Characterization and Compositional Studies of the Oils from Some Legume Cultivars, *Phaseolus vulgaris*, Grown in Southern Africa

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ABSTRACT: Seed oils from six legume cultivars of *Phaseolus vulgaris*, grown in the Kingdom of Lesotho, were extracted and their physicochemical properties and FA compositions were determined in order to compare their dietary lipids with those in *P. vulgaris* cultivars grown in other parts of the world. The oil content of the beans was very low, ranging from 1.5 to 2.0% (w/w). The acid values ranged from 11.0 to 19.2 mg KOH/g, whereas a combination of the PV and the *p*-anisidine values in Holm's equation gave oxidation values that ranged from 11.0 to 15.0. Thus, considerable enzymatic hydrolysis and oxidation had taken place in the beans during storage. Iodine values ranged from 80.5 to 92.3 (Wijs method), indicating moderate unsaturation in the oils. However, capillary GC analysis, supported by proton NMR analysis of the FAME, gave a total unsaturation range from 79.67 to 84.24%. The dominant FA were α-linolenic acid (36.47–48.81%) and linoleic acid (20.96–36.10%), with appreciable amounts of palmitic acid (14.33–18.23%). This FA composition pattern is quite similar to the FA distribution reported for low oil-bearing legume seeds. Thus, notwithstanding the different climatic and soil conditions, the general properties of lipids in the southern African legume cultivars were quite similar to those of lipids in *P. vulgaris* cultivars grown in other parts of the world. The high content of α-linolenic acid in the cultivars of *P. vulgaris* could very likely play a beneficial role in reducing the risk of coronary heart disease among the large populations consuming them in the southern African region.

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The common bean, *Phaseolus vulgaris*, one of about 100 species of the genus *Phaseolus* of the Fabaceae (Leguminosae) family, is reportedly native to the New World, probably originating between Central Mexico and Guatemala. The Spaniards and Portuguese are believed to have taken it to Africa, Europe, and other parts of the world (1). Numerous cultivars of *P. vulgaris* have now been developed, the seeds of which differ in size, shape, and color and are called by a variety of names, depending on where they are cultivated.

In the small Kingdom of Lesotho in southern Africa, red speckled beans (RSB), small white beans (SWB), and large and small pinto beans (PLB and PSB) are the most common cultivars of *P. vulgaris* grown. These and others, such as pink watcher beans (PW), golden beans (GB), and black oval beans, are all cultivated on the highlands and in the foothills of Lesotho.

Beans of the numerous cultivars of *P. vulgaris* are used extensively as an important food source for humans as well as a feed for livestock in many parts of the world. These legumes serve as a major source of protein for vegetarians and for low- to middle-income groups throughout the world. In Lesotho and other countries in southern Africa, the bean is cooked to prepare one of the traditional dishes served at funeral celebrations. With an average protein content of 22.5%, carbohydrate content of 62%, and a rather small oil content of 1.8% (w/w), the common bean is indeed an important source of nutrition. Its protein content compares favorably with such other food sources as fish (23%), meat (30%), and peanuts (24%) (2). In addition, some cultivars of the common bean are a good source of mineral nutrients. For example, the kidney bean provides such minerals as calcium (86 mg/100 g sample), phosphorus (240 mg/100 g sample), and iron (700 mg/100 g sample) (3). Thus, the common bean is a valuable food source for many people around the world, not the least because of its availability and affordability.

The oil content of the beans of the cultivars of *P. vulgaris* grown in the Americas, Europe, and India is reportedly only about 1.6% (w/w) on average. Thus, by all standards the common bean cannot be described as an oil-bearing seed. However, the reported FA profiles of the seed oils of *P. vulgaris* cultivars suggest that these oils may be more valuable than can be inferred from their very low oil content. The oils from the beans of a number of important *P. vulgaris* cultivars have been reported to consist principally of linoleic (18:2n-6) and linolenic (18:3n-3) acids to a combined level of about 80% (w/w), with linolenic acid being the dominant FA in most cases (4). The predominance of linolenic and linoleic acids, the precursors of the long-chain n-3 and n-6 polyunsaturated EFA, certainly adds an extra dimension to the nutritional value of *P. vulgaris* beans. Over the last 10 yr, the scientific literature has drawn much attention to the health benefits of dietary n-3 and n-6 PUFA due to their therapeutic and preventive effects on coronary heart disease and other diseases (5,6).

The immense interest in the n-3 and n-6 EFA as good nutritional agents and nutraceuticals encouraged the authors of this article to conduct characterization and compositional

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studies to ascertain the lipid profiles of the seeds of the *P. vulgaris* cultivars grown in southern Africa, which constitute an important food source in the diet of the people in the region. As far as the authors are aware, such a study on the lipid content of the seeds of the *P. vulgaris* cultivars grown in southern Africa has not been reported.

EXPERIMENTAL PROCEDURES

Materials. Samples of dry and edible beans from six different cultivars of *P. vulgaris* were purchased from the main market (Pitso Ground) in Maseru, the capital city of the Kingdom of Lesotho. The six cultivars were: RSB, SWB, PLB, PW, GB, and PSB.

Extraction. The solvents (Fisher Scientific, Loughborough, United Kingdom) used were of analytical grade and were not further purified. After thorough cleaning and removal of all visible impurities, the seed samples were macerated in a Waring blender and the powders were extracted with a mixture of *n*-hexane/2-propanol (3:1, vol/vol) in a Soxhlet apparatus (6 h). Another batch of powders was extracted with the same solvent mixture but at room temperature by shaking in an orbital shaker (5 × 2 h).

General properties. The bulk physical and chemical properties (Table 1) were determined according to standard IUPAC methods for the analysis of fats and oils (7). All experiments were conducted in triplicate.

Lipid class composition. Separation of the lipid classes in each oil sample was accomplished by adsorption column chromatography using florisil (7% H_2O, w/w; Saarchem Pty. Ltd., Muldersdrift, Republic of South Africa) and gradient elution as follows: hydrocarbons (*n*-hexane, 100%), sterol esters (*n*-hexane/ether, 95:5% vol/vol), TAG + FFA (*n*-hexane/ether, 85:15% vol/vol), free sterols (*n*-hexane/ether, 75:25% vol/vol), DAG (*n*-hexane/ether, 50:50% vol/vol), MAG (ether/methanol, 90:10% vol/vol), glycolipids (acetone, 100%) and phospholipids (methanol, 100%) (8).

Separation of acylglycerols. TAG, DAG with FFA (DAG + FFA), and MAG in the oil samples were further separated by gradient elution on silica gel (Saarchem Pty. Ltd.) using benzene (100%), benzene/ether (90:10% vol/vol), and ether (100%), respectively (8).

FA composition—sample preparation. Portions of the extracted oil samples were transesterified by refluxing in dry methanol that contained ethanoyl chloride to yield FAME. These were used for chromatographic and ^{1}H NMR analyses.

Instrumentation and separation conditions. FA compositions were determined using a PerkinElmer Autosystem gas chromatograph (Norwalk, CT) with on-column injection and FID interfaced with a PE Nelson computer. The column was an Omegawax TM 320 capillary column (30 m × 0.32 mm × 0.25 mm i.d.; Supelco, Bellefonte, PA). The carrier gas was nitrogen at a pressure of 15.0 psi. The oven, injection, and detection temperatures were fixed at 200, 250, and 260°C, respectively. Reference compounds were standard FAME mixtures from Supelco and Sigma (Sigma Chemical Co., St. Louis, MO).

NMR analysis. Proton NMR spectra of the FAME were acquired at 300 MHz using a Bruker Avance DPX300 spectrometer.

Statistical analysis. All experiments were carried out in triplicate unless otherwise stated; results are expressed as mean values ± SD. Statistical analysis was carried out using a one-way ANOVA with a significance level of $P < 0.05$. The software used for statistical analysis was the SPSS for Windows statistical package (v. 10.0.6; SPSS, Chicago, IL).

RESULTS AND DISCUSSION

The oil yields obtained from Soxhlet extraction, the more efficient of the two extraction methods, were very low and thus upheld the view that, with the exception of groundnuts and soybeans, legumes are generally not oil-bearing seeds. The oil yields for the six cultivars ranged from 1.5% for PSB to 2.0% for PLB. The low yields reported here are in close agreement with those reported for the same or other cultivars of *P. vulgaris* grown in the Americas, India, and Europe (4).

The physicochemical parameters determined for the oils from the six legume cultivars are shown in Table 1. The refractive indices (RI) fell within a close range, from 1.474 to 1.478. Although this range compares quite favorably with the RI for grapeseed oil (1.473–1.477), the range is higher than the RI for soybean (1.466–1.470) and groundnut (1.460–1.465) oils, the two legumes with the highest oil content (9). These relatively high RI are an indication of substantial unsaturation in the oils of the six legume cultivars (10). The range of relative densities shown in Table 1, 0.943–0.984, is again higher than the relative densities for groundnut (0.914–0.917) and soybean (0.919–0.923) oils.

TABLE 1
Physicochemical Properties of Golden Beans (GB), Small White Beans (SWB), Red Speckled Beans (RSB), Large Pinto Beans (PLB), Small Pinto Beans (PSB), and Pink Watcher Beans (PW)[a]

Physicochemical Properties	GB	SWB	RSB	PLB	PSB	PW
Refractive index (40°C)	1.478 ± 0.004	1.474 ± 0.006	1.477 ± 0.005	1.475 ± 0.004	1.475 ± 0.003	1.476 ± 0.006
Relative density (30°C) (g/cm^3)	0.984 ± 0.012	0.943 ± 0.011	0.960 ± 0.015	0.961 ± 0.012	0.953 ± 0.013	0.953 ± 0.016
Saponification value (mg KOH/g)	196.6 ± 2.1	173.3 ± 2.4	173.9 ± 2.2	175.8 ± 2.4	182.6 ± 2.1	172.2 ± 2.6
Iodine value (Wijs method)	86.5 ± 3.0	92.3 ± 3.3	81.2 ± 3.6	80.8 ± 3.8	82.1 ± 3.6	80.5 ± 3.5
PV (meq/kg)	3.9 ± 0.1	1.8 ± 0.1	2.9 ± 0.1	2.5 ± 0.1	10.7 ± 0.4	1.8 ± 0.1
p-Anisidine value	4.4 ± 0.2	10.3 ± 0.3	8.7 ± 0.4	10.0 ± 0.3	9.8 ± 0.4	7.4 ± 0.3
Unsaponifiable matter (% w/w)	6.0 ± 0.2	5.6 ± 0.1	5.7 ± 0.3	5.5 ± 0.1	4.9 ± 0.2	5.6 ± 0.2
Acid value (mg KOH/g)	13.6 ± 0.5	12.4 ± 0.4	11.4 ± 0.2	13.1 ± 0.5	19.2 ± 0.5	11.0 ± 0.2

[a]Values represent the average of three replicate analyses ± SD.

Bulk chemical properties such as acid value (AV), saponification value (SV), iodine value (IV), PV, and *p*-anisidine value (*p*AV) give structural, stability, and quality information about oils and fats. Thus, although the range of SV from 172.2 to 196.6 mg KOH/g indicates the absence of lauric oils in the six legume seed oils, this range is indicative of oils characterized by medium chain-length FA. Indeed, the range of SV for the six legume cultivars is quite similar to those of olive (184–196 mg KOH/g), soybean (189–195 mg KOH/g), and sunflowerseed (188–194 mg KOH/g) oils, which are typical C_{16} and C_{18} oils. The range of IV of 80.5 to 92.3 (Wijs method) (Table 1) is rather similar to the IV for groundnut oil (80–106, Wijs method), suggesting medium unsaturation for the test oils. The seed oils from the six legume cultivars must thus contain significant amounts of saturated FA, likely to be palmitic acid (16:0), a common feature of legume seed oils (4).

The oxidation states of the oils from the six legume cultivars were indicated by PV and *p*AV as shown in Table 1. Except for PSB (10.7 meq/kg), PV for all the cultivars fell well within the Codex recommended maximum (10 meq/kg) for edible oils (9). In fact, the PV range of 1.8 to 3.9 meq/kg would initially suggest the presence of only small amounts of hydroperoxides, indicating that not much oxidative activity had taken place in the oils. However, the *p*AV range of 4.4 to 10.3 anisidine units suugests the presence of significant amounts of secondary oxidation products in the test oil samples. By combining PV and *p*AV in Holm's equation for oxidation value, OV = *p*AV +2(PV), to describe the degree of oxidation of the oils, the values in Table 2 were obtained (11). The OV shown in Table 2 indicate that considerable oxidative activity, catalyzed by the lipoxygenase present in the seeds of the *P. vulgaris* cultivars and also by autoxidation, had taken place prior to extraction. Table 2 shows theoretical flavor scores (*F*) obtained from an equation by List and coworkers (11), $F = 7.7 - 0.35(OV)$, for predicting flavor scores. Although the equation was developed for a particular set of experiments, the flavor scores given in Table 2 indicate that the oils from the test legumes would receive rather low acceptance as edible oils without further refinement.

The *p*AV reported in Table 1 are rather high, which is another reported characteristic of legumes. The *p*AV ranged from 11.0 to 19.2 mg KOH/g. This range of *p*AV is much higher than the Codex recommended maximum *p*AV for virgin olive (6.6 mg KOH/g) and palm (10 mg KOH/g) oils, indicating that an appreciable amount of enzymatic hydrolysis must have taken place in the seeds during storage. It is quite possible that secondary oxidation products also contributed to the high level of FFA found in the legumes. It is worth noting that the high amount of oxidation and hydrolysis of the legume lipids did not appear to affect the edibility of the seeds, probably due to the long cooking times that the dried seeds require. The range of unsaponifiable matter (UM) given in Table 1, 4.9 to 6.0% (w/w), appears to be consistent with the pattern observed for low oil-bearing seeds. Nevertheless, the high levels of UM determined in this work prompted a separate study to investigate the components of the UM.

The compositional studies carried out in this work included determination of the lipid classes and the FA composition of the oils. Table 3 shows that neutral lipids, dominated by TAG, were the predominant lipid compounds in the oils. The significant amounts of sterols and sterol esters found (Table 3) indicate that sterols constitute a prominent component of the UM. The phospholipid content is quite significant, whereas glycolipids are present only in trace amounts, indicating that phospholipids form the principal components of the cell membranes in the seeds.

The principal FA components of legumes generally are palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6), and linolenic (18:3n-3) acids, the distribution of which varies according to species, variety, and geographical conditions. Generally, oleic and linoleic acids tend to dominate in high oil-bearing legumes, as exemplified by groundnut oil (47–50% oleic and 28–30% linoleic) and soybean oil (50–57% linoleic and 18–26% oleic), whereas linolenic and linoleic acids are the dominant FA in low oil-bearing legumes, as in pinto bean oil (49% linolenic and 28% linoleic) and kidney bean oil (51% linolenic and 27% linoleic) (4). All six *P. vulgaris* cultivars under study here had low oil-bearing seeds. Indeed, their FA composition followed the general pattern, with a linolenic acid content ranging from 36.47% (for PW) to 48.81% (for RSB), followed by a linoleic acid content ranging from 20.96% (for RSB) to 36.10% (for PW) (Table 4). It is worth noting in Table 4 that the palmitic acid content (14.33–18.23%) in the test samples was higher than that of

TABLE 2
Oxidation Values and Flavor Scores Calculated from the PV and *p*-Anisidine Values of the Oils from the Selected Cultivars of *Phaseolus vulgaris*[a]

P. vulgaris cultivar	PV (meq/kg)	*p*-Anisidine value	Oxidation value	Flavor score
GB	3.9 ± 0.1	4.4 ± 0.2	12.2 ± 0.5	3.4 ± 0.1
SWB	1.8 ± 0.1	10.0 ± 0.3	13.6 ± 0.6	2.8 ± 0.1
RSB	2.9 ± 0.1	8.7 ± 0.4	14.5 ± 0.5	2.6 ± 0.1
PLB	2.5 ± 0.1	10.0 ± 0.3	15.0 ± 0.7	2.5 ± 0.1
PSB	10.7 ± 0.4	9.8 ± 0.4	31.2 ± 1.4	–3.2 ± 0.1
PW	1.8 ± 0.1	7.4 ± 0.3	11.0 ± 0.4	3.9 ± 0.1

[a]Values represent the average of three replicate analyses ± SD. For abbreviations of cultivars see Table 1.

TABLE 3
Percentage Composition of Lipid Classes in the Oils from the Beans of Selected Cultivars of *P. vulgaris* Estimated from Adsorption Column Chromatography[a]

P. vulgaris	Neutral lipids				Polar lipids			
cultivars	HC	TAG	STE + FFA	FST	DAG	MAG	GL	PL
GB	0.6	59.6	2.9	2.9	0.4	2.7	0.3	19.6
SWB	0.01	60.6	3.0	3.0	1.0	2.6	0.4	17.0
RSB	0.2	59.7	2.9	2.9	0.8	2.9	0.3	18.4
PLB	0.1	56.4	2.8	2.8	0.9	2.8	0.2	20.3
PW	0.5	61.1	3.0	3.0	0.4	2.8	0.1	16.8

[a]Values represent the average of two replicate analyses. Abbreviations: HC, hydrocarbons; STE, sterol esters; FST, free sterols; GL, glycolipids; PL, phospholipids. For abbreviations of cultivars, see Table 1.

TABLE 4
FA Composition (%, w/w) of Oils from the Beans of Selected Cultivars of *P. vulgaris* as Estimated from Capillary GC[a] and Proton NMR Integrals[b]

FA	GB	SWB	RSB	PLB	PSB	PW
16:0	15.89 ± 0.30	15.70 ± 0.31	14.33 ± 0.30	16.44 ± 0.33	18.08 ± 0.32	18.23 ± 0.30
18:0	2.30 ± 0.05	1.74 ± 0.04	1.44 ± 0.02	1.73 ± 0.06	2.17 ± 0.03	2.10 ± 0.02
18:1n-9	11.07 ± 0.14	7.32 ± 0.10	14.47 ± 0.15	4.09 ± 0.12	10.94 ± 0.16	7.10 ± 0.14
18:2n-6	27.06 ± 0.35	34.01 ± 0.38	20.96 ± 0.36	33.18 ± 0.37	28.11 ± 0.34	36.10 ± 0.36
18:3n-3	43.69 ± 0.46	41.23 ± 0.2	48.81 ± 0.41	44.56 ± 0.44	40.70 ± 0.42	36.47 ± 0.39
Total saturated	18.19 ± 0.35	17.44 ± 0.35	15.77 ± 0.32	18.17 ± 0.36	20.25 ± 0.36	20.33 ± 0.34
Total unsaturated	81.82 ± 0.95	82.56 ± 0.92	84.24 ± 0.92	81.83 ± 0.93	79.75 ± 0.92	79.67 ± 0.89
FA classes	Estimates from proton NMR integrals (% mol)					
α-Linolenic	43	43	45	39	39	38
Diunsaturated	42	46	42	22	40	41
Monounsaturated	7.5	5.5	6.5	19.5	10.5	10.5
Saturated	7.5	5.5	6.5	19.5	10.5	10.5
Average carbon number	17	18	17	15	17	17

[a]Values represent the average of three replicate analyses ± SD. For abbreviations of cultivars, see Table 1.
[b]Composition of the FA classes was estimated from one proton NMR experiment for each cultivar.

oleic acid (4.09–14.47%), and that the stearic acid content was very low (1.44–2.30%). This is another distribution pattern consistent with low oil-bearing legumes (4).

The FA classes in the seed oils of the legume cultivars were estimated by comparing integrals of proton NMR signals in the olefinic region. As shown in Table 4, the pattern is similar to the FA composition determined by capillary GC. The two methods of analysis agree on α-linolenic acid being the predominant FA component in all the seed oils. The proton NMR experiments overestimated diunsaturation, i.e., linoleic acid, and hence underestimated the total saturation of the oils. Nevertheless, the two methods of analysis agreed on α-linolenic and linoleic acids being the dominant FA in the seed oils.

The FA compositions of the oils from *P. vulgaris* cultivars estimated from capillary GC and proton NMR analyses (Table 4) largely corroborate measurements of the physicochemical characteristics of the oils (Table 1). The rather high RI values were an indication of the presence of considerable amounts of PUFA in the oils (10). The IV would appear to have been underestimated in view of the total percentage unsaturation determined from the GC and ^{1}H NMR analyses; however, the total saturation of about 20% in the oils could account for the average IV of 83.9 (Wijs method). The high content of linolenic acid, 18:3n-3, would increase the susceptibility of the oils to oxidation and hence result in high OV, as shown in Table 2. The average FA chain length of C_{17} calculated from the ^{1}H NMR experiments agrees very well with the indications from the SV and the FA composition of the test samples estimated by GC.

The results of this study showed that the FA compositions of oils from the beans of *P. vulgaris* cultivars grown in the highlands and the foothills of Lesotho in southern Africa are quite similar to the general FA composition of the oils from cultivars of *P. vulgaris* grown in other parts of the world. Thus, different climatic, soil, and general environmental conditions do not appear to affect the general properties of the lipids in the seeds of *P. vulgaris* cultivars. In particular, being low oil-bearing seeds, the six cultivars in this study contained α-linolenic and linoleic acids as their dominant FA. Therefore, consumption of the beans of these cultivars, in addition to providing such nutrients as proteins, carbohydrates, and minerals, must also impart some of the widely acclaimed health benefits of the n-3 and n-6 EFA to the people of southern Africa. In particular, notwithstanding their low lipid content, the populations in southern Africa obtain a good amount of α-linolenic acid from consuming these beans, which would help reduce the risk of coronary heart disease in the region (5,6).

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