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Effect of Processing Methods on Anti-nutritional contents of Selected Lentils (*Lens Culinuris*) Varieties (Derash and Alemaya) Grown in Ethiopia

Daniel Asfaw[1] Solomon Abera[2]

Department of Food Science and Nutrition, Wollega University[1]

School of Food Science, Postharvest Technology and Processing Engineering, Haramaya University[2]

*Corresponding author: Daniel Asfaw, Department of Food Science and Nutrition, Wollega University, Ethiopia. Email: daniasfaw10@gmail.com

Abstract

Phytic acid and tannic acid are the two main anti-nutrients present in legumes. The aim of this study was to investigate the effects of various processing methods (autoclaving, boiling, dehulling, germinating and soaking) on anti-nutritional contents of the two selected lentil varieties (Alemaya and Derash). Anti-nutritional factors were reduced by processing methods as indicated in this finding. Dehulling, autoclaving, boiling, germinating and soaking process reduced tannin contents by 84.69, 61.73, 56.12, 36.22 and 12.24%, respectively. Phytic acid was reduced during boiling, autoclaving, germination and soaking process by 78.09, 76.32, 51.39 and 7.81%, respectively. Processing methods improved the anti-nutritional contents of studied lentil varieties.

Key words: *Lentil, Alemaya variety, Derash variety, Anti-nutritional contents, Processing methods*

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1. Introduction

The anti-nutrients like trypsin inhibitors, phytic acid, saponins, heamagglutinins and tannins are some of the undesirable components in legumes that could hinder utilization of important minerals including calcium, magnesium, iron and zinc by interfering with their absorption and utilization and thereby contributes to mineral deficiency (Vasagam and Rajkumar, 2011). Phytic acid and tannic acid are the two main anti-nutrients present in legumes. Tannins inhibit the digestibility of protein, whereas phytic acid reduces the bioavailability of some essential minerals (Van der Poel, 1990; Rehman and Shah, 2006).

The nutritionally important minerals, such as calcium, magnesium, copper, iron (Fe^{2+} and Fe^{3+}) and others form complexes with phytic acid, resulting in reduced solubility of the metals. Phytic acid and its salts represent the majority of the phosphorus in plant legume seeds and monogastric species have a limited ability to hydrolyse phytates and release phosphate for absorption. In dicotyledonous seeds such as legumes, phytic acid is found closely associated with proteins and is often isolated or concentrated with protein fraction of these foods. Phytic acid or phytate when in salt form is the principal storage form of phosphorus in plant tissues (Kumar *et al.*, 2010). It was formed during maturation of the plant seed and represents 60-90% of total phosphate in dormant seeds (Loewus, 2002).

Tannin in plant is involved in defense mechanism to environmental attack (Okuda *et al.*, 1992). It exists in mixtures with many other classes of plant phenolic compounds. Tannins are usually subdivided into two groups: hydrolyzable tannins (HT**)** and proanthocyanidins (PA**).** Hydrolyzable tannins are more susceptible to enzymatic and non-enzymatic hydrolysis than proanthocyanidins, and usually are more soluble in water. The ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects (Hagerman and Butler, 1981).

In Ethiopia people consume lentils after passing through different traditional processing. However, there is a research gap on the effect of these processing methods on anti-nutritional contents of lentil. Therefore, the objective of the present study was to determine the effect of processing methods on anti-nutritional contents of lentils varieties.

2. Materials and Methods

2.1. Experimental Location

The study was conducted at Haramaya University. Chemical analysis such as crude protein, crude fat, crude fiber, ash content, moisture content and anti-nutritional content, were determined at School of Food Science, Post Harvest Technology and Processing Engineering laboratory. Mineral analysis was carried out at School of Food Science, Post Harvest Technology and Processing Engineering and Soil laboratories.

2.2. Experimental Materials

The samples for investigation i.e 6 kg seed of each of the lentil varieties Derash and Alemaya, were collected from Bishoftu Agricultural Research Centre (BARC), national legumes improvement program.

2.3. Experimental Design

Completely randomized design (CRD) with a 2×6 factorial experiment with three replication was implemented. Two selected lentil varieties, Derash and Alemaya, were tested under five different processing methods (dehulling, soaking, germination, autoclaving, and boiling) with one untreated sample (control) for each variety (Table 1).

Table 1. Experimental plan

Factor-2, Processing	Factor -1, Variety	
	De	Al
Au	AuDe	AuAl
Bo	BoDe	BoAl
Dh	DhDe	DhAl
Ge	Ge De	Ge Al
Ra	RaDe	RaAl
So	SoDe	SoAl

Where Al = Alemaya variety, De = Derash variety, Au = Autoclaving, Bo = Boiling, Dh = Dehulling, Ge = Germinating, Ra = Raw/Control sample, So = Soaking

2.4. Sample Preparation

Samples of each of Derash and Alemaya lentil varieties were cleaned manually by removing any foreign material, damaged and broken seeds. The seeds were processed by dehulling, soaking, germination, boiling and autoclaving.

The processed samples including the control sample were dried in an oven at 50°C for about 24 hr. All samples were milled by laboratory miller (cyclo sample mill model no: 3010-081p) and pass through 75 µm sieve. The flours were packed in moisture proof plastic bags and stored in airtight tin containers at 4°C until required for analysis.

2.5. Processing Techniques

2.5.1. Direct grinding

Cleaned seed of 600 g each of Derash and Alemaya varieties were directly ground by mill.

2.5.2. Dehulling

A 600 g cleaned seed of each of Derash and Alemaya varieties were dehulled by pestle and mortar. The hull was separated using traditional tool (gundoo) from split seeds. The split lentil was milled as indicated above.

2.5.3. Soaking

The 600 g of clean seed each of the two lentil varieties were soaked in distilled water for 12 hr at room temperature (Osama *et al.,* 1985). The soaked samples were drained and rinsed three times with 600 ml distilled water. The samples were oven dried at 50ºC for 24 hr. The dried samples were milled and passed through 75 µm sieve. The flours were packed in moisture proof plastic bags and stored in airtight tin containers at 4ºC until required for analysis.

2.5.4. Autoclaving

The 600 g cleaned seed each of the two lentil variety were soaked in distilled water (1:10, w/v) for 12 hr at room temperature (25 °C). The soaked samples were drained and rinsed three times with 600 ml distilled water. The rinsed soaked samples were autoclaved at 121°C under 15 lb pressure in distilled water (1:10, w/v) until they became soft when felt between the fingers (35 min) (Hefnawy, 2011) and immediately dried in oven drying at 50°C for 24 hr. The dried sample were milled and pass through 75 µm sieve. The flours were packed in moisture proof plastic bags and stored in airtight tin containers at 4ºC until required for analysis.

2.5.5. Germination

The 600 g seed of each of Derash and Alemaya varieties were cleaned and soaked in distilled water at room temperature for 12 hr. The soaked samples were drained and rinsed three times with 600 ml distilled water. The water was drained off and the samples covered in trays lined with absorbent paper and left to germinate for 72 hr (Mashair *et al*., 2008). At the end of the germination process, the samples were oven dried at 50ºC for 24 hr, milled and passed through 75 µm sieve. The flours were packed in moisture proof plastic bags and stored in airtight tin containers at 4ºC until required for analysis.

2.5.6. Boiling

The 600 g cleaned samples of each of the two varieties were soaked in distilled water (1:10, w/v) for 12 hr at room temperature (25°C). The soaked seeds were drained and rinsed three times with 600 ml distilled water. The samples were then boiled in distilled water in the ratio of 1:10 (w/v) until they became soft when felt between the fingers (90 min) (Hefnawy, 2011) and immediately dried in oven at 50°C for 24 hr. The dried samples were milled and passed through 75 µm sieve. The flours were packed in moisture proof plastic bags and stored in airtight tin containers at 4ºC until required for analysis.

2.6. Anti-nutritional Factors Analysis

2.6.1. Tannin content

Condensed tannin was analyzed by vanillin-HCl method of Price *et al.,* (1980) using the modified Vanillin-HCl methanol method. Tannins content was expressed as catechin equivalent as follows:

$$Tannin\ (\%) = \frac{C \times 10 \times 100}{200} \qquad (4)$$

Where,
C = Concentration corresponding to the optical density.
10 = Volume of the extract (mL).
200 = Sample weight (mg)

2.6.2. Phytic acid

Phytic acid content was determined according to Wheeler and Ferrel, (1971). The amount of phytic acid was estimated by multiplying phytate-phosphorous with 3.55 based on empirical formula $C_6P_6O_{24}H_{18}$.

2.7. Statistical Analysis

Anti-nutrients of the raw and processed samples were statistically analyzed using analysis of variance (ANOVA) and least significant difference (LSD). The statistical package used was (SAS Institute and Cary, NC). Significant differences were determined at the $P \leq 0.05$ level using Fisher LSD to identify significant differences among mean effects of the varieties and processing methods.

3. Results and Discussions

3.1. Anti-nutritional Contents in Raw Lentil Varieties

As indicated in Table 2, anti-nutritional factors of the two varieties are significantly ($P \leq 0.05$) different from each other. The average values of tannin were 1.91 and 2.01 mg/gm for Alemaya and Derash, respectively. As observed in this finding the value of tannin in Derash is greater than that of Alemaya variety. Both values were lower than those obtained in red lentil varieties (5 – 6.5 gm/kg) (Wang, 2008). However, they were close to that obtained by El-Adawy, (2003) on Giza 9 lentil variety. The difference in tannin content between the two selected lentil varieties may be due to difference in their seed coat thickness as large portion of the tannin in pulses is found in the seed coat pigments (Vasic *et al*., 2011).

The phytic acid content of the two selected lentil varieties were also significantly ($P \leq 0.05$) different from each other. The average values of the two selected varieties were 3.84 and 4.10 mg/gm for Alemaya and Derash, respectively. These values were less than the values obtained by Wang, (2008) and El-Adawy, (2003) on different lentil varieties.

Table 2. Anti-nutritional factors of the two lentil varieties

Variety	Tannin (mg/g)	Phytic acid (mg/g)
Al	1.91 ± 0.01^b	3.84 ± 0.04^b
De	2.01 ± 0.002^a	4.10 ± 0.06^a
CV	0.43	2.27
LSD	0.02	0.20

Al = Alemaya variety, De = Derash variety, CV = coefficient of variation; LSD = least significant difference; values followed by the same letter in a column are not different at 5% level of significance.

3.2. Effect of Processing Methods on Anti-nutritional Contents

3.2.1. Tannin

Tannin contents were significantly ($P \leq 0.05$) different between processed sample of the two varieties (Table 3). The average values were 1.09 and 1.19 mg/gm for Alemaya and Derash, respectively, and are lower than those found in the raw lentil viz., 1.91 and 2.01 mg/g for Alemaya and Derash, respectively (Table 2.).

Processing methods resulted in significantly ($P \leq 0.05$) different amount of tannin contents (Table 4.). The tannin content decreased by 12.24, 36.22, 56.12, 61.73 and 84.69% due to soaking, germinating, boiling, autoclaving and dehulling processing methods, respectively. The highest reduction was noted after dehulling, followed by autoclaving, boiling, germinating and soaking. The highest reduction in dehulled sample might be due to the fact that predominant amount of tannin is found in seed coat which has been removed during dehulling process. Autoclaving and boiling also decreased tannin content to significant level. The reduction of tannins after soaking, boiling and autoclaving is due to the fact that the large part of the tannin in seed coats are water soluble and consequently leached into the liquid medium (Reddy and Pierson 1994). Wang and Hatcher, (2009) reported that cooking lentil in boiling water significantly reduced phytic acid and tannins. The reduction of tannin content by autoclaving and boiling was also reported by Hefnawy, (2011); Mubarak, (2005) and Khattab *et al.* (2009).

Germination and soaking also reduced tannin content. Many workers (Mubarak, 2004; Kayembe and Rensburg, 2013; Kakati *et al*., 2010) reported that tannin content and anti-nutritional factors (Vidal-Valverde and Frias, 1992) are reduced in legumes by germination.

Table 3. Anti-nutritional factors in processed lentil samples

Variety	Tannin (mg/g)	Phytic acid (mg/g)
Al	1.09 ± 0.12^{a}	2.41 ± 0.35^{b}
De	1.19 ± 0.17^{a}	2.81 ± 0.35^{a}
CV	14.72	11.77
LSD	0.23	0.36

Al = Alemaya variety, De = Derash variety, CV = coefficient of variation; LSD = least significant difference; values followed by the same letter in a column are not different at 5% level of significance.

The interaction of both varieties and processing methods resulted in significantly ($P \leq 0.05$) difference on tannin contents (Table 5). The maximum (2.01 mg/gm) and minimum (0.17 mg/gm) values were recorded in raw Derash and dehulled Derash samples, respectively. Higher tannin contents were found in raw Derash, soaked Derash, raw Alemaya and germinated Derash with average values of 2.01, 1.94, 1.91 and 1.50 mg/g, respectively. The low tannin contents were found in dehulled Derash, dehulled Alemaya and authoclaved Derash with average values of 0.17, 0.44 and 0.55 mg/g, respectively, in which processing methods dominates in the interaction of the two factors.

Table 4. Effect of processing methods on the anti-nutritional factors

Processing methods	Tannin (mg/gm)	Reduction (%)	Phytic acid (mg/gm)	Reduction (%)
Au	0.75 ± 0.09^{d}	61.73	0.94 ± 0.05^{d}	76.32
Bo	0.86 ± 0.06^{d}	56.12	0.87 ± 0.05^{d}	78.09
Dh	0.30 ± 0.06^{e}	84.69	4.29 ± 0.01^{a}	-8.06
Ge	1.25 ± 0.11^{c}	36.22	1.93 ± 0.33^{c}	51.39
Ra	1.96 ± 0.02^{a}	----	3.97 ± 0.07^{ab}	----
So	1.72 ± 0.10^{b}	12.24	3.66 ± 0.14^{b}	7.81
CV	14.72		11.77	
LSD	0.23		0.36	

Al = Alemaya variety, Derash variety, Au = autoclaving, Bo = boiling, Dh = dehulling, Ge = germinating, Ra = raw, So = soaking, CV = coefficient of variation; LSD = least significance difference; values followed by the same letter in a column are not different at 5% level of significance

Table 5. Interaction effect of variety and processing methods on Tannin content

Variety	Processing methods					
	Au	Bo	Dh	Ge	Ra	So
Al	0.96 ± 0.01^{f}	0.73 ± 0.01^{g}	0.44 ± 0.03^{i}	1.01 ± 0.04^{e}	1.91 ± 0.01^{c}	1.49 ± 0.01^{d}
De	0.55 ± 0.01^{h}	0.99 ± 0.04^{ef}	0.17 ± 0.04^{j}	1.50 ± 0.04^{d}	2.01 ± 0.01^{a}	1.94 ± 0.03^{b}
CV	1.45					
LSD	0.03					

Al = Alemaya variety, Derash variety, Au = autoclaving, Bo = boiling, Dh = dehulling, Ge = germinating, Ra = raw, So = soaking, CV = coefficient of variation; LSD = least significance difference; values followed by the same letter in a column are not different at 5% level of significance

3.2.2. Phytic acid

Table 3, indicated that the two varieties showed significantly (P≤0.05) different in phytic acid contents. The average values for Alemaya and Derash were 2.41 and 2.81 mg/gm, respectively. These values were lower than those of the untreated samples with average values of 3.84 and 4.10 mg/g, respectively (Table 2.).

Processing methods, exhibited significant (P≤0.05) effect on phytic acid contents (Table 4.). The phytic acid was reduced by 7.81, 51.39, 76.32 and 78.09% for soaking, germinating, autoclaving and boiling methods, respectively. Except dehulling all processing methods reduced phytic acid contents. The highest reduction was found in boiling followed by autoclaving, germinating and soaking processing methods. Cooking lentil in boiling water significantly reduced phytic acid and this was also reported by Wang and Hatcher, (2009). Level of phytic acid in untreated faba bean was 8.36 mg/g, while it ranged from 1.34 to 7.70 mg/g in treated samples (Luo *et al*., 2013).

Germination reduced phytic acid significantly. This may be because of utilization of phytate as a source of inorganic phosphate during seed germination and the inorganic form becomes available for purposes of plant growth and development. Germination reduces and/or eliminates considerable amounts of phytate from the seeds or grains as reported by Sudermadji and Markakis, (1977). Soaking cereal and most legume in water can result in passive diffusion of water soluble phytate, which can then be removed by decanting the water (*Hotz and Gibson, 2001; Perlas and Gibson, 2002).* Soaking of millet, soya bean, maize, sorghum, and mung bean at 30°C for 24 h decreased the contents of phytic acid by 4-51% (Lestienne *et al*., 2005a; Lestienne *et al*., 2005b), and soaking of sorghum flour (80% extraction) at room temperature for 24 hr reduced phytic acid levels by 16-21% (Mahgoub *and* Elhag, 1998). Similarly the reduction of the phytic acid content during soaking, cooking or germination has been reported by many investigators, (Kakati *et al*., 2010; El-Adawy *et al*., 2003; Alonso *et al.,* 1998 and Alonso *et al.,* 2000) on different legumes. Processes, such as soaking and germination, activate the endogenous phytases which are able to hydrolyse IP6 to free myo-inositol and inorganic phosphate via lower inositol phosphate esters (IP5–IP1) (Kozlowska *et al*., 1996; Honke *et al*., 1998).

Dehulling increased the phytic acid content of lentil. This might be due to the high concentration of phytate in cotyledon as tannin concentrated in legumes' seed coats. Wang and Hatcher, (2009) reported that dehulling resulted in a significant increase in phytic acid. The increase of phytic acid during dehulling is also reported by Wang, (2008).

The interaction of the two factors significantly (P<0.05) affected the phytic acid contents. As indicated in Table 6, the phytic acid content values were significantly different from one another varying between 0.77 mg/gm for boiled Alemaya and 4.32 mg/gm for dehulled Derash.

Table 6. Interaction effect of variety and processing methods on phytic acid content

Variety	Processing methods					
	Au	Bo	Dh	Ge	Ra	So
Al	1.04 ± 0.03^{h}	0.77 ± 0.01^{i}	4.27 ± 0.01^{a}	1.18 ± 0.01^{g}	3.84 ± 0.04^{d}	3.35 ± 0.01^{e}
De	0.83 ± 0.03^{i}	0.98 ± 0.01^{h}	4.32 ± 0.01^{a}	2.67 ± 0.03^{f}	4.10 ± 0.06^{b}	3.96 ± 0.01^{c}
CV	1.82					
LSD	0.08					

Al = Alemaya variety, Derash variety, Au = autoclaving, Bo = boiling, Dh = dehulling, Ge = germinating, Ra = raw, So = soaking, CV = coefficient of variation; LSD = least significance difference; values followed by the same letter in a column are not different at 5% level of significance

4. Conclusion and Recommendation

4.1. Conclusion

According to this finding processing methods reduced the anti-nutritional factors into different levels. The highest reduction of tannin content was observed in dehulled samples and the lowest reduction was found in soaked samples. Dehulling process increased the content of phytic acid, however, boiling process was the highest reduction of phytic acid.

4.2. Recommendation

The issues which may be considered in the future study are the following:

- ✓ The effect of combination of these processing methods on anti-nutritional contents.
- ✓ The effect of these processing on anti-nutrients such as trypsin inhibitor, hemagglutinin, saponin and lecetins.

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