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Selected Nutritional Values and Physicochemical Properties of Traditionally Prepared Ethiopian Fresh Butter Intended for Direct Consumption

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Abstract

The level of selected nutritional values, physicochemical properties and metal contents of butter samples collected from Dire Enchini and Ejere Districts in West Showa Zone, Oromia Regional State, Ethiopia have been investigated to ensure food safety. Properly optimized sample preparation methods and calibrated and verified analytical procedures have been employed for the analysis. The result of the study indicated that, the butter sample collected from Dire Enchini district have shown average ash, fat, protein, carbohydrate and solid not fat contents of 0.10, 82.73, 2.32, 1.18 and 4.45 %, respectively, while the corresponding values for butter sample from Ejere district were 0.13, 84.71, 1.87, 0.86 and 2.19 %, respectively. The results showed there was significant differences in fat, crude protein and solid-not-fat contents of butter from the two districts. The determined physicochemical properties of butter samples from Dire Enchini have shown the average moisture (%), Melting point (°C), Free fatty acids (as oleic) (%), titratable acidity (butyric acid) (%), peroxide value (meq/ kg), Iodine value (I_2g /100g), saponification value (mg KOH/ g) and cholesterol in butter (mg/ 100 g) contents of 12.82, 34.5-35.40, 0.82, 0.28, 0.48, 3.76, 95.3 and184.13±12.19, respectively, while the corresponding values for butter sample obtained from Ejere were 11.77, 35.0-35.70, 0.62, 0.23, 0.41, 3.97, 103 and 280.88, respectively. The optimized wet digestion method for Butter sample analysis was found effective for all of the minerals as it was evaluated through the recovery experiment and a good percentage recovery range from 91 – 100 % was obtained with %RSD ≤ 12 for the minerals analyzed. The average levels of metals investigated followed the order of K > Ca > Na > Mg > Fe > Zn > Cu >Mn (mg/ Kg) in Butter sample from Dirre Enchini district. Similar order was obtained in butter sample from Ejere, except Cu > Zn. The concentrations of the metals, nutritional value and Physicochemical property of butter were found to be in a good agreement with the international standards and indicating no risk exposure of using the butter from the investigated areas.

Keywords: food safety, butter, physicochemical, nutritional values, optimization, recovery study

Introduction

Butter is a traditional natural food used worldwide and is essential for human nutrition. Due to its high fat content; it is an important source of energy and contains many other nutritionally important components, such as minerals and vitamins (Hermida *et al.*, 2001; Dvorák et al., 2016). Indigenous dairy products made from different milk sources (cow, buffalo, sheep and goats) are traditionally produced and consumed in a majority of African and Arabian countries (Mourad and Nour-Eddine, 2006; Mattiello *et al.*, 2018). In Africa, milk is produced in most agricultural production systems. It is either sold fresh, consumed as fermented milk or manufactured into products such as butter, ghee and cheese. Sour milk is the most common product, and milk is usually soured before further processing.

While there are several milk processing plants in Africa, much of the milk produced by rural smallholders is processed on-farm using traditional technologies(O'Connor, 1995).

The main reasons for souring milk before processing as stated by O'Connor (1995) are associated with high ambient temperatures, small daily quantities of milk (1-4 lit/day), consumer preference and increased keeping quality of sour milk. In addition, souring milk has a number of advantages including retardation of the growth of undesirable micro-organisms, such as pathogens and putrefactive bacteria and makes the milk easier to churn. The equipment required for processing sour milk is also simple and available locally. Therefore, processing sour milk will continue to be important in this sector (O, Connor, 1995).

Dairy processing in Ethiopia is generally based on ergo (fermented milk), without any defined starter culture, with natural starter culture (Mogessie, 2002). Traditional Ethiopian butter is always made from Ergo and not from cream (O,Connor, 1995). However, it is more efficient to make butter from cream, than from whole milk (O'Mahony, 1988). Butter (also called locally "Dhadha" in Afan Oromo or Kibe in Amharic) has an attractive appearance with a white to yellowish color. Like factory processed butter, it is semi-solid at room temperature. It has a pleasant taste and odor when fresh, but with increased storage, changes occur in odor and taste, unless refrigerated or further processed into Neter kibe (traditional Ghee) by boiling with spices (CARE-Ethiopia, 2009).

In Ethiopia, smallholder farmers and pastoralists together produce and supply 98% of the total annual milk production of the country (CARE-Ethiopia, 2009). The vast majority of milk produced outside urban centers in the country is processed into milk products at household level using traditional technologies (Tegegne *et al.*, 2013). In the rural areas of Ethiopia, it is estimated that 40% of the milk produced is converted to butter, while only 9% is converted to cheese. Traditional butter ferments slowly at room temperature, offering rural consumers a readily storable and durable dairy product (Shapiro *et al.*, 2015).

In Ethiopia, at national level, out of the total butter production, 80% is used as food ingredient and the rest used as cosmetics for hairdressing. Out of the 80% used for food 70% was used in rural and nearby urban areas; while 30% is channeled into Addis Ababa market (Zelalem et al., 2011). Though the processing of milk in to butter is nutritionally and economically important for rural peoples and saves the milk from spoilage and diversifies its use, the production processes, handling, traditional preservatives and preservation techniques of butter requires great care. In different rural areas of Ethiopia, producers use different traditional preservatives and preservation methods to increase butter shelf life (Alganesh and Yetenayet, 2017; Bereda et al., 2013. These traditional preservatives used as a principle of acidification and moisture reduction and can thus make butter of good storage stability (O'Mahony and Peters, 1987).

Dire Enchini and Ejere woreds, located in the West Shewa Zone of Oromia regional state, Ethiopia, are very famous in producing butter of high quality. However, scientific data on nutritional values, physicochemical properties and levels of essential metals in butter samples from these two districts is currently not available. So, in the current study, we aimed at filling this gap by generating a reliable data on nutritional values, physicochemical properties and concentration levels of selected essential metals in traditionally prepared butter samples from the two aforementioned districts.

Materials and methods

Description of the Study Area

This study was conducted in West Shewa administrative zone, Oromia regional state, Ethiopia. The butter samples used in this investigation were collected from Dire Enchini and Ejere districts of West Shewa Zone. Dire Enchini is located at a distance of 40 km from Ambo (the capital of West Shewa Zone) with altitude ranging from 2505 to 2634 m.a.s.l., while Ejere is located at a distance of 70 km from Ambo (the capital of West Shewa Zone) with altitude ranging from 2500 to 3200 m.a.s.l. Both districts are well known for their production of high quality butter. The communities of these two districts are highly dependent on their butter for food security and other benefits. The geographical locations of these two woredas are specified in Figure 1

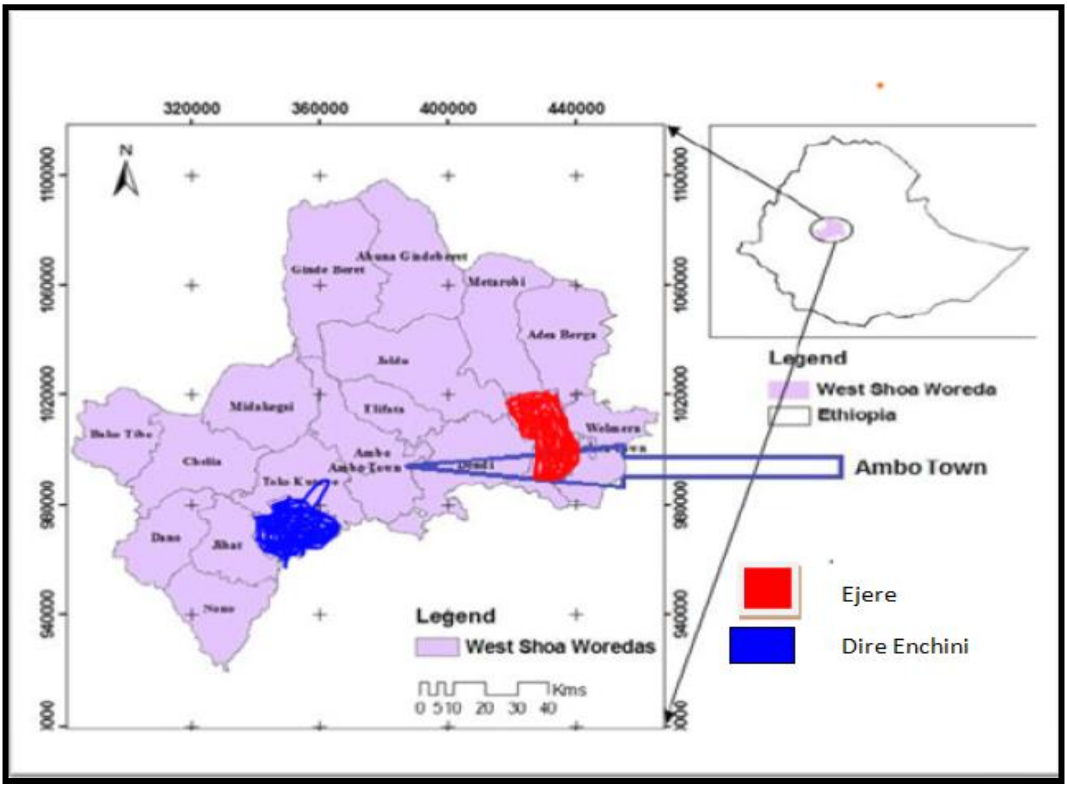

Figure 1. Location map of Dire Enchini and Ejere Districts

Apparatus and Instruments

A drying oven (Tianjin Taisite instrument, 101-0, China) was used to dry butter samples for determination of moisture content. Electronic analytical balance (AA-200DS, Deriver Instrument Company, Germany) was used for weighing the samples. Precleared and sterilized polyethylene bags were used for collecting butter samples. A porcelain crucible was used to dry the butter sample in the electric furnace followed by cooling the extracted butter samples using desiccator. A 250 mL round bottomed flasks fitted with reflux condensers in Kjeldahl apparatus (KDN-20C, china) hot plate were used to digest the butter samples. Various volume borosilicate volumetric flasks were used during sample dilution and crude protein analysis. Flame atomic absorption spectrometer (Agilent Technology 200 Series AA), equipped with deuterium arc back ground corrector and hollow cathode lamps with air/acetylene flame atomizer was used for the analysis of metals (Mn, Zn, Fe, Mg, Ca and Cu) and Flame Photometer (ELICO, CL-

378, India) was used for Na and K metals analysis.

Chemicals and Reagents

All chemicals and reagents used were of either analytical or laboratory reagent grade and obtained from Uni-Chem chemical reagents, India, unless otherwise stated. HNO_3 (65-68 %); H_2SO_4 (98 %) and extra pure hydrogen peroxide, H_2O_2 (30 %). NaOH (40 %) and H_2SO_4 (98 %) were used for protein digestion. A mixture of K_2SO_4, $CuSO_4.5H_2O$ and TiO_2 (NICE CHEMICALS, COHIN) were used to catalyze the protein digestion and as a reagent blank. Boric acid (H_3BO_3) (99.5%) was used as indicator and NaOH (99%) were used for determination of protein. Petroleum ether was used for determination of crude fat. $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$ were used for the determination of total Ca and Mg in the butter samples. Glacial acetic acid (99.8%-100%), Hannus iodine solution (99.5%), potassium iodide (98.5%) ethanol (97%), sodium thiosulphte (99%) and phenolphthalein indicator were used for estimation of iodine and acid values. Ferric chloride (99.0%) and Glacial acetic acid (99.8%-100.5%), and 98% concentrated (H_2SO_4) were used for determination of cholesterol in butter. Stock standard solution of concentration 50 ppm of metals Na, K, Fe, Zn, Ca and Mg standard solution, copper and zinc metals (Fluka, Switzerland), NaCl (99 %) and $MnSO_4$ (99 %) (both from NICE, Chemical pvt. Ltd) were used as stock standard and intermediate standard solutions.

Analytical Procedures

Butter Sample Collection and Pretreatment

Traditionally prepared Cow's milk butter samples were bought from known open local market for the analysis of both nutritional values, physicochemical properties and levels of metals. The purchased butter samples were immediately rapped with aluminum foil and kept in an ice box at 4 °C and transported to Ambo University laboratory without delay for further treatment and analysis.

Determination nutritional values of the butter

The moisture content, solid not fat (SNF) and fat contents of the butter samples were determined by following the procedure used by Evers and co-workers (Evers et al., 2003). The total ash content of the butter was determined by following standard procedures. Protein content was determined by Kjeldahl method according to the AOAC official method 920.87 (AOAC, 2000). For nutritional labeling purposes, carbohydrates were calculated by difference after determination of ash, protein, moisture and fat contents of the butter.

Determination of Some Physicochemical Properties of Butter

The melting point of butter samples was determined by following the method described by Nahm (Nahm, 2011). Titratable Acidity (Butyric Acid %) was determined by following standard procedure. The free fatty acid; peroxide value; iodine value and saponification value were determined by titration method following the standard method outlined in AOAC 940.28; AOAC 965.33; AOAC 920.158 and AOAC 920.160, respectively (AOAC, 2000). The cholesterol contents of the butter samples were also determined by using standard technique.

Optimization of digestion method

Different digestion procedures were assessed to figure out an optimal condition for digestion of the butter samples using HNO_3, $HClO_4$ and H_2O_2 acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature. The optimum digestion procedure was selected based on the usage of lesser reagent volume, shorter digestion time and reasonably mild temperature for obtaining clear solutions of the resulting digests.

Quality control procedures

A practical approach to study the accuracy and precision of the method used for digestion of the butter samples is to spike the known concentrations of each metals followed by digesting the spiked sample using the optimized procedure. From the results obtained percentage recovery (%R) was calculated to estimate the accuracy of the method employed while percentage relative standard deviation (%RSD) was calculated to estimate precision of the method used. To verify the sensitivity of our method, the method detection and quantification limits (MDL and MQL) was calculated based up on seven replicate measurements of a series of calibration blanks (reagent blank) employed through the entire sample preparation scheme following the standard procedures used by Miller (2010). The MDL was calculated by Eq. 1 while Eq. 2 was used to calculate MQL.

$$\mathbf{MDL = S\ x\ t - test} \qquad \mathbf{Eq.\ 1}$$

Where, S is standard deviation of the replicated analysis with n-1 degree of freedom, t = 3.71 (t- test value for a 99% of confidence level for six degrees of freedom) reagents (n = 7).

$$\mathbf{MQL} = X_{\mathbf{blank}} + \mathbf{10Sd_{blank}} \qquad \mathbf{Eq.\ 2}$$

Where, X_{blank} = mean for the blank measurement and Sd_{blank} = standard deviation of the blank measurement

Digestion Method

After proper optimization of the digestion procedure (wet digestion) (*cf.* Table 1), triplicate of 0.5g of each butter sample was weighed in 50 mL conical flasks. About 20 mL of a freshly prepared mixture of concentrated 4 mL of HNO_3, 1 mL of H_2O_2 and 6 mL of H_2SO_4 solution was added to each flask and kept for 10 min at room temperature. Then, the sample solution was heated on kejldahl (KDN-20) digestive furnace at 350 ℃ until clear solutions was obtained. Then, the samples were evaporated and the semidried mass was dissolved in 5 mL of 0.2 M HNO_3 then filtered through Whatman with No.42 filter paper and made up to final volume of 50 mL in volumetric flasks with ultrapure water. The metal contents were determined in the diluted solution by AAS.

Statistical Analysis

The recorded data were subjected to ANOVA to investigate the effect of sample origin on the concentration of heavy metals. As the level of metal contamination might vary with sample site, one-way ANOVA was used to test the existence of significant difference between the means. In all statistical analyses, confidence level was held at 95% (unless otherwise indicated) and the statistical calculations were made using SPSS software (Version 21).

Results and Discussion

Physicochemical Parameters of Analyzed Butter Samples

The physicochemical parameters of butter samples from Dire Enchini and Ejere districts have been investigated. The data obtained are presented in Table 1. The results of study revealed that, butter samples obtained from Dire Enchini Woreda of West Shewa Zone in central Ethiopia, has shown the average Moisture (%), Melting point (℃), Free fatty acids (as oleic)%, Titratable acidity (butyric acid %), peroxide value (meq/kg), iodine value (I_2/100g) and Cholesterol in butter (mg/100g) contents of 12.82, 34.5-35.40, 0.82, 0.28, 0.48, 3.76, 95.3 and 184.13, respectively. Similarly, the corresponding values for butter sample from Ejere Woreda has shown the average values of 11.77, 35.0-5.70, 0.62, 0.23, 0.41, 3.97, 103.00 and 280.88 for moisture (%), melting point (℃), free fatty acids (as oleic %), titratable acidity (butyric acid %), peroxide value (meq/kg), iodine value (I_2 /100g) and cholesterol, respectively.

Table 1 Physicochemical properties of butter samples from Dire Enchini and Ejere districts (n=3)

Parameters	Dire Enchini	Ejere	Permissible limit or standard reference
Moistures (%)	12.82±1.26	11.77±1.19	16[a]
Melting point (°C)	34.50-35.40	35.00-35.70	NA
Free fatty acids (as oleic) %	0.82±0.05	0.62±0.10	0.3[b]
Titratable acidity (butyric acid %)	0.28±0.02	0.23±0.03	NA
Peroxide value (meq/kg)	0.48±0.14	0.41±0.04	0.6[b]
Iodine value (I_2 g/100g)	3.76±0.14	3.97±0.07	NA
Saponification value (mg KOH/g)	95.3±0.42	103.00±0.01	NA
Cholesterol in butter(mg/100g)	184.13±12.19	280.88±0.53	215[c]

NA: not available, a = GB/19646-2010, b = FAO/WHO (1999), c = USDA (2018)

As can be seen from Table 1, the moisture contents of butter samples from both Dire Enchini and Ejere districts are lower than the standard reference set by National Standard of Food Safety of People's Republic of China (GB/19646-2010). The percentage total moisture contents in Ejere and Dire Enchini districts were 11.77 and 12.82, respectively. The % moisture contents of butter samples studied were found to be lower than reported value, which is 17.2%, for similar study as reported by Ashenafi (Ashenafi, 2008). Observation suggests that the low level of moisture content in butter sample were attributed to its long shelf life. The data obtained in this investigation for % moistures contents of butter from both districts were in the range of the permissible level value of ≤16 % (GB/19646-2010). From the statistical data analysis, the values for the moisture content of butter samples from the two woreds have showed no significant difference at $P > 0.05$. The melting point of butter sample from Ejere was found to be in the range of 35.0-35.7 °C, while the corresponding value for Dire Enchini butter was ranged from 34.5-35.4 °C.

The total free fatty acids (as oleic acids) contents in butter sample from Dire Enchini and Ejere woreds were 0.82 and 0.62 %, respectively. The free fatty acid contents (as oleic acid) obtained in this study were found to be higher than the reported value of 0.17% by Simon (2012) in similar study. However, the results obtained from this study were higher than the permissible level of free fatty acid value, 0.3%, reported by FAO/WHO as per CODEX STAN 279-1971 document (FAO/WHO, 2007). It is important to mention that; acid value determination is often used as a general indication of the condition of the butter. In line with this, there are arguments that an increase in an acid value is accompanied by development of insufferable flavors and odors of butter.

The titratable acidity content of Butter from Dire Enchini and Ejere were 0.28 and 0.23 %, respectively. There were no significant differences in titratable acidity contents between butter samples ($P>0.05$, at 95 %CI) from the two districts. The titratable acidity contents obtained from both samples are very comparable with the literature values of 0.26 and 0.37 % (Simon, 2012). However, the result of this study for % titratable acidity contents of butter samples from both districts are lower than standard reference value of 0.5 % (USDA, 2018).

The peroxide value contents of butter samples from Dire Enchini and Ejere districts were 0.48 and 0.41 meq/kg, respectively (*cf.* Table 1). The peroxide values obtained from both butter samples are very comparable with the reported values of 0.46 meq/kg (Simon, 2012). In the results of this study are lower than FAO/WHO maximum value of 0.6 meq/ kg according to CODEX STAN 234-1999 (2006). There is no significant differences in peroxide value contents of studied butter samples (P>0.05at 95%CI) from the two districts. As per the reports of McGinely (1991), the peroxide value is a measure of the content of hydro-peroxides in butter, which are the primary reaction product formed in the initial stages of oxidation of butter and therefore gives an indication of the likely occurrence of the process of lipid per oxidation (Onwuka, 2005).

The saponification value of butter samples from Dire Enchini and Ejere districts were 95.3 and 103 mg KOH/g, respectively, as can be seen from Table 2. There were no significant differences in saponification values of the butter samples (P > 0.05 at 95 %CI) from the two districts. The iodine value content of butter from Dire Enchini was 3.76 I_2g/100g while the corresponding value for butter sample from Ejere was 3.97 I_2g/100g, respectively. There were no significant differences in iodine value contents of butter samples (P>0.05 at 95%CI) from the two districts. According to Thomas (2002), iodine value suggests degree of unsaturation present in butter and the higher the iodine number the more C=C bonds are present in the fat.

Nutritional Compositions of Butter Samples Analyzed

The Nutritional compositions of butter samples collected from Dire Enchini and Ejere district of West Shewa Zone of Oromia regional state in Ethiopia are presented in Table 2. The results of study revealed that, butter samples from the two districts have shown the average Ash, fat, protein, carbohydrate and solid not fat contents of 0.10, 82.73, 2.32, 1.18, 4.45 %, respectively, for butter samples from Dire Enchini and 0.13, 84.71, 1.87, 0.86, 2.19 %, respectively, for butter samples from Ejere district.

As indicated in Table 2, the average ash content of 0.128 and 0.100 % in butter sample from Ejere and Dire Enchini districts, respectively. The ash contents obtained from both samples are very comparable with the reported value of 0.12 - 0.2 % (Mekdes, 2008). The data obtained in this investigation for %Ash contents of butter from Dire Enchini and Ejere districts are in agreement with the standard reference value of 0.09 % (USDA, 2018). From the statistical analysis result, there were no significant differences in ash contents of butter samples (P>0.05, at 95 %CI) from the two districts.

Table 2 Nutritional values of butter from Dire Enchini and Ejere Districts

Parameters (%)	Dire Enchini	Ejere	permissible limit or Standard reference
Ash	0.1003±0.0087	0.128±0.0281	0.09[a]
Fat	82.73±0.097	84.71±0.075	82[b]; 81.11[a]
Protein	2.32±0.045	1.87±0.094	0.85[a]
Carbohydrate	1.18±0.055	0.86 ±0.012	0.06[a,b]
Solid not fat	4.45±0.050	2.19±0.110	2[b]

a. Standard reference value (USDA, 2018)
b. Permissible limit (FAO/WHO, 2007)

The average fat content in Dire Enchini butter sample was 82.73 % and the corresponding value for butter sample from Ejere was 84.71 %, respectively (*cf.* Table 2). It has been found that there was significant difference in fat contents between butter samples ($P < 0.05$, at 95 %CI) from the two districts. A slightly higher fat contents in both butter samples were obtained as compared with reports from similar investigation with fat content ranges from 81.2 to 81.7 % (Zelalem,1999; Ashenafi, 2006). The result indicates that butter in two districts is rich in fat. The result of this study is in good agreement with the permissible level of fat contain in butter, which indicate the recommended minimum level should be 82 % as reported by FAO/WHO (2007).

The solid not fat (SNF) content of butter from Dire Enchini and Ejere were 4.45 and 2.19 %, respectively. Twice as high solid not fat content was recorded for the Dire Enchini butter sample compared with butter samples from Ejere district. Likewise, high content of solid not fat was observed in this study for the Dire Enchini butter sample when compared with values obtained in similar research (2 %) (Van denBerg, 1988). There were significant differences in solid not fat contents of butter samples ($P<0.05$, at 95 %CI) from the two districts. From the analysis of solid not fat value from Dire Enchini and Ejere butter samples were above the permissible limit (2 %).

The crude protein contents of butter samples from Dire Enchini and Ejere districts were 2.32 and 1.87 %, respectively. The results indicated that Dire Enchini butter contained appreciable amount of crude protein content of 2.32 % compared with the butter sample from Ejere and statistically significantly different at 95 %CI. The protein contents of butter samples investigated have showed comparatively higher values than the reported value of 1.3 % as reported by Ashenafi (2006). The values of crude protein obtained in this study were found to be higher than the permissible level set by FAO/WHO (2007), which states that, the crude protein level in animal fat (butter) should not exceed 0.85 %.

The Carbohydrate by difference contents from the Dire Enchini and Ejere were 1.18 and 0.86 %, respectively. Comparatively higher Carbohydrate by difference contents were obtained in this study than data obtained in similar research with Carbohydrate content values of 0.1% carbohydrate (Ashenafi, 2006). The standard reference value of Carbohydrate by difference containing of butter should be 0.06 % (USDA, 2018). However, the result obtained from this study is much higher than the indicated standard value.

The Cholesterol contents of the butter samples from two districts were determined using UV-vis spectroscopic techniques at the entire wave length range. The cholesterol level content of Butter from Dire Enchini and Ejere were 184.13 and 280.5 mg/100g, respectively. According to the analysis, Ejere butter samples has recorded high cholesterol level compared with butter samples from Dire Enchini butter sample. A study by Seckin and his coworkers (Seçkin *et al.*, 2005) on Turkish dairy products have reported cholesterol contents of butter varying from 251.27 to 369.04 mg/100g, while the report by Souci *et al.* (2000) have showed cholesterol contents varying from 180 to 295 mg/100g. The Cholesterol level in butter sample should not exceed 215 mg/100g according to standard reference (USAD, 2018). The result of this study indicated that the cholesterol level in butter samples from Ejere district was found to be higher than the standard level indicated.

Quality Control and Method Validation

Optimizations of Digestion Procedure for Butter Samples

The digestion procedure employed in this study has been optimized to establish appropriate digestion procedure in terms of acid volume to be used, optimum temperature and time required for the digestion process.

The data obtained in the optimization procedure is as presented in Table 3. From the result of the optimization procedure, an optimum volume of 6, 4 and 1 mL for H_2SO_4, HNO_3 and H_2O_2, respectively, have been established at optimum temperature of 350 °C.

Method of Validation

Method Precision and Accuracy

The accuracy of the method was determined by matrix spike recovery. The recovery values the matrix spike of Dire Enchini and Ejere samples were calculated and the results are presented in Table 4. As it can be seen from Table 4 that, the mean percent recovery values ranged from 90.5 to 100 %, all lied in the acceptable range (80–120%) for metal analysis. This showed that the analytical method used has showed the required level of accuracy. The precision of the method used were estimated by calculating % RSD values, which has ranged from 0.01 to 12.0 %, and all lied under the required limit ≤15 % (Csuros and Csuros, 2002). This indicated that the analytical method used have provided an acceptable repeatability or precision.

Table 3 Result for the optimizations of digestion procedure for 0.5 g butter sample

T/No	Mass of sample (g)	Mixture of reagent (mL) H_2SO_4	HNO_3	H_2O_2	Temperature (°C)	Time (hr.)	Observation
1	0.5	7	5	2	350	0:45	Clear and light yellow
2	0.5	6.5	4.5	1.5	350	0:45	clear and colorless
3	0.5	**6**	**4**	**1**	350	0:45	***clear and colorless**
4	0.5	6	4	0.5	350	0:45	Clear and light yellow
5	0.5	5	3	1	350	0:45	Clear and light yellow
6	0.5	5	3	0.5	350	0:45	Clear and deep yellow
7	0.5	4	3	1	350	0:45	Yellow
Optimization of Temperature							
1	0.5	6	4	1	310	0:45	Yellow
2	0.5	6	4	1	330	0:45	Light yellow
3	0.5	6	4	1	**350***	0:45	***Clear and colorless**
4	0.5	6	4	1	370	0:45	Clear and colorless
Optimization of Time							
1	0.5	6	4	1	350	0:15	Yellow
2	0.5	6	4	1	350	0:30	Light yellow
3	0.5	6	4	1	350	**0:45***	***Clear and colorless**
4	0.5	6	4	1	350	1:00	Clear and colorless
5	0.5	6	4	1	350	1:15	Clear and colorless

Table 4 Recovery and precision test for the optimized procedure from sample spike (n= 3)

Metals	Concentration in sample (mgL^{-})	Amount of added (mgL-)	Concentration in spike (mgL^{-})	Accuracy Recovery (%)	Precision (%RSD)
Na	1.083±0.0060	2.00	3.042±0.128	97 .98±6.66	0.53
K	1.097±0.0050	1.00	2.044±0.039	94 .7 ± 4.31	0.46
Ca	2.353±0.0026	1.00	3.346±0.138	99.36 ± 4. 13	0.11
Mg	0.178±0.0014	1.00	1.155±0.094	97.63 ± 9.32	0.65
Fe	1.140±0.0002	2.00	2.195±0.146	96.42 ± 8.24	0.01
Mn	0.0005±0.0001	2.00	2.005±0.001	90.52 ± 6.42	12.0
Zn	0.0075±0.0004	1.00	1.005±0.005	91 ± 0.51	5.40
Cu	0.0005±0.0001	2.00	1.996±0.005	100 ± 0.26	1.85

Method Detection and Quantification Limit

Method detection limit and method quantification limit for each metal analyte were calculated from the instrument response of seven replicates of the method blank using the respective regression equation of the calibration curve equation and the values are presented in Table 5.

Table 5 Instrument detection limits, Method detection limit and method quantification limit for each meta

Metals	IDL(mgL^{-})	MDL(mg/kg)	MQL(mg/kg)
Na	0.0017	2.185	2.526
K	0.0016	9.103	9.477
Ca	0.0138	8.965	9.180
Mg	0.0032	9.106	9.854
Fe	0.0016	0.087	0.094
Mn	0.0017	0.006	0.007
Zn	0.0050	0.051	0.064
Cu	0.0024	0.059	0.068

IDL= Instrument Detection Limit; MDL=Method Detections Limit and MQL = Method Quantification Limit

As it can be observed in Table 5, the method detection values ranged from 0.006 mg/kg for Mn determination in butter to 9.103 mg/kg for K determination. The MQL values on the other hand lied in range from 0.007 mg/kg for Fe determination and 9.477 for K. The results revealed the both MDL and MQL values were greater than the IDL; hence, the results of the analysis expected to be reliable.

Levels of Metals Analyzed in Butter

The levels of micro and macro metals in butter samples from Dire Enchini and Ejere districts have been analyzed and the data obtained is as presented in Table 6. The levels of metals analyzed in Dire Enchini butter sample were found to be 265.4, 107.6, 235.3, 17.9, 1.92, 0.53, 0.05 and 0.73 mg/kg for K, Na, Ca, Mg, Fe, Cu, Mn and Zn, respectively. While the corresponding values for butter sample from Ejere district were found to be 254.8, 102.7, 229.5, 14.1, 1.81, 0.9, 0.062 and 0.57 mg/kg for K, Na, Ca, Mg, Fe, Cu, Mn and Zn, respectively. From the data we can see that the metal contents have followed the order of **K > Ca > Na > Mg > Fe > Cu > Mn** for butter samples from both districts.

Table 6 Mean concentration (n = 3) of metals (mg/kg) in butter samples from the two districts

Metals	Sample concentration (mg/kg)		USDA (2018)
	Dire Enchini butter	Ejere butter	
K	265.4 ± 4.860	254.8 ± 4.260	240
Na	107.6 ± 0.115	102.7 ± 0.130	110
Ca	235.3 ± 0.265	229.5 ± 0.550	240
Mg	17.9 ± 0.116	14.1 ± 0.064	20
Fe	1.92 ± 0.006	1.81 ± 0.010	0.2
Cu	0.53 ± 0.005	0.9 ± 0.006	0.16
Mn	0.05 ± 0.001	0.062 ± 0.006	0.04
Zn	0.73±0.0015	0.57 ±0.0015	0.9

As it can be seen from Table 6 and Figure 2, the mean concentrations of potassium (K) in both butter samples was higher than all metals analyzed followed by Ca metal. The statistical tests showed that there were no significant differences (P > 0.05, at 95% CI) among the mean concentrations of K metal in the butter samples. The levels of K obtained from this study is slightly higher than the standard value set by USAD (2018). The analyzed sodium concentration in butter samples Dire Enchini and Ejere butter samples in were ranged from 102.7 to 107.56 mg/kg, respectively. The Mg contents of Dire Enchini and Ejere butter sample were 17.87 and 14.07 mg/kg, respectively. The concentration of Mg in butter samples were not significantly different (P > 0.05, at 95% CI).

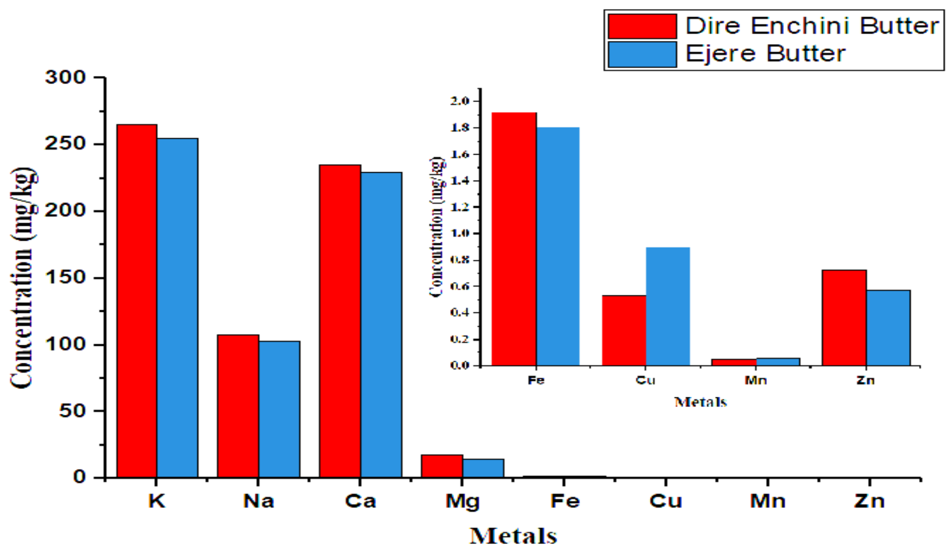

Figure 2. Concentration of Metals in Butter Sample

The average concentration of Calcium in Dire Enchini and Ejere butter sample were found to be 235.3 and 229.5 mg/kg, respectively. The concentrations of Calcium in butter samples found in this study were less than reported value of 240 mg/kg (Ashenafi, 2006). From the statistical F- and ANOVA test, it is evidenced that, there is no significant differences (P > 0.05, at 95 % CI) in Ca concentration among the butter samples analyzed. On the other hand, the concentrations of iron (Fe) analyzed in butter samples from Dire Enchini and Ejere butter samples were 1.92 nd 1.81 mg/kg, respectively. The result of Fe concentration obtained from this study is much lower than values reported by Meshref (6.69 mg/kg) (Meshref et al., 2014). However, Vahedi and his coworkers (Vahedi et al., 2015) have reported a very comparable Fe concentration (1.27 mg/kg) in butter sample collected from local market in Tehran with the result of this study. The report of Ashenafi (2006) on the level of Fe in butter sample was also very similar to our finding with reported value of 1.5 mg/kg.

The Concentration of Manganese in Dire Enchini and Ejere butter samples were found to be 0.051 and 0.0617 mg/kg, respectively. There was no significant difference (P > 0.05, at 95% CI) in concentrations of Mn among the butter samples studied from the results of statistical F-test and ANOVA test. On the the other hand, the concentration of Zn accumulated in Dire Enchini and Ejere butter samples were found to be 0.75 and 0.566 mg/kg, respectively. From the statistical F- and ANOVA test, it has been found out that there is no significant variation (p > 0.05, at 95% CI) in concentration of Zn between the tested butter samples. The level of Zn metal in the studied butter samples were found to be much lower than reported value, 5.98 mg/kg as reported by Meshref and his coworkers (Meshref et al., 2014).

The concentrations of Copper in Dire Enchini and Ejere butter samples were 0.53g and 0.9 mg/kg, respectively (*cf.* Table 6). From the statistical F-test, no significant differences (P>0.05, at 95% CI) in Cu concentration were observed among the butter samples analyzed. A very similar copper content of butter has been reported (Meshref et al., 2014) with Cu level of 0.60 mg/kg, while Vahedi and his coworkers (Vahedi et al., 2015) have reported a lower value of Cu content, 0.18 mg/kg, compared with the result of this study.

Conclusions

From the results of the current study it can be concluded that, the concentration of K, Ca, Na, Mg and Fe were found to be present in higher concentration in Dire Enchini and Ejere butter sample. In the same manner, Cu, Mn and Zn concentration were found to be less accumulated in butter samples taken from the two districts. When, the distribution of the selected essential metals over butter was observed, they were found to vary in the decreasing order as K >Ca>Na > Mg > Fe > Cu> Zn>Mn in butter samples of the two districts.

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