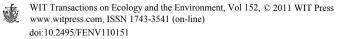
Nutrient constituents, functional attributes and *in vitro* protein digestibility of the seeds of the *Lathyrus* plant

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Abstract

Lathyrus seeds are a major component of human diets especially in regions with marginal soils and during drought-induced famine. 20 lines comprising ten of Lathyrus sativus (4 local and 6 improved), 5 lines of Lathyrus cicera and 5 lines of *Lathyrus ochrus* were analysed for proximate constituents, energy values, nutritionally valuable minerals, functional properties and *in vitro* multi-enzyme protein digestibility. The mean values for crude protein, crude fibre, ether extract, ash, nitrogen-free extract and gross energy in L. sativus (local) were 24.9, 4.0, 9.8, 3.7, 51.7g/100g DM and 441.6kcal/100g respectively, while the corresponding values for the improved varieties were 22.9, 5.1, 6.7, 3.9, 55.9g/100g/DM and 417.6 kcal/100g. The corresponding proximate values for L. cicera were 20.4, 4.1, 3.8, 3.0, 63.3g/100g DM and 403.2kcal/100g; and L. ochrus were 22.9, 7.0, 6.1, 3.5, 54.8g/100g DM and 407.7kcal/100g respectively. Mg, K, Na, Ca and P were the most abundant minerals in all the species analysed. Mn was not detected in some lines of L. sativus (improved), L. cicera and L. ochrus while none was found in local L. sativus lines. Water absorption capacity (WAC) ranged from 120% in L. sativus (improved), L.cicera and L. ochrus to 250% in lines 527, 508 and 504 of L. sativus. Among the improved lines, Oil absorption capacity (OAC) ranged from 138% to 294.4% in L. sativus and between 184% and 294.4% in L. ochrus. Foaming capacity and Foaming stability at 30mins were similar in L. cicera and L. ochrus while wide variations were observed in local and improved varieties of L. sativus as



shown by the high coefficients of variation of 31.7 and 36.2% respectively. The emulsion capacity and emulsion stability of all the species showed little interspecies variabilities. The seed flours from all the species had varying protein solubilities with changes in pH. The proteins generally had multiple maxima and minima solubilities with pH changes. The mean *in vitro* multi-enzyme protein digestibility ranged from 75.0% in *L. sativus* (local) to 77.4% in *L. cicera*.

Keywords: lathyrus seeds, nutritive characteristics, functional properties, in vitro protein digestibility.

1 Introduction

Efforts to increase the availability of protein in man's diet have encouraged the use of high-protein plant materials such as the legumes. They are considered as a good source of proteins for a large segment of the world's population, especially for the people of the developing countries (Altchul and Wilcke [1]). Most of the legumes fix their required nitrogen, and thereby reduce the demand for chemical fertilizers.

The genus *Lathyrus* is classified under the family leguminosae which includes about 150 species distributed within the West Africa and North Africa regions (Aletor *et al.* [2]). The seeds may be eaten raw as snacks or the flour used to adulterate the more expensive flours. The attraction of *Lathyrus* in these regions derives from its desirable agronomic characteristics, ease of cultivation, drought tolerance, low fertilizer requirements, high resistance to pests and diseases and reasonably good yields. It is often referred to as "insurance crop" as it produces reliable yields when all other crops fail especially during drought-induced famine. The major limitations to their full utilization as food is, however, the presence of the potent, low-molecular weight neurotoxin named either β -Noxalyamino-L-alanine (BOAA) or β -N-oxaly- α , β -diaminopropionic acid (ODAP) (Aletor *et al.* [2]). This non-protein has been implicated in the development of the neurodegenerative condition – Lathyrism – in humans [2, 3].

While most studies on the quality of *Lathyrus* have centred mainly on the anti-nutrients and neurotoxins ,there is limited information on the nutritive characteristics of these seeds. This paper therefore reports the relative nutritive potentials of these seeds as well as their *in vitro* multi-enzyme protein digestibility.

2 Materials and methods

2.1 Materials

The legume seeds analysed were local or their improved comprising *Lathyrus sativus* (common chickling or grass pea, 10 lines) *Lathyrus cicera* (dwarf chickling, 5 lines) and *Lathyrus ochrus* (ochrus chickling, 5 lines). The samples were improved lines maintained in the germplasm collection at International Centre for Agricultural Research in the Dry Areas (ICARDA) where the breeding programme entailed the multiplication of seeds in nursery rows, and



evaluates selected genotypes in microplots and promising genotypes in advanced yield trials. Clean mature seeds from individual lines were ground to a fine flour using a UD Cylone sample mill with a 1 mm mesh sieve (UD Corporation, Fort Collins, CO, USA) before analysis. All reagents were of analytical grade.

2.2 Methods

2.2.1 Chemical and physico-chemical analyses

Proximate analysis of the samples were carried out in triplicate, using the method described by [4]. Nitrogen was determined by the micro-kjeldahl method, described by [4] and percentage nitrogen was converted to crude protein by multiplying by 6.25. The minerals were analysed after dry-ashing at 550°C in a Muffle furnace and dissolved in deionized water to standard volume. Sodium and phosphorus were determined by flame photometry while phosphorus was determined by the vanado-molybdate method [4]. The other minerals: Mg, Ca, Zn, Mn, Fe and Cu were determined using an atomic absorption spectrophotometer (AAS) [5]. The gross energy content of the different samples was computed from the proximate constituents as described by Ng and Wee [6].

2.2.2 Determination of the functional properties

The protein solubilities (PS) of the seeds flour were determined as described by Oshodi and Aletor [7] and Adeyeye *et al.* [8] while the water absorption capacity and fat emulsion stability were determined by the procedure of [9]. The oil absorption capacity (OAC) was determined as described by [10]. Similarly, the lowest gelation concentration (LGC), forming capacity (FC) and foaming stability (FS) of the LPCs were analysed using the technique of Coffman and Garcia [11].

2.2.3 Determination of *in vitro* protein digestibility (IVPD)

The IVPD was determined using the procedure of Hsu *et al.* [12]. The multienzyme used contained (mg/cm³) 1.6 mg porcine pancreatic trypsin (EC 3.4.21.4), 3.1 mg bovine chymotrypsin (EC 3.4.21.1) and 1.3 mg bovine intestinal peptidase (EC.3.4.23.1) which were purchased from Sigma-Aldrich, Dublin, Ireland. 50 cm³ of the aqueous suspension of the respective seed flours (6.25 mg/sample/cm³) in distilled water was adjusted to pH 8 with 0.1M HCl and or 0.1M NaOH while stirring in water bath maintained at 37°C. A 5 cm³ aliquot of multi-enzyme solution was added to the sample suspension with constant stirring at $37 \pm 2^{\circ}$ C. The pH of the suspension was recorded 15 mins afterwards. The *in vitro* digestibility was thereafter calculated using the regression equation of Hsu *et al.* [12]: Y = 210.46 – 18.10X, where Y is the *in vitro* digestibility in (%) and X is the pH of the sample after 15 mins digestion with the multi-enzyme solution.



2.2.4 Data analysis

All data used were means of triplicate determinations. The coefficient of variation (CV) among the different species were also determined as described by Steel and Torrie [13].

Energy (ccal/100g) 466.86 452.25 424.21 422.93 441.56 18.73 4.24 406.24
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417.56
13.18
3.16
419.30
392.28
397.46
376.00
431.02
403.21
19.62
4.87
465.67
358.87
357.25
422.78
433.81
407.68
42.89
10.52

Table 1:	Proximate composition (g/100 g DM) of the seeds of Lathyrus
	sativus, Lathyrus cicera and Lathyrus ochrus.

Means are for triplicate determinations. DM, Dry matter; CP, Crude Protein; CF, crude fibre; EE, ether extract; NFE, nitrogen free extract; S.D, standard deviation; C.V, coefficient of variation.



3 Results and discussions

The dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash, nitrogen free extract (NFE) and energy values are shown in Table 1. The mean DM values of all lines analysed were similar and ranged from 95.10 ± 1.54 g/100g DM in *L. sativus* (improved) to 94.03 ± 0.90 g/100g DM in *L. sativus* (local). The mean CP content was lowest in *L. cicera* 20.04 \pm 0.68 g/100g DM and highest in *L. sativus* (improved) 24.85 ± 1.15 g/100g DM. The mean values of CF ranged from 4.02 ± 1.22 g/100g DM in *L. sativus* (local) to 6.95 ± 4.78 g/100g DM in *L. ochrus* with low intra and inter species variations. There were large variabilities among and within species in EE values as indicated by respective coefficient of variation (CV) 25.56; 39.98; 88.33; and 76.51% in *L. sativus* local; *L. sativus* improved; *L. cicera* and *L. ochrus*, respectively. The mean values for ash were similar and ranged from 2.96 \pm 0.68 g/100g DM in *L. cicera* to 3.89 ± 0.20 g/100g DM in *L. sativus* (improved).

The mean gross energy (GE) values were similar and ranged from 403.21 ± 19.62 k cal/100 g in *L. cicera* to 441.56 ± 18.73 k cal/100 g in *L. sativus* (local). Intra specie variations in GE were generally low ($\leq 4.87\%$) in all *L.* specie except in *L.ochrus* with a relatively high coefficient of variation (CV) of 10.52%.

Analysis of the proximate constituents clearly indicate the potential of Lathyrus seeds as rich sources of nutrient for both humans and animals. For example, the mean CP, CF and EE content of all the species analysed compared favourably with that reported for cowpeas (Aletor and Aladetimi [14]) and some other leafy vegetables reported by [15, 16] but lower than those for more conventional legumes such as Parkia biglobosa seeds (Aletor et al. [17]) and some of conventional protein concentrates/isolates [18]. The ash content were generally lower than those reported for some leafy vegetables (Aletor *et al.* [19]) but compare well with those reported for soya bean protein isolate, potato protein isolate and corn glutein [20]. Higher NFE values which are a measure of the carbohydrates in foods were recorded for all the species especially in L. ochrus of lines 450. These values surpass those reported for some conventional protein concentrates or isolates reported by [20]. Of nutritional significance, particularly with regard to proximate constituents and energy value, is the relatively high nutrient density of all the seeds. Of the major nutritionally important minerals (Table 2) Mg, K and Na were the most abundant in all the lines analysed followed by P and Ca. There were similarities in the mean values of Mg as indicated by low coefficients of variations. Of the trace minerals, Fe and Zn was detected across board, Zn and Cu exhibited sporadic occurrences across lines. Mn and Co were not detected in L. sativus.

The concentration of the major minerals were generally high, and compared well with those reported by [21, 22] on some grain legumes and surpassed those reported for some leafy vegetables [23]. While these grain legumes represent useful sources of desirable minerals, they are usually less biologically available than those from animal origin [24]. The poor availability of minerals from plant sources derives largely, from the anti-nutritional effects of certain inherent antimetal agents such as phytates and oxalates which form chelates particularly with



			Major					Trace		
(Local)	Mg	Ca	K	Na	Р	Fe	Zn	Cu	Mn	Co
L. sativus										
527	404.4	75.40	461.59	264.99	71.89	1.85	7.00	4.86	ND	ND
508	399.36	60.37	445.49	334.09	91.89	28.57	155.46	0.39	ND	ND
504	458.78	79.79	487.19	319.82	75.86	3.98	70.28	ND	ND	ND
520	435.24	85.45	442.58	365.81	87.69	5.31	33.45	ND	ND	ND
Mean	424.38	75.25	459.21	106.25	81.83	9.93	66.55	2.63	-	-
S.D	24.17	9.30	17.70	29.04	8.22	10.83	56.04	2.24	-	-
C.V %	5.70	12.36	3.86	27.3	10.04	109.10	84.21	85.17	-	-
Improved L. sativus										
578	449.63	44.91	490.28	289.18	63.75	21.82	5.01	0.87	ND	ND
435	432.36	48.24	484.51	304.20	69.79	29.97	35.28	ND	0.60	ND
483	399.98	50.07	430.69	280.91	107.91	20.72	21.78	ND	0.60	ND
536	433.13	58.16	479.63	323.33	75.37	17.47	63.66	ND	ND	ND
535	401.75	50.93	439.02	321.46	81.67	16.11	36.12	ND	ND	ND
434	469.21	47.38	487.47	301.67	51.82	2.12	ND	ND	ND	ND
Mean	431.01	51.28	468.60	303.46	75.05	18.04	32.37	0.87	0.60	-
S.D	24.60	5.14	24.20	15.47	17.41	8.38	19.30	0.00	0.00	-
C.V %	5.71	10.02	5.16	5.10	23.20	46.45	59.62	0.00	0.00	-
L. cicera										
490	406.76	77.71	451.66	304.24	69.55	10.71	ND	ND	ND	ND
457	413.34	86.08	461.09	327.20	61.89	5.55	ND	ND	ND	ND
489	412.67	74.07	437.40	246.71	65.75	1.77	ND	ND	ND	ND
450	429.58	119.01	462.19	313.12	73.84	3.73	2.35	ND	0.65	ND
397	436.06	135.09	481.65	335.23	55.92	1.92	210.95	ND	0.67	ND
Mean	419.68	98.39	458.80	305.30	65.39	4.74	106.65	-	0.66	-
S.D	11.16	24.26	14.48	31.21	6.15	3.29	104.30	-	0.01	-
C.V %	2.66	24.66	3.16	10.22	9.45	69.41	97.80	-	1.52	-
L. ochrus										
539	418.36	94.86	450.36	320.23	75.35	25.14	45.31	ND	0.62	ND
545	439.29	92.00	458.28	332.75	69.57	32.08	4.85	0.42	ND	ND
543	430.35	61.78	447.74	339.71	71.78	20.08	ND	ND	ND	ND
538	443.54	88.66	467.94	346.19	75.94	1.89	ND	ND	ND	ND
104	424.02	94.38	442.07	281.41	69.85	1.77	ND	ND	ND	ND
Mean	431.11	88.34	453.38	324.06	72.50	16.19	25.08	0.42	0.62	ND
S.D	9.33	12.47	8.97	23.00	2.69	12.33	20.23	0.00	0.00	ND
C.V %	2.16	14.12	1.98	7.10	3.71	76.16	80.66	0.00	0.00	ND
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Table 2:Mineral compositions (mg/kg) of the seeds of Lathyrus sativus,
Lathyrus cicera and Lathyrus ochrus.

divalent minerals such as Ca, Zn, Mg and Fe thereby making them metabolically unavailable [25].

Table 3 presents the water absorption capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC) and foaming stability (FS), emulsion capacity (EC) and emulsion stability (ES), bulk density (BD) and least gelation concentration (LGC) of all the seed flours. The mean values (%) of WAC was $250 \pm 30.82\%$ in *L. sativus* (local); $180 \pm 42.82\%$ in *L sativus* (improved) while *L. cicera* and *L. ochrus* had the same value of $138\pm14.70\%$. The mean oil absorption capacity varied from $176.6 \pm 15.83\%$ in *L. cicera* to $239.20 \pm 41.8\%$ in *L. sativus* (local). The foaming capacity and stability at 30 mins were similar in *L. cicera* and *L. ochrus* ($8.20\pm0.75\%$; $8.60\pm1.02\%$), respectively while wide variations were observed in local varieties of *L. sativus* as shown by high coefficient of variations of 31.7 and 36.2% respectively. The emulsion capacity and emulsion stability of all the species at 1 hr showed little intra and inter-

Means are for duplicate determinations. S.D, standard deviation; C.V, coefficient of variation. ND, None detected.

(Local)	WAC	OAC	FC	FS (at 30	EC	ES (at	BD	LGC
(Eccul)	wite	one	10	mins)	LC	1 hr.	DD	100
L. sativus				mmo)		1		
527	250.00	230.00	9.00	10.20	44.88	43.59	0.60	0.20
508	250.00	266.80	4.00	10.20	41.56	38.96	0.64	0.20
504	250.00	276.00	9.00	10.20	39.74	38.46	0.62	0.16
520	180.00	184.00	5.00	10.20	37.98	35.44	0.07	0.20
Mean	250.00	239.20	6.50	10.20	41.04	39.11	0.48	0.19
S.D	30.82	41.8	2.06	0.00	2.55	2.92	0.28	0.02
C.V %	12.3	17.5	31.72	0.00	6.22	7.45	58.3	10.57
Improved	L. sativus							
578	210.00	239.00	4.00	10.20	43.21	43.21	0.68	0.20
435	200.00	184.00	5.00	10.20	37.50	37.50	0.72	0.20
483	240.00	138.00	6.00	10.20	43.75	41.25	0.64	0.14
536	120.00	184.00	5.00	10.20	40.00	40.00	0.70	0.20
535	180.00	276.00	4.00	10.20	40.00	35.00	0.70	0.20
434	130.00	294.40	10.00	10.20	37.04	34.57	0.70	0.20
Mean	180.00	219.27	5.67	10.20	40.25	38.59	0.69	0.19
S.D	42.82	55.31	2.06	0.00	2.55	3.18	0.25	0.02
C.V %	23.79	21.22	36.26	0.00	6.34	8.24	3.65	10.53
L. cicera								
490	140.00	184.00	8.00	10.30	43.75	43.75	0.71	0.18
457	160.00	174.80	8.00	10.30	37.98	37.80	0.68	0.18
489	140.00	193.20	7.00	10.30	37.18	39.74	0.68	0.20
450	130.00	184.00	9.00	10.30	36.71	44.30	0.61	0.20
397	120.00	147.20	9.00	10.30	37.50	40.00	0.73	0.20
Mean	138.00	176.64	8.20	10.30	38.62	41.12	0.68	0.19
S.D	14.70	15.83	0.75	0.00	2.60	2.50	0.04	0.01
C.V %	10.65	8.96	9.15	0.00	6.73	0.06	5.85	5.26
L. ochrus								
539	130.00	294.00	8.00	10.30	40.00	50.00	0.74	0.19
545	120.00	184.00	10.00	10.30	40.00	39.74	0.72	0.12
543	160.00	257.60	7.00	10.30	40.00	37.50	0.75	0.20
538	130.00	220.80	9.00	10.30	40.00	43.20	0.71	0.10
104	150.00	193.20	9.00	10.30	37.97	37.98	0.63	0.10
Mean	138.00	230.00	8.60	10.30	39.74	39.68	0.71	0.14
S.D	14.70	41.14	1.02	0.00	0.93	7.35	0.04	0.05
C.V %	10.65	17.89	11.86	0.00	0.02	0.19	5.64	35.71

Table 3:Functional properties (%) of the seeds of Lathyrus sativus, Lathyrus
cicera and Lathyrus ochrus.

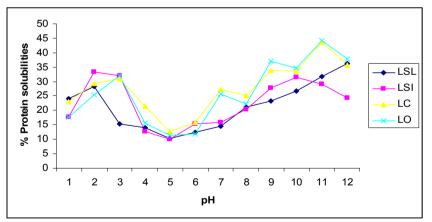
Means are triplicate determination. WAC, water absorption capacity; OAC, oil absorption capacity; FC, foaming capacity; FS, foaming stability; EC, emulsion capacity; ES, emulsion stability; BD, bulk density; LGC, least gelation concentration.

species variabilities. The mean bulk density in all the species analysed showed similar values except in *L. sativus* (local) which had a high intra specie variation as indicated by a (CV) of 58.3%. The mean least gelation concentration values were similar in *L. sativus* (local and improved) and *L. cicera* $0.19 \pm 0.1\%$ while $0.14\pm0.05\%$ was recorded for *L. ochrus*.

Results of the functional properties of these seeds clearly indicate their potential for the development of different food products. The results of water absorption capacity and oil absorption capacity showed that the *Lathyrus* seeds generally compared favourably with those of soya bean meal and flours from whole and rejected cashew nut reported by [16,17] but lower than those reported for soya bean protein isolate [20]. These functional attributes suggest their potential use in viscous foods such as soups and gravies as well as flavour retainers.

The values on foaming capacity and stability in all the species were rather low (4.00-10.00% and 10.20-10.30%), respectively, than the values of 6-12% and 104-108%) respectively reported by [18] for some conventional protein concentrates, but higher than those reported for *Glyricidia* leaf protein concentrate 3.7-4.9% (Aletor *et al.* [23]. The foaming capacity and stability are important since the success of whipping agents depends on their ability to maintain whip as long as possible. Results on emulsion capacity and emulsion stability were comparable with those reported for cashew nuts [10, 16]. This suggests their potential for use as additives for the stabilization of emulsions in the production of soups sausages and cakes. [1, 10]

Values for the least gelation concentration were low and similar (0.14%-0.20%) in all the species as compared with 8% reported for unfermented locust and soya bean seeds but higher than the fermented seeds [17]. Modification of proteins and carbohydrate generally occur during drying or processing which often cause denaturation. Such denatured proteins often coagulate and aggregate randomly instead of the desired continuous ordered net-work required for progel and gel formation [10, 16, 25]. The pooled mean protein solubility of the



Key: LSL, *L. sativus* (Local); LSI, *L. sativus* (improved); LC, *L. cicera*; LO, *L. ochrus*.

Figure 1: Pooled mean protein solubility of different *Lathyrus species* as a function of pH.

Lathyrus seed flour analysed (Fig. 1) were generally higher in both the acid and alkaline media indicating their usefulness in the manufacture/preparation of both acidic and alkaline foods such as gravies and mayonnaise.

The *in vitro* multi-enzyme protein digestibility (Table 4) were similar and ranged from 75.0% in *L. sativus* (local) to 77.4% in *L. cicera*. These digestibility data were quite similar to the 73.8% reported for unfermented locust and soya bean seeds Aletor *et al.* [17] and 74.3% for pumpkins reported by Fagbemi *et al.* [10] but lower than 85.9% reported for fermented locust and soya bean seeds (Aletor *et al.* [17].

(Local)	Digestibility after 15 mins.	(Local)	Digestibility after 15 mins.
L. sativus	unter re mins.	Improved	
527 508 504 520 Mean S.D C.V %	74.71 73.99 75.98 75.43 75.03 0.75 1.00	L. sativus 578 435 483 536 535 434 Mean S.D C.V %	75.25 73.62 72.90 78.87 75.43 76.16 75.37 1.92 2.64
L. cicera 490 457 489 450 397 Mean S.D C.V %	77.97 78.15 77.43 78.15 75.43 77.43 1.15 1.49	L. ochrus 537 545 543 538 104 Mean S.D C.V %	75.98 73.81 76.34 76.70 77.43 76.05 1.22 1.60

Table 4:In vitro protein digestibility (%) of the seeds of Lathyrus sativus,
Lathyrus cicera and Lathyrus ochrus.

Means are for duplicate determinations.

4 Conclusion

In all, the study clearly shows the high potential of Lathyrus in the provision of adequate levels of crude protein, energy, nutritional minerals, suitable functionality in food processing and highly digestible protein. Considering the desirable nutritive characteristics, functional attributes of the flour and the desirable agronomic traits of *Lathyrus* plants, it is conceivable that it represents a



veritable intervention crop to mitigate the food security challenge especially in regions with marginal soils and resource-poor base.

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