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Physicochemical and nutritional properties of khat (*Catha edulis* forsk) substituted orange (*Citrus sinensis*) and mango (*Mangifera indica*) juices

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Abstract
Khat is widely grown and it is part of livelihood of many communities in Hararghe. Khat has never been tested as component of food. This study was initiated to assess the physicochemical and nutritional properties of fruit juices substituted with khat types grown at three different growing areas of Hararghe, eastern Ethiopia. Three different khat types (Awaday, Galamso and Kobo) and two fruit juices of orange and mango were purchased from open market in Harar and brought to Haramaya University, Central Laboratory. Khat types were processed to powder forms. Phytochemical, particle size, water absorption index (WAI) and water solubility index (WSI) of khat types were made. The three khat types and two fruit juices were combined in 100:0, 97.5:2.5, 95:5.0, 92.5:7.5 and 90:10 of khat: fruit juice ratios. Proximate compositions, mineral contents, vitamin C, total carotenoids (TC), antioxidants, total soluble solids (TSS%), Titratable acidity (TTA%), Viscosity, condensed tannins (CT), total phenols and heavy metals were determined from all the combinations of orange and mango juice. Means separation was made using least significant differences (LSD) at $p \leq 0.05$. The result showed that all khat types had alkaloids, flavonoids, phenols, tannins, proteins, glycosides and terpenoids. All nutrient compositions and anti-nutrients values were increased but vitamin C and antioxidants decreased as khat substitutions increased. Khat as a wild could be tamed and made part of the food security of the country as it is contributing increasing amount of nutrients to the diet by being part of fruit juices.

Keywords: phytochemical, nutritional, anti-nutritional, khat, awaday, galamso, kobo

Introduction

Khat (*Catha edulis* Forsk), is evergreen, glabrous shrub or tree 2–25 m high with reddish stems, shiny green leaves and white flowers. There are several names for the plant, depending on its origin: chat-Ethiopia, qat-yemen, and qaad/jab- Somalia [1]. Khat is mainly limited to, until recently, in Ethiopia and Yemen where it has been cultivated for a very long time. However, recently its cultivation has been spread to most of the eastern African countries and further to South Africa [2]. There is an ever-growing demand both for domestic consumption and for the export market. Most of the exported khat is grown in the eastern Ethiopia and mainly exported to the neighbouring and the Middle East countries, and in recent years, the market for khat has grown to Europe and America [3]. Khat use is widespread and cultivated in most parts of Ethiopia, where its use is socially sanctioned and even prestigious [4]. To date, it is a common practice among many individuals particularly in the eastern, southern, and some parts of northern of the country. Because of increase in population, khat production and consumption is increasing in an alarming rate. Report indicates that over 20 million people in Arabian Peninsula and east Africa are chewing this plant daily [5].

In Ethiopia, khat prefers mid altitudes ranging from 1500 to 2100 m in almost all regions of the country. Khat is grown on well-drained soil [6]. From planting a stand of khat to regular harvesting normally takes 2–3 years. It is harvested by breaking off the young branches from the main branches and trimming it to about 40 cm. Young and soft shoots are detached with the bare hands, while hardy shoots are cut off by hand tools. Depending up on the geographical location, the variety of khat is enormous. They differ in colour, size and height of the leaves and size and height of the plant as a whole and the most favoured part of the plant is its leaves, particularly the young shoots near the top [7]. In Ethiopia, the best time for harvest is the rainy season; June–August with secondary harvest in November – February for irrigation users [6].

In Ethiopia, the plant is marked under different names where some of them are commonly exported to the neighbouring countries while the remaining is chewed by the local people around. Most names of khat are derived from the name of the place where the plant is growing. Example, Awaday khat is cultivated around Awaday, Galamso khat is cultivated in Galamso, Bahir Dar khat is cultivated in Bahir Dar, etc. Hararghe is the principal region of khat cultivation in particular in east and west Hararghe zones: Deder/Kobo, Awaday and Galamso districts with Awaday town as the most important khat market [6].

Among the various compounds in the plant, the two phenyl alkyl amines namely cathine and cathinone seem to account for most of the stimulating effect when ingested users get feeling of well-being mental alertness and excitement then after effects are usually numbness and lack of concentration [8].

To date, extensive work has been done with respect to phytochemical studies and pharmacological as well as social effects of khat. However, no adequate investigations have been made and documented regarding the functional properties of Khat grown in different agro - ecological areas. In addition, although the most common way of

obtaining the stimulating effect of khat is by chewing fresh leaves and soft twigs, consuming in the form of dried, and pounded and mixing with other fruit products particularly in the form of juice is not known. Furthermore, the content and composition of such juice is not determined. Therefore, this study was initiated to analyse the physicochemical, functional and anti - nutrient properties of khat powder supplemented with fruit drinks.

Materials and Methods

Khat collection areas

Fresh khat samples were randomly collected from Awaday, Galamso and Kobo farmers' plots. The collected khat samples were wrapped in pre cleaned polythene bags and transported from these areas to Haramaya University laboratory for further processing [9].

Laboratory Analysis

The product development and laboratory analysis were conducted at the Central Laboratory and Food science laboratory of Haramaya University, Ethiopia.

Experimental Materials

For the purpose of the study, non-wilted (fresh) khat samples were collected from Awaday, Galamso, and Kobo of Hararghe zones. These areas were known for their high khat production and demand throughout the country. The ripened orange and mango were obtained from Harar open market.

Sample preparation

Each khat sample was collected in a pre-cleaned polyethylene bag. It was sorted by cutting the tender leaves and twigs which could be used for chewing purpose. The sorted samples were washed thoroughly with de-ionized water and dried using an oven drying method at 70 °C for 72 h. Dried khat samples were finely ground using a mortar and pestle and the flour was sealed in plastic bags and stored in a refrigerator at 4 ^{0}C until further use.

Preparation of the khat substituted juice

The collected khat samples were sorted by cutting the tender leaves and twigs which can be used for chewing purpose. The sorted samples were washed thoroughly with clean water and dried using an oven drying method. Dried khat samples were finely ground using a mortar and pestle. Khat powder was mixed with water and filtered by using muslin cloth. Furthermore, the sound and ripened mango and orange fruits were selected and washed by water. The cleaned sample of fruits were peeled by using a sharp knife and sliced for juice extraction. The juice was extracted separately from each fruit by using a juice extractor and filtered by muslin cloth. Finally, the extracted juice was blended with filtered khat in the ratios of (100%:0%, 97.5%:2.5%, 95%:5%, 92.5%:7.5% and 90%:10%, respectively). The produced juices were then ready for quality analysis.

Laboratory analysis of khat and khat substituted juice

The particle size of khat powder was determined by allowing the powder to be agitated through a fixed, non - worn out and calibrated sieve. Those powder particles passed through the sieve were considered as equal as or less than the hole of the sieve and considered as the particle size of the khat powder. The viscosity of the fruit juices were determined using Ostwald Viscometer in which fixed amounts of juices and standard liquid (distil water) were allowed to pass through the Ostwald viscometer bulbs and from the flow rates and densities of the two liquids, viscosity of the juices were determined.

All procedures for the determination of moisture, ash, crude protein, crude fat, total carbohydrates and crude fibre were based on [10]. Total carbohydrates (%) were estimated by difference: 100 - (% Moisture +% Crude protein +% Crude fat +% Crude fibre +% Ash) [11-13]. Energy value (kcal per 100 g) was calculated using the Atwater conversion factors [14-16]. E (kcal per 100 g) = [9 x Lipids (%) + 4 x Proteins (%) + 4 x Carbohydrates (%)]

The concentrations of Ca, Mg and Zn in the sample were determined by flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP, East Norwalk, USA) using an air -acetylene flame. Quantitative estimation of condensed tannins was carried out using the modified vanillin–HCl method [17]. A 200 ml sample was extracted using 10 mL of 1% (v⁄v) concentrated HCl in methanol for 20 minutes in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 minutes at 30^{o}C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg 100 g-1) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then, tannin content was calculated and expressed in mg 100 g-1. Water absorption index (WAI) and water solubility index (WSI) of the khat sample were measured as described in [18]. Total soluble solid (TSS) content was determined using digital refractometer which had been calibrated, the lid closed and TSS read directly from the digital scale at 20 °C±1 and results expressed in ^{o}Brix. Ascorbic acid content (vitamin C) was determined using the [19] method. Total Titratable Acidity (TTA) content was determined following the [20, 21]. The total phenolics content was determined colorimeterically using the Folin-Ciocalteu method as described by [22, 23]. Carotenoid was determined according to the method of [24, 25]. Total carotenoids (TC) were made according to [26, 27]. Antioxidant was made according to [28, 29].

Experimental design and data analysis

The quality analysis of juice sample made of fruits and khat were undertaken in triplicates. Completely Randomized design (CRD) was used. Data analysis was carried out using one way analysis of variance (ANOVA) using SAS statistical package (version 9.1, SAS Institute Inc., Cary, NC, USA). Means separation was done using the Fischer's least significant differences (LSD) at $p \leq 0.05$.

Results and Discussions

Phytochemical and functional properties of khat

Phytochemical profiles and functional properties of khat are shown in Table 1 and 2. The assessment of the phytochemicals in the khat types revealed the presence of alkaloid, flavonoid, phenols, tannin, saponins, glycosides and terpenoids in all Awaday, Galamso and Kobo khat types. However, steroids were not present in all khat types and saponins were not presented in Galamso and Kobo khat types (Table 1). There are more than forty compounds present in khat and some of these compounds are health promoting, but the stimulant action of khat is related to the presence of amphetamine-like alkaloids of cathine and

cathinone [30-32].

Table 1: Phytochemical profile of Khat types

Phytochemical Compounds	Khat Type		
	Awaday	Galamso	Kobo
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Saponins	+	-	-
Proteins	+	+	+
Glycosides	+	+	+
Steroids	-	-	-
Terpenoids	+	+	+

Where + = present and - = absent

There were high values of 2.10 g/g and 3.45% recorded for WAI and WSI, respectively, for Galamso khat. Similarly, 3.81% of WSI was recorded for Kobo type. The amounts of WSI and WAI recorded in this study were higher compared to other products like amaranths which were 1.2g/g and 1.5%, respectively [13].

Table 2: Particle size and functional properties of Khat types

Khat type	Particle size (nm)	WAI (g/g)	WSI (%)
Awaday	<200	1.66±0.03^{b}	1.80±0.02^{b}
Galamso	<200	2.10±0.10^{a}	3.45±0.02^{a}
Kobo	<200	1.22±0.02^{c}	3.81±0.02^{a}

Where, values are mean ± SD and mean values followed by the same letters in a column are not significantly different at 5% level of significance.

There high WSI reported in this study was related to the presence of soluble molecules and measure of starch degradation. WSI plays an important role in the food preparation as it influences other functional and sensory properties [33, 34].

Nutrient compositions of khat substituted orange and mango juice

The proximate composition of khat types substituted orange and mango juice is shown in Table 3. Fruit juice substitution with khat powder up to 10 percent) had a significant ($p \leq 0.05$) effect on the proximate compositions and energy values of orange and mango juices. The moisture content decreased as khat substitution increased and ranged from 87.86% to 75.19% for khat substituted orange juice and from 88.75% to 73.22% for mango juice. It is obviously known that juices are known for their high moisture content which makes them ideal for satisfying thirst. Therefore, the high moisture content of the juices was expected because of the raw materials from which the juices were made, were high in moisture content. However, the degree of reduction of the moisture content due to added khat types was different ($p \leq 0.05$) between orange and mango juices. The lowest moisture content of 75.19% was in orange juice substituted with Galamso khat at 90:10 ratio whereas the lowest moisture content of 73.22% was achieved in Mango juice substituted with Kobo khat at 90:10 ratios. This could be due to the presence of higher water solubility indices in both khat types as indicated in Table 2.

Table 3: Proximate nutrient composition of khat substituted fruit juice of orange and mango

Juice Type	Khat type	Ratio % (juice: khat)	Moisture (%)	Ash (%)	CP (%)	Fat (%)	CF (%)	CHO (%)	Energy (Kcal)
Orange	Awaday	100:0	87.86±0.01^{a}	0.53±0.01^{gh}	0.46±0.02^{g}	0.42±0.00^{f}	0.12±0.00^{f}	10.61±0.01^{k}	48.06±0.01^{f}
		97.5 :2.5	85.32±0.03^{c}	0.61±0.02^{efg}	0.82±0.02^{ef}	0.56±0.01^{e}	0.14±0.00^{f}	12.55±0.02^{j}	58.52±0.01^{g}
		95:5.0	83.26±0.01^{e}	0.64±0.00^{edfg}	1.34±0.01^{c}	0.63±0.01^{d}	0.32±0.00^{e}	13.81±0.01^{h}	66.39±0.02^{f}
		92.5:7.5	78.79±0.02^{g}	0.75±0.01^{bcd}	1.63±0.01^{b}	0.86±0.01^{c}	0.53±0.01^{d}	17.44±0.00^{e}	84.02±0.01^{d}
		90:10	75.32±0.02^{h}	0.83±0.01^{abc}	1.93±0.02^{a}	0.95±0.01^{b}	0.64±0.00^{c}	20.33±0.01^{c}	97.59±0.01^{c}
	Galamso	100:0.0	87.89±0.21^{a}	0.53±0.02^{gh}	0.46±0.03^{g}	0.40±0.15^{f}	0.12±0.02^{f}	10.60±0.10^{k}	47.84±0.10^{f}
		97.5 :2.5	85.72±0.10^{d}	0.54±0.01^{fgh}	0.73±0.01^{f}	0.52±0.06^{f}	0.18±0.01^{f}	12.31±0.06^{j}	56.84±0.04^{g}
		95:5.0	82.54±0.21^{f}	0.63±0.10^{edfg}	0.97±0.02^{de}	0.68±0.10^{h}	0.73±0.10^{b}	14.45±0.10^{f}	67.80±0.10^{e}
		92.5:7.5	78.55±0.07^{ih}	0.73±0.15^{cde}	1.40±0.10^{c}	0.84±0.02^{i}	0.94±0.02^{a}	17.54±0.26^{e}	83.32±0.03^{d}
		90:10	75.19±0.15^{j}	0.85±0.03^{a}	1.72±0.21^{b}	0.91±0.08^{c}	0.97±0.02^{a}	20.36±0.26^{b}	96.51±0.25^{b}
	Kobo	100:0	87.72±0.25^{a}	0.53±0.20^{gh}	0.46±0.10^{g}	0.47±0.20^{f}	0.12±0.02^{f}	10.70±0.10^{k}	48.87±0.21^{f}
		97.5 :2.5	85.49±0.15^{c}	0.57±0.10^{fg}	0.68±0.21^{f}	0.58±0.02^{e}	0.18±0.03^{f}	12.50±0.02^{i}	57.94±0.20^{g}
		95:5.0	82.84±0.12^{f}	0.66±0.02^{def}	1.13±0.02^{d}	0.74±0.02^{c}	0.27±0.02^{e}	14.36±0.03^{g}	68.62±0.01^{e}
		92.5:7.5	78.71±0.03^{ij}	0.84±0.03^{abc}	1.63±0.03^{b}	0.85±0.15^{b}	0.51±0.10^{d}	17.46±0.20^{d}	84.01±0.15^{c}
		90:10	75.35±0.17^{k}	0.86±0.03^{ab}	1.73±0.26^{b}	0.87±0.15^{a}	0.64±0.10^{c}	20.55±0.25^{a}	96.95±0.12^{a}
Mango	Awaday	100:0	88.75±0.06^{a}	0.41±0.51^{g}	0.52±0.00^{h}	0.54±0.00^{h}	0.15±0.00^{e}	9.63±0.03^{i}	45.46±0.02^{j}
		97.5 :2.5	86.76±0.02^{b}	0.46±0.01^{fg}	0.65±0.00^{fg}	0.69±0.00^{g}	0.35±0.01^{cd}	11.09±0.00^{h}	53.17±0.02^{k}
		95:5.0	82.98±0.01^{e}	0.54±0.01^{def}	0.99±0.00^{d}	0.78±0.00^{e}	0.43±0.01^{bc}	14.28±0.00^{e}	68.10±0.02^{h}
		92.5:7.5	78.76±0.05^{g}	0.61±0.01^{cde}	1.53±0.02^{b}	0.87±0.00^{d}	0.43±0.01^{bc}	17.80±0.00^{d}	85.15±0.00^{d}
		90:10	75.48±0.02^{j}	0.73±0.01^{ab}	1.73±0.01^{a}	0.94±0.02^{b}	0.52±0.01^{b}	20.60±0.02^{b}	97.78±0.02^{b}
	Galamso	100:0.0	88.75±0.01^{a}	0.41±0.02^{g}	0.52±0.01^{h}	0.53 ±0.10^{h}	0.15±0.01^{e}	9.64±0.01^{j}	45.41±0.06^{j}
		97.5 :2.5	86.22±0.10c	0.46±0.01^{efg}	0.56±0.02^{g}	0.63±0.06^{f}	0.17±0.02^{e}	11.96±0.10^{h}	55.75±0.94^{g}
		95:5.0	84.36±0.10^{d}	0.53±0.10^{def}	0.92±0.02^{ed}	0.72±0.10^{d}	0.26±0.01^{de}	13.21±0.15^{g}	63.00±0.21^{i}
		92.5:7.5	79.42±0.15^{h}	0.63±0.04^{bcd}	1.26±0.10^{c}	0.88±0.10^{d}	0.39±0.10^{bcd}	17.42±0.15^{d}	82.64±0.25^{d}
		90:10	76.54±0.12^{i}	0.77±0.02^{a}	1.69±0.05^{ab}	0.95±0.10^{b}	0.45±0.10^{bc}	19.60±0.06^{c}	93.71±0.10^{c}
	Kobo	100:0	88.75±0.15^{a}	0.42±0.02^{g}	0.51±0.31^{g}	0.53±0.15^{h}	0.15±0.02^{e}	9.64±0.04^{k}	45.37±0.21^{j}
		97.5 :2.5	85.03±0.20^{d}	0.45±0.02f^{g}	0.76±0.10^{ef}	0.60±0.10^{f}	0.37±0.10^{cd}	12.79±0.21^{h}	59.60±0.21^{f}
		95:5.0	81.79±0.35^{f}	0.52±0.03^{def}	1.01±0.00^{d}	0.80±0.14^{c}	0.52±0.10^{b}	15.36±0.04^{f}	72.68±0.30^{e}
		92.5:7.5	77.24±0.10^{h}	0.66±0.15^{bc}	1.52±0.15^{b}	0.87±0.03^{b}	0.72±0.15^{a}	18.99±0.35^{e}	89.87±0.35^{c}
		90:10	73.22±0.21^{k}	0.74±0.15^{ab}	1.76±0.21^{a}	0.96±0.03^{a}	0.70±0.21^{a}	22.62±0.25^{a}	106.16±0.15^{a}

Where, values are mean ± SD and mean values followed by the same letter in a column are not significantly different at 5% level of significance. CP = Crude protein, CF = Crude fibre, CHO = Carbohydrates

Table 4: Mineral contents of khat substituted fruit juice of orange and mango

Juice Type	Khat Type	Ratio % (juice: khat)	Ca(mg/l)	Mg(mg/l)	Fe(mg/l)	Zn(mg/l)	P(mg/l)
Orange	Awaday	100:0	1067.71±0.12^{m}	6100.00±0.00^{e}	260.00±0.00^{h}	82.00±0.00^{j}	35.42±0.68^{e}
		97.5 :2.5	2338.24±1.00^{k}	7400.00±0.00^{d}	273.33±1.00^{i}	110.00±0.00^{i}	43.47±1.53^{c}
		95:5.0	2371.59±1.00^{j}	10566.66±3.00^{c}	316.33±1.53^{g}	120.00±0.00^{h}	44.59±1.00^{c}
		92.5:7.5	2737.90±0.58^{i}	10966.67±2.00^{b}	333.33±2.00^{f}	154.00±1.00^{f}	51.13±0.58^{a}
		90:10	4125.80±2.08^{f}	12000.00±0.00^{a}	381.67±2.89^{e}	161.67±0.58^{e}	52.60±2.52^{a}
	Galamso	100:0.0	325.29±1.00^{o}	2600.0±00.00^{m}	125.66±1.00^{m}	160.00±0.00^{e}	36.91±1.53^{e}
		97.5 :2.5	847.06±0.00^{n}	2900.00±0.00^{l}	146.99±1.53^{l}	270.00±0.00^{d}	40.79±0.21^{d}
		95:5.0	2035.29±0.00^{l}	3700.00±0.00^{j}	150.00±0.00^{l}	340.00±5.00^{c}	43.31±1.53^{c}
		92.5:7.5	3277.75±2.08^{h}	4301.67±2.89^{i}	181.67±1.53^{j}	377.67±1.53^{b}	46.74±0.32^{b}
		90:10	4271.59±1.00^{e}	4866.66±2.00^{f}	173.66±1.53^{k}	396.33±1.53^{a}	52.77±1.00^{a}
	Kobo	100:0	3742.51±1.53^{g}	1435.00±1.53^{o}	1374.66±1.53^{c}	126.33±1.53^{g}	14.70±1.00^{g}
		97.5 :2.5	4578.41±1.00^{d}	2500.00±0.00^{n}	1221.67±1.53^{d}	148.00±1.00^{k}	15.80±0.15^{g}
		95:5.0	6575.90±1.53^{c}	3201.33±2.31^{k}	1220.00±0.00^{d}	142.00±0.00^{l}	18.65±1.15^{f}
		92.5:7.5	7263.43±1.53^{b}	4634.33±1.00^{g}	1393.33±1.00^{b}	138.00±1.00^{l}	18.06±1.00^{f}
		90:10	9185.29±0.00^{a}	4600.00±0.00^{h}	1467.00±1.00^{a}	131.67±11.55^{m}	19.83±0.15^{f}
Mango	Awaday	100:0	344.42±0.26^{o}	1066.33±2.52^{m}	411.67±4.73^{j}	171.33±6.03^{g}	35.47±0.40^{g}
		97.5 :2.5	1176.57±0.36^{m}	2901.33±1.53^{o}	456.36±0.15^{i}	216.33±0.58^{e}	38.84±0.15^{ef}
		95:5.0	2117.84±0.10^{k}	4500.67±1.15^{j}	490.33±0.58^{h}	248.33±0.58^{b}	42.28±0.15^{d}
		92.5:7.5	2858.72±0.26^{h}	10100.00±0.00^{b}	496.83±0.15^{g}	250.00±0.00^{b}	45.63±0.21^{c}
		90:10	3870.65±0.12^{e}	14500.00±0.00^{a}	570.00±0.00^{f}	276.00±0.00^{a}	52.38±0.43^{a}
	Galamso	100:0.0	350.73±0.38^{n}	3900.00±0.0^{0k}	140.00±0.00^{o}	133.33±1.53^{i}	37.33±1.00^{f}
		97.5:2.5	1253.27±1.53^{l}	7764.99±1.53^{f}	180.00±0.00^{n}	156.00±0.00^{h}	39.41±0.50^{e}
		95:5.0	2332.35±0.00^{i}	7700.00±0.00^{g}	196.33±3.51^{m}	188.00±0.00^{f}	42.91±1.53^{d}
		92.5:7.5	3052.94±0.00^{g}	8065.66±1.00^{e}	240.00±0.00^{l}	232.33±1.53^{d}	44.79±1.00^{c}
		90:10	4328.41±1.00^{d}	8800.00±0.00^{c}	250.00±0.00^{k}	244.00±1.00^{c}	50.56±0.35^{b}
	Kobo	100:0	2238.92±1.00^{j}	2766.66±2.00^{n}	1554.33±2.65^{d}	191.67±1.53^{j}	14.48±0.25^{k}
		97.5 :2.5	3742.18±1.00^{f}	3502.67±2.52^{l}	1740.67±2.08^{a}	174.00±1.00^{l}	19.54±0.30^{i}
		95:5.0	4953.33±5.77^{c}	5200.00±0.00^{i}	1606.66±3.00^{c}	171.00±3.00^{l}	18.46±1.53^{ij}
		92.5:7.5	5076.47±3.00^{b}	6000.00±0.00^{h}	1653.66±2.52^{b}	162.67±2.08^{m}	17.57±1.53^{j}
		90:10	7523.77±3.00^{a}	8603.33±1.53^{d}	1295.66±2.52^{e}	183.33±1.5^{3k}	24.42±1.53^{h}

Where, values are mean ± SD and mean values followed by the same letter in a column are not significantly

The ash content of khat substituted orange and mango juices ranged from 0.53% to 0.86% and from 0.41% to 0.77%, respectively, which significantly (p≤0.05) different either from no khat substituted orange juice or no khat substituted mango juice. The higher ash content observed in both juices was contributed from the substitution of the fruit juices by khat types.

Protein contents of khat substituted juices ranged from 0.46% to 1.93% for orange and 0.52% to1.73% for mango juice. Awaday type of khat at 90:10 ratios had highest protein content (1.93%) for orange juice. For mango juice, however, at 90:10 ratios for all types of khat, the highest crude protein was recorded with no statistical parity. The Fat% in both orange and mango juice increased as the level of khat substitution increased in the juices. These values were significantly (p≤0.05) different among khat types and between orange and mango juices. The CF% increased as the levels of khat substitutions increased in the juices and these values were significantly (p≤0.05) different among the khat types and between orange and mango juices. The CHO values also increased as the levels of khat substitution increased and these values were significantly (p≤0.05) different among the khat types and between orange and mango juices. Since the value of fat and CHO increased as the level of khat substitution increased, the energy of the juices also increased and these values were significantly (p≤0.05) different among the khat types and between orange and mango juices. Generally, in both orange and mango juices, the nutrient compositions were increased with increased level of khat substitution. This might be due to the presence of higher chemical composition in the khat types [9].

Khat substitution had significant (p≤0.05) effect on the mineral compositions of orange and mango juices (Table 4). The mineral content of orange and mango juice were increased as the levels of khat substitution increased, which was due to the presence of many minerals and different compounds in the khat types. Vitamin C, TC and antioxidant of khat substituted fruit juices are presented in Table 5. These values were significantly (p≤0.05) different among khat types and between orange and mango juices. The TC content increased as the level of khat substitutions increased in the juices. But vitamin C (Ascorbic acid) and antioxidants were decreased as the level of khat substitutions increased in the fruit juices. As vitamin C values decreased in the fruit juices so were the antioxidants which could be due to the replacement of ascorbic acid by khat compounds.

The %TSS, %TTA, viscosity, CT and total phenolics are presented in Table 6. %TSS of orange and mango substituted by Awaday type khat was significantly (p≤0.05) different either from Galamso or Kobo khat substituted orange or mango juices. There were no significant (p≤0.05) differences between Galamso and Kobo khat substituted orange or mango juices. The %TTA was significantly (p≤0.05) different between khat type substituted orange and mango juices. The highest and lowest viscosities were recorded in Kobo khat substituted orange juice. These values were 1.05±0.04 g/cm.sec and 1.37±0.02g/cm.sec, respectively. In general, the viscosities were decreased as the amount of khat substitutions increased the fruit juices.

Natural foods especially citrus fruits play major roles in

human nutrition as they are good sources of vitamin C, carotenoids, antioxidants, proteins, carbohydrates, minerals and many other important compounds needed for healthy human life [35,36]. The nutrient composition of fruit juices substituted by khat decreased in moisture contents whereas the other nutrient components were increased as the substitution of khat increased in the juices. Normally, the proximate composition of freshly squeezed orange and mango juices would have had more moisture, vitamin C and minerals contents than the processed juices [12]. But the current data showed that minerals, proteins, fats, carbohydrates and energy contents were increased as the khat substitutions increased in the juices.

Ant-nutrient content of khat types substituted orange and mango juices

The heavy metals contents of khat substituted orange and mango juice are presented in Table 7. There were only two heavy metals namely Cd (Cadmium) and Co (Cobalt) measured in khat substituted orange and mango juices. There were no values detected for Cu, Ni, Pb, Cr, or Mn for all khat types substituted orange or mango juices. The lowest Cd value of 18.19±0.02 mg/100g was obtained from 97.5:2.5 ratios of Galamso khat and mango juice. The highest Cd value of 137.89±0.10 mg/100g was obtained from 97.5:2.5 ratios of Galamso khat and orange juice. The lowest Co value of 45.89±0.77 mg/100g was obtained from 100:0 ratios (no khat) Mango juice. The highest Co value of 341.87±0.12 mg/100g was obtained from 92.5:7.5 ratios of Kobo khat and mango juice. These heavy metals are great concern for human health although some heavy metals may have health importance as trace elements [37].

The amount of CT and total phenolics increased significantly ($p \leq 0.05$) as the khat substitution increased in orange and mango juices. The lowest CT value of 1.26±0.04 mg/100g was obtained in no Awaday khat substituted (100:0) mango juice. The highest CT value of 108.35±0.56 mg/100g was obtained in Galamso khat substituted (90:10) orange juice. This value was not statistically significant ($p \leq 0.05$) from the value of CT obtained from Kobo khat substituted (90:10) orange juice. The lowest total phenols value of 175.33±0.58 mg/100g was obtained in no Awaday khat (100:0) substituted mango juice which was significantly ($p \leq 0.05$) different from other values at the same ratios. The highest total phenols value of 5554.72±0.14 mg/100g was obtained in Awaday khat substituted (90:10) mango juice which was not statistically significant ($p \leq 0.05$) from 4575.33±0.58 mg/100g of total phenolics obtained from Awaday khat substituted (90:10) orange juice. This means Awaday khat had higher phenolics than the other types of khat used in the study.

Anti-nutrients are naturally found plants are compounds designed to protect plants from insect attack and bacterial infections. Tannins may be able to reduce the absorption of iron but Phenols may also be able to reduce the absorption of important metals such as Iron, Zinc, Magnesium and Calcium [38, 39]. The increase in CT content might be ascribed to the presence of high tannin content in khat types. Tannins form a complex with proteins rendering the protein less available for body utilization [15]. Some phenolics compounds have the ability to bind cations such as magnesium, calcium, iron, zinc, and molybdenum and make them unavailable for human body utilization and can cause the deficiency of micro nutrients [40]. It also forms stable bond with protein and may inhibit the activity of some enzymes, such as amylase and proteases [41]. However, Phytic acids also have antioxidant and ant carcinogenic properties. Indeed, Phytic acids can reduce free ion radical generation and thus peroxidation of membranes by complexing iron, and phytate may protect against colon cancer [42].

Table 5: Vitamin C, total carotenoid (TC) and antioxidant contents of fruit juice substituted with different levels of Khat types

Juice Type	Khat Type	Ratio % (juice: khat)	Vitamin C (mg/100g)	TC (mg/g)	Antioxidant (%)*
Orange	Awaday	100:0	19.32±0.28^{b}	5.71±0.16^{j}	80.06±0.15^{a}
		97.5 :2.5	4.35±0.03^{de}	8.38±0.16^{i}	71.32±0.19^{f}
		95:5.0	4.56±0.05^{cd}	10.67±0.28^{h}	73.03±0.34^{e}
		92.5:7.5	4.25±0.02^{e}	14.43±0.29^{e}	74.92±0.34^{d}
		90:10	4.26±0.01^{e}	19.35±0.13^{a}	63.28±1.10^{i}
	Galamso	100:0.0	22.32±0.21^{a}	5.71±0.16^{j}	80.06±0.15^{a}
		97.5 :2.5	4.47±0.02^{cde}	8.54±0.39^{i}	77.51±0.52^{b}
		95:5.0	3.85±0.06^{f}	10.39±0.15^{h}	74.58±0.64^{d}
		92.5:7.5	4.66±0.20^{c}	11.65±0.32^{g}	71.62±0.35^{f}
		90:10	4.34±0.09^{de}	15.39±0.33^{d}	74.11±0.43^{d}
	Kobo	100:0	22.47±0.35^{a}	5.71±0.16^{j}	80.06±0.15^{a}
		97.5 :2.5	4.53±0.06^{cd}	8.57±0.17^{i}	74.46±0.41^{d}
		95:5.0	3.92±0.05^{f}	12.46±0.24^{f}	68.23±0.39^{h}
		92.5:7.5	4.47±0.16^{cde}	16.23±0.13^{c}	70.39±0.49^{g}
		90:10	4.26±0.02	17.32±0.50^{b}	76.02±0.89^{c}
Mango	Awaday	100:0	6.85±0.01^{c}	9.82±0.17^{j}	77.02±1.01^{ab}
		97.5 :2.5	3.93±0.01^{j}	10.57±0.22^{i}	65.74±1.15^{g}
		95:5.0	5.39±0.08^{d}	15.25±0.20^{f}	70.46±0.78^{e}
		92.5:7.5	4.88±0.10^{f}	19.55±0.36^{c}	68.44±0.16^{f}
		90:10	5.37±0.06^{d}	22.92±0.28^{a}	69.60±1.13^{ef}
	Galamso	100:0.0	9.73±0.16^{a}	9.82±0.17^{j}	75.69±2.67^{bc}
		97.5 :2.5	5.03±0.08^{e}	10.30±0.45^{i}	77.83±0.57^{a}
		95:5.0	4.36±0.02^{h}	12.47±0.39^{h}	74.20±1.13^{cd}
		92.5:7.5	4.39±0.06^{h}	14.18±0.14^{g}	74.33±0.20^{cd}
		90:10	4.18±0.0^{1i}	18.46±0.19^{d}	65.05±0.04^{g}
		100:0	8.45±0.14^{b}	9.82±0.17^{j}	75.69±2.67^{bc}

	Kobo	97.5 :2.5	4.64±0.03^{g}	10.39±0.44^{i}	77.06±0.66^{ab}
		95:5.0	4.16±0.0^{4i}	15.46±0.19^{f}	73.94±0.47^{cd}
		92.5:7.5	4.44±0.0^{1h}	17.12±0.14^{e}	65.80±0.70^{g}
		90:10	4.75±0.01^{g}	22.32±0.32^{b}	72.67±0.46^{d}

TC = total carotenoids, Vitamin C = Ascorbic acid, * % antioxidant activity (DPPH radical scavenging activity) Where, values are mean ± SD and mean values followed by the same letter in a column are not significantly different at 5% level of significance.

Table 6: %Total soluble solid (TSS), % total titratable acidity (TTA), viscosity, condensed tannins (CT) and total phenols of fruit juice substituted with different levels of khat types

Juice Type	Khat Type	Ratio % (juice: khat)	TSS (%)	TTA (%)	Viscosity (g/cm.sec)	CT (mg/100g)	Total Phenols (mg/100g)
Orange	Awaday	100:0	9.10±0.10^{e}	2.53±0.01^{c}	1.24±0.03^{b}	5.75±0.03^{i}	611.36±0.55^{i}
		97.5 :2.5	10.03±0.06^{d}	2.33±0.05^{d}	1.23±0.01^{b}	17.18±0.10^{gh}	1322.93±0.90^{g}
		95:5.0	11.13±0.15^{c}	2.84±0.01^{a}	1.15±0.01^{c}	41.63±0.06^{e}	2717.91±0.14^{d}
		92.5:7.5	12.10±0.10^{b}	2.49±0.01^{c}	1.13±0.01^{c}	67.93±0.05^{d}	3892.50±0.50^{b}
		90:10	13.10±0.10^{a}	2.31±0.01^{de}	1.12±0.02^{c}	93.45±0.00^{b}	4575.33±0.58^{a}
	Galamso	100:0.0	8.55±0.05^{f}	1.66±0.01^{i}	1.35±0.01^{a}	6.46±0.01^{i}	344.45±0.39^{j}
		97.5 :2.5	8.51±0.02^{f}	2.23±0.01^{fg}	1.27±0.02^{b}	14.99±1.35^{h}	827.72±0.25^{h}
		95:5.0	8.52±0.02^{f}	2.05±0.02^{h}	1.27±0.03^{b}	35.48±0.45^{f}	1464.54±0.50^{f}
		92.5:7.5	8.52±0.03^{f}	2.17±0.01^{g}	1.25±0.02^{b}	75.33±0.58^{c}	2183.45±0.51^{e}
		90:10	8.50±0.00^{f}	2.66±0.01^{b}	1.16±0.01^{c}	108.35±0.56^{a}	2882.30±0.61^{c}
	Kobo	100:0	8.53±0.06^{f}	1.67±0.01^{i}	1.37±0.02^{a}	6.47±0.02^{i}	344.56±0.51^{j}
		97.5 :2.5	8.50±0.00^{f}	2.25±0.04^{ef}	1.26±0.05^{b}	21.51±0.50^{g}	827.17±1.04^{h}
		95:5.0	8.50±0.00^{f}	2.05±0.05^{h}	1.15±0.05^{c}	35.03±0.08^{f}	1464.54±0.50^{f}
		92.5:7.5	8.53±0.06^{f}	2.16±0.15^{g}	1.14±0.01^{c}	74.67±0.58^{c}	2183.12±1.02^{e}
		90:10	8.50±0.00^{f}	2.66±0.01^{b}	1.05±0.04^{d}	107.68±0.59^{a}	2881.63±0.55^{c}
Mango	Awaday	100:0	8.13±0.23^{f}	1.02±0.00^{f}	1.26±0.02^{b}	1.26±0.04^{m}	175.33±0.58^{o}
		97.5 :2.5	8.53±0.06^{de}	1.22±0.0^{1c}	1.25±0.03^{b}	20.80±0.22^{k}	1647.05±1.00^{i}
		95:5.0	9.11±0.20^{c}	1.23±0.01^{c}	1.16±0.07^{de}	33.24±0.02^{i}	2662.08±0.88^{h}
		92.5:7.5	10.08±0.13^{b}	1.02±0.01^{f}	1.12±0.01^{e}	47.68±0.10^{d}	3876.83±0.76^{d}
		90:10	11.30±0.26^{a}	1.84±0.01^{a}	1.06±0.05^{f}	58.63±0.55^{b}	5554.72±1.11^{a}
	Galamso	100:0.0	8.10±0.17^{f}	0.97±0.02^{g}	1.27±0.02^{b}	5.39±0.10^{l}	443.33±1.53^{m}
		97.5 :2.5	8.30±0.30^{ef}	1.02±0.01^{f}	1.23±0.00^{bc}	21.33±0.28^{j}	363.58±0.52^{n}
		95:5.0	8.53±0.06^{de}	1.25±0.05^{c}	1.25±0.05^{bc}	38.67±0.11^{g}	2955.06±0.92^{g}
		92.5:7.5	8.52±0.03^{de}	1.15±0.03^{de}	1.12±0.02^{e}	48.41±0.06^{c}	3346.00±1.00^{f}
		90:10	8.53±0.03^{de}	1.33±0.01^{b}	1.05±0.04^{f}	64.82±0.17^{a}	4283.50±1.32^{b}
	Kobo	100:0	8.53±0.03^{de}	0.63±0.02^{i}	1.37±0.03^{a}	5.59±0.07^{l}	462.14±0.80^{l}
		97.5 :2.5	8.58±0.08^{d}	0.88±0.01^{h}	1.26±0.02^{b}	21.33±0.01^{j}	619.59±0.53^{k}
		95:5.0	8.60±0.10^{d}	1.14±0.01^{e}	1.23±0.02^{bc}	33.83±0.21^{h}	1459.14±1.03^{j}
		92.5:7.5	8.60±0.10^{d}	1.18±0.03^{d}	1.26±0.04^{b}	40.49±0.42^{f}	3464.44±0.51^{e}
		90:10	8.50±0.10^{de}	1.25±0.04^{c}	1.20±0.01^{cd}	47.03±0.06^{e}	4166.00±1.00^{c}

Where, values are mean ± SD and mean values followed by the same letter in a column are not significantly different at 5% level of significance.

Table 7: Heavy metal contents of khat substituted fruit juice of orange and mango

Juice Type	Khat type	Ratio % (juice: khat)	Cd(mg/l)	Co(mg/l)	Cu (mg/l)	Ni (mg/l)	Pb (mg/l)	Cr (mg/l)	Mn (mg/l)
Orange	Awaday	100:0	40.42±0.15^{g}	73.60±0.20^{n}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	35.67±0.23^{i}	84.61±0.39^{m}	Nd	Nd	Nd	Nd	Nd
		95:5.0	36.63±0.23^{h}	87.70±0.41^{l}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	45.59±0.34^{f}	84.63±0.37^{m}	Nd	Nd	Nd	Nd	Nd
		90:10	36.67±0.28^{h}	100.29±0.60^{k}	Nd	Nd	Nd	Nd	Nd
	Galamso	100:0.0	63.58±0.43^{e}	126.38±0.33^{h}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	98.66±0.57^{c}	120.51±0.51^{i}	Nd	Nd	Nd	Nd	Nd
		95:5.0	96.49±0.49^{d}	115.81±0.25^{j}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	137.89±0.10^{a}	130.59±0.27^{g}	Nd	Nd	Nd		Nd
		90:10	135.82±0.20^{b}	146.42±0.12^{f}	Nd	Nd	Nd	Nd	Nd
	Kobo	100:0	Nd	305.88±0.11^{a}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	Nd	294.80±0.26^{b}	Nd	Nd	Nd	Nd	Nd
		95:5.0	Nd	285.82±0.30^{c}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	Nd	273.94±0.07^{d}	Nd	Nd	Nd	Nd	Nd
		90:10	Nd	263.64±0.56^{e}	Nd	Nd	Nd	Nd	Nd
	Awaday	100:0	100.26±0.25^{a}	45.89±0.77^{m}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	89.63±0.55^{b}	73.77±0.18^{k}	Nd	Nd	Nd	Nd	Nd
		95:5.0	77.72±0.01^{c}	63.26±0.07^{l}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	73.90±0.16^{d}	78.25±0.23^{j}	Nd	Nd	Nd	Nd	Nd
		90:10	55.08±0.11^{e}	84.56±0.23^{i}	Nd	Nd	Nd	Nd	Nd
		100:0.0	36.37±0.12^{h}	84.48±0.50^{i}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	18.19±0.02^{i}	110.54±0.21^{g}	Nd	Nd	Nd	Nd	Nd

Mango		95:5.0	54.12±0.10^{g}	105.08±0.13^{h}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	54.66±0.19^{f}	125.33±0.58^{f}	Nd	Nd	Nd	Nd	Nd
	Galamso	90:10	54.58±0.28^{f}	151.19±0.05^{e}	Nd	Nd	Nd	Nd	Nd
		100:0	Nd	305.77±0.23^{d}	Nd	Nd	Nd	Nd	Nd
		97.5 :2.5	Nd	331.74±0.23^{b}	Nd	Nd	Nd	Nd	Nd
		95:5.0	Nd	326.80±0.29^{c}	Nd	Nd	Nd	Nd	Nd
		92.5:7.5	Nd	341.87±0.12^{a}	Nd	Nd	Nd	Nd	Nd
	Kobo	90:10	Nd	326.43±0.50^{c}	Nd	Nd	Nd		Nd

Nd = not detected Where Nd = Not detected, values are mean ± SD and mean values followed by the same letters in a column are not significantly different at 5% level of significance.

Conclusion

The phytochemical, functional and anti-nutrient properties of khat types (Awaday, Galamso and Kobo) substituted fruit juice (orange and mango) were analyzed. The phytochemical profile showed that khat types were rich in alkaloids, flavonoids, phenols, tannins, proteins, glycosides and terpenoids. Except the moisture content, which decreased as the Khat substitution increased, the other nutrient compositions were increased as khat substitution increased in the juices. Vitamin C and antioxidants decreased and TC and Phenols were increased as the khat substitutions increased in all orange and mango juices. Except for Awaday khat, TSS% did not have significant differences compared to the control values. TTA% was higher in orange juice when compared to mango juices in all khat substitutions. Viscosity values were higher in mango juices compared to orange juices in all khat substitutions. CT and total phenols increased as khat type substitutions increased in both orange and mango juice. Cd (mg/l) and Co (mg/l) were the two heavy metals found in both orange and mango juices. Both heavy metals increased as the khat substitutions were increased in orange and mango juices. The other heavy metals of Cu, Ni, Pb, Cr, and Mn were not detected in both orange and mango juices. Food security is the main issue around the world. Khat is not part of the diet although it is chewed by the community as part of habit forming situations. However, it could be more useful if it included in the diet since it increases the nutritional values of foods specially fruit juices.

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