation systems. The selected varieties are highly valued by farmers for their productivity and economic benefits, as well as by markets for their competitive prices, which are driven by strong consumer demand. Most of the pulse varieties were developed by ICARDA and National Research Systems in the Mashreq countries and have been widely adopted by farming communities. Meanwhile, local cultivars are either the result of institutional breeding programs or derived from seasonal multiplication by local producers.

2.2. *Extraction method, Samples preparation, Running conditions and Instrumentation*

The dry seed samples were ground using an electric grinder to produce a homogeneous powder capable of passing through a 35-mesh stainless steel sieve with a 0.5 mm opening size. Dry matter content was determined by drying the powdered sample in static oven at 105°C until constant weight reached according to the AACC Method 44-17.01. Ash content as percentage was determined according to the AACC Method 08-16.01 by incinerating the residual obtained for dry matter content in muffle at 550°C. The extraction method, sample preparation, running conditions and the instrumentation were explained separately in the next sections.

2.2.1. *Centesimal composition determination of total Protein and Fat*

Total proteins, expressed in g per 100 g of seeds, was determined according to the AOAC Method 992.23 based on the Dumas method and using a carbon/nitrogen analyzer PRIMACS ™ SNC-100 (Scalar, The Netherlands) and the conversion factor nitrogen-proteins of 5.71. Total fat content was determined by solid liquid extraction using a Soxtec™ system model 2050 (Foss, Denmark), petroleum ether 40-60° as extraction solvent, and expressed as gram per 100 gram of dry seeds.

2.2.2. *Amino acids content*

The quantitative determination of proteinogenic amino acids was performed by the application of three different types of hydrolysis: HCl 6N after oxidation with performic acid for the determination of sulfur containing amino acids (i.e. cysteine and methionine); NaOH 4N for tryptophan determination and HCl 6N for all remaining amino acids. All hydrolysis processes were performed in amber borosilicate vials under N_2 in presence of pyrogallol as antioxidant at 110°C per 24hr using a block heater with temperature control. After hydrolysis, 100nmol of norleucine was added as internal standard, then samples were neutralized, diluted with ultrapure water, analyzed by ions chromatography and post column derivatization with ninhydrin using the amino analyzer Biochrom 30+ (Harvard Bioscience, USA). The concentration of each amino acid was calculated using internal calibration method. The calibration curves were obtained by the injection of 5 levels of calibration from 5 -500 nmol/mL of a standard mix (Protein hydrolysate standard mix, Biochrom) in triplicate.

2.2.3. *Total polyphenols, flavonoids and antioxidants contents*

Polyphenols compounds were extracted from the powder of dry seeds obtained as described in sample preparation and extraction methods section (2.2). The extraction solution was acetone: water: acetic acid (70:29.5:0.5 by volume). An aliquot of 2.0 g of powder samples was weighed in 50 mL centrifuge tubes, then 20 mL of the extraction solution was added, sonicated for 15 min at ambient temperature, orbitally shaken for 2hr, and finally centrifuged at 4000 RCF for 10 min at 10°C. After centrifugation, the clear upper phase was recovered, whereas the pellet was re-suspended in 20 mL of the extraction solution and the whole procedure repeated again. The two extracts were mixed, filtered at 0.45 µm regenerated cellulose filters and stored at 4°C for the next assays.

Twenty microliters of sample extract were used for the Folin micro assay according to (Wrolstad *et al.*, 2005). Calibration was done using gallic acid standard in the range 10-400 mg/L. Results were expressed as mg of gallic acid equivalent per 100 g of dry seeds. The antioxidant activity was performed using the extract obtained for the determination of total polyphenols. The assay used was the ABTS calibrated with Trolox. The ABTS radical solution was obtained by mixing 10 mL of ABTS 7mM with an equal volume of persulphate 4.95 mM. The mixture was left at room temperature in the dark for 12h, then stored in refrigerator for a maxim of 3 days. Using the stock solution of ABTS radical, a dilution was prepared, obtaining an absorbance at 730nm of approximately 0.7 absorbance units. The calibration range was 25-800 nmol/mL of TROLOX. In plastic cuvettes of 1.5 mL total volume, 980 µL of ABTS diluted radical solution was placed and added to 20 µL of sample extract or standard solution. The cuvettes were closed using parafilm, mixed for 25 min and then, the absorbance was measured. The difference in absorbance between the cuvette containing the sample extract and that containing only the extraction solvent as blank was calculated and used to express the final concentration as µmol of Trolox Equivalent Antioxidant Capacity (TEAC) per 100 g of dry seeds.

Total flavonoids determination was performed by colorimetric method using $AlCl_3$ and $NaNO_2$ reagent, according to (Heimler *et al.*, 2005). Catechin was used as reference standard with a calibration range 10-500 mg/L. Results were expressed as mg of catechin equivalent per 100 g of dry seeds.

2.2.4. *Vitamin E*

Vitamin E as total tocopherol was determined according to the method ISO 9936, by alkaline hydrolysis in presence of pyrogallol as antioxidant under nitrogen, followed by liquid – liquid extraction with petroleum ether at 40-60°C. The obtained extract was concentrated under vacuum and re-suspended in 1.0 mL of isooctane containing 0.2% of butylhydroxytoluene. Twenty microliters of this solution were injected into a High Performance Liquid Chromatography (HPLC) system. The HPLC system consisted of a normal phase isocratic pump LC-40D equipped with a fluorescence detector RF-20A XS (Shimadzu, Kyoto, Japan). The mobile phase used was hexane with 1% dioxane under isocratic elution of 1.0 mL/min flowrate. The stationary phase was Zorbax RX-Sil of 100 mm × 3.0 mm I.D with 1.8 µm particles diameter. A calibration curve was obtained by the injection of five concentration solutions of α-tocopherol pure standard in a range 0.1-10 µg/mL. Results were expressed as mg of α-tocopherol equivalent per 100 g of dry seeds.

2.2.5. *Sterols*

The unsaponifiable matter was obtained from the total fat extracted with Soxtec apparatus as described before, submitted to a saponification with ethanolic KOH 0.2N and then to liquid-liquid extraction according to the AOAC Official Method 933.08. A 5α-cholestanol as an internal standard was added to the unsaponifiable matter, then the sample was analyzed by tin layer chromatography (TLC) to separate and purify the sterols fraction.

Finally, sterol's fraction obtained by TLC was derivatized using a silylating mixture and subjected to gas chromatographic-flame ionization (GC-FID) analysis. The Nexis 2030 GC system was equipped with autosampler AOC-20i Plus (Shimadzu), column SPB-5 (Supleco 30 m × 0.25 mm I.D, 0.25 µm film thickness). The separation of sterols was performed at the following conditions: isothermal condition of 265°C, injector temperature at 280°C, split mode of 1:25, detector at 300°C and hydrogen as carrier gas at constant velocity of 35 cm/s. Results were expressed in mg/100g of dry seeds.

2.2.6. *Fatty acids*

The total fat extracted by the soxtec apparatus was trans methylated by cold method with KOH 2N in methanolic solution. The methylated fatty acids were injected into the GC system described above, equipped with a DB-Fast FAME column (Agilent 20 m × 0.18 mm I.D, 0.20 µm film thickness). The chromatographic conditions were: H_2 as carrier gas at constant velocity of 35cm/s, injector temperature of 230°C, split ratio 1:50, initial temperature 80°C increased to 175°C at 65°C/min, then increased to 185°C at 10°C/min, increased to 230°C at 7°C/min, finally isothermal for 3 min. The quantitative results expressed in mg/100g of dry seeds were obtained by applying an external calibration method using FAME 37 standard mix at five levels of concentrations analyzed using the same chromatographic conditions of samples.

2.2.7. *Mineral elements and phosphorus*

Mineral elements were analyzed accordingly to the method AOAC 984.27 with wet mineralization process using Ethos Easy Microwave Digestion System -Milestone (Bergamo, Italy), followed by Inductively Coupled Plasma Atomic Emission Spectroscopy analysis using a spectroscope model iCAO 6000 Thermo Fisher Scientific (Waltham, Massachusetts, U.S.).

2.3. *Statistical software*

Data acquisition and processing were conducted using MS Workstation software version 6.6 (SP1). The statistical analysis including PCA and HCA were performed using Chemoface 1.61 software which work under Matlab® (Mathworks, 8.6, USA) and *XL*STAT software (Excel, Microsoft®).

3. Results

3.1. *Multivariate analysis of legumes samples using unsupervised clustering methods*

Initially, the analytical results obtained from the whole analysis (Appendix 1) was arranged in a data matrix $X_{n\times l}$, where n is the number of samples (i.e. number of locations for each species) and l is the number of measured variables (i.e. solutes: faba beans, beans, chickpea and lentil). The total number of samples was 47 represents 47 locations of 5 countries; Jordan, Lebanon, Syria, Egypt and Palestine. For the current system, matrix X had the size of (12×18), (17×18), (14×18), (4×18) for faba beans, lentils, chickpeas and bean, respectively. Matrix X was subjected to clustering methods as will be discussed below. None of the collected samples were not priori assigned to class membership; hence, unsupervised clustering methods will be applied.

3.2. *PCA and HCA*

The main adopted unsupervised methodologies in analytical chemistry for grouping/clustering objects are PCA and HCA (Abu Mualla and Al Bakain, 2023; Al Bakain *et al.*, 2020). In this study, HCA and PCA were implemented to: 1) confirm whether the legumes obtained from different cities would be grouped together based on their 18 chemical contents, or 2) the resulted chemical contents would be grouped together according to their similar/different concentrations in each species. In fact, PCA reveals the chemical contents that are responsible for grouping the samples. This classification results may have value in the discrimination and selection of legumes species in certain locations. The results may help to show the similarities and the differences between legumes profiles (faba, lentil, chickpea and beans) cross the big producers and consumers of these legumes (i.e. Lebanon, Jordan, Palestine, Syria and Egypt).

3.3. *Legumes characterization by PCA and HCA*

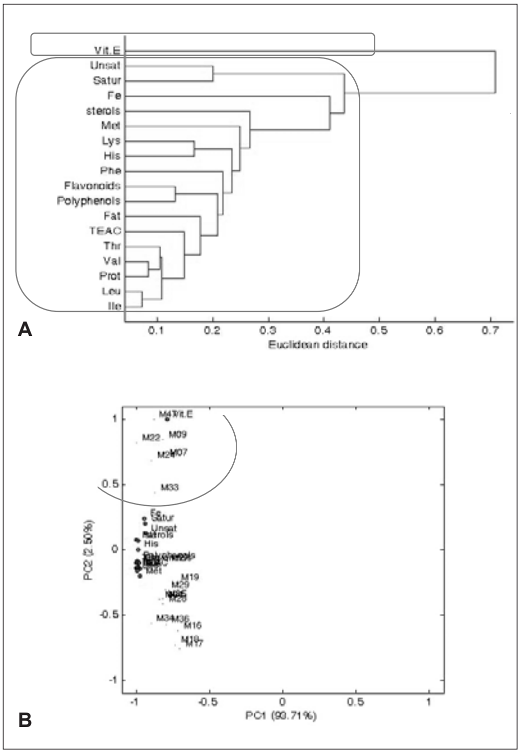
Both PCA and HCA are performed to confirm whether the legumes species obtained from different locations would be grouped together based on the 18 nutritional parameters measured (total fat, protein, Fe, sterols, vitamin E, polyphenols, flavonoids, TEAC, histidine, methionine, lysine, leucine, phenylalanine, valine, threonine, isoleucine, saturated and unsaturated fat). PCA can reveal the variables that is/are responsible in grouping legumes. Legumes classification will be the foundation for industrial production, and informative guidance for individual growers. Indeed, these results would help to show the similarities and the differences between legumes-origin samples of some Arab countries that are considered as big producers and consumers of legumes.

- *Arrangement of analytical data*: Analytical data can be arranged as a data matrix X of n samples or location rows and l variables. For the current case, matrix X has the size of 18 (solutes) × n locations (12, 17, 14, and 4 for faba, lentil, chickpea and beans, respectively), and matrix X has the size of 18 (solutes) × 4 species (faba, lentil, chickpea and beans). Matrix X was subjected into HCA and PCA as discussed below. Data was preprocessed using normalizing methodology, which allowed for better and interpretably PCA outputs.

- *Quantitative legumes classification by PCA and HCA*

In this study, two data matrices where built. The data matrix X (18×n) (i.e. 18 solutes from n locations) is decomposed into two matrices, T (score matrix) and L (loading matrix) using suitable PCA algorithm. The first step in PCA is the computation of loadings. Mathematically, the loadings are the Eigen vectors of the matrix (XXT). There are several methods to estimate the eigenvectors, such as singular value decomposition (SVD) and NIPALS (non-linear iterative partial least-squares) in the order of explained proportion of the variations in X, until a certain pre-established number of components (Al Bakain *et al.*, 2021). The loadings are

Figure 1 - A) Dendrogram based on average linkage clustering and B) PCA bi-plot obtained from the 17 origin- lentil components from Syria, Egypt, Palestine, Jordan and Lebanon.



grouped into a matrix L. The collected loadings are orthonormal, meaning that they are both orthogonal and normalized. The relationship between the original matrix X, the loading matrix L and the score matrix T is described as:

$$X = TLT \qquad \text{Eq. 1}$$

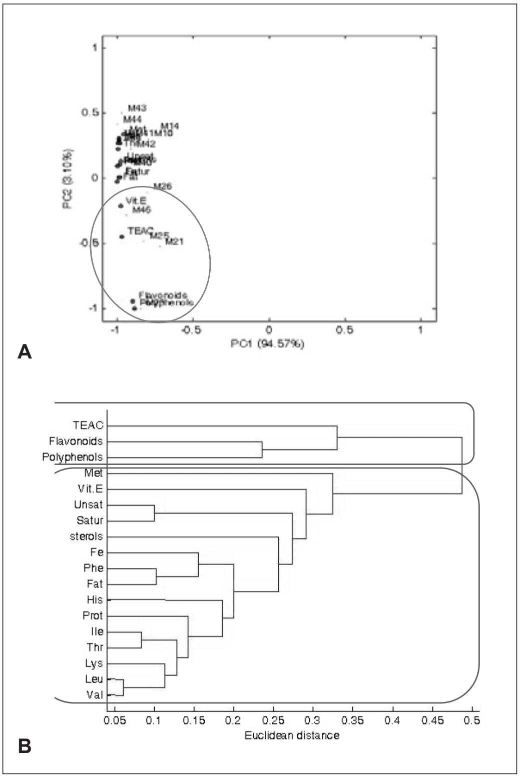
Mathematically, matrix X is decomposed in the product of two matrices, T and L, on the condition that L is formed by orthonormal columns. T is the obtained as: $T = XTL$. In this work, size of X is 18×18 while size T is 18×h and L is h×18, where h is the number of factors needed to decompose matrix X. The optimum number of factors (h) is necessary to create optimum number of loadings and scores and produce informative discrimination among samples/legumes. The analytical scan data were subjected to HCA and PCA analysis. PCA and HCA are commonly employed to reduce the complexity of multivariate data sets without losing important information, observe variance in data sets, and visualize data clustering (Al Bakain *et al.*, 2020). In this study, 18 chemical contents are the original variables (18 dimensions) in PCA. By calculating the covariance matrix between these 18 dimensions, PCA can generate 18 PCs that are orthogonal to each other and can explain 100% of the total variance of the orthogonal data. Each PC is correlated with the original 18 variables. All detected chemical contents were rather necessary for legumes clustering. Accordingly, the number of variables used in clustering was 18 (detected solutes) × *n* (locations) for each species.

As noticed in the 17 lentil samples results (Figure 1), Vitamin E is the main content responsible for grouping samples M7, M9, M22, M24, M33 and M47. Referring to Appendix 1, these locations showed distinguished contents of vitamine E. Two main clusters collect the 18 contents; cluster A collects only vitamin E, and cluster B collects the remaining variables.

Regarding the faba samples, the PCA and HCA outcomes in Figure 2, reveal that (M21, M25) (M23) and (M46) have distinct contents of (TEAC), (polyphenols, flavonoids, TEAC) and (vitamin E), respectively.

According to chickpea, the outcomes of PCA in Figure 3, reveal that M30, M31 and M45 have distinguished content of vitamin E, in addition to flavonoids in M30 and histidine in M45.

Figure 2 - A) PCA bi-plot and B) Dendrogram of the 18 chemical contents obtained from the 12 origin- faba from Syria, Egypt, Palestine, Jordan and Lebanon.



For Bean, M5 showed distinguished contents of vitamin E, polyphenols, flavonoids and TEAC, whereas M1 have distinct content of TEAC as presented in Figure 4.

Figure 3 - PCA outcomes: A) score and B) loading plots obtained from the 14 origin- chickpea components from Syria, Egypt, Palestine, Jordan and Lebanon.

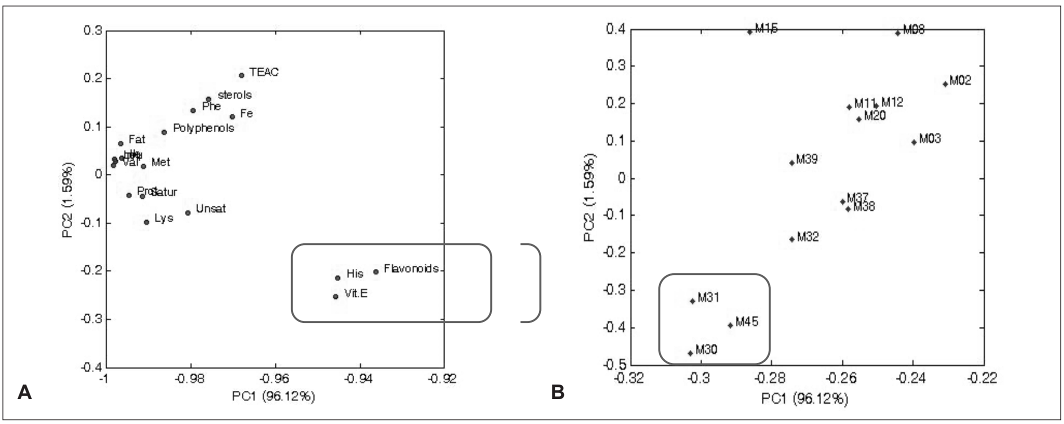
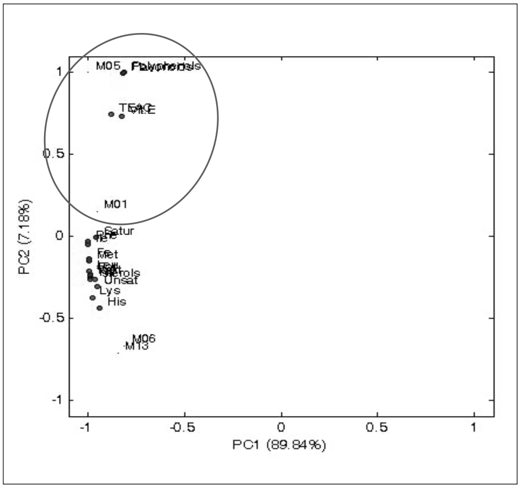


Figure 4 - PCA bi-plot obtained from the 4 origin-bean contents.



As shown in the results of the PCA outcomes, 2 PCs account 96.21%, 97.67%, 97.71% and 97.02% of the total variance in data for lentil, faba, chickpea and beans, respectively.

3.2. *Nutritional profile*

From the statistical analysis outcomes presented in the previous section, the distinguished varieties responsible for grouping and clustering were selected to reveal numerically their distinct contents, as shown in Table 1.

For beans, M01 and M05 originated from Lebanon have distinct content of TEAC reaching approximately above 50% of average effective dose. Moreover, M05 has prominent content of vitamin E that is almost close to dietary reference intake (DRI) of Alpha-tocopherols, polyphenols, and flavonoids close to or higher than the estimated effective levels of these antioxidants. Regarding M01, it had the highest protein content, contributing around one-third or more of the protein recommended dietary allowance (RDA) for males and females, respectively. For lysine content, the selected varieties could provide approximately 50% or more of the RDA. On the other hand, M05 had the highest methionine content, providing close to one-fifth or more of the requirement.

As for faba beans, four varieties were selected: M21, M23, M25, from Syria, and M46 from Palestine. M46 had 1) distinguished contents of vitamin E that is above 50% of the DRI of Alpha-tocopherols, 2) the highest protein content of around 50% or more of the RDA, 3) highest methionine content, which is around 15% of its requirement, and 4) highest lysine content, which satisfies most of the lysine RDA. M23 had prominent contents of total polyphenols and flavonoids, both higher than the estimated effective levels of these antioxidants, whereas M23, M21, and M25 have distinguished content of TEAC reaching above 50% of average effective dose.

Regarding chickpea, three varieties out of 14 were selected for their antioxidant and amino acid profiles; M30 and M31 from Syria and M45 from Palestine. These three varieties had significant contents of vitamin E both close to or higher than the DRI of Alpha-tocopherols. M30 had significant content of flavonoids approximately 30% of the effective dose of these antioxidants, the highest protein content, contributing around 50% or more of the RDA, and highest methionine content providing around 25% or more of the requirements. While M45 had significant content of histidine surpassing the recommended dietary allowance (RDA) of this essential amino acid, and highest contribution to lysine providing around 80% and 99% of the RDA for males and females, respectively.

As for lentil, two varieties from Lebanon (M7, M9), three from Syria (M22, M24, M33), and one from Palestine (M47) had distinguished vitamine E content, which is around third to half the DRI of Alpha-tocopherols, and the highest contribution to methionine, providing around 15% of the requirement. M33 had the highest contribution to protein, providing more than 50% of the RDA, and the highest lysine content close to or higher than the RDA for males and females, respectively.

Among the tested legume species, Vitamin E and histidine were the highest in chickpea, whereas total polyphenols and TEAC were the highest in faba beans, and flavonoids were the highest in beans. Protein and lysine were the highest in lentil, while methionine was the highest in chickpea.

Table 1 - Antioxidant and nutritional profiles of the selected significant varieties of legume species from Lebanon (LB), Syria (SY), and Palestine (PA) (results are expressed per 100g raw seeds).

Requirement of Nutrient or Effective Dose of Nutraceutical		*Dietary Reference Intake: 15mg/d alpha-tocopherol*	*Effective dose: >1170mg/d[*]*	*Effective dose: 500mg/d[†]*	*Effective dose: >5540µmol/d[‡]*	*Recommended Dietary Allowance: Females: 798mg/d Males: 980mg/d[§]*	*Recommended Dietary Allowance: Females: 46g/d Males: 56g/d[¶]*	*Requirement: Females: 593mg/d Males: 728mg/d[#]*	*Recommended Dietary Allowance: Females: 2166mg/d Males: 2660mg/d[§]*
Beans		*mg/100g*	*mg/100g*	*mg/100g*	*mg/100g (µmol/100g)*	*mg/100g*	*g/100g*	*mg/100g*	*mg/100g*
LAB code	*Orig. Code#*	*Total tocopherol Vitamin E*	*Total Polyphenols gallic acid Equivalents (GAE)*	*Flavonoids catechin equivalent (CE)*	*Trolox equivalent antioxidant capacity (TEAC)*	*Histidine*	*Protein*	*Methionine*	*Lysine*
M01	LB01	2.15	829.97	548.67	**721.09 (2881)**	466.01	18.65	122.24	1332.29
M05	LB05	**12.77**	**1165.01**	**831.03**	**720.50 (2879)**	470.88	17.54	133.27	1061.59
Faba bean									
M21	SY 007	4.23	950.60	380.61	**926.87 (3703)**	578.15	25.15	96.41	1519.58
M23	SY009	5.45	**1387.14**	**588.11**	**924.25 (3693)**	415.93	22.93	84.99	1189.96
M25	SY011	6.30	881.29	423.70	**756.75 (3023)**	596.78	24.00	96.90	1593.13
M46	PA02	**8.40**	954.63	370.68	658.80 (2632)	916.75	25.83	99.68	1717.53
Chickpea									
M30	SY016	**14.85**	88.05	**136.28**	53.57 (214)	741.37	27.72	185.33	1853.81
M31	SY017	**16.31**	88.01	99.04	67.28 (269)	753.90	24.43	173.99	1802.79
M45	PA01	**17.42**	116.72	46.20	73.94 (295)	**1391.54**	20.93	178.37	2138.61

Requirement of Nutrient or Effective Dose of Nutraceutical		*Dietary Reference Intake: 15mg/d alpha-tocopherol*	*Effective dose: >1170mg/d**	*Effective dose: 500mg/d†*	*Effective dose: >5540µmol/d‡*	*Recommended Dietary Allowance: Females: 798mg/d Males: 980mg/d§*	*Recommended Dietary Allowance: Females: 46g/d Males: 56g/d¶*	*Requirement: Females: 593mg/d Males: 728mg/d#*	*Recommended Dietary Allowance: Females: 2166mg/d Males: 2660mg/d§*
Lentil		*mg/100g*	*mg/100g*	*mg/100g*	*mg/100g (µmol/100g)*	*mg/100g*	*g/100g*	*mg/100g*	*mg/100g*
LAB code	*Orig. Code#*	*Total tocopherol Vitamin E*	*Total Polyphenols gallic acid Equivalents (GAE)*	*Flavonoids catechin equivalent (CE)*	*Trolox equivalent antioxidant capacity (TEAC)*	*Histidine*	*Protein*	*Methionine*	*Lysine*
M07	LB07	**6.99**	1193.52	592.52	707.83 (2828)	613.24	21.55	101.54	1440.43
M09	LB09	**7.63**	1002.88	459.45	694.60 (2775)	672.11	22.14	87.76	1470.98
M22	SY008	**5.34**	1323.71	491.36	930.16 (3716)	930.21	27.25	89.88	1900.01
M24	SY010	**4.92**	947.56	384.62	936.20 (3740)	789.62	24.40	97.65	1522.07
M33	SY019	**5.25**	865.65	484.30	752.72 (3007)	1018.63	30.69	91.21	2347.66
M47	PA03	**7.63**	889.56	394.99	695.94 (2780)	1244.29	24.83	102.83	1717.03

Note: Values in bold are significant. Protein and amino acid requirements for adults are based on reference weights used by the Institute of Medicine: 70kg for males and 57kg for females (Medicine, 2005).
** Overall inverse association between total polyphenol intake (above 1170 mg/day) and cardiovascular (CV) risk events and mortality (Del Bo' et al., 2019).*
† Higher dietary intake of total flavonoids is associated with decreased cardiovascular disease (CVD) risk in a linear manner, with the highest intake calculated at 500 mg/day (Micek et al.*, 2021).*
‡TEAC is inversely associated with colorectal cancer risk (La Vecchia et al.*, 2013).*
§ Histidine and Lysine recommended dietary allowance (RDA) for adults is 14 and 38mg/kg/d, respectively (Medicine, 2005).
¶ Protein recommended dietary allowance (RDA) for adults is 0.8g/kg/d (Medicine, 2005).
 # Methionine requirement for adults is 10.4 mg/kg/d (Joint et al., 2007).

4. Discussion

To the best of our knowledge, this is the first study to identify varieties of legume species (i.e., bean, faba bean, chickpea, and lentil) with superior antioxidant and nutritional profiles from several countries in the Mediterranean region, particularly Lebanon, Jordan, Egypt, Syria, and Palestine. This marks a significant step toward maximizing the health benefits of legumes and addressing malnutrition, especially in impoverished communities. Legumes are an affordable source of protein, micronutrients, and antioxidants, which are associated with reduced disease risk (Grewal *et al.*, 2022). Identifying varieties with significantly enhanced nutritional and antioxidant profiles offers a means to alleviate malnutrition and enhance public health. Additionally, bioactive compounds in legumes, such as polyphenols and flavonoids, contribute to their medicinal properties and potential as drug sources (Vijayakumar, 2021). The identified varieties could serve as parental material for breeding cultivars with enhanced nutritional profiles (Grewal *et al.*, 2022).

For beans, two varieties from Lebanon stood out for their distinguished antioxidant content, with M05 demonstrating significant levels of vitamin E near the recommended daily allowances (RDAs). Vitamin E is a potent antioxidant that has been shown to support cardiovascular health, cancer prevention, and immune function by reducing the oxidation of low-density lipoprotein (LDL) cholesterol and preventing blood clot formation (Rizvi *et al.*, 2014). Additionally, vitamin E protects cell membranes from free radical damage, inhibits carcinogen formation in the stomach (Wood and Grusak, 2007) contributes to immune function (Lewis *et al.*, 2019), facilitates DNA repair (Kaźmierczak-Barańska *et al.*, 2020), and supports various metabolic processes (Wood and Grusak, 2007). The M05 variety also exhibited high levels of polyphenols, flavonoids, and TEAC, offering antioxidant, anti-obesity, anti-diabetic, anti-inflammatory, and anti-carcinogenic properties (Ganesan and Xu, 2017). Polyphenols play a critical role in protecting organisms from external stressors and neutralizing reactive oxygen species (ROS) (Rana *et al.*, 2022). Flavonoids, a class of dietary polyphenols, are associated with numerous health benefits, including anticancer, anti-inflammatory, antiviral, neuroprotective, and cardioprotective effects (Ullah *et al.*, 2020). The flavonoid content in M05 was significantly higher than previously reported levels in other legumes and varieties (Rodríguez Madrera *et al.*, 2021).

Regarding faba beans, this study selected four varieties of faba beans from 12 different cultivars for their distinguished antioxidant profiles. The M46 variety from Palestine had the highest vitamin E content among the tested varieties, while M23 exhibited superior antioxidant capacity, including high levels of TEAC, polyphenols, and flavonoids. These findings align with studies showing that faba beans are rich in polyphenols (Johnson *et al.*, 2024) and are a good source of natural antioxidants (Chaieb *et al.*, 2011), offering protective effects against conditions such as hypertension and cancer (Turco *et al.*, 2016). Concerning chickpea, three varieties of chickpeas were selected out of 14 for their antioxidant and amino acid profiles. These varieties had significant levels of vitamin E, consistent with studies identifying chickpeas as a good source of tocopherols [50]. The M30 variety from Syria exhibited a flavonoid content higher than those reported in Turkish varieties (Macar *et al.*, 2017). Finally, five varieties of lentils from Lebanon, Syria, and Palestine were selected out of 17 for their vitamin E content, corroborating research that lentils are rich in bioactive phytochemicals, including tocopherols. Gamma-tocopherol accounts for over 92% of the total tocopherols in commercial lentil samples from Italy (Boschin and Arnoldi, 2011). These varieties could be used to improve nutritional qualities in lentil breeding lines (Riaz *et al.*, 2024).

Variations in polyphenol content among the selected varieties may be attributed to genetic factors, climatic conditions, storage, and coat color differences between cultivars (Carbas *et al.*, 2020; Yang *et al.*, 2018). For instance, dark-colored beans tend to have higher phenolic content and antioxidant capacity than uncolored varieties (Carbas *et al.*, 2020; Yang *et al.*, 2018). Environmental factors such as soil composition, annual rainfall, altitude, and humidity also influence the nutraceutical properties of legumes (Yegrem, 2021).

The protein content of the selected bean, faba bean, chickpea, and lentil varieties was consistent with ranges reported in the literature (Grewal *et al.*, 2022; Martineau-Côté *et al.*, 2022). Among the legumes, chickpeas exhibited the highest methionine content, a limiting amino acid in legumes. Meanwhile, lentils stood out for their lysine and protein content, making them ideal for creating complementary proteins when paired with grains.

By fixing nitrogen in the soil, legumes reduce the need for synthetic fertilizers, enhancing soil health and contributing to sustainable agricultural practices that align with SDG Goal 12 (Responsible Consumption and Production). Their cultivation supports local biodiversity by providing habitat and food for a variety of organisms, promoting ecosystem resilience (SDG Goal 15, reduce biodiversity lost). Additionally, the integration of legumes into agricultural systems can address SDG Goal 13 (Climate Action) by reducing the carbon footprint of farming. Policies that incentivize legume cultivation and consumption, especially among small and medium enterprises (SMEs), can drive economic growth by empowering local farmers, processors, and food producers across the Mediterranean. These SMEs are key players in developing innovative legume-based products, such as functional foods and nutraceuticals, catering to the growing demand for health-conscious and sustainable dietary options. The antioxidant, anti-inflammatory, and cardio-protective properties of legumes make them functional foods, contributing to the prevention of non-communicable diseases and aligning with global health goals (SDG Goal 3, promote healthy lives and well-being).

5. Conclusion

This study highlights the nutritional and nutraceutical richness of legumes - faba beans, lentils, chickpeas, and beans - collected from Lebanon, Syria, Jordan, Palestine, and Egypt, emphasizing their critical role in food security, sustainability, and public health. Through detailed chemical profiling and multivariate analysis, the study revealed the distinct nutritional and bioactive compound profiles of these legumes. Being rich in protein, fiber, vitamins, minerals, and bioactive compounds such as polyphenols and flavonoids, legumes are confirmed as valuable alternatives to meat, particularly for resource-constrained communities. Furthermore, the study identifies superior legume varieties that could serve as parental material for breeding programs aimed at enhancing nutritional profiles and strengthening food system resilience. Beyond their nutritional benefits, legumes play a pivotal role in sustainable agricultural practices by supporting local biodiversity and improving soil fertility. Their cultivation contributes to environmental sustainability while reducing dependence on synthetic fertilizers. By integrating legumes into national food security strategies and promoting their inclusion in the Mediterranean Diet, countries in the region can achieve nutritional, economic, and environmental resilience. These efforts reaffirm the Mediterranean Diet as a global model for health and sustainability, ensuring food sovereignty and fostering a sustainable future for generations to come.

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Appendix 1 - Chemical profiling for Faba beans, Chickpea, Beans and Lentils from Lebanon, Jordan, Egypt, Syria, and Palestine

			mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
Code	**Legume**	**Country**	**Protein**	**Fat**	**Fe**	**Saturated fat**	**Unsaturated fat**	**Total sterols**	**Vit. E**	**Tot. Polyphenols gallic ac. Eq**	**Flavonoids catechin eq**	**Trolox equivalent antioxidant capacity (TEAC)**	**Threonine**	**Valine**	**Methionine**	**Isoleucine**	**Leucine**	**Phenylalanine**	**Histidine**	**Lysine**
M01	Bean	Lebanon	18652.3	1259.28	6.1	112	711	69.5	**2.1**	830	549	721	860.27	735.19	122.24	430.10	1232.59	1189.44	466.01	1332.29
M02	Chickpea	Lebanon	20840.4	5067.26	6.2	265	973	74.8	**4.1**	82	54	101	542.88	575.99	176.48	312.44	1046.76	972.96	604.05	1486.09
M03	Chickpea	Lebanon	18406.7	5054.45	5.7	404	3089	97.9	**10.4**	81	54	97	527.28	520.18	116.74	272.02	934.04	856.38	515.81	1333.39
M04	Lentil	Lebanon	22753.5	772.49	16.0	50	155	68.1	**1.3**	1185	645	675	726.38	579.86	132.93	433.60	1394.09	1092.77	878.90	2045.64
M05	Bean	Lebanon	17543.8	1050.10	5.8	174	325	42.8	**12.8**	1165	831	720	704.34	598.09	133.27	496.14	1149.17	1342.61	470.88	1061.59
M06	Bean	Lebanon	22148.7	996.42	4.8	193	396	40.2	**3.6**	100	84	133	818.43	647.94	149.95	406.91	1205.49	1032.75	892.57	1583.56
M07	Lentil	Lebanon	21554.6	750.01	8.2	48	243	51.4	**7.0**	1194	593	708	672.06	676.80	101.54	439.48	1291.59	936.53	613.24	1440.43
M08	Chickpea	Lebanon	17464.1	5255.38	6.7	429	2931	68.3	**1.9**	118	43	79	577.67	572.30	172.29	405.51	1123.50	1429.81	416.15	1175.04
M09	Lentil	Lebanon	22141.5	764.14	12.0	37	168	49.5	**7.6**	1003	459	695	684.11	676.86	87.76	449.90	1286.38	1373.12	672.11	1470.98
M10	Faba	Lebanon	26616.7	1063.16	7.5	84	337	65.5	**5.8**	324	138	524	808.48	800.19	114.91	529.75	1568.78	967.13	570.79	1606.77
M11	Chickpea	Jordan	19127.0	5182.05	7.1	432	3361	76.7	**10.2**	78	42	84	611.34	592.30	160.23	408.83	1164.77	1456.43	452.43	1259.92
M12	Chickpea	Jordan	16016.5	5826.12	5.0	415	2902	78.5	**10.9**	93	51	113	616.15	597.91	128.03	380.57	1091.24	1128.14	410.72	1220.11
M13	Bean	Jordan	18549.6	1434.56	6.8	89	608	71.9	**3.5**	89	72	161	891.28	774.48	118.22	458.23	1347.37	1261.64	536.94	1454.33
M14	Faba	Jordan	25215.5	1151.88	6.1	80	321	40.7	**3.4**	279	105	665	858.32	868.50	121.28	525.16	1587.43	861.29	585.92	1734.41
M15	Chickpea	Syria	21182.5	6194.11	10.7	453	2951	88.1	**6.3**	121	58	107	674.77	700.36	169.48	428.79	1227.73	1190.64	477.89	1354.17
M16	Lentil	Syria	24118.0	649.67	6.2	13	72	56.3	**1.3**	1239	586	923	677.01	671.92	92.51	436.49	1309.39	974.47	614.72	1424.18
M17	Lentil	Syria	25879.0	501.99	5.4	42	138	39.6	**0.4**	886	359	917	651.34	640.46	121.15	419.34	1231.92	1313.03	770.99	1391.08
M18	Lentil	Syria	24977.3	596.91	7.8	27	150	41.8	**0.7**	849	328	917	756.61	744.90	160.61	447.10	1315.34	833.54	789.67	1627.00
M19	Lentil	Syria	25003.6	730.12	8.4	27	110	45.0	**2.6**	924	395	933	760.44	713.90	76.98	439.33	1301.13	1086.44	627.65	1567.18
M20	Chickpea	Syria	21325.3	5907.90	4.2	355	2412	73.6	**10.7**	102	52	82	631.93	582.90	174.56	411.58	1183.04	1502.49	440.01	1224.39
M21	Faba	Syria	25150.9	964.57	4.1	45	218	54.0	**4.2**	951	381	927	607.53	591.38	96.41	301.70	1072.73	721.89	578.15	1519.58
M22	Lentil	Syria	27253.5	941.07	16.7	129	511	77.0	**5.3**	1324	491	930	670.49	706.51	89.88	351.16	1222.38	951.27	930.21	1900.01
M23	Faba	Syria	22933.9	1177.86	5.9	126	502	45.9	**5.4**	1387	588	924	590.72	561.89	84.99	354.72	1045.68	799.81	415.93	1189.96
M24	Lentil	Syria	24397.7	874.10	20.0	88	368	55.5	**4.9**	948	385	936	716.80	717.35	97.65	412.49	1245.14	806.01	789.62	1522.07
M25	Faba	Syria	24002.6	1087.13	7.4	111	487	60.3	**6.3**	881	424	757	625.15	585.60	96.90	315.44	1111.17	752.89	596.78	1593.13
M26	Faba	Syria	28760.8	1046.82	5.1	100	421	46.5	**5.4**	443	434	535	716.15	723.06	112.90	372.55	1354.99	903.95	732.81	1887.55
M27	Lentil	Syria	26642.8	599.18	6.8	42	275	44.9	**3.4**	1143	664	757	687.50	706.81	112.13	336.60	1233.74	916.50	937.50	1964.51
M28	Lentil	Syria	26501.9	745.50	8.5	79	455	56.9	**0.6**	1025	529	754	725.12	712.96	88.57	443.63	1326.88	1011.27	647.51	1505.75
M29	Lentil	Syria	25751.4	636.64	6.1	71	323	47.1	**2.0**	1163	595	754	748.69	740.56	88.73	460.97	1403.39	1092.40	563.57	1525.78
M30	Chickpea	Syria	27724.7	5588.69	5.3	561	4060	72.2	**14.9**	88	136	54	674.96	641.32	185.33	417.70	1246.92	1009.30	741.37	1853.81
M31	Chickpea	Syria	24431.2	5427.52	8.0	564	4049	65.9	**16.3**	88	99	67	639.53	750.52	173.99	437.53	1225.67	992.58	753.90	1802.79
M32	Chickpea	Syria	24189.4	5771.86	6.2	568	4129	55.4	**8.2**	94	96	64	647.30	623.71	165.93	386.61	1086.87	995.20	642.62	1551.99
M33	Lentil	Syria	30694.9	693.95	7.6	82	373	50.3	**5.3**	866	484	753	746.17	705.68	91.21	441.57	1204.46	894.46	1018.63	2347.66
M34	Lentil	Syria	27227.6	725.66	7.5	60	437	74.3	**0.8**	1256	654	753	867.61	818.02	92.54	464.48	1365.71	945.08	950.34	2514.12
M35	Lentil	Syria	25902.0	824.86	9.1	81	459	55.1	**0.5**	876	439	749	767.12	710.77	84.80	432.60	1314.68	706.60	903.58	2086.02
M36	Lentil	Syria	30844.0	670.06	7.6	76	364	24.5	**0.5**	954	346	755	781.98	781.04	101.99	395.45	1373.65	1004.97	935.84	2177.33
M37	Chickpea	Syria	23518.0	5248.19	4.8	417	3032	60.6	**10.4**	77	46	69	689.33	618.16	215.77	408.95	1224.64	984.04	668.52	1707.05
M38	Chickpea	Syria	22806.8	5101.20	4.8	420	3008	67.7	**11.2**	79	57	77	659.92	641.93	165.20	389.78	1192.85	915.15	654.92	1667.14
M39	Chickpea	Syria	21832.2	5914.41	4.8	501	3653	77.4	**12.1**	78	42	80	675.65	712.78	190.01	408.38	1233.20	1341.49	550.77	1607.52
M40	Faba	Egypt	31182.0	1389.59	6.2	169	789	58.5	**6.3**	589	243	616	807.11	766.69	112.30	478.01	1479.65	1086.41	684.66	1956.67
M41	Faba	Egypt	28908.0	1251.91	6.3	137	650	69.1	**5.2**	485	196	530	930.72	870.50	98.93	520.37	1616.15	926.95	1092.60	2333.11
M42	Faba	Egypt	29560.6	1207.32	5.4	132	677	72.0	**5.3**	531	219	578	926.24	867.04	93.39	572.20	1671.69	911.66	898.19	2133.28
M43	Faba	Egypt	27755.3	1256.34	6.8	131	681	69.7	**5.8**	286	196	568	979.62	950.80	176.51	574.98	1732.76	1013.45	998.99	2494.70
M44	Faba	Egypt	37317.0	1085.08	5.5	100	567	102.4	**3.7**	586	237	622	981.72	1026.01	239.60	661.50	1658.74	849.64	896.09	2565.96
M45	Chickpea	Palestina	20934.3	5348.75	6.2	413	2978	44.4	**17.4**	117	46	74	617.88	658.05	178.37	388.47	1183.63	1178.55	1391.54	2138.61
M46	Faba	Palestina	25832.1	1207.83	5.4	110	514	99.6	**8.4**	955	371	659	949.40	633.16	99.68	596.14	1282.13	716.26	916.75	1717.53
M47	Lentil	Palestina	24825.8	849.54	7.8	66	386	74.3	**7.6**	890	395	696	898.31	616.23	102.83	396.60	1092.07	713.66	1244.29	1717.03