

Effects of Natural Fermentation and Toasting on Nutritional Composition and Antinutrient Contents of Ethiopian Oat Grains

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Received: October 14, 2022

Accepted: May 22, 2023

Published: May 26, 2023

Citation: Alemayehu GF, Forsido SF, Tola YB, Amare E. 2023. Effects of Natural Fermentation and Toasting on Nutritional Composition and Antinutrient Contents of Ethiopian Oat Grains. *J Food Chem Nanotechnol* 9(2): 54-62.

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Abstract

Traditional processing methods, such as toasting and natural fermentation, are widely used in Ethiopia to improve the flavor, texture, and palatability of food products. However, the nutrient value of oat foods can be altered because processing has both a qualitative and quantitative impact on the oat matrix, and processing is also responsible for the cleavage of antinutrient-nutrient complexes, resulting in free nutrients. This research examined the effects of varieties and cultural processing methods on the proximate composition and antinutrients of two local and one improved variety of oats. Oat grains were toasted for 3 hrs at 115 °C, ground into flour, and fermented naturally for 24 and 48 hrs, with raw oat flour serving as a control. Raw and processed oats were evaluated following standard analytical methods. The results showed that toasting reduced phytate (0.5 - 2.0%) and tannin (1.7 - 5.3%) while increasing carbohydrates (5.7 - 8.4%). Natural fermentation of oats for 24 and 48 hrs resulted in a significant ($p < 0.05$) reduction in crude fat (5.8 - 17.5%), carbohydrate (2.9 - 12.9%), phytate (26.9 - 37.6%), and tannin (35.0 - 53.8%), while it improved crude protein (11.0 - 30.3%). In all oat varieties, natural fermentation for 48 hrs had the highest protein content than raw (control), toasted, and 24 hrs fermented oat flour slurry. Similarly, toasted flour had the highest total carbohydrate and gross energy values compared to raw and fermented oats flour slurry. Fermentation and toasting are low-cost methods of cultural processing that should be valued and encouraged.

Keywords

Antinutrients, Food composition, Natural fermentation, Nutrition, Oat, Toasting

Introduction

Oat (*Avena sativa*) is a nutritious cereal grain with high functional food potential. Oats are high in proteins with essential amino acids, fats with high unsaturated fatty acids, dietary fiber with high beta-glucan, phytochemicals, and micronutrients [1]. Oats' nutritional interest is primarily due to their high content of soluble dietary fiber, β -glucan, and a unique antioxidant, avenathramide [2].

In Ethiopia, oat production and utilization are limited to a few districts in northwest Ethiopia [3]. The high altitude (1000 - 3200 m) and rainy areas of northwest Ethiopia cultivated local varieties of oats for human consumption for many years. However, most people in other parts of the country do not still know its importance as a human food [4]. The same authors assess the food significance and document the associated traditional food processing techniques from oats in the Gozamin district, East Gojjam zone of northwest Ethiopia [3]. Oats are commonly processed in Ethiopian households and communities into a wide

range of foods such as *Injera*, *Anebabiro*, *Kitta*, *gruel*, *Enket*, porridge, and *Tella* [3]. Some of the foods are lightly heated (toasted), and some are fermented naturally.

Local populations in developing countries including Ethiopia incorporate low costs processing techniques such as fermentation and toasting to improve the nutritional values of readily available cereals such as oats. These techniques are encouraged in communities greatly affected by malnutrition and micronutrient deficiencies. Previous studies reported that heat treatment and natural fermentation of cereals significantly reduced the antinutrients [5, 6]. Antinutrients are abundantly present in cereals including oats [1]. They are well known to limit iron absorption among others, especially in infants with developing immune systems.

Processing is one of the methods used to reduce the antinutrient contents and increase the nutritional value of cereals. Thermal processing of oats improves oat foods' texture, palatability, and nutritive value by gelatinization of starch, denaturation of proteins, increasing nutrient availability, increasing the extractable β -glucan content, inactivation of heat-labile toxic compounds, and other enzyme inhibitors [7-10]. While fermenting cereal grains also improve their nutritional value and significantly reduces antinutrient content [11, 12]. According to Das and Maria [13], fermented oats are higher in carbohydrates, protein, fat, fiber, amino acids, vitamins, and minerals than unfermented oats.

There have been some findings regarding the effects of modern pretreatments on the nutritional value of oats. However, Ethiopian local oat varieties were not studied for their nutritional values, or the changes associated with traditional processing methods as practiced at home. Therefore, this study examined the effect of natural fermentation and toasting on the nutritional composition and antinutrient content of one improved and two local oat varieties cultivated in the Gozamin district, Ethiopia.

Materials and Methods

Sample collection

One improved (Goslin) and two local (black and white-colored) oat varieties were obtained from Adet Agricultural Research Center and Gozamin district, respectively. The grains were cleaned to remove foreign particles and ruptured ones. They are then dehulled to remove the husk, packed into polyethylene bags, and stored at room temperature until further analysis.

Experimental design and treatments

The experiment had two factors, namely oat variety (white-colored oat, black-colored oat, and Goslin) and traditional processing methods (raw or untreated, toasting, 24 hrs fermentation, and 48 hrs fermentation). The effect of fermentation on *Anebabiro* fermented oat slurry (common oat food for the locals) was chosen as the study's subject. This natural fermentation experiment was carried out like the local *Anebabiro* fermentation, not only in terms of fermentation time but also of ingredient mixing ratios (flour to water). Natural

fermentation of oat flour for *Anebabiro* food has traditionally taken between 12 and 24 hrs; thus, these two extreme fermentation times were chosen to investigate the changes in nutrient and antinutrient content. A 3 x 4-factorial design was employed to study the main and interaction effects of oat varieties and processing methods on the proximate composition and antinutrients of oats. A completely randomized design was employed, and the experiment was carried out in triplicate.

Toasting and natural fermentation

The oat grains (toasted and control) were ground into flour (RRH-200, Zhejiang, China) and sieved with a 0.05 mesh sieve, and stored at 4 °C until the next experiments. Toasting was done using the method described by Sandhu et al. [14]. Oat grains (300 g) were toasted in an oven (DHG-9203A, Shanghai, China) at 115 °C for 3 hrs. Natural fermentation of raw oat grains flour was conducted per the procedure outlined in Ibrahim et al. [15]. Briefly, in a 1000 ml beaker, 250 g raw oat grain flour was combined with 500 ml distilled water and naturally fermented for 24 and 48 hrs at ambient temperature (22 ± 2 °C). The fermented slurry was then homogenized with a glass stirring rod, placed in the aluminum dish, and then dried in the oven (DHG-9203A, Shanghai, China) at 45 °C for 20 hrs. After drying, it was milled and sieved through a 0.05 mm hole sieve and stored at 4 °C until the next analysis.

Analysis of proximate compositions

Moisture content

The AOAC [16] official method 925.10 was used to determine the moisture content of the sample flour. After weighing the pre-cleaned aluminum plate (W_1), 5 g (W_2) of the sample was added to it and heated in a 105 °C oven (LABQUIP, LEICESTER LE67 5FT, England) for 3 hrs. The plate and its contents were cooled in a desiccator (CSN-SIMAX) for 30 min and reweighed until a constant weight (W_3) was achieved. The moisture content was then calculated using weight loss by difference ($W_3 - W_1$), and the percentage of moisture was calculated using equation 1.

$$\text{Moisture (\%)} = \frac{(W_3 - W_1)}{W_2} \times 100 \quad (1)$$

Crude protein content

The crude protein content of the samples was determined using the automatic Velp Scientifica Kjeldahl analyzer (UDK 159) according to the AOAC [16] official method 979.06. In brief, 1.0 g of sample flour was mixed with a catalyst (K_2SO_4 and $CuSO_4 \cdot 5H_2O$) and digested in 12 ml of concentrated H_2SO_4 at 420 °C for 60 min to release bound nitrogen in the form of $(NH_4)_2SO_4$. The ammonia in the digested $(NH_4)_2SO_4$ was then distilled off using 50 ml of distilled water, 30 ml of H_3BO_3 , and 50 ml of NaOH, and then automatically titrated with 0.2 N HCl. The total nitrogen content (%) was calculated as:

$$\text{Nitrogen (\%)} = \frac{(V_s - V_b)_{HCl} \times N_{HCl} \times 14.01}{\text{gram of sample}} \times 100 \quad (2)$$

Where, V_s is the volume of HCl consumed by the sample, V_b is the volume of HCl consumed by the blank, N_{HCl} is the normality of the HCl, and 14.01 is the molecular weight of nitrogen.

The percentage of crude protein was calculated by multiplying the percent (%) nitrogen by a conversion factor of 5.36 [17]. Most proteins contain 16% N, so the conversion factor is 6.25 ($100/16 = 6.25$); however, the conversion factors for various foods vary, and oat has a conversion factor of 5.36 [17].

$$\text{Crude protein (\%)} = \% \text{ N} \times 5.36 \quad (3)$$

Crude fat content

The crude fat was determined using the Soxhlet extraction according to AOAC [16] official method 920.39. Briefly, 2 g of sample flour (W_1) was transferred to a thimble, covered with fat-free cotton, and then fitted into the Soxhlet extraction apparatus. In the pre-cleaned extraction cylinder (W_2), 50 ml of diethyl ether was added. After 4 hrs of extraction, the samples were dried in an oven (Blast Air Oven, DHG-9240A, China) set to 70 °C for 30 min. After cooling in the desiccator for 30 min, the extraction cylinder and its contents (crude fat) were weighed (W_3). The crude fat percentage was then obtained as follows:

$$\text{Crude fat (\%)} = \frac{(W_3 - W_2)}{W_1} \times 100 \quad (4)$$

Where, W_1 represents the sample flour weight, W_2 represents the extraction cylinder weight, and W_3 represents the crude fat and extraction cylinder weight.

Crude fiber content

The AOAC [16] official method 962.09 was used to de-

termine the crude fiber content. One gram of sample flour (W_1) was digested in 50 ml of dilute (2.5%) H_2SO_4 for 40 min. After draining the acid with a vacuum pump, the residue was washed with distilled water and boiled in 50 ml of 2.5% NaOH for 40 min. The residue was washed twice with 20 ml 99.8% ethanol, twice with 20 ml diethyl ether, and three times with 20 ml acetone. The insoluble residue (crude fiber and ash) was dried in a Blast Air Oven (DHG-9240A, China) and weighed (W_2). This residue was ignited and carbonized (W_3) for 3 hrs in a 550 °C furnace (Nabertherm, D-6072 Dreieich, Germany). The crude fiber percentage was calculated as follows:

$$\text{Crude fibre (\%)} = \frac{(W_2 - W_3)}{W_1} \times 100 \quad (5)$$

Where, W_1 represents the weight of the sample, W_2 represents the weight of the dried residue (crude fiber + ash), and W_3 represents the weight of the ash.

Ash content

The ash content was determined using AOAC [16] official method 923.03. After determining the weight of the clean crucible (W_1), 5 g of sample (W_2) was added and charred on the hot plate under the hood. The charred sample was ignited to 550 °C in a muffle furnace (CARBOLITE, S336RB, England) for 5 hrs until it turned white/gray. The crucibles and their contents (ash) were weighed (W_3) after cooling in a desiccator. The percentage of the ash was calculated as:

$$\text{Ash (\%)} = \frac{(W_3 - W_1)}{W_2} \times 100 \quad (6)$$

Where, W_1 is the crucible weight, W_2 is the sample weight, and W_3 is the combined weight of the crucible and sample after ashing.

Table 1: Effect of varieties and traditional processing methods on proximate composition (g/100 g, DB) and gross energy (kcal/100 g) of white-colored, black-colored, and Goslin oat varieties.

Oat varieties	Processing methods	Moisture	Crude protein	Crude fat	Crude fiber	Ash	Crude CHO	Gross energy
White	Raw (control)	9.8 ± 0.3 ^{ab}	13.7 ± 0.02 ^c	8.6 ± 0.02 ^c	2.3 ± 0.50 ^a	1.2 ± 0.00 ^c	64.5 ± 0.90 ^b	394.6 ± 2.4 ^{gh}
	Toasted	4.4 ± 0.16 ^d	13.7 ± 0.04 ^c	8.4 ± 0.16 ^c	2.4 ± 0.54 ^a	1.2 ± 0.02 ^{bc}	69.9 ± 0.64 ^a	415.2 ± 1.97 ^{ab}
	Fermented - 24 hrs	10.1 ± 0.30 ^{ab}	15.2 ± 0.04 ^b	8.1 ± 0.13 ^{cd}	1.7 ± 0.25 ^a	1.2 ± 0.02 ^c	62.6 ± 0.52 ^{bc}	398.1 ± 1.38 ^{efg}
	Fermented - 48 hrs	10.2 ± 0.29 ^a	16.4 ± 0.03 ^b	7.3 ± 0.13 ^{de}	1.7 ± 0.31 ^a	1.1 ± 0.01 ^c	60.3 ± 0.67 ^{cd}	403.3 ± 1.28 ^{def}
Black	Raw (control)	8.5 ± 0.04 ^c	11.9 ± 0.05 ^d	10.3 ± 0.26 ^a	3.5 ± 0.44 ^a	1.3 ± 0.00 ^a	64.5 ± 0.66 ^b	405.0 ± 0.72 ^{cde}
	Toasted	4.5 ± 0.13 ^d	11.9 ± 0.16 ^d	10.6 ± 0.28 ^a	3.3 ± 0.35 ^a	1.27 ± 0.06 ^{ab}	68.2 ± 0.46 ^a	425.0 ± 2.92 ^a
	Fermented - 24 hrs	10.2 ± 0.37 ^a	15.1 ± 0.45 ^{bc}	9.4 ± 0.12 ^b	3.1 ± 0.61 ^a	1.3 ± 0.01 ^a	57.9 ± 0.18 ^{de}	409.8 ± 2.96 ^{bcd}
	Fermented - 48 hrs	10.4 ± 0.33 ^a	15.5 ± 0.27 ^b	8.5 ± 0.10 ^c	3.2 ± 0.5 ^a	1.3 ± 0.02 ^a	56.2 ± 0.60 ^c	413.8 ± 1.36 ^{bc}
Goslin	Raw (control)	8.9 ± 0.14 ^{bc}	15.8 ± 0.53 ^b	6.7 ± 0.01 ^{ef}	3.0 ± 0.37 ^a	1.2 ± 0.00 ^{bc}	64.4 ± 0.30 ^b	387.3 ± 0.19 ^h
	Toasted	4.2 ± 0.15 ^d	15.6 ± 0.31 ^b	6.5 ± 0.25 ^{ef}	3.0 ± 0.32 ^a	1.2 ± 0.01 ^{bc}	69.4 ± 0.20 ^a	404.9 ± 1.11 ^{cde}
	Fermented - 24 hrs	10.8 ± 0.09 ^a	18.2 ± 0.47 ^a	6.1 ± 0.04 ^{fg}	3.0 ± 0.36 ^a	1.2 ± 0.00 ^{bc}	58.4 ± 1.41 ^{de}	388.6 ± 2.50 ^{gh}
	Fermented - 48 hrs	10.7 ± 0.08 ^a	18.8 ± 0.15 ^a	5.6 ± 0.13 ^g	2.5 ± 0.29 ^a	1.1 ± 0.02 ^c	56.7 ± 0.2 ^{ce}	398.6 ± 2.05 ^{ef}
CV (%)		29.9	13.9	19.5	30.82	6.08	7.7	2.77

Data are expressed as mean ± standard error of replicates (n = 3). Means that do not share the same letter are significantly different. CHO - carbohydrate

Total carbohydrate

Total carbohydrate content was obtained by adding up all proximate percentages and subtracting from 100.

$$\text{Total Carbohydrate (\%)} = 100 - \% \text{ of (Crude protein + Moisture + Ash + Crude fat)} \quad (7)$$

Energy value

Gross energy content was calculated using Atwater's conversion factors of 4 kcal/g for protein, 9 kcal/g for fat, 4 kcal/g for carbohydrates, and 2 kcal/g for fiber, all expressed in kilocalories [18].

$$\text{Gross energy (kcal/100 g)} = ((9 \times \text{Crude fat}) + (4 \times \text{Crude protein}) + (4 \times \text{Utilizable carbohydrate}) + (2 \times \text{Crude fiber})) \quad (8)$$

Determination of anti-nutritional factors

Phytate content

The phytate content was determined using the Latta and Eskin [19] method. To obtain the extract, 0.1 g of flour samples were agitated for 1 hr at room temperature with 10 ml of 2.4% HCl and centrifuged for 30 min at 3000 rpm. The supernatant liquid was poured and used to quantify phytates, 3 ml of extract was mixed with 2 ml of wade reagent (containing 0.3% sulfosalicylic acid and 0.03% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in water), then mixed for 5 seconds in a Vortex. A series of phytic acid standard solutions of varying concentrations (0.0, 10, 20, 30, 40, 50, 60, 90, and 100 $\mu\text{g/ml}$, $Y = -0.0058X + 0.593$, and $R^2 = 0.996$) were prepared in 0.2 N HCl, with pure deionized water serving as a blank. A UV-Vis spectrophotometer was used to measure the absorbance of the sample and standard solutions at 500 nm.

Tannin content

The tannin content of the oat samples was determined using the Burns [20] method. The sample extract was obtained by mixing 2 g of flour with 10 ml of 1% HCl in methanol and shaking it for 24 hrs on a mechanical shaker before centrifug-

ing it for 5 min at 1000 rpm. One ml of the supernatant was then mixed with 5 ml of vanillin-HCl reagent (8% HCl in methanol with 4% vanillin). A series of D-catechin standard solutions (0, 1, 2, 4, 6, 8, 10, and 12 mg/100 ml, $Y = 0.0049X + 0.0265$, and $R^2 = 0.995$) were prepared, and after 20 min, the extracted sample and standards absorbance at 500 nm were measured using a UV-Vis spectrophotometer.

Statistical Analysis

The effects of oat varieties (Goslin, white-colored oat, and black-colored oat) and traditional processing methods (raw, toasting, 24 hrs fermentation, and 48 hrs fermentation) on proximate and antinutrient content of oats were investigated using a two-way ANOVA. Tukey's test was used to separate significant values, which were reported as mean \pm SE (standard error) of triplicate results at $p < 0.05$. Minitab®, Version 19 software was used for data analysis.

Results and Discussion

Effect of toasting and fermentation on proximate compositions

Table 1 shows the effect of oat varieties and cultural processing methods on oats' proximate composition (g/100 g, DB), whereas table 2 shows the calculated percent increase or decrease in proximate and gross energy values after toasting, 24 and 48 hrs of natural fermentation for each oat variety compared to their corresponding raw oat flour (control). These two factors (oat varieties and traditional processing methods) significantly ($p < 0.05$) influenced the oats' proximate compositions. Oats are processed to produce food products with health-beneficial properties [21].

Moisture content

The moisture ranges between 4.2 and 10.8 g/100 g for all the test samples (Table 1). The effects of oat varieties on the moisture content were not significant ($p > 0.05$), but significant changes were observed through traditional process-

Table 2: The calculated percent increase or decrease in proximate and gross energy values after toasting, 24 and 48 hrs of natural fermentation for each oat variety compared to their corresponding raw oat flour (control).

Oat varieties	Processing methods	Moisture	Crude protein	Crude fat	Crude fiber	Ash	Crude	Gross
							CHO	energy
White	Toasted	-55.1	0.0	-2.3	4.3	0.0	8.4	5.2
	Fermented - 24 hrs	3.1	10.9	-5.8	-26.1	0.0	-3.0	0.9
	Fermented - 48 hrs	4.1	19.7	-15.1	-26.1	-8.3	-6.5	2.2
Black	Toasted	-47.1	0.0	2.9	-5.7	0.0	5.7	4.9
	Fermented - 24 hrs	20	26.9	-8.7	-11.4	0.0	-10.2	1.1
	Fermented - 48 hrs	22.4	30.3	-17.5	-8.6	0.0	-12.9	2.1
Goslin	Toasted	-52.8	-1.3	-3.0	0.0	0.0	7.8	4.5
	Fermented - 24 hrs	21.3	15.2	-9.0	0.0	0.0	-9.3	0.3
	Fermented - 48 hrs	20.2	19	-16.4	-16.7	-8.3	-12	2.9

Percentage increments/decrements after toasting and natural fermentation were calculated as (final (processed) value - initial (raw) value)/initial (raw) value x 100. - (negative) results showed a decrease in percentage, while positive results showed an increase.

ing methods (Table 3). As expected, toasting significantly ($p < 0.05$) lowered the moisture level of all samples. It decreased by 55.1% for white oat, 47.1% for black oat, and 52% for Goslin (Table 2). It could be because of the drying effect of the toasting treatment. Toasting reduces water activity and is usually applied to prevent rapid enzymatic deterioration of oat-based foods to ensure safe storage stability [22]. The lesser moisture content in food is proportional to a lesser amount of water activity. Hence, toasted oat with low moisture content can be stored for a more extended period without spoilage as compared to raw oat. Black-colored and Goslin oats showed a significant increase in their moisture contents upon natural fermentation (24 and 48 hrs), while increases in white-colored oat were not significant ($p > 0.05$). The moisture increase in fermented slurry could be a result of the absorption of water during natural fermentation. A substantial improvement in the moisture of pearl millet via natural fermentation was also reported by Chinenye et al. [23].

Crude protein content

The crude protein contents in the Goslin, white-colored, and black-colored oat varieties toasted and fermented at different times ranged between 11.9 and 18.8 g/100 g (Table 1). As it is shown in Table 3, the effects of oat varieties and traditional processing methods (24 and 48 hrs natural fermentation) on the crude protein contents were significant ($p < 0.05$). Moreover, the interaction between the two factors demonstrated a significant effect ($p < 0.05$) on the crude protein. The crude protein contents did not show significant ($p > 0.05$) change upon toasting in all oat varieties used in this study.

Natural fermentation increased the crude protein content of white oat by 11%, black oat by 26.9%, and Goslin by 15.2% (Table 2). The maximum crude protein content went to the 48 hrs-fermented flours, followed by the 24 hrs-fermented flours. The percentage of crude protein in the first 24 hrs fermentation period was greater than that of the second 24 hrs. The improvement in protein could be attributed to the massive microbial population or the presence of protein already present in the medium, as carbohydrates are utilized during fermentation. Bacterial fermentation is also documented to increase lysine content in fermented flour [24].

The mechanism of enhancement in protein depends on the substrate system and the organism used [25]. Osman [5] and Nelofer [26] reported similar results for pearl millet and barley after 24 hrs of fermentation, respectively. According to Amare et al. [27], fermentation increases not only the amount of crude protein but also the number of amino acids. For example, amaranth grain flour fermented naturally for 48 hrs increased the level of nearly all free amino acids except glutamic acid, proline, and tyrosine. Improvement of amino acids after natural fermentation was also reported in cowpea by Bocchi et al. [28]. However, the effect of natural fermentation on proteins and amino acids has produced inconclusive results, which are probably due to the various research fermentation periods and variability in food protein or amino acid profiles at the initial stage [29].

Crude fat content

Both oat varieties and traditional processing methods

(natural fermentation) ($p < 0.05$) significantly affected the crude fat content of oat flour (Table 3). In addition to the two main factors, the interaction effects also showed a significant effect on the fat content (Table 3). Traditional processing methods did not significantly change the fat content except for the black-colored oat from traditional processing methods. But high-temperature roasting on quality protein maize increased the crude fat from 1.3 to 4.7%, as Chukwu-ma [30] reported. Raw oats used in this study contained 6.7 - 10.6% crude fat, which is somewhat rare in that they have high fat compared to the 2.0 - 3.0% fat content of most other grains and thereby higher in lipase and lipoxygenase activity than other grains [31]. However, oats' high-fat content can lead to a variety of processing issues, including poor flavoring and too much discoloration of toasted products [32]. The high oat lipoxygenase and lipase activity are one of the most difficult aspects of oat processing. Since they can act in reduced moisture and induce lipid oxidation in oat food products if not monitored [22]. According to Angelov et al. [33], the lipid content of intact oat kernels did not change after one year of storage. However, oat flour and oats without kernels are affected more rapidly and negatively [22]. Head et al. [34] state that heat treatment inactivates lipases and lipoxygenases. According to Ovando-Martínez et al. [35], heating oat grains at 88 - 115 °C for 90 - 120 min inactivated the lipase by 60 - 80%. Similarly, Ndungu et al. [36] demonstrated that heating cowpea seeds reduced phytase activity by 40%.

Commercial oat processing techniques, including superheated steam, extrusion cooking, and microwave heating are used widely in the food industry to produce oat-based foods [21]. Hence, avoiding lipid hydrolysis in oats is the primary goal of industries producing oat-based products. Comparably, households in developing countries can use toasting as a processing/pre-processing method in place of expensive commercial processing for oat grains to prolong the shelf life of traditional oat grains food products.

Natural fermentation significantly ($p < 0.05$) decreased the crude fat content in all oat varieties (Table 1). Fermentation for 24 hrs decreased the white-colored oat by 5.8%, the black-colored oat by 8.7%, and the Goslin by 9%. Similarly, fermentation for 48 hrs decreased the white-colored oat by 15.1%, the black-colored oat by 17.5%, and the Goslin by 16.4% (Table 2). The minimum crude fat content for all oat varieties was in the 48 hrs fermented flour. The maximum was registered for raw oats of white-colored and Goslin variety and the toasted black-colored oat showed the highest content of crude fat. The decrease in crude fat is in line with the works of Ojokoh and Bello [37], who reported a 2.4 - 5.7% decrease in the crude fat content of pearl millet flour after 48 hrs of natural fermentation. Fat oxidation during prolonged fermentation may cause fat loss over time [38]. It could also be attributed to microorganisms consuming lipids for metabolic uses during fermentation [39]. The low-fat content may help to extend shelf life by reducing lipid oxidation, but it will also influence the fermented foods' low energy value.

Crude fiber content

As indicated in Table 3, differences in oat varieties and traditional processing methods did not significantly affect the

Table 3: Two-way ANOVA p-value for the effect of processing and variety on their proximate composition, gross energy, and antinutrient contents.

	Moisture	Crude protein	Crude fat	Crude fiber	Ash	Crude CHO	Gross energy	Phytate	Tannin
Effect of varieties	0.649	0.000	0.000	0.060	0.000	0.054	0.000	0.031	0.000
Effect of processing	0.000	0.000	0.002	0.064	0.532	0.000	0.000	0.000	0.000
Interaction effect of varieties*processing	0.015	0.070	0.032	0.969	0.485	0.011	0.699	0.000	0.000

crude fiber contents of oat flours. Since toasting requires slight heating, significant alteration in crude fiber might not be expected. According to Oz yurt and Otles [40], various thermal treatments changed dietary fiber's physicochemical properties by altering the ratio between soluble and insoluble dietary fiber and total fiber content. The natural fermentation insignificantly ($p > 0.05$) decreased the crude fiber contents for all oat varieties. This slight decrease during fermentation might be attributed to the fermenting microorganism's ability to degrade cellulose and hemicellulose into short-chain hydrolyzable carbohydrates. Previous research reported a significant reduction in fibers after 4 days of corn fermentation [41]. Currently, health and nutritional policies encourage the consumption of foods high in dietary fiber [42]. Fiber-rich diets have been linked to a lower risk of gastrointestinal disorders, obesity, hypertension, diabetes, and cardiovascular disease [24, 40].

Ash content

The effect of traditional processing methods did not show a significant ($p > 0.05$) change in ash content; however, differences in oat varieties showed a significant effect ($p < 0.05$) on the ash content (Table 3). Contrary to this result, other studies showed a significant increase of total ash content from 1.9 to 2.0% during the first 24 hrs and a decrease from 2.0 to 1.8% in the 2nd consecutive 24 hrs spontaneous fermentation of maize food, *Doklu* [43].

Total carbohydrate content

The traditional processing methods (toasting, 24 hrs, and 48 hrs fermentation) significantly ($p < 0.05$) affect carbohydrate content, but differences in oat varieties were not (Table 3). However, the interaction effect of the two factors was significant. Toasting showed a significant ($p < 0.05$) increase in the carbohydrate content in all oat varieties. Toasting increased the crude carbohydrate content by 8.4% for white oat, 5.7% for black oat, and 7.8% for Goslin (Table 2). However, natural fermentation decreased significantly ($p < 0.05$) the carbohydrate content, as observed for all oat varieties. Natural fermentation lowered the carbohydrate content of white-colored oat by 2.9%, black-colored oat by 10.2%, and Goslin by 9.3% upon 24 hrs fermentation (Table 2). Fermentation for 48 hrs further reduced the carbohydrate by 6.5% for white oat, 12.9% for black oat, and 12% for Goslin (Table 2). This reduction could be primarily due to consuming glucose, a favorite substrate, by fermenting microorganisms [5]. Secondly, the enzymes released during fermentation can break down carbohydrates into other simple sugars like maltose. In turn, the microorganisms change simple sugars into macromolecules like protein and fat, or other metabolites [44]. Similar decreases in the total carbohydrates were shown by Osman [5] and Chinenye et al. [23]

for pearl millet and finger millet upon natural fermentation, respectively. This result is contrary to that of Assohoum et al. [43], who reported no significant decrease in carbohydrates in the course of 72 hrs of spontaneous fermentation of *Doklu*, a fermented maize food.

Energy values

The mutual effect of differences in oat varieties and traditional processing methods on the gross energy contents of oats flour is shown in table 3. Both factors significantly ($p < 0.05$) enhanced the gross energy. But the interaction effect of the two factors was not significant (Table 3). Toasting increased the gross energy content of Goslin by 4.5%, white-colored oat by 5.2%, and black-colored oat by 4.9%. Whereas natural fermentation increased the gross energy content of all oats significantly. White-colored oat increased by 0.9% through the 24 hrs fermentation and 2.2% through the 48 hrs fermentation. Black-colored oat increased by 1.2% during the 24 hrs variety was increased by 0.3% and 2.9% through 24 hrs and 48 hrs fermentation, respectively (Table 2). Upon these traditional methods in general, the gross energy value was highest in the toasted oats, followed by 48 hrs and 24 hrs fermented oats, respectively. Enhancement in the total energy could be attributed to the high increase of crude protein compared to

Table 4: Effect of varieties and traditional processing methods on antinutrients (mg/100 g, DB) of white-colored, black-colored, and Goslin oat varieties.

Oat varieties	Processing methods	Phytate	Tannin
White	Raw (control)	276.2 ± 0.45 ^b	51.5 ± 0.28 ^a
	Toasted	270.5 ± 0.64 ^c	49.7 ± 0.27 ^a
	Fermented – 24 hrs	186.1 ± 0.45 ^e	31.6 ± 0.55 ^c
	Fermented – 48 hrs	172.2 ± 0.42 ^h	25.7 ± 0.48 ^f
Black	Raw (control)	293.0 ± 0.06 ^a	45.9 ± 0.12 ^b
	Toasted	291.5 ± 0.28 ^a	43.5 ± 0.30 ^c
	Fermented – 24 hrs	201.4 ± 0.32 ^d	29.8 ± 0.43 ^c
	Fermented – 48 hrs	186.2 ± 0.20 ^e	21.2 ± 0.20 ^h
Goslin	Raw (control)	272.1 ± 0.22 ^c	41.1 ± 0.53 ^d
	Toasted	271.4 ± 0.30 ^c	40.4 ± 0.36 ^d
	Fermented – 24 hrs	198.8 ± 0.49 ^d	23.3 ± 0.16 ^e
	Fermented – 48 hrs	189.5 ± 0.36 ^f	19.5 ± 0.24 ^h
CV (%)		19.95	31.53

Data are expressed as mean ± standard error of replicate (n = 3) determinations. Means that do not share the same letter are significantly different.

Table 5: The calculated percent increase or decrease in the phytate and tannin after toasting, 24 and 48 hrs of natural fermentation for each oat variety compared to their corresponding raw oat flour (control).

Oat varieties	Processing methods	Phytate	Tannin
White	Toasted	-2.1	-3.5
	Fermented - 24 hrs	-32.6	-38.7
	Fermented - 48 hrs	-37.6	-50.1
Black	Toasted	-0.5	-5.3
	Fermented - 24 hrs	-31.3	-35
	Fermented - 48 hrs	-36.5	-53.8
Goslin	Toasted	-0.2	-1.7
	Fermented - 24 hrs	-26.9	-43.4
	Fermented - 48 hrs	-30.4	-52.7

Percentage increments/decrements after toasting and natural fermentation were calculated as (final (processed) value - initial (raw) value)/initial (raw) value x 100. - (negative) results showed a decrease in percentage, while positive results showed an increase.

the counter decreases of other macronutrients (crude fat, carbohydrate, and fiber) during fermentation, as the total energy computed from the sum of these constituents. Chemed and Bussa [45] reported a similar increase in the total energy while studying the effect of roasting on the nutritional value of amaranth grain (364.5 kcal/100 g to 369.8 kcal/100 g) fermentation and 2.1% during the 48 hrs fermentation.

Effect of natural fermentation and toasting on antinutrients

Table 3 shows the mutual effect of differences in oat varieties and traditional processing methods on oats' phytate and tannin contents. The outcome revealed a significant ($p < 0.05$) decrease in the antinutrients upon differences in oat varieties and traditional processing methods. The interaction between the two factors also aided in the decrease of antinutrients (phytate and tannin) significantly (Table 3).

Phytate content

The phytate contents were significantly ($p < 0.05$) decreased for the local oats (white and black-colored) upon toasting, at the same time, it did not show any significant decreases in the case of Goslin (Table 4). It showed a decrease of 0.2 - 2.0%, with a reduction more pronounced for white-colored oats. Natural fermentation decreased the phytate contents significantly. Fermentation for 24 hrs decreased by 26.9 - 32.6%, with white-colored oats showing an extreme decrease, and 48 hrs fermentation leads to a decrease of 30.4 - 37.6%, with an extreme decrease, marked to white-colored oat (Table 5). The minimum phytate content exhibited in the 48 hrs fermented oat flours. In the course of 48 hrs natural fermentation, significant decreases in the phytate were observed in the first 24 hrs compared to the second consecutive 24 hrs. This reduction could be related to microbial enzymatic (phytase) degradation of phytate. In addition to grain phytases, microbial phytases can diminish the phytate during fermentation [46]. The low pH condition induced during fermentation stimulates endogenous phytases and the production of microbial phytases [47]. Phytases exhibit broad substrate specificity with the highest

affinity for phytic acid [48]. During fermentation, enzymes such as decarboxylases, reductases, esterases, and glucosidases have also been recognized as phytic acid converters [49]. Alemayehu et al. [50] reported the phytate content of oat-based composite beverages decreased by 12.4 to 17.4% after 24 hrs of natural fermentation. A similar reduction of phytate by 20% within two days of the natural fermentation of pearl millet was reported by Onyango et al. [51]. Debabandya et al. [52] also showed a reduction in phytic acid levels by 57 - 80% for different varieties of sorghum flour after 12 hrs of fermentation. A reduction of phytate by more than 50% after spontaneous fermentation has also been reported during the preparation of sorghum and millet-based African porridges [53]. This study's difference in the extent of reductions in phytates might be due to the changes in variety, processing, and fermentation periods.

Tannin content

Tannin content was significantly ($p < 0.05$) decreased by 5.3% through toasting in the black-colored oats, while white-colored oat and Goslin varieties were not significantly ($p > 0.05$) affected (Table 4). Natural fermentation decreased the tannin content by 35.0 - 43.4% after 24 hrs fermentation and 50.1 - 53.8% after 48 hrs fermentation (Table 5). Minimum tannin content was observed in the 48 hrs of fermented Goslin flour. This decrease might be attributed to microbial activities of the breakdown of tannin into smaller units by the action of amylolytic enzymes, and microbial enzyme hydrolysis (alpha-galactosidases, phenyl oxidase, and tannin acyl hydrolase enzymes) mobilized during the fermentation [54]. The fermenting microbes are also responsible for the dissociation of tannic acid-starch, tannin-iron complexes, and tannin-protein bonds, resulting in the availability of free nutrients [55]. Other investigators have also reported similar observations on decreases in tannins. Osman [56] found that fermentation of sorghum genotypes naturally reduced tannin content by 52.7 - 92.0%, while Shimelis and Rakshit [57] observed that fermentation of bean varieties reduced tannin content by 26 to 47%. Tannin-rich foods can cause kidney inflammation, abdominal pain, liver cirrhosis, and stomach irritation [36]. Tannins are also known to be responsible for the decrease in mineral bioavailability. Hence, reducing tannin by natural fermentation can result in a relative increase in the amount of soluble iron, zinc, and calcium, thereby enhancing the uptake of essential dietary minerals in the body.

Conclusion

The effect of oat variety differences and traditional processing methods significantly changed their proximate composition and antinutrients of oat grains. Toasting showed a positive impact on nutritional composition by improving calorific energy and by reducing the antinutrients. Natural fermentation enhanced the crude protein prominently and decreased the antinutrients (phytate and tannin) extensively. It is recommended that toasting and fermentation be considered when preparing oat-based foods. The nutrient content of both raw and processed oats suggests their nutritional importance for health, and it is therefore recommended that oats be included in daily meals and in the functional food formulating industries.

Acknowledgements

I would like to acknowledge Adet Agricultural Research Center for supplying a sample of improved variety. I'd like to express my gratitude to the Ethiopian Public Health Institute (EPHI) for their support with nutritional analysis.

Conflict of Interest

None.

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