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Impact of Storage on Selected Nutrients of '*Oromo dinich*' (*Plectranthus edulis*) Tubers

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Abstract

The impact of storing tubers of *Plectranthus edulis* from four landraces grown in Ethiopia at 21°C in a shaded underground pit and at 2°C in a refrigerator for four weeks on selected nutrients was investigated. Changes in the proximate composition, mono- and di-saccharides and free amino acid content of the tubers were determined at weekly intervals. The results showed a significant interaction effect ($p < 0.05$) of storage facility and duration on reducing sugars, sucrose and most of the proximate composition parameters, except for the crude fibre content of the tuber from one landrace (*DHSew*). In all the landraces, an increase in crude fat, crude protein and reducing sugar content was observed when tubers were stored for up to four weeks in both the pit and refrigerator. The increase in reducing sugar content was significantly higher ($p < 0.05$) for tubers stored in a refrigerator than for those stored in the pit. Pit storage is preferred over refrigerator storage but may be better at temperatures lower than 20°C.

Keywords: *Plectranthus edulis; Oromo Dinich; Pit Storage; Refrigerator Storage; Reducing Sugar; Protein; Proximate Composition; Fiber; Free Amino Acids*

Introduction

The nutritional composition of '*Oromo dinich*' (*Plectranthus edulis*) (Vatke) Agnew (syn. *Coleus edulis*) tubers from different landraces and their potential contribution to the human diet was evaluated [1]. According to them, the tubers have a considerable potential to contribute to the diets of Ethiopian farming communities as a staple food.

P. edulis tubers are harvested in the southern and south western parts of Ethiopia between September and November. Seasonal production patterns, inadequate cold storage capacity and limited alternative market outlets (e.g. processing and export) often force farmers to use traditional storage methods. Consequently, farmers typically leave physiologically matured *'Oromo dinich'* tubers *in situ* (in the soil where they are grown) until needed [2]. The duration of such *in situ* storage depends on the intended end use of the tubers. Farmers claim that tubers intended for consumption can be stored for up to 5 months, whereas those intended for use as seeds can be stored for up to 7 months [3]. However, prolonged *in situ* storage of tubers for consumption is considered less desirable by farmers because of undesirable changes in flavour, fibrousness of the cooked tubers and longer cooking times that are presumed to be linked to the storage period [3]. [3] reported in his study on two cultivars *Lofua* (*LWsh*) and *Chenqowa* (*CWsh*), a 20% decrease in average fresh weight of tubers with no

visible signs of rotting during 6 weeks of *in situ* storage. Additionally, *in situ* storage makes land unavailable for the subsequent cropping. Therefore, farmers generally consider storage of tubers in pits as useful alternative. They store tubers in a shaded pit until needed for consumption, sale or use as seeds. In both *in situ* and pit storage, water loss is inevitable as the optimal storage conditions are not yet well known and rarely maintained.

Temperature and relative humidity (RH) are the two critical factors to be controlled during postharvest handling and storage of roots and tubers. They are critical in reducing the water vapour pressure deficit (VPD) between the intracellular tissue of roots and tubers and storage environment. VPD is a driving force for transpiration (loss of water in the form of water vapour from plants to the atmosphere Therefore, exposure of harvested roots and tubers to non-optimal conditions can lead to an increase in VPD, increasing the transpiration rate and resulting in increased water loss [4,5].

Water loss during storage of roots and tubers is one of the most critical parameters affecting their marketability, storage potential, processing, sensory quality and nutritional composition [6-9]. Moreover, during storage of tropical roots and tubers, physiological disorders may occur due to increased respiration rate, breakage of dormancy and temperature-induced changes that depend on the initial status of roots and tubers, storage duration and conditions [10].

The impact of relative humidity and temperature in storage is interrelated. The impact of relative humidity depends on the temperature difference between the stored product and storage. At a given temperature and rate of air movement, the rate of water loss from the stored commodity depends on the RH. At a given RH, water loss increases with increase in temperature [11]. When the RH during storage is too low, transpiration is enhanced, resulting in loss of moisture and excessive tissue softening. Thus, high RH will not prevent moisture loss if the product temperature is not near the storage temperature as a small fluctuation in temperature (< 0.5°C) can result in condensation on cool product surfaces [12].

Tropical roots and tubers exhibit "chilling injury" when stored at temperatures below a critical level that is usually between 10 and 15°C [10]. Storing them with in this optimal rage, slow metabolic process, prolong the dormancy period by slowing down the sink-to-source transition process necessary for sprouting and prolong the market life [13,14]. However, if the temperature is too low and below the optimal range, depending on the sensitivity of roots and tubers, low temperature injuries such as freeze and chilling injury will occur [15].

Due to the interdependence of impact of temperature and relative humidity on the shelf life and quality of roots and tubers in storage, several researchers recommended different temperature relative humidity combinations as an ideal storage condition (Table 1).

Table 1: *Ideal storage conditions, optimum temperature T (°C) and relative humidity (RH%) for selected roots and tubers.*

Roots/tubers	T (°C)	RH (%)	Storage life	References
fresh potato Processing	4 - 7 7 - 20	90 - 95 95	4 - 10 months nv	[16,24] [26]
Sweet potato	13 - 16 15 13 - 15	80 - 90 95 80 - 95	4 - 7 months nv 13 months	[24] [19] [25,27]
Cassava	0 - 5 10	85 - 90 80	1 - 6 months 14 days	[10,25] [28,29]
Taro	7 - 10	85 - 90	4 - 5 months	[25]
Yam	16 14 - 16	70 - 80 70 - 80	6 - 7 months 1 - 7 months	[25] [10,30]
Aroids	24 - 29	86 - 98	2 weeks	[23,31]

(nv) indicates no values given.

The impact of both traditional and other storage conditions on nutrient composition of roots and tubers such as potato (*Solanum tuberosum*) [13,16-18], sweet potato (*Ipomoea batatas*) [19], cassava (*Manihot esculenta*), yams (*Dioscorea* spp.) [20,21], taro (*Colocasia esculenta*) [22] and aroids cormels (*Colocasia, Xanthosoma, Amorphophallus*) [23] have been well documented.

However, optimal storage condition for *P. edulis* tubers is not known yet. Besides, unlike for temperate underground storage organs, use of mechanically refrigerated storage facilities for subtropical and tropical underground storage organs such as *P. edulis* is limited due to economic constrains. Consequently, apart from the rather indicative report of Taye [3], the impact of storage condition on the overall quality of *P. edulis* tuber has not well documented. Hence, this research was conducted to evaluate the impact of low temperature under refrigerated storage on the nutrient composition (proximate, mono- and disaccharides and free amino acids) of the tuber in comparison with a reference traditional storage system (pit storage) commonly used by the local farmers in Ethiopia.

Materials and Methods

Mature and cured tubers from four '*Oromo dinich'* landraces, namely *Dinnichaa - habesha - white* (*DHSew*), *Inuka* - Wolayta *donuwa* (*IWsh*), *Lofua* - Wolayta *donuwa* (*LWsh*) and *Chenqo* - Wolayta *donuwa* (*CWsh*) were used for the storage experiment. The tubers were cured in the field by decreasing the watering frequency one month before harvesting. Five kilogrammes of tubers from each landrace were collected from the field and a composite sample was prepared. After sorting for the absence of visual defects and disease, 60 healthy tubers (approximately 25 - 50 g/tuber) were collected from each landrace for storage. The tubers were divided into two groups and stored separately in a pit and in refrigerator for four consecutive weeks. Replications were made on the composite sample but not on the storage facilities.

Storage facility

Thirty tubers from each landrace were stored in a refrigerator and spread over a rack. The remaining 30 tubers were stored in the modified pit measuring 1.5m x 1m x 1m (LxWxD) (Figure 1). Unlike typical farm pits, the bottom of the pit used in the study was cemented with a mixture of clay and sand to prevent tubers from contacting the soil.

Figure 1: *Pit storage facility covered with shade (A), and 'Oromo dinich' tubers stored in the pit (B).*

To imitate the farmers' practices, the pit was constructed under a temporary plastic shade and the bottom part was covered with a layer of dry grass mulch. Then, the tubers were spread uniformly on top of the dry grass mulch and covered with another layer of mulch to restrict moisture loss. The pit was closed with a wire mesh to protect from rats, leaving approximately 25 cm headspace for air circulation. Throughout the storage period, the pit was kept dark, by covering the top wire mesh with layers of dry grass mulch. A separate pit was used for each landrace. For the refrigerator, the tubers from the four landraces were stored on different racks in the same refrigerator.

At the end of the storage period, the tubers were peeled and analysed for their proximate composition and mono-and disaccharides in triplicate and for free amino acids in duplicate (analytical replications).

The temperature and relative humidity of the storage facilities were measured throughout the storage duration using a mini temperature (T) and relative humidity (RH) measurement logger (Testo 174H, Lenzkirch, Germany). The data were retrieved to a computer using ComSoft Basic-5 software and the minimum, maximum and average temperature and relative humidity were computed.

Sample preparation

Five tubers of each landrace were drawn from the two storage facilities every week for four weeks. The drawn samples were peeled and their dry matter was determined on a fresh weight basis. The remaining samples were then freeze-dried at -50°C and 0.04 mbar and subsequently ground using a pestle and mortar at Hawassa University, College of Agriculture, School of Animal and Range Sciences' Animal Nutrition Laboratory. The resulting flour for each landrace sample was sealed in a Ziplock plastic bag (double packaging) and stored at 6 ± 4°C until analysis.

Reagents

All solutions were prepared from analytical reagent grade chemicals and deionized water (18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Burlington, USA) as the solvent.

Nutrient analysis

The freeze-dried tuber flours were analysed for their contents of crude fat, crude fibre, total nitrogen, mono- and disaccharides and free amino acids both before and after storage.

Moisture and total ash

Moisture and total ash were determined gravimetrically according to AOAC method [32] on a fresh weight basis. Moisture was determined in triplicate by drying 5 ± 0.05g of peeled and chopped tubers in a BINDER forced convection oven (model BF 115, Germany) at 100 ± 5°C until constant weight was obtained. Percent moisture content was computed by weight difference after oven drying to that of the original sample weight.

Total ash was determined by incinerating the residue of moisture determination at 500°C in a Nabertherm muffle furnace (model B 170, Germany) for 2 - 4h until the residue was uniformly white. Ash content was reported on a dry matter basis.

Crude fat content by ANKOM

Crude fat was analysed from freeze dried flour in triplicate according to ANKOM Technology Method Version 1/30/09. Fat was extracted following the procedure on the manufacturers' user guide manual for ANKOM^{XT10} extractor according to AOCS procedure Am 5-04 [33]. 0.5 ± 0.05g of partially oven-dried tuber flour was weighed in a filter bag and sealed. Fat/oil was extracted by refluxing the filter bags

containing the samples for 60 min in petroleum ether. After extraction, filter bags were dried in the oven at 102 ± 2°C for 30 minutes and crude fat content was computed by the weight difference to that of the initial sample weight.

Crude fibre

Crude fibre was determined in triplicates by ANKOM^{200} Fibre Analyser and F57 ANKOM filter bags (Porosity of 25 µm), following the filter bag technique [34]. 0.5 ± 0.05g of freeze dried tuber flour was weighed in a filter bag and sealed leaving 4.0 mm at the top. Before digestion, fat/oil was extracted by soaking the bags in 250.0 mL petroleum ether for 10 minutes and bags were subsequently air-dried. Samples were digested in two steps by agitating for 40 minutes at each step, first with 1.8L of 0.255N sulphuric acid (H_2SO_4), followed by 1.8L 0.313N sodium hydroxide (NaOH) solution.

After each digestion, bags were rinsed by agitating with 2.0L of boiling water for 5 minutes (three times) until water attained neutral pH. Remaining excess water was gently pressed out from the bags into the beaker and decanted. Then bags were soaked for 5 minutes in a 250.0 mL beaker filled with acetone until all bags were covered. Acetone was then decanted and the remaining excess was gently pressed out of the bags. Bags were air-dried and further dried in an oven at 102 ± 2°C for 2h, cooled to room temperature in a desiccator and weighed. Crude fibre was calculated based on the weight difference after digestion to that of the initial sample weight.

Mono- and disaccharide contents

Mono- and disaccharides were determined by gas chromatographic analysis after an aqueous extraction in triplicate from the freeze-dried tuber flour using gas chromatograph after conversion of the reducing sugars to oximes with hydroxylamine and further derivatisation to silyl ethers as described earlier in detail [35]. Total reducing sugar was expressed as a summation of glucose, fructose, and maltose on dry matter basis.

Nitrogen and protein content

Total nitrogen content of approximately 300.0 ± 0.05 mg freeze dried tuber flour was determined triplicate by the standard Kjeldahl method [36]. The sample was transferred into a Kjeldahl tube to which 10.0 mL of H_2SO_4 and 1.0 Kjeltab CX (catalyst compound) were added. Decomposition was done in a destruction block at 420°C until a clear solution was obtained. Distillation was carried out with a 2200 Kjeltec Auto (FOSS Tecator, Sweden). The obtained distillate was titrated with 0.05M HCl. Protein content was calculated by multiplying total nitrogen with a factor of 6.25.

Free amino acid contents of the tubers by HPLC

Proteins were acid hydrolyzed to their constituent amino acids [37] using 0.1 ± 0.05g of the freeze-dried tuber flour as described earlier [38]. Free amino acids were analyzed by mixing 0.1 ± 0.05g of freeze dried tuber flour in to 17.5 ml of 15% trichloroacetic acid (TCA) [39] and further diluted to exactly 25.0 ml with 15% TCA. Both primary and secondary amino acids were derivatized by o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) respectively, at room temperature in the injector of an Agilent 1100 system (Agilent Technologies, Switzerland) and separated on a Zorbax Eclipse AAA Rapid Resolution column (4.6 × 150 mm, Agilent Technologies) as described earlier [38]. Results were quantified on 100 g dry matter basis using external calibration curves (R = 0.99) prepared using freshly prepared derivatized amino acid standards.

Data analysis

The data were analysed using IBM SPSS Statistics for Windows (version 22.0, IBM Corp., New York, USA). The normality of the data and homogeneity of variance within a subject were checked by the Shapiro-Wilk and Leven's test for equality of error variance at $p < 0.05$, respectively. Five by two (two-way) factorial repeated measure ANOVA was used to determine the significance of five storage durations at 0, 1, 2, 3 and 4 weeks and two storage facilities (pit and refrigerator) on proximate, mono- and disaccharides and free amino acid content of the tubers ($p < 0.05$). The error covariance matrix of nutrients (dependent variables) was checked using Mauchly's test of sphericity at $p < 0.05$. The significant value at Greenhouse-Giessen was used when the assumption of sphericity was not met. When significant interaction existed between the two factors (storage facility and storage duration), a paired sample t-test was conducted to determine the significance of a single factor, with a 95% confidence interval at Least Significant Difference (LSD) value of $p < 0.05$.

Result and Discussion

The pit had an average temperature and relative humidity of 20.71 ± 2.40°C and 75.91 ± 8.30%, while the refrigerator had an average temperature and relative humidity of 2.16 ± 2.60°C and 20.47 ± 14.40%, respectively. In the refrigerator, a larger standard deviation of temperature and relative humidity measurements was noted due to a few occasional electric power cuts and delay for the generator to start by an operator. Despite the fact that the pits were not actively thermally regulated, the temperature inside remained fairly stable.

Effects of storage facility and storage duration on proximate and free amino acid contents

At both pit and refrigerator storage facilities, tubers remained turgid and did not sprout throughout the experiment. In general, in all the four landraces, the factorial repeated measure Analysis of Variance (ANOVA) revealed a significant interaction ($p < 0.05$) between storage facility and duration on most of the proximate composition parameters except for crude fibre content of tubers from a particular landrace (*DHSew*) as indicated in table 2.

Table 2: *Effect of pit (20.71°C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on proximate composition of 'Oromo dinich' tubers as a function of storage durations for four landraces (g/100 g dm ± SD, unless specified otherwise).*

Landrace	Proximate Composition	Storage Facility (SF)	Storage Duration (SD*)					SD*	SF	SD* × SF
			0 week	1 week	2 weeks	3 weeks	4 weeks			
DHSew	Moisture (g/100 g fw)	Pit	$83.33^{a(c)} \pm 0.94$	$80.93^{b(c)} \pm 1.49$	$80.13^{b(c)} \pm 0.89$	$79.66^{b(d)} \pm 0.68$	$80.19^{b(d)} \pm 0.50$	**	***	***
		Refrigerator	$83.33^{abc(c)} \pm 0.94$	$81.54^{abc(c)} \pm 1.74$	$81.61^{c(c)} \pm 0.41$	$84.91^{a(c)} \pm 0.18$	$84.23^{b(c)} \pm 0.33$			
	Crude fibre	Pit	$1.80^{ab(c)} \pm 0.07$	$2.22^{a(c)} \pm 0.17$	$1.64^{b(c)} \pm 0.29$	$1.76^{ab(c)} \pm 0.06$	$1.61^{ab(c)} \pm 0.40$	***	*	NS
		Refrigerator	$1.80^{b(c)} \pm 0.07$	$2.08^{a(c)} \pm 0.09$	$1.64^{abc(c)} \pm 0.15$	$1.31^{bc(c)} \pm 0.19$	$1.40^{c(c)} \pm 0.16$			
	Crude fat	Pit	$7.58^{c(c)} \pm 0.13$	$12.31^{b(c)} \pm 0.24$	$11.77^{b(d)} \pm 0.24$	$12.34^{ab(d)} \pm 0.08$	$13.19^{a(c)} \pm 0.44$	***	NS	***
		Refrigerator	$7.58^{d(c)} \pm 0.13$	$12.22^{b(c)} \pm 0.10$	$12.82^{a(c)} \pm 0.2$	$13.13^{a(c)} \pm 0.11$	$11.85^{c(d)} \pm 0.05$			
	Crude protein	Pit	$6.77^{c}(c) \pm 0.01$	$11.93^{b(c)} \pm 0.18$	$12.48^{ab(c)} \pm 0.18$	$12.65^{b(c)} \pm 0.18$	$13.18^{a(c)} \pm 0.20$	***	***	***
		Refrigerator	$6.77^{c}(c) \pm 0.01$	$11.76^{a(c)} \pm 0.18$	$11.63^{b(d)} \pm 0.18$	$12.06^{ab(c)} \pm 0.32$	$11.28^{b(d)} \pm 0.19$			
IWsh	Moisture (g/100g fw)	Pit	$82.11^{ac(c)} \pm 0.05$	$82.68^{ab(c)} \pm 0.55$	$81.44^{bd(c)} \pm 0.00$	$81.27^{cde(d)} \pm 0.83$	$80.14^{e(d)} \pm 0.1$	NS	***	***
		Refrigerator	$82.11^{b(c)} \pm 0.05$	$82.54^{a(c)} \pm 0.36$	$83.13^{a(c)} \pm 0.75$	$82.49^{a(c)} \pm 0.67$	$83.71^{a(c)} \pm 0.46$			
	Crude fibre	Pit	$1.65^{c(c)} \pm 0.15$	$1.67^{bc(c)} \pm 0.20$	$2.74^{a(c)} \pm 0.04$	$2.48^{ab(c)} \pm 0.22$	$1.52^{c(c)} \pm 0.30$	***	**	***
		Refrigerator	$1.65^{b(c)} \pm 0.15$	$2.47^{a(c)} \pm 0.38$	$1.34^{b(d)} \pm 0.10$	$1.82^{a(d)} \pm 0.18$	$1.47^{b(c)} \pm 0.17$			
	Crude fat	Pit	$6.33^{c(c)} \pm 0.12$	$7.79^{b(c)} \pm 0.12$	$10.10^{a(c)} \pm 0.251$	$8.03^{b(c)} \pm 0.35$	$9.33^{a(c)} \pm 0.38$	***	*	***
		Refrigerator	$6.33^{c(c)} \pm 0.12$	$7.51^{b(c)} \pm 0.43$	$7.94^{b(d)} \pm 0.27$	$8.56^{b(c)} \pm 0.35$	$10.13^{a(c)} \pm 0.13$			
	Crude protein	Pit	$9.00^{d(c)} \pm 0.01$	$12.17^{b(c)} \pm 0.19$	$11.88^{b(d)} \pm 0.21$	$10.81^{c(d)} \pm 0.20$	$13.09^{a(c)} \pm 0.23$	***	*	***
		Refrigerator	$9.00^{d(c)} \pm 0.01$	$12.28^{b(c)} \pm 0.22$	$12.49^{b(c)} \pm 0.01$	$13.07^{a(c)} \pm 0.20$	$10.88^{c(d)} \pm 0.19$			

Landrace	Proximate Composition	Storage Facility (SF)	Storage Duration (SD*)					SD*	SF	SD* × SF
			0 week	1 week	2 weeks	3 weeks	4 weeks			
LWsh	Moisture (g/100 g fw)	Pit	$82.14^{a(c)} \pm 0.21$	$81.99^{ab(c)} \pm 0.74$	$84.04^{a(c)} \pm 0.63$	$80.78^{b(d)} \pm 0.19$	$78.73^{c(d)} \pm 0.17$	***	***	***
		Refrigerator	$82.14^{c(c)} \pm 0.21$	$83.27^{ab(c)} \pm 0.30$	$83.89^{abc(c)} \pm 0.52$	$84.40^{a(c)} \pm 0.31$	$82.56^{bc(c)} \pm 0.83$			
	Crude fibre	Pit	$1.57^{c(c)} \pm 0.09$	$1.79^{bc(c)} \pm 0.14$	$2.26^{a(c)} \pm 0.14$	$2.33^{ab(c)} \pm 0.17$	$1.75^{bc(c)} \pm 0.15$	***	***	***
		Refrigerator	$1.57^{b(c)} \pm 0.09$	$1.56^{b(c)} \pm 0.09$	$1.36^{b(d)} \pm 0.21$	$2.05^{a(c)} \pm 0.05$	$1.72^{ab(c)} \pm 0.17$			
	Crude fat	Pit	$5.59^{d(c)} \pm 0.12$	$11.83^{ab(c)} \pm 0.41$	$8.55^{c(c)} \pm 0.17$	$9.50^{bc(c)} \pm 0.63$	$13.15^{a(c)} \pm 0.28$	***	***	***
		Refrigerator	$5.59^{c(c)} \pm 0.12$	$11.36^{a(d)} \pm 0.35$	$7.49^{b(c)} \pm 0.34$	$10.25^{a(c)} \pm 0.19$	$10.42^{a(d)} \pm 0.33$			
	Crude protein	Pit	$9.23^{d(c)} \pm 0.19$	$13.01^{c(c)} \pm 0.18$	$10.56^{bc(d)} \pm 0.18$	$11.24^{b(c)} \pm 0.18$	$11.64^{a(c)} \pm 0.24$	***	NS	***
		Refrigerator	$9.23^{d(c)} \pm 0.19$	$12.95^{a(c)} \pm 0.19$	$11.55^{b(c)} \pm 0.18$	$10.94^{c(d)} \pm 0.20$	$11.23^{bc(c)} \pm 0.16$			
CWsh	Moisture (g/100 g fw)	Pit	$83.87^{a(c)} \pm 0.43$	$82.45^{a(c)} \pm 0.95$	$81.56^{a(c)} \pm 1.15$	$81.64^{a(d)} \pm 0.97$	$77.31^{b(d)} \pm 0.14$	***	***	***
		Refrigerator	$83.87^{a(c)} \pm 0.43$	$84.10^{a(c)} \pm 0.33$	$83.85^{a(c)} \pm 0.14$	$82.94^{ab(c)} \pm 0.68$	$81.66^{b(c)} \pm 0.72$			
	Crude fibre	Pit	$1.25^{c(c)} \pm 0.15$	$1.55^{bc(c)} \pm 0.21$	$2.25^{abc(c)} \pm 0.29$	$2.21^{ab(c)} \pm 0.13$	$2.51^{a(c)} \pm 0.14$	***	NS	*
		Refrigerator	$1.25^{b(c)} \pm 0.15$	$1.18^{ab(c)} \pm 0.06$	$1.56^{ab(c)} \pm 0.54$	$1.62^{ab(d)} \pm 0.32$	$1.51^{a(d)} \pm 0.24$			
	Crude fat	Pit	$5.99^{d(c)} \pm 0.06$	$7.67^{c(c)} \pm 0.20$	$13.59^{a(c)} \pm 0.17$	$7.76^{c(d)} \pm 0.39$	$11.95^{b(c)} \pm 0.61$	***	***	***
		Refrigerator	$5.99^{c(c)} \pm 0.06$	$7.72^{b(c)} \pm 0.16$	$12.78^{a(d)} \pm 0.26$	$13.01^{a(c)} \pm 0.09$	$13.02^{a(c)} \pm 0.14$			
	Crude protein	Pit	$8.98^{d(c)} \pm 0.01$	$11.36^{ab(d)} \pm 0.19$	$11.30^{b(c)} \pm 0.18$	$11.44^{a(c)} \pm 0.17$	$10.10^{c(d)} \pm 0.30$	***	***	***
		Refrigerator	$8.98^{d(c)} \pm 0.01$	$12.61^{ab(c)} \pm 0.19$	$12.00^{b(c)} \pm 0.18$	$11.17^{c(d)} \pm 0.18$	$12.12^{acd(c)} \pm 0.18$			

SD × SF indicate significance of interactions between storage durations (SD) and storage facilities (SF), NS = not statistically significant, *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.*

Different superscript letters in a row indicate significant differences between storage durations based on LSD value (p < 0.05), SD standard deviation among analytical replicates.

( ) superscript letters in parentheses in a column indicate significant difference between storage facilities based on LSD value (p < 0.05).

There was a significant reduction ($p < 0.05$) in the moisture content of tubers stored in the pit for up to three or four weeks. This was not observed in tubers stored in the refrigerator. Consequently, between the storage facilities, there was a significantly higher moisture loss in tubers stored in the pit than in those stored in the refrigerator for the same period, despite the higher relative humidity in the pit. This greater reduction in moisture content of tubers stored in the pit could be related to an increase in the respiration and transpiration (evaporation of water) rate of the tubers [16] because the temperature in the pit were higher than in the refrigerator. According to [7,41], temperature-mediated variations in plant respiration and water loss are demonstrated as a simple exponential function of temperature with a Q_{10} value of approximately 2.0. This means that the rate doubles for every 10°C increase in temperature over the range of 5°C to 25°C.

The moisture loss in tubers stored for up to four weeks was below 5% for all storage facilities in all landraces, except landrace *CWsh*, which lost 7.83% water when stored in the pit. According to [42], percentage moisture losses above 10% in tubers cause greater peeling loss and affect cooking quality due to the resulting shrivelled texture of the skin. This suggests that tubers from either storage facility will not present such processing difficulties since the percentage moisture loss was less than 10% in each case, at least within the 4 weeks storage period. Unfortunately, the limited data points as a function of time did not allow us to reliably predict the further evolution of the dry matter content of the tubers stored in the pit, making it not possible to estimate the time at which a critical moisture loss above 10% would be reached.

The losses reported by [3] during *in situ* storage of tubers from *Lofua* (*LWsh*) and *Chenqoua* (*CWsh*) landraces, were considerably higher during six weeks of storage (> 40%) than we found. Although a reliable prediction of the further evolution of the dry matter content of the tubers stored in the pit was not possible, it seems not realistic that such a dramatic moisture loss would have been obtained in the present study. In fact, Taye did not protect *in situ* stored tubers from exposure to solar radiation and rodents' access. This suggests that storing tubers in the pit where entry of rodents and solar radiation is prevented is a better approach to reduce postharvest losses than *in situ* storage and may alleviate at least for a particular time the issue of land use. It should be stressed that the applied pit storage can easily be applied locally and does not require major investments. Due to the observed changes in moisture content during the storage period, all other proximate parameters were expressed on a dry matter basis.

Changes in crude fibre content of tubers during storage were not consistent among landraces. For tubers stored in the pit, crude fibre content in landraces *IWsh* and *LWsh* significantly increased by 50.30% and 48.41% ($p < 0.05$) at three weeks of storage, respectively. However, no further changes are observed. For landrace *CWsh,* crude fibre content increased by 76.80% ($p < 0.05$) at three weeks of storage. Landrace *DHSew* recorded an increase of 23.33% during the first week of storage but did not change further during the remaining storage period.

On the other hand, when tubers were kept in the refrigerator for up to four weeks, crude fibre content was significantly reduced ($p < 0.05$) by 22.22% for *DHSew,* whereas for landrace *CWsh* it increased by 20.80%. For the remaining landraces, the change was insignificant. This suggests that the change in crude fibre content of fresh tubers during storage did not only depend on storage facility and storage duration but also on the type of landraces. The increase in crude fibre content in all storage facilities is in agreement with a previous finding of [43] who reported a significant increase in both acid and neutral detergent fibre content in yam (*D. dumetorum*) cultivars stored for 72h at 4°C and 28°C. As stated in their work, an increase in crude fibre content was due to an increase in lignin, cellulose and hemicellulose contents, which contributed to the hardening of the tuber. This might be the case for the observed increase in the crude fibre content of *'Oromo dinich*' tubers as well. However, further investigations are required to confirm this hypothesis.

In all landraces, during the four weeks of storage, crude fat content of tubers significantly increased ($p < 0.05$) irrespective of the storage facilities used. The increase varied in the range between 47.39% to 100.00% and 56.33% to 100.00% in the pit and refrigerator, respectively (Table 2). The range was narrower for tubers stored in the refrigerator than for those stored in the pit. This might be due to a change in the fatty acid content of tubers during low temperature storage. [44] reported a change in plant membrane lipids from the gel to the liquid-crystalline phase and an increase in the level of lipid desaturation in response to low temperature stress. Fatty acid desaturases are responsible for this change. The attribution of modifications in membrane lipid unsaturation to freezing tolerance of two potato species (*S. tuberosum* and *S. commersonii*) had been reported [45]. [46] reported greater changes in the fatty acid content of sweet potato (*I. batatas*) tubers stored at low temperatures (4.5°C) and suggested *de novo* synthesis of lipids from non-lipid components. The increase in crude fat content of the tubers during storage is in agreement with the findings of [47]. According to them, total lipids significantly increased in sweet potato tubers stored at 15.5°C and 85% relative humidity for four weeks. Similarly, [48] reported an increase in fatty acid content in yam (*D. alata, rotundata and esculenta*) tubers during 4 weeks of storage at 25°C and 80% RH. According to [49] the activity of fatty acid synthase increases and pattern of *de novo* synthesis of fatty acids changes in aging potato discs. As reported by [1], a considerable proportion of crude fat consists of non-fatty acids. It is not clear to what extent the observed increase is due to an increase in fatty acid or non-fatty acid substances. Thus, further investigations are required to clarify this.

Similar to crude fat, the change in crude protein content of fresh tubers as a function of storage facility and duration was consistent among landraces. The crude protein content of tubers stored in both the pit and refrigerator significantly increased ($p < 0.05$) after one week of storage and remained steady during the subsequent four weeks storage with slight variations.

In all landraces, the crude protein content of tubers stored for up to four weeks in both the pit and refrigerator was significantly ($p < 0.05$) higher than that of fresh tubers. The increase varied in the range of 12.47% to 94.98% and 21.11% to 66.91% for tubers stored for four weeks in the pit and refrigerator, respectively. The range was broader and the increase was higher in the pit storage at a higher temperature compared to refrigerator storage. The increase in protein content might be due to changes in the physiological activities of the tubers. [50] reported that due to physiological activities during storage, the insoluble nitrogen content of protein in the eye tissue of potato tuber increased by 47% compared to the rest of potato tubers (*S. tuberosum*). According to him at the end of the dormancy stage, nitrogen fractions were temporarily accumulated near the bud due to translocation of nitrogen from the other parts of tubers to the terminal bud where it may be utilized as a material for the formation of a new sprout.

He also explained that some of the nitrogen fractions accumulated near the terminal bud are synthesized to proteins and stored temporarily [50]. Similarly, a significant increase in amide-N content is reported in the same region which may be utilized as a nitrogen source during the formation of new sprouts in potato tubers [51]. Although these earlier observations seem to suggest that increases in crude protein content of tubers during storage could be due to upcoming sprouting, no sprouts were observed throughout the storage period of this experiment. Moreover, it should be noted that our findings are in contrary to those findings reported by [52] and [20] in potato and yam, respectively. [20] reported a 26.9% and 35% decrease in the crude protein content of yam (*Dioscorea* spp.) tubers stored for up to three months in a traditional barn with and without a fan at an average temperatures of 27.8°C and 31.4°C, respectively. Species differences may account for this disparity. [52] showed a 9% decrease in potato (*S. tuberosum*) protein nitrogen due to a decreased capacity for protein synthesis, an increase in proteolytic activity and an increase in free amino nitrogen during storage of seed tubers at 4°C and 95% RH for five months.

Because of the changes in crude protein content observed, it was considered relevant to preliminarily evaluate the changes in the free amino acid profiles of stored tubers.

Although a major increase in crude protein content of fresh tubers as a function of storage facility and duration was observed in all the landraces, the change in total free amino acids varied among the landraces. An increase in a broader range (1.91% to 36.94%) was observed only for one landrace (*IWsh*) in both storage facilities, although the free amino acid content seemed to fluctuate a bit as function of storage time (Table 3B). The increase in cold storage for this landrace corresponded for a large part to the increase in the crude protein content, but this was not the case for the pit storage. On the contrary, a decrease in total free amino acid content was observed for two of the landraces (*LWsh* and *CWsh*). For *LWsh,* it was minor from the first week onward in a range of 14.13% to 26.09% in both storage facilities (Table 3C). For *CWsh,* the decrease was in the range of 40.91% to 55.85% in both storage facilities (Table 3C and 3D). For *DHSew* the change was not consistent in pit storage while in a refrigerator a minor decrease was observed from the second week onward (Table 3A). Hence because of the discrepancy between the increase in crude protein content in general and the drop in free amino acids (for 3 of the 4 landraces), it implies that the importance of free amino acids in the crude protein content (which you can compute by considering total protein/total free amino acids) dropped as well quite considerably in fact.

Table 3A: *The effect of pit (20.71 °C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on free amino acid contents of 'Oromo dinich' tubers for landrace DHSew stored for four weeks.*

Storage Duration (weeks)	Storage Facility	Non-essential free amino acids (g/100 g dm)									
		Asp	Glu	Asn	Ser	Gln	Gly	Cit	Arg	Ala	Tyr
W0	at storage	0.19, 0.20	0.21, 0.22	0.19, 0.20	0.15, 0.16	0.21, 0.22	0.11, 0.11	ND	0.25, 0.26	0.13, 0.13	0.26, 0.27
W1	Pit	0.22, 0.19	0.21, 0.24	0.22, 0.19	0.15, 0.17	0.21, 0.24	0.11, 0.12	0.25, 0.25	0.25, 0.29	0.13, 0.15	0.25, 0.25
W2	Pit	0.21, 0.22	0.24, 0.23	0.21, 0.22	0.17, 0.17	0.24, 0.23	0.12, 0.12	ND	0.28, 0.28	0.14, 0.15	ND
W3	Pit	0.20, 0.20	0.22, 0.23	0.20, 0.20	0.16, 0.16	0.22, 0.23	0.11, 0.12	0.27, 0.26	0.26, 0.27	0.13, 0.14	0.27, 0.28
W4	Pit	0.16, 0.10	0.11, 0.18	0.16, 0.10	0.13, 0.08	0.11, 0.18	0.06, 0.09	0.14, 0.22	0.14, 0.22	0.07, 0.11	0.14, 0.22

Storage Duration (weeks)	Storage Facility	Non-essential free amino acids (g/100 g dm)									
		Asp	Glu	Asn	Ser	Gln	Gly	Cit	Arg	Ala	Tyr
W1	Refrigerator	0.20, 0.120	0.22, 0.23	0.20, 0.20	0.16, 0.16	0.22, 0.23	0.11, 0.12	ND	0.26, 0.27	0.13, 0.14	0.27, 0.28
W2	Refrigerator	0.13, 0.17	0.14, 0.19	0.13, 0.17	0.10, 0.13	0.14, 0.19	0.07, 0.10	0.17, 0.17	0.22, 0.17	0.11, 0.09	0.18, 0.18
W3	Refrigerator	0.16, 0.15	0.16, 0.18	0.16, 0.15	0.12, 0.13	0.16, 0.18	0.08, 0.10	0.20, 0.20	0.22, 0.20	0.11, 0.10	0.23, 0.20
W4	Refrigerator	0.16, 0.20	0.22, 0.18	0.16, 0.20	0.13, 0.16	0.22, 0.18	0.09, 0.11	ND	0.26, 0.21	0.13, 0.11	ND
		Essential and semi-essential free amino acids (g/100 g dm)									∑ (free amino acid means)
		His	Thr	Val	Trp	Phe	Ile	Leu	Lys		
W0	At storage	0.23, 0.22	0.17, 0.18	0.17, 0.18	0.31, 0.29	0.23, 0.25	0.19, 0.20	ND	ND		3.02
W1	Pit	0.22, 0.26	0.17, 0.20	0.16, 0.19	0.34, 0.29	0.23, 0.23	0.18, 0.18	ND	ND		3.35
W2	Pit	0.25, 0.25	0.19, 0.19	0.19, 0.19	0.33, 0.32	0.27, 0.26	ND	ND	ND		2.84
W3	Pit	0.23, 0.24	0.18, 0.18	0.18, 0.17	0.31, 0.30	0.25, 0.24	0.20, 0.19	0.20, 0.19	ND		3.60
W4	Pit	0.12, 0.19	0.15, 0.09	0.14, 0.09	0.25, 0.16	0.13, 0.20	0.16, 0.10	0.16, 0.10	0.18, 0.11		2.51
W1	Refrigerator	0.23, 0.24	0.18, 0.18	0.18, 0.18	0.31, 0.30	0.25, 0.24	0.20, 0.19	ND	ND		3.13
W2	Refrigerator	0.15, 0.20	0.12, 0.15	0.12, 0.15	0.20, 0.26	0.16, 0.21	0.13, 0.17	ND	ND		2.49
W3	Refrigerator	0.19, 0.17	0.15, 0.13	0.15, 0.13	0.25, 0.23	0.21, 0.18	0.16, 0.15	ND	ND		2.64
W4	Refrigerator	0.23, 0.19	0.15, 0.18	0.17, 0.14	0.25, 0.30	0.20, 0.25	ND	ND	ND		2.38

n = 2 (analytical duplicates), W0 = at storage, W1 = week 1, W2 = week 2, W3 = week 3, and W4 = week 4, ND = not detected, Methionine was not detected in any storage facilities or any storage durations.

His = Histidine, Thr = Threonine, Val = Valine, Trp = Tryptophan, Phe = Phenylalanine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Asp = Aspartic acid, Glu = Glutamic acid, Asn = Asparagine, Ser = Serine, Gln = Glutamine, Gly = Glycine, Cit = Citrulline, Arg = Arginine, Al = Alanine, Tyr = Tyrosine.

Table 3B: *The effect of pit (20.71°C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on free amino acid contents of 'Oromo dinich' tubers for landrace IWsh stored for four weeks (g/100 g dm).*

Storage Duration (weeks)	Storage Facility	Non-essential free amino acids (g/100 g dm)									
		Asp	Glu	Asn	Ser	Gln	Gly	Cit	Arg	Ala	Tyr
W0	At storage	0.16, 0.17	0.18, 0.18	0.16, 0.17	0.13, 0.13	0.18, 0.18	0.09, 0.09	0.22, 0.22	0.22, 0.22	0.11, 0.11	0.23, 0.22
W1	Pit	0.25, 0.25	0.28, 0.28	0.25, 0.25	0.20, 0.20	0.28, 0.28	0.14, 0.14	0.34, 0.33	0.33, 0.34	0.17, 0.17	0.34, 0.35
W2	Pit	0.26, 0.25	0.28, 0.29	0.26, 0.25	0.20, 0.20	0.28, 0.29	0.15, 0.14	ND	0.34, 0.34	0.17, 0.17	0.35, 0.35
W3	Pit	0.26, 0.23	0.26, 0.29	0.26, 0.23	0.18, 0.20	0.26, 0.29	ND	ND	0.31, 0.34	0.17, 0.16	0.35, 0.32
W4	Pit	0.25, 0.26	0.28, 0.29	0.26, 0.25	0.20, 0.21	0.28, 0.29	ND	ND	0.33, 0.35	0.17, 0.18	0.34, 0.36
W1	Refrigerator	0.25, 0.26	0.28, 0.28	0.25, 0.25	0.20, 0.20	0.28, 0.28	0.14, 0.14	ND	0.33, 0.33	0.17, 0.17	0.34, 0.35
W2	Refrigerator	0.27, 0.26	0.30, 0.30	0.27, 0.27	0.22, 0.21	0.30, 0.31	0.15, 0.16	ND	0.36, 0.36	0.18, 0.18	0.37, 0.38
W3	Refrigerator	0.26, 0.26	0.29, 0.29	0.26, 0.26	0.20, .00	0.29, 0.29	0.15, 0.15	ND	0.34, 0.34	0.17, 0.17	ND
W4	Refrigerator	0.27, 0.26	0.30, 0.29	0.26, 0.26	0.21, 0.22	0.30, 0.29	0.15, 0.15	0.32, 0.37	0.35, 0.36	0.18, 0.18	0.33, 0.37
		Essential and semi-essential free amino acids (g/100 g dm)									∑ (free amino acid means)
		His	Thr	Val	Trp	Phe	Ile	Leu	Lys		
W0	At storage	0.19, 0.19	0.15, 0.15	0.15, 0.15	0.25, 0.26	0.21, 0.20	0.16, 0.16	0.16, 0.16	0.18, 0.18		3.14

		Essential and semi-essential free amino acids (g/100 g dm)								∑ (free amino acid means)
		His	Thr	Val	Trp	Phe	Ile	Leu	Lys	
W1	Pit	0.29, 0.30	0.23, 0.22	0.22, 0.22	0.39, 0.38	0.31, 0.32	ND	ND	0.28, 0.28	4.30
W2	Pit	0.30, 0.30	0.23, 0.23	0.23, 0.23	0.40, 0.39	0.32, 0.32	ND	ND	ND	3.76
W3	Pit	0.27, 0.30	0.23, 0.21	0.23, 0.21	0.36, 0.40	0.32, 0.29	ND	ND	ND	3.46
W4	Pit	0.31, 0.29	0.22, 0.24	0.23, 0.22	0.39, 0.40	0.31, 0.33	ND	ND	ND	3.61
W1	Refrigerator	0.29, 0.30	0.23, 0.22	0.22, 0.22	0.39, 0.39	0.31, 0.32	0.25, 0.25	ND	ND	3.94
W2	Refrigerator	0.32, 0.32	0.24, 0.25	0.24, 0.24	0.42, 0.41	0.34, 0.34	0.27, 0.27	ND	ND	4.25
W3	Refrigerator	0.30, 0.30	0.23, 0.23	ND	0.40, 0.40	0.32, 0.32	ND	ND	ND	3.20
W4	Refrigerator	0.31, 0.32	0.24, 0.24	0.21, 0.24	0.40, .042	0.34, 0.33	ND	ND	ND	4.25

n = 2 (analytical duplicates), W0 = at storage, W1 = week 1, W2 = week 2, W3 = week 3, and W4 = week 4, ND is not detected, Methionine was not detected in all storage facilities and all storage durations.

His = Histidine, Thr= Threonine, Val = Valine, Trp = Tryptophan, Phe = Phenylalanine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Asp = Aspartic acid, Glu = Glutamic acid, Asn = Asparagine, Ser = Serine, Gln = Glutamine, Gly = Glycine, Cit = Citrulline, Arg = Arginine, Al = Alanine, Tyr = Tyrosine.

Table 3C: *The effect of pit (20.71°C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on free amino acid contents of 'Oromo dinich' tubers for landrace LWsh stored for four weeks (g/100 g dm).*

Storage Duration (weeks)	Storage Facility	Non-essential free amino acids (g/100 g dm)									
		Asp	Glu	Asn	Ser	Gln	Gly	Cit	Arg	Ala	Tyr
W0	At storage	0.19, 0.19	0.21, 0.22	0.19, 0.19	0.15, 0.15	0.21, 0.22	0.11, 0.11	0.26, 0.26	0.26, 0.25	0.13, 0.13	0.26, 0.26
W1	Pit	0.18, 0.17	0.20, 0.19	0.18, 0.17	0.14, 0.14	0.20, 0.19	0.01, 0.10	0.23, 0.23	0.23, 0.24	0.12, 0.12	0.24, 0.25
W2	Pit	0.16, 0.16	0.18, 0.18	0.16, 0.17	0.13, 0.13	0.18, 0.18	0.09, 0.09	0.22, 0.22	0.22, 0.22	0.11, 0.11	0.22, 0.23
W3	Pit	0.16, 0.16	0.18, 0.18	0.16, 0.16	0.13, 0.13	0.18, 0.18	0.09, 0.09	0.21, 0.22	0.21, 0.21	0.11, 0.11	0.22, 0.22
W4	Pit	0.16, 0.18	0.18, 0.20	0.18, 0.16	0.14, 0.13	0.18, 0.20	0.10, 0.09	0.22, 0.24	0.23, 0.22	0.11, 0.12	0.23, 0.24
W1	Refrigerator	0.17, 0.16	0.18, 0.18	0.16, 0.17	0.13, 0.13	0.18, 0.18	0.09, 0.09	0.22, 0.22	0.22, 0.22	0.11, 0.11	0.23, 0.23
W2	Refrigerator	0.18, 0.17	0.20, 0.19	0.17, 0.18	0.14, 0.14	0.20, 0.19	0.10, 0.01	ND	0.23, 0.24	0.12, 0.12	0.24, 0.25
W3	Refrigerator	0.18, 0.18	0.20, 0.20	0.18, 0.18	0.15, 0.14	0.20, 0.20	0.10, 0.10	0.25, 0.24	0.24, 0.24	0.12, 0.12	0.25, 0.25
W4	Refrigerator	0.17, 0.17	0.19, 0.19	0.17, 0.17	0.14, 0.13	0.19, 0.19	0.09, 0.90	0.23, 0.23	0.23, 0.22	0.12, 0.11	0.23, 0.24
		Essential and semi-essential free amino acids (g/100 g dm)								∑ (free amino acid mean)	
		His	Thr	Val	Trp	Phe	Ile	Leu	Lys		
W0	At storage	0.23, 0.23	0.17, 0.17	0.17, 0.17	0.30, 0.30	0.24, 0.24	0.19, 0.19	0.19, 0.19	0.21, 0.21	3.68	
W1	Pit	0.20, 0.21	0.16, 0.16	0.16, 0.15	0.27, 0.28	0.22, 0.23	ND	ND	ND	2.83	
W2	Pit	0.19, 0.18	0.15, 0.15	0.15, 0.14	0.25, 0.25	0.21, 0.20	0.16, 0.16	ND	ND	2.79	
W3	Pit	0.19, 0.19	0.14, 0.14	0.14, 0.14	0.25, 0.24	0.20, 0.20	ND	ND	0.18, 0.18	2.73	
W4	Pit	0.20, 0.19	0.16, 0.14	0.15, 0.16	0.25, 0.27	0.21, 0.22	ND	ND	ND	2.72	
W1	Refrigerator	0.19, 0.19	0.15, 0.15	0.15, 0.15	0.26, 0.25	0.21, 0.21	0.16, 0.16	0.16, 0.16	0.18, 0.18	3.16	
W2	Refrigerator	0.21, 0.20	0.16, 0.16	0.16, 0.15	0.27, 0.28	0.22, 0.23	0.18, 0.17	ND	ND	2.77	
W3	Refrigerator	0.21, 0.22	0.16, 0.17	0.16, 0.16	0.28, 0.28	0.23, 0.23	ND	ND	0.02, 0.02	2.95	
W4	Refrigerator	0.20, 0.20	0.15, 0.16	0.15, 0.15	0.26, 0.27	0.21, 0.22	ND	ND	0.19, 0.19	2.92	

n = 2 (analytical duplicates), W0 = at storage, W1 = week 1, W2 = week 2, W3 = week 3, and W4 = week 4, ND is not detected, Methionine was not detected in all storage facilities and all storage durations.

His = Histidine, Thr = Threonine, Val = Valine, Trp = Tryptophan, Phe = Phenylalanine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Asp = Aspartic acid, Glu = Glutamic acid, Asn = Asparagine, Ser = Serine, Gln = Glutamine, Gly = Glycine, Cit = Citrulline, Arg = Arginine, Al = Alanine, Tyr = Tyrosine.

Table 3D: *The effect of pit (20.71°C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on free amino acid contents of 'Oromo dinich' tubers for landrace CWsh stored for four weeks (g/100 g dm).*

Storage Duration (weeks)	Storage Facility	Non-essential free amino acids (g/100 g dm)									
		Asp	Glu	Asn	Ser	Gln	Gly	Cit	Arg	Ala	Tyr
W0	At storage	0.20, 0.19	0.22, 0.21	0.20, 0.19	0.16, 0.15	0.22, 0.22	0.11, 0.11	ND	0.26, 0.27	0.13, 0.14	0.27, 0.26
W1	Pit	0.05, 0.05	0.16, 0.16	0.04, 0.04	0.01, 0.01	0.16, 0.16	ND	ND	1.13, 1.13	0.04, 0.04	0.01, 0.01
W2	Pit	0.06, 0.13	0.11, 0.12	0.02, 0.03	0.01, 0.02	0.06, 0.07	ND	ND	0.74, 1.10	0.01, 0.05	0.01, 0.01
W3	Pit	0.09, 0.09	0.20, 0.19	0.06, 0.06	< 0.01	0.09, 0.09	0.03, 0.03	ND	0.86, 0.89	0.04, 0.04	ND
W4	Pit	0.04, 0.05	0.17, 0.18	0.15, 0.15	0.02, 0.02	0.15, 0.15	< 0.01	ND	1.08, 1.10	0.08, 0.08	< 0.01
W1	Refrigerator	0.12, 0.18	0.17, 0.15	0.04, 0.05	0.01, 0.02	0.10, 0.13	ND	< 0.01	0.95, 1.02	0.02, 0.03	0.01, 0.01
W2	Refrigerator	0.06, 0.10	0.11, 0.15	0.02, 0.04	0.01, 0.02	0.06, 0.12	< 0.01	< 0.01	0.84, 0.99	0.04, 0.03	0.01, 0.00
W3	Refrigerator	0.07, 0.07	0.14, 0.14	0.01, 0.01	0.02, 0.02	0.08, 0.08	ND	ND	0.90, 0.91	0.04, 0.04	ND
W4	Refrigerator	0.14, 0.14	0.16, 0.16	0.07, 0.07	0.01, 0.02	0.15, 0.15	ND	ND	0.93, 0.94	0.02, 0.03	0.01, 0.01
Storage duration (weeks)	**Storage facility**	**Essential and semi-essential free amino acids (g/100 g dm)**								**∑ (free amino acid means)**	
		His	**Thr**	**Val**	**Trp**	**Phe**	**Ile**	**Leu**	**Lys**		
W0	At storage	0.23, 0.24	0.18, 0.17	0.17, 0.18	0.30, 0.31	0.25, 0.24	0.19, 0.20	ND	ND	3.08	
W1	Pit	0.03, 0.03	0.02, 0.02	ND	0.06, 0.06	< 0.01	ND	ND	0.01, 0.01	1.72	
W2	Pit	0.03, 0.02	0.01, 0.03	ND	0.04, 0.06	< 0.01	ND	ND	0.01, 0.01	1.37	
W3	Pit	0.02, 0.02	< 0.01	ND	0.07, 0.07	ND	ND	ND	0.01, 0.01	1.48	
W4	Pit	0.03, 0.03	0.02, 0.02	ND	0.06, 0.06	ND	ND	ND	0.01, 0.01	1.82	
W1	Refrigerator	0.03, 0.03	0.02, 0.02	ND	0.05, 0.06	< 0.01	ND	ND	0.01, 0.01	1.61	
W2	Refrigerator	0.03, 0.03	0.02, 0.03	ND	0.06, 0.04	< 0.01	ND	ND	0.01, 0.01	1.41	
W3	Refrigerator	0.02, 0.02	0.03, 0.03	ND	0.06, 0.05	ND	ND	ND	0.01, 0.01	1.36	
W4	Refrigerator	0.03, 0.03	0.02, 0.02	ND	0.05, 0.05	< 0.01	ND	ND	0.01, 0.01	1.59	

n = 2 (analytical duplicates), W0 = at storage, W1 = week 1, W2 = week 2, W3 = week 3, and W4 = week 4, ND is not detected, Methionine was not detected in all storage facilities and all storage durations.

His = Histidine, Thr = Threonine, Val = Valine, Trp = Tryptophan, Phe = Phenylalanine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Asp = Aspartic acid, Glu = Glutamic acid, Asn = Asparagine, Ser = Serine, Gln = Glutamine, Gly = Glycine, Cit = Citrulline, Arg = Arginine, Al = Alanine, Tyr = Tyrosine.

Some remarkable changes could be observed for particular free amino acids. Especially for the landrace *CWsh,* a major increase in the free arginine content combined with a major drop in most of the other detected free amino acids, except glutamine and glutamate (Table 3D). This might indicate the released ammonia from the degradation of other amino acids, partially be captured in arginine. The increase in arginine content could be due to the physiological activities of the tubers associated with stress conditions. These stresses contribute to the accumulation of ammonia in the plant tissues. Consequently, the *de novo* synthesis of arginine provides a mechanism to detoxify excess ammonia from the tissue and protect tubers from its lethal effect [53]. The fact that this was only observed for landrace *CWsh* may be a contributory factor for the reported lower susceptibility of landrace *CWsh* to diseases and their relatively longer shelf life than the other landraces [54]. This finding could be a possible explanation for the preference of farmers in the Wolayta zone for this landrace than the other landraces [55].

Generally, variation among landraces in response to total free amino acid content could be due to the change in the physiology of the tubers in protein metabolism relative to their stage of dormancy as reported for potato tubers [51]. Moreover, the presence of these amino acids at different concentrations can affect taste of the final product [56]. Therefore, further study is necessary in this context.

Effects of storage facility and duration on reducing sugars and sucrose

The factorial repeated measure Analysis of Variance (ANOVA) revealed a significant interaction effect ($p < 0.05$) between storage facility and duration on reducing sugars and sucrose content of the tubers, except for the maltose content in landrace *CWsh*. Generally, in all the landraces, reducing sugars content of the tubers stored both in the pit and in the refrigerator, for up to four weeks showed an increasing trend throughout the storage duration (Table 4). For landraces *DHSew, IWsh* and *LWsh,* the reducing sugars content of tubers stored in refrigerator for up to four weeks was significantly higher than for those stored in the pit. For landrace *CWsh,* although the reducing sugars content of tubers significantly increased after one, two and three weeks of storage in the refrigerator, indeed, toward four weeks of storage, both glucose and fructose content dropped significantly ($p < 0.05$). For landrace *DHSew,* the observed increase in reducing sugars content in four weeks of refrigerator storage was due to the parallel increase in fructose content of tubers, whereas for landrace *IWsh* and *LWsh* the increase was due to an increase in both fructose and glucose content (Table 4).

Table 4: *The effect of pit (20.71°C, 75.91% RH) and refrigerator (2.16°C, 20.47% RH) storage facilities on mono- and disaccharides compositions of 'Oromo dinich' tubers as a function of storage duration for four landraces (g/100 g dm).*

Landrace	Mono- and disaccharides (g/100 g dm)	Storage Facility	Storage Duration					SD*	SF	SD × SF
			0 week	1 week	2 weeks	3 weeks	4 weeks			
DHSew	Fructose	Pit	$1.88^{bc(c)} \pm 0.06$	$1.90^{bc(d)} \pm 0.03$	$2.49^{a(d)} \pm 0.07$	$1.88^{c(d)} \pm 0.03$	$2.84^{a(d)} \pm 0.22$	***	***	***
		Refrigerator	$1.88^{e(c)} \pm 0.06$	$3.15^{d(c)} \pm 0.01$	$3.84^{c(c)} \pm 0.10$	$4.96^{ab(c)} \pm 0.34$	$4.27^{abc(c)} \pm 0.38$			
	Glucose	Pit	$1.08^{b(c)} \pm 0.03$	$0.99^{c(c)} \pm 0.02$	$1.78^{abc(c)} \pm 0.7$	$1.64^{a(d)} \pm 0.90$	$1.72^{a(c)} \pm 0.15$	***	**	*
		Refrigerator	$1.08^{c(c)} \pm 0.03$	$1.01^{c(c)} \pm 0.05$	$1.81^{b(c)} \pm 0.12$	$2.51^{a(c)} \pm 0.16$	$2.05^{a(c)} \pm 0.09$			
	Maltose	Pit	$0.04^{ab(c)} \pm 0.04$	$0.07^{ab(c)} \pm 0.02$	$0.07^{a(c)} \pm 0.01$	$0.02^{b(c)} \pm 0.02$	$0.07^{ab(c)} \pm 0.01$	**	***	**
		Refrigerator	$0.04^{ab(c)} \pm 0.04$	$0.08^{ab(c)} \pm 0.00$	$0.08^{ab(c)} \pm 0.01$	$0.11^{b(c)} \pm 0.03$	$0.13^{a(c)} \pm 0.03$			
	Reducing sugars	Pit	$3.00^{ac(c)} \pm 0.12$	$2.95^{c(d)} \pm 0.03$	$4.35^{abc(c)} \pm 0.72$	$3.54^{b(d)} \pm 0.06$	$4.63^{a(d)} \pm 0.14$	***	***	***
		Refrigerator	$3.00^{d(c)} \pm 0.12$	$4.24^{c(c)} \pm 0.06$	$5.72^{b(c)} \pm 0.15$	$7.58^{a(c)} \pm 0.35$	$6.51^{ab(c)} \pm 0.41$			
IWsh	Sucrose	Pit	$1.28^{b(c)} \pm 0.06$	$2.24^{a(c)} \pm 0.06$	$2.88^{a(c)} \pm 0.53$	$1.41^{b(d)} \pm 0.04$	$2.34^{a(c)} \pm 0.09$	***	NS	***
		Refrigerator	$1.28^{c(c)} \pm 0..06$	$1.92^{b(d)} \pm 0.12$	$2.40^{a(c)} \pm 0.19$	$2.55^{a(c)} \pm 0.20$	$2.36^{ab(c)} \pm 0.19$			
	Fructose	Pit	$2.02^{a(c)} \pm 0.09$	$2.45^{a(c)} \pm 0.47$	$2.18^{a(c)} \pm 0.17$	$2.01^{a(d)} \pm 0.08$	$1.60^{a(d)} \pm 0.01$	***	***	***
		Refrigerator	$2.02^{d(c)} \pm 0.09$	$2.26^{c(c)} \pm 0.02$	$0.92^{e(d)} \pm 0.03$	$5.64^{b(c)} \pm 0.01$	$5.88^{a(c)} \pm 0.13$			
	Glucose	Pit	$1.18^{b(c)} \pm 0.03$	$0.95^{abc(c)} \pm 0.21$	$0.84^{c(c)} \pm 0.05$	$1.05^{a(d)} \pm 0.02$	$1.05^{ab(d)} \pm 0.03$	***	***	***
		Refrigerator	$1.18^{c(c)} \pm 0.03$	$1.08^{d(c)} \pm 0.10$	$0.57^{e(d)} \pm 0.03$	$3.39^{b(c)} \pm 0.04$	$3.95^{ad(c)} \pm 0.03$			
	Maltose	Pit	$0.39^{a(c)} \pm 0.03$	$0.12^{bc(c)} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.11^{c(d)} \pm 0.01$	$0.13^{b(c)} \pm 0.01$	***	***	***
		Refrigerator	$0.39^{a(c)} \pm 0.03$	$0.13^{c(c)} \pm 0.01$	ND	$0.17^{b(c)} \pm 0.00$	$0.12^{bc(c)} \pm 0.00$			
	Reducing sugars	Pit	$3.59^{a(c)} \pm 0.09$	$3.52^{ab(c)} \pm 0.69$	$3.17^{ab(c)} \pm 0.16$	$3.16^{b(d)} \pm 0.09$	$3.00^{b(d)} \pm 0.02$	***	***	***
		Refrigerator	$3.59^{c(c)} \pm 0.09$	$3.48^{d(c)} \pm 0.05$	$1.49^{e(d)} \pm 0.21$	$9.20^{b(c)} \pm 0.05$	$10.50^{a(c)} \pm 0.16$			
	Sucrose	Pit	$0.82^{c(c)} \pm 0.06$	$1.29^{abc(c)} \pm 0.27$	$1.56^{ab(c)} \pm 0.12$	$1.25^{b(c)} \pm 0.12$	$1.61^{a(c)} \pm 0.05$	***	**	***
		Refrigerator	$0.82^{b(c)} \pm 0.06$	$0.73^{c(c)} \pm 0.04$	$0.36^{d(d)} \pm 0.02$	$1.55^{a(c)} \pm 0.06$	$1.52^{a(c)} \pm 0.01$			
LWsh	Fructose	Pit	$1.46^{b(c)} \pm 0.02$	$0.87^{c(d)} \pm 0.02$	$0.57^{d(d)} \pm 0.00$	$1.55^{a(d)} \pm 0.03$	$0.46^{e(d)} \pm 0.04$	***	***	***
		Refrigerator	$1.46^{c(c)} \pm 0.02$	$1.03^{d(c)} \pm 0.01$	$1.88^{abc(c)} \pm 0.35$	$1.69^{b(c)} \pm 0.02$	$2.81^{a(c)} \pm 0.25$			
	Glucose	Pit	$0.79^{a(c)} \pm 0.01$	$0.25^{d(d)} \pm 0.03$	$0.21^{d(c)} \pm 0.01$	$0.70^{b(d)} \pm 0.02$	$0.32^{c(d)} \pm 0.04$	***	***	***
		Refrigerator	$0.79^{c(c)} \pm 0.01$	$0.60^{d(c)} \pm 0.04$	$1.09^{abcd(c)} \pm 0.36$	$0.89^{b(c)} \pm 0.03$	$1.55^{a(c)} \pm 0.13$			
	Reducing sugars	Pit	$2.25^{a(c)} \pm 0.01$	$1.12^{b(d)} \pm 0.05$	$0.78^{c(d)} \pm 0.00$	$2.24^{a(d)} \pm 0.04$	$0.78^{c(d)} \pm 0.07$	***	***	***
		Refrigerator	$2.25^{c(c)} \pm 0.01$	$1.63^{d(c)} \pm 0.04$	$2.98^{abcd(c)} \pm 0.71$	$2.58^{b(c)} \pm 0.01$	$4.36^{a(c)} \pm 0.37$			
	Sucrose	Pit	$0.70^{ab(c)} \pm 0.03$	$0.64^{bc(c)} \pm 0.04$	$0.60^{cd(c)} \pm 0.00$	$0.55^{d(d)} \pm 0.03$	$1.00^{a(c)} \pm 0.10$	**	NS	*
		Refrigerator	$0.70^{ab(c)} \pm 0.03$	$0.49^{c(d)} \pm 0.02$	$0.63^{abc(c)} \pm 0.14$	$0.67^{b(c)} \pm 0.01$	$0.81^{a(d)} \pm 0.03$			

Landrace	Mono- and disaccharides (g/100 g dm)	Storage Facility	Storage Duration					SD*	SF	SD × SF
			0 week	1 week	2 weeks	3 weeks	4 weeks			
CWsh	Fructose	Pit	$0.89^{c(c)} \pm 0.01$	$2.11^{b(c)} \pm 0.10$	$1.71^{b(d)} \pm 0.22$	$2.45^{ab(d)} \pm 0.21$	$3.40^{a(c)} \pm 0.38$	***	***	***
		Refrigerator	$0.89^{e(c)} \pm 0.01$	$2.37^{c(c)} \pm 0.12$	$4.91^{a(c)} \pm 0.33$	$4.18^{b(c)} \pm 0.22$	$1.69^{d(d)} \pm 0.03$			
	Glucose	Pit	$0.41^{d(c)} \pm 0.02$	$1.15^{c(c)} \pm 0.09$	$1.13^{bc(d)} \pm 0.20$	$1.64^{b(d)} \pm 0.09$	$2.62^{a(c)} \pm 0.21$	***	***	***
		Refrigerator	$0.43^{d(c)} \pm 0.02$	$1.40^{c(c)} \pm 0.23$	$3.18^{a(c)} \pm 0.15$	$2.51^{b(c)} \pm 0.16$	$1.32^{c(d)} \pm 0.05$			
	Maltose	Pit	ND	$0.12^{a(c)} \pm 0.01$	$0.07^{a(c)} \pm 0.06$	$0.10^{a(c)} \pm 0.04$	$0.10^{a(c)} \pm 0.01$	***	*	NS
		Refrigerator	ND	$0.14^{a(c)} \pm 0.04$	$0.12^{a(c)} \pm 0.03$	$0.12^{a(c)} \pm 0.01$	$0.13^{a(c)} \pm 0.01$			
	Reducing sugars	Pit	$1.31^{c(c)} \pm 0.02$	$3.38^{b(c)} \pm 0.18$	$2.95^{b(d)} \pm 0.44$	$4.19^{b(d)} \pm 0.33$	$6.15^{a(c)} \pm 0.44$	***	***	***
		Refrigerator	$1.31^{c(c)} \pm 0.02$	$3.91^{b(c)} \pm 0.34$	$8.21^{a(c)} \pm 0.40$	$6.81^{a(c)} \pm 0.39$	$3.13^{b(d)} \pm 0.06$			
	Sucrose	Pit	$0.35^{b(c)} \pm 0.01$	$0.29^{c(c)} \pm 0.03$	$0.41^{b(c)} \pm 0.07$	$0.93^{a(c)} \pm 0.13$	$0.28^{bc(c)} \pm 0.06$	**	**	**
		Refrigerator	$0.35^{ab(c)} \pm 0.01$	$0.24^{c(c)} \pm 0.02$	$0.31^{b(c)} \pm 0.03$	$0.34^{ab(d)} \pm 0.03$	$0.38^{a(c)} \pm 0.02$			

n = 3 (analytical replicates), Maltose was not detected in any storage facilities or storage durations in landrace LWsh, ND was not detected, and the limits of detection (LOD) was 0.005 g/100g.

SD × SF indicates significance of the interaction between storage duration (SD*) and storage facility (SF), *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001, NS = not significant.*

Different superscript letters in a row indicate significant differences between storage duration based on LSD values (p < 0.05).

( ) superscript letters in parentheses in a column indicate significant differences between the storage facilities based on LSD values (p < 0.05).

SD standard deviation among analytical replicates.

In all the landraces, the clear increase in reducing sugars content of tubers stored in the refrigerator indicated, the susceptibility for cold-induced sweetening or accumulation of reducing sugars in *'Oromo dinich'* tubers during low temperature storage (2.16°C), which could be due to an increase in starch hydrolysing enzymes (α-amylase, β-amylase and debranching enzymes). These enzymes have been reported to accelerate the rate of conversion of starch to reducing sugars (glucose and fructose) in potato (*S. tuberosum*) tubers stored below 10°C [57-59]. According to [16], the amount of reducing sugars a potato accumulates is cultivar-dependent. Cold-sensitive cultivars accumulate a higher amount of reducing sugars when exposed to low temperatures than cold-tolerant cultivars [59]. Thus, the increase in the reducing sugars content of tubers stored in the refrigerator indicates the susceptibility of all landraces evaluated to cold-induced sweetening. The observation that for landrace *CWsh* reducing sugar content dropped again from the fourth week was in this context quite unexpected.

On the other hand, the significant increase in reducing sugar content of the tubers stored in the pit for up to four weeks, indicated the presence of senescence sweetening in '*Oromo dinich*' tubers during high temperature storage (20.71°C). According to [60], when sweet potato (*I. batatas*) roots stored at a high temperature (20.00°C), the rate of development of senescence sweetening increased due to increased activity of α-amylase.

The amount of sucrose in landraces *DHSew* and *IWsh* significantly increased when tubers were stored for up to four weeks in both storage facilities, without a clear impact of the storage facility used. For the landraces *LWsh* and *CWsh* no clear trend of the sucrose content could be observed.

From the processing point of view, in all the landraces, the amount of reducing sugars in both fresh and stored tubers was above the limit (1 g/kg fresh weight) to be used for roasting and frying [61]. Consequently, both fresh and stored tubers from these landraces are not suitable for processing chips and fries, as increases in reducing sugars will lead to an increase in the brown colour of fried tubers and

in combination with the observed presence of free asparagine, an increase in acrylamide concentration [58,62,63]. Acrylamide is a "probable human carcinogen" [64-66]. However, the amount of accumulated sugars in tubers stored in refrigerator may not be a problem if the accumulated sugars due to cold-induced sweetening can be reduced through reconditioning at higher temperatures. This needs further study on the behaviour of reducing sugar in '*Oromo dinich*' during conditioning. The amount of accumulated sugars in tubers stored in the pit storage can be reduced by blanching/ boiling. Our previous findings showed that boiling significantly reduced the reducing sugars content of '*Oromo dinich*' tubers by 44.00% [67] indicating the possibility of producing quality fried products. Based on the current experiment, the ideal storage temperature for '*Oromo dinich*' tubers favouring a stable reducing sugars content, remains unknown and further storage experiments are recommended.

Conclusion

This study provides considerable amounts of information about the changes in the nutrient composition of '*Oromo dinich'* tubers as a function of storage facility and duration. Losses of moisture from tubers stored for up to four weeks both in the pit and refrigerator were below 10%, much lower than expected based on earlier report on yields from *in situ* stored tubers in the field. Thus, in terms of moisture loss, storing tubers in both storage facilities, do not present shrivelling difficulties for processing. However, in both storage facilities, a rise in the reducing sugars content of tubers was observed due to senescence and cold sweetening. This potentially limits further processing of stored tubers for baking, roasting, frying and chips preparation. On the other hand, the accumulated sugars contribute to the sweet taste of boiled and steamed tubers. For all landraces, irrespective of the storage facilities used, crude fat content of the tubers increased consistently up to four weeks, while the changes in other nutrient composition varied amongst landraces and storage facilities. From an economic point of view, pit storage is preferred over refrigerators but may be better at a temperatures lower than 20°C. Farmers can take advantage of storing tubers in the pit for up to four weeks to prepare a final boiled or steamed product with a sweeter taste and improved nutrition, such as increased protein and fibre contents. Nevertheless, the changes in other tuber chemical components including antinutritional factors and anti-oxidants need further investigation. Based on our findings, tubers from landrace *CWsh* showed a better tendency to tolerate the prevailing stress condition in both storage facilities. Compared with other landraces, the increase in crude fibre content in the *CWsh* could contribute to the hardening of the tuber. Besides, its ability to detoxify excess ammonia from the tissue through the *de novo* synthesis of arginine during storage makes it less susceptible to diseases during storage.

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Volume 18 Issue 1 January 2023