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## Review

# Prevention and treatment of different health problems by common people's diet (Haleem)

Ghazala H. Rizwani<sup>1\*</sup>, Khizar Abbas<sup>1,2</sup> and Hina Zahid<sup>1</sup>

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi Pakistan.

<sup>2</sup>Department of pharmacy, Faculty of Pharmacy, Bahauddin Zakeriya University, Multan, Pakistan.

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Malnutrition is the major problem in many countries of the world including Pakistan. Due to inadequate macro and micro nutrients, prevalence of diseases is increasing day by day. Haleem is a common diet that can be used by people of all age group. It is prepared by different methods and consists of *Lens culinaris* L., *Hordeum vulgare* L., *Phaseolus vulgaris* L., *Vigna mungo* L., *Vigna radiate* L., *Cicer arietinum* L., *Oryza sativa* L., *Triticum aestivum* L., *Gallus gallus domesticus*, *Brassica napus* L., *Zingiber officinale* Rosec., *Curcuma longa* L., *Allium sativum* L., *Allium cepa* L., *Capsicum annuum* L., *Cinnamomum zeylanicum* L., *Cuminum cyminum* L., *Myristica fragrans* Houtt, *Piper longum* L., *Mentha piperita* L., *Amomum subulatum* Roxb., *Coriandrum sativum* L., *Mangifera indica* L., *Citrus lemon* L. and rock salt. These materials either from plant, animal and mineral origin contain variety of substances such as dietary fibers, energy, proteins, carbohydrates, lignins, tannins, flavonoids, phytosterols, squalene, tocopherols, saponins, pre-biotics, plant acids, sterol, crude fibre, fats, vitamin and minerals.

**Key words:** Neurological disorder, nutrition, micronutrients, macronutrients, bioactive compounds.

## INTRODUCTION

Consumption of food that contains large quantity of plant material provides abundant phyto-chemicals and non-nutritive material that has protective effect on human health. Natural dietary agents such as fruits, vegetables, spices and biologically active compounds have drawn a great attention from scientific community and general public due to their health promoting effects (Shukla and Singh, 2007; Rizwani and Zahid, 2014). Plant derived foods have potential health benefits as their consumption is increasing by 5 to 10% annually. Health organizations

are recommending increasing the intake of plant-derived foods to prevent chronic diseases and improve health status worldwide (Tang et al., 2014). Patients suffering from chronic inflammatory diseases are turning to dietary supplements for prophylactic treatments and relief of symptoms (Jolad et al., 2004). Malnutrition of micronutrient is influencing greater than one-half of the world's population, especially women, teenage girls and preschool children. Occurrence of imbalance food is in abundance in developed and developing countries.

\*Corresponding author. E-mail: rizwanix2@yahoo.com.

Human health depends on the availability, affordability and acceptability of balanced diet (Ramakrishnan et al., 2015). People awareness to the health benefits of foods is increasing day by day and are paying attention to disease prevention and health promoting compounds of food (Nyau, 2015). Haleem is a tempting combination of lentils and meat that is widely made on special occasion and it is also used as a breakfast, lunch and dinner. It is commonly prepared in the month of Muharram in Pakistan.

However, there is no updated compilation on the importance of Haleem and its composition. The present review therefore, endeavors to provide for the first time an updated compilation of phyto-constituents and nutritional value which can subsequently open new perspectives for further research.

## COMPOSITION OF HALEEM

Haleem is one of the most popular and favorite dish in Middle East, Central Asia and in Indian Subcontinent. It is a very healthy traditional recipe and its composition varies from region to region. It is slowly cooked for seven to eight hours and results in a paste-like consistency. Haleem is normally enjoyed with naan or with any type of bread as such eaten with spoon in Pakistan. It is a high-calorie dish, provides protein from the meat and fibre and carbohydrates from the various combinations of grains and pulses.

## PHYTOCONSTITUENTS AND NUTRITIONAL VALUE

### *Allium sativum* L.

*A. sativum* (Family Liliaceae) is known as garlic (English) or lehsan (Urdu). Chemical constituent of garlic are alliin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide, ajoenes, methyl allyl di- and trisulfides, vinyl dithiols, c-glutamylcysteine, steroidal glycosides, essential oil, flavonoids, lectins, prostaglandins, fructan, pectin, adenosine, vitamins B1, B2, B6, C, E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, essential amino acids, arginine, alliin, alliin, germanium, calcium, copper, iron, potassium, magnesium, selenium, zinc, allinase, vitamins A, B1 and C (Pantoja et al., 1996; Bozin et al., 2008; Gaafar, 2012). *A. sativum* contain energy 1109 KJ, tryptophan 58 mg, water 53.6 g, lysine 549 mg, protein 12.0 g, threonine 376 mg, lipid 5.1 g, valine 1.040 mg, methionine 116 mg, dietary fiber 1.2 g, phenylalanine 534 mg, ash content 2.3 g, leucine 737 mg, sodium 53.9 mg, iso leucine 404 mg, calcium 13.0 mg, cystine 318 mg, iron 2. mg, tyrosine 592 mg, vitamin B2 0.1 mg, arginine 1964 mg, vitamin B6 10.7 mg, histidine 318 mg, nicotinic acid 14.0 mg, alanine 722 mg, aspartate 1 560 mg, glutamate 2456 mg, glycine 563 mg,

proline 318 mg and serine 477 mg and also contain manganese, selenium, calcium, vitamins B1 and B6 (Suleria et al., 2015).

### *Allium cepa* L.

*A. cepa* (Family Liliaceae) is known as onion (English) or piyaz (Urdu). It contain carbohydrates which include glucose, fructose, sucrose, low-molecular weight fructo-oligosaccharides, quercetin, quercetin 3,4'-diglucoside, quercetin-4'-glucoside, flavonoids, glycosides, proteins, alkaloids, saponins, reducing sugars, oils, ceposides A, ceposides B, ceposides C, flavonoids, vitamins and organosulphur compounds are also present. It contain vitamin C, vitamin B6, folic acid, energy 40 kcal per 100 g, sodium 16.15 mg, potassium 185.05 mg, phosphorus 19.24 mg, calcium 375.15 mg, iron 2.60 mg, magnesium 232.05 mg and manganese 213.65 mg (Edet et al., 2015; Ige and Akhigbe, 2013; Russo et al., 2012).

### *Amomum subulatum* Roxb.

*A. subulatum* (Family Zingiberaceae) is called large cardamom (English) or bari ilaichi (Urdu). It contains essential oils, anthocyanins, aurone, chalcone, flavanone, 1,8-Cineole,  $\alpha$ -pinene,  $\beta$ -pinene, geraniol, 1,8-cineole, terpenyl acetate, monoterpene hydrocarbon, that is, limonene, sabinene, terpinenes, pinenes, cineol, limonene, myrcene,  $\alpha$ -terpinene, 4-terpinene, petunidin-3,5-diglucoside, leucocyanidin-3-O- $\beta$ -D glucopyranoside, subulin, 1-8 cineole and  $\alpha$ -terpinyl acetate. The fruit consists of 70% seeds and 30% skin from which seed contain 8.6% moisture, 5% total ash, 4% non-volatile, 91.4% of total solid, volatile oil content varies between 1.95 and 3.32%, copper 7.4 mg/kg, nickel 0.006 mg/kg, zinc 57.6 mg/kg, lead 0.015 mg/kg, cobalt 5.4 mg/kg, cadmium 0.2 mg/kg, iron 111.2 mg/kg and chromium 0.003 mg/kg (Vivaiya et al., 2012; Bisht et al., 2011).

### *Brassica napus* L.

*B. napus* (Family Brassicaceae) is called rape-seed (English) or Rai (Urdu). It is third largest source of vegetable oil in the world, providing high quality edible oils and raw materials for industry and biodiesel (Liu et al., 2015). It contains protein, polyphenols, phytosterols, tocopherol, canolol, flavonoid glycosides and glucopyranosyl sinapate, having antioxidant activity (Jing et al., 2008). Each 100 g of canola oil contain omega-3-linolenic acid 10 to 12%, oleic acid 59 to 62%, linoleic acid 18 to 22%, linolenic acid 10 to 12%, calories 884, vitamin E 17.46 mg, vitamin K 71.30 mcg, monounsaturated fat 63.28 g, polyunsaturated fat 28.14 g, saturated fat 7.37 g, stigma sterol 3 mg, campesterol

241 mg, beta sitosterol 413 mg.

### ***Capsicum annuum* L.**

*C. annuum* (Family Solanaceae) is known as chilli (English) or mirch (Urdu). It is used as spice due to its flavor, color and pungency. It contains ascorbic acid, carotenoids, tocopherols, flavonoids, capsaicinoids, polysaccharide, phenols, flavonoids, capsaicinoids, sterols, triterpenes, organic acids, fatty acids, volatile compounds, furostanol saponin derivative, vitamins C, E, carotenoids, phenolics compounds, capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nonivamide and L-asparaginase (Majee et al., 2015; Loizzo et al., 2015).

### ***Cicer arietinum* L.**

*C. arietinum* (Family Leguminaceae) is called as chickpea (English) or Channa (Urdu). It is the oldest and widely consumed legume in the world due to its good source of energy, protein, minerals, vitamins, fiber and health-beneficial phytochemicals. It contains proteins 21.70 – 23.40%, carbohydrates 41.10 – 47.42% from which starch is 83.9%, minerals, trace elements (Zia-Ul-Haq et al., 2007; Esmat, 2010), phenolic acids, isoflavones, aliphatic acids, aromatic acids, flavonoids, volatile compounds, polyamines, coumarins, phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, amino acids, fixed oils, fats, dietary fiber (Mamta and Parneet Kaur, 2013), quercetin, kaempferol, flavonols, flavanones, isoflavones, hydroxybenzoic and hydroxycinnamic acids. It contain carbohydrate such as sucrose 4.3%, raffinose 1.0%, stachyose 2.8%, fructose 0.1%, galactinol 0.5%, glucose 0.1%, manninotriose 3.4%, pinitol 0.2%, lysine 45-79 mg, methionine 7-31 mg, cystine 7-18 mg, phenylalanine 30-68 mg, tyrosine 20-35 mg, isoleucine 44-60 mg, leucine 49-80 mg, threonine 28-48 mg, valine 38-63 mg, tryptophan 2-12 mg, sterol, tocopherol, fatty acids, copper 1.18 µg, iron 4.60 µg, zinc 6.11 µg, manganese 1.21 µg, calcium 220 µg, magnesium 119 µg, phosphorus 398 µg, chromium 0.08 µg, vitamin C 2.15-6.00 mg, thiamin 0.028-0.40 mg, riboflavin 0.15-0.30 mg, niacin 1.6-2.90 mg, pyridoxine 0.55 mg, folic acid 150.0 µg and vitamin K 120.0 µg in each 100 g (Jukanti et al., 2012).

### ***Cinnamomum zeylanicum* L.**

*C. zeylanicum* (Family Lauraceae) is known as cinnamon (English) or dar chini (Urdu). It is used as spice and flavoring agent in food due to characteristic pleasant odour. The bark contains L-arabino-D-xylan, D-glucan, phenolic compounds and Type-A procyanidine

polyphenols (Sarathy, 1987; Kitazuru et al., 2004).

### ***Citrus lemon* L.**

*C. lemon* (Family Rutaceae) is called lemon (English) or limo (Urdu). It contains flavanones, flavones, flavonols, anthocyanins, coumarins, limonene, β- pinen, flavanone glycosides, hydroxycinnamic acids, vitamin C, carotenoids, flavonoids, citric acid, vitamin C, potassium, citric acid, carboxylic acid. 100 g of raw lemon contains carbohydrates 9.32 g, sugars 2.50 g, dietary fiber 2.8 g, fat 0.30 g, protein 1.10 g, thiamine 0.040 mg, riboflavin (vitamin B2) 0.020 mg, niacin 0.100 mg, pantothenic acid 0.19 mg, vitamin B6 0.080 mg, folate 11 µg, vitamin C 53.0 mg, calcium 26 mg, iron 0.60 mg, magnesium 8 mg, phosphorus 16 mg, potassium 138 mg and zinc 0.06 mg (Guimaras et al., 2010; Lorente et al., 2014).

### ***Coriandrum sativum* L.**

*C. sativum* (Family Umbelliferae) is called coriander (English) or dhania (Urdu). The fruit contain proteins, vitamins, calcium, phosphorus, iron, fibers, carbohydrates, petroselinic acid and the oil is composed of limonene, β-phellandrene, eucalyptol, linalool, borneol, β-caryophyllene, citronellol, thymol, linalyl acetate, geranyl acetate, caryophyllene oxide, elemol, methyl heptenol, pinenes, γ-terpinene, myrcene, geraniol and borneol, limonene, p-cymene, camphor, coriandrin, coriandrins A-E, dihydrocoriandrin, flavonoids, oleic, petroselinic and linolenic, linalool 60-80%, geraniol 1.2-4.6%, terpineol 0.5%, terpinene 1-8%, cymene 3.5%, limonene 0.5-4%, pinene 0.2-8.5%, camphene 1.4%, myrcene 0.2-2%, ketones 7-9%, camphor 0.9-4.9%, geranyl acetate 0.1-4.7%, linalyl acetate 0-2.7%, (E)-2-decenal, dodecenal, (E)-2-tridecenal, dodecanal (Bhuiyan and Sultana, 2009; Nadeem et al., 2013).

### ***Cuminum cyminum* L.**

*C. cyminum* (Family Umbelliferae) is known as cumin (English) or zeera (Urdu). It contains terpenes, glycosides, myrcene, α-phellandrene, α-pinene, β-pinene, cyminal, α-terpene, γ-terpinene, p-cymene, cyminum, cumaldehyde, cuminaldehyde, cymene, terpenoids, 2-ethoxy-3-isopropylpyrazine, 2-methoxy- 3-s-butylpyrazine and 2-methoxy-3-methylpyrazine, contain fixed oil (about 10%), protein, cellulose, sugar, mineral elements, volatile oil (1.5%) and phenolics compounds (Akrami et al., 2015; Hajlaoui et al., 2010).

### ***Curcuma longa* L.**

*C. longa* (Family Zingiberaceae) known as turmeric (English) or haldi (Urdu). Major chemical constituent are



curcumin (Selvam et al., 1995), demethoxycurcumin (DEMC), bisdemethoxycurcumin (BDEMC) (Li et al., 2014) and turmerone. Turmeric contain 0.76% alkaloid, 0.45% saponin, 1.08% tannin, 0.03% sterol, 0.82% phytic acid, 0.40% flavonoid, 0.08% phenol, 8.92% moisture, 2.85% ash, 4.60% crude fiber, 6.85% fat, 9.40% crude protein, 67.38% carbohydrate, 0.89% thiamine, 0.16% riboflavin, 2.30% niacine, 0.20% calcium, 0.63% phosphorus, 0.46% potassium and 0.05% iron (Ikpeama and Nwankwo, 2014). Each 100 g of turmeric contain ascorbic acid 50.0 mg, ash 6.8 g, calcium 0.2 g, carbohydrate 69.9 g, fat 8.9 g, energy 390.0 K Cal, iron 47.5 g, niacin 4.8 mg, potassium 200.0 mg, phosphorus 260.0 mg, protein 8.5 g, riboflavin 0.19 mg, sodium 30.0 mg, thiamine 0.09 mg and water 6.0 g.

### ***Gallus gallus domesticus***

*G. gallus domesticus* is commonly known as chicken (English) or Murghi (Urdu). Its meat is important in people's daily life as it provide abundant protein, fat, trace elements (Xiong et al., 2015), low proportion of saturated fatty acids, higher proportion of polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic acid (Almeida et al., 2006). Each 100 g of chicken contain energy 916 kJ, fat 12.56 g, saturated 3.5 g, mono saturated 4.9 g, protein 24.68 g, tryptophan 0.276 g, threonine 1.020 g, isoleucine 1.233 g, vitamin A equiv. 44 µg, pantothenic acid (B5) 0.667 mg, iron 1.16 mg, sodium 67 mg, water 63.93 g, serine 0.870 g, proline 1.190 g, glycine 1.583 g, leucine 1.797 g, lysine 2.011 g, methionine 0.657 g, cystine 0.329 g, arginine 1.545 g, phenylalanine 0.959 g, tyrosine 0.796 g, valine 1.199 g, histidine 0.726 g, alanine 1.436g, aspartic acid 2.200 g and glutamic acid 3.610 g.

### ***Hordeum vulgare* L.**

*H. vulgare* (Family Gramaniae) is commonly known as barley (English) or Jao (Urdu). It contains protein that constitute glutamine, proline (Fatemeh, et al., 2015), B-D-glucan, phenolic compounds, B-complex vitamins, tocotrienols, tocopherols (Asima et al., 2015), benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, chalcones, flavones, flavanones and amino phenolic compounds (Gallegos-Infante et al., 2010). Each 100 g of barley contain energy 370 kcal, protein 10.5 g, fat 3.7 g, carbohydrate 73.6 g, starch 68.2 g, total fiber 17.5 g, soluble fibre 5.8 g, insoluble fiber 11.7 g, β-glucan 3.2 g, resistant starch 0.09 g, lignin 1.0 g, magnesium 110 mg, phosphorus 410 mg, potassium 410 mg, chloride 117 mg, iron 6.1 mg, zinc 2.4 mg, calcium 40 mg, copper 0.59 mg, sulphur 120 mg, manganese 1.3 mg, iodine 60 µg, thiamin 0.50 mg, riboflavin 0.06mg, niacin 0.50 mg, tryptophan 2.5 mg,

vitamin B6 0.22 mg, total folates 107 µg, pantothenic acid 1.0 mg, biotin 1.7 g and vitamin E 0.51 mg (Theobald et al., 2006).

### ***Lens culinaris* L.**

*L. culinaris* L. (Family Leguminaceae) is commonly called lentil (English) or masoor dal (Urdu). Seeds are source of calcium, iron, vitamin B, protein, carbohydrates, fibers contents (Kripi, 2012), lectins, defensin protein, Bowman-birk trypsin inhibitors (Mo'ez Al-Islam et al., 2012), phytosterols, squalene, tocopherols, saponins, flavonoids, tannins, phytic acid, oligosaccharides (Jameel and Ali, 2015), hydroxybenzoic acid, hydroxycinnamic acids, glycosides of flavonols and flavones, trans-resveratrol-3-O-glucoside and proanthocyanidins (Fратиани, et al., 2014). Each 100 g of lentil constitute moisture 10.4 g, energy 353 Kcal, protein 25.8 g, fat 1.1 g, carbohydrate 60.1 g, fiber 30.5 g, sugar 2.03 g, calcium 55 mg, iron 7.5 mg, magnesium 122 mg, phosphorus 451 mg, potassium 955 mg, sodium 6 mg, zinc 4.8 mg, vitamin C 4.4 mg, thiamin 0.9 mg, riboflavin 0.2 mg, niacin 2.6 mg, vitamin B6 0.5 mg, folate 479 µg, vitamin A 39 IU, vitamin E 0.5 mg, vitamin K 5 µg, saturated fatty acids 0.2 g, monounsaturated fatty acids 0.2 g and polyunsaturated fatty acids 0.5g (Zahra, 2015).

### ***Mangifera indica* L.**

*M. indica* (Family Anacardiaceae) is called mango (English) or aam (Urdu). Each hundred gram of fruit pulp contain 250 kJ energy, prebiotic dietary fiber, vitamin C, polyphenols and pro vitamin A and carotenoids. Seed is a single flat oblong with fiber or hairy on the surface. Seed kernel contain 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash, high in potassium, magnesium, phosphorus, calcium and sodium, essential and non-essential amino acids, stearic, palmitic acids, oleic and linoleic acids oleic, polyphenols, phytosterols, campesterol, sitosterol and tocopherols. Each 100 g of seed contain vitamin A 15.27 IU, 1.30 mg vitamin E, 0.59 mg vitamin K, 0.08 mg vitamin B1, 0.03 mg vitamin B2, 0.19 mg vitamin B6, 0.12 mg vitamin B12 and 0.56 mg vitamin C (Olorunaiye et al., 2012; Fahimdanesh and Bahrami, 2013).

### ***Mentha piperita* L.**

*M. piperita* (Family Lamiaceae) is known as mint, peppermint (English) or podina (Urdu). It is rich sources of iron and magnesium. It contain menthol, menthone, menthyl acetate, neomenthol, isomenthone, menthofuran, ascorbic acid, β-carotene, acetylmenthol, neomenthone, pulegone, limonene, pulegone, alpha and beta pinene,

trans-sabinene hydrate, caffeic acids, flavonoids, tannins, 50-78% free menthol, monoterpene, menthofurane and traces of jasmine (0.15%) that improve the quality of oil (Valmorbidia and Boaro, 2007; Shah and Mello, 2004; Saeed et al., 2014).

### ***Myristica fragrans* Houtt.**

*Myristica fragrans* (Family Myristicaceae) known as nutmeg (English) or jaiphal (Urdu). It contains oleoresin hydrocarbon monoterpenes 61-88%, that is,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene monoterpenes acid 5-15%, aromatic ether 2-18% such as myristicin, elemicin, safrole. Kernel contains volatile oil, fats, starch, mucilage, myristicin, myristic acid, while volatile oil contains pinene, sabinene, camphene, elemicin, isoelemicin, eugenol, methoxyeugenol, isoeugenol and safrole (Rodianawati et al., 2015; Sonavane et al., 2002).

### ***Oryza sativa* L.**

*O. sativa* (Family Graminae) is called rice (English) or Chawal (Urdu). It is a source of bioactive non-nutrient compounds known as phytochemicals and major food for the rural population and household food security (Calpe, 2006). Rice is good source of complex carbohydrates, thiamine, niacin, riboflavin, vitamin D, calcium and fibers (Umadevi et al., 2012). Endosperm of rice consists mostly of starch which is 90% of total weight of rice (Morales-Martinez et al., 2014). Each hundred gram contains moisture 10.20 g, energy 361.00 kcal, carbohydrates 82.00 g, protein 6.00 g, total fat 0.80 g, dietary fiber 0.60 g, calcium 8.00 mg, phosphorus 87.00 mg, potassium 111.00 mg, sodium 31.00 mg, vitamin B1 (Thiamine) 0.07 mg, vitamin B2 (Riboflavin) 0.02 mg and vitamin B3 (Niacin) 1.80 mg. Rice grains consist of protein 8%, iron 1.2 mg/100 g, zinc 0.5 mg/100 g, glutamic acid, aspartic acid and lysine 3.8% (Babu et al., 2013).

### ***Phaseolus vulgaris* L.**

*P. vulgaris* (Family Leguminaceae) is known as common bean (English) or Lal lobia (Urdu). It is considered as perfect food because of high protein, fiber and complex carbohydrates content (Rosales et al., 2012). Seed contains 20–25% phaseolin 40–50%, lectins 10–27%, phytohemagglutinin (PHA), polyphenolic compounds, alkaloids, fiber, saponins, steroids, lectins and terpenoids. Common bean contains fructo oligosaccharides, raffinose, phenolic acids and flavonols. It has energy of 347.00 kcal, protein 21.42 g, total fat 1.23 g, carbohydrate 62.55 g, fiber 15.50 g, total sugar 2.11 g, calcium 113 mg, iron 5 mg, magnesium 176 mg, phosphorus 411 mg, potassium

1393 mg, sodium 12 mg, zinc 2 mg, vitamin C 6.30 mg, thiamin 0.71 mg, riboflavin 0.21 mg, niacin 1.17 mg, vitamin B6 0.47 mg, folate 0.53 mg, vitamin E 0.21 mg, vitamin K 5.6  $\mu$ g, saturated fatty acids 0.24 g, monounsaturated fatty acids 0.23 g and polyunsaturated fatty acids 0.41g (Romero-Arenas et al., 2013).

### ***Piper longum* L.**

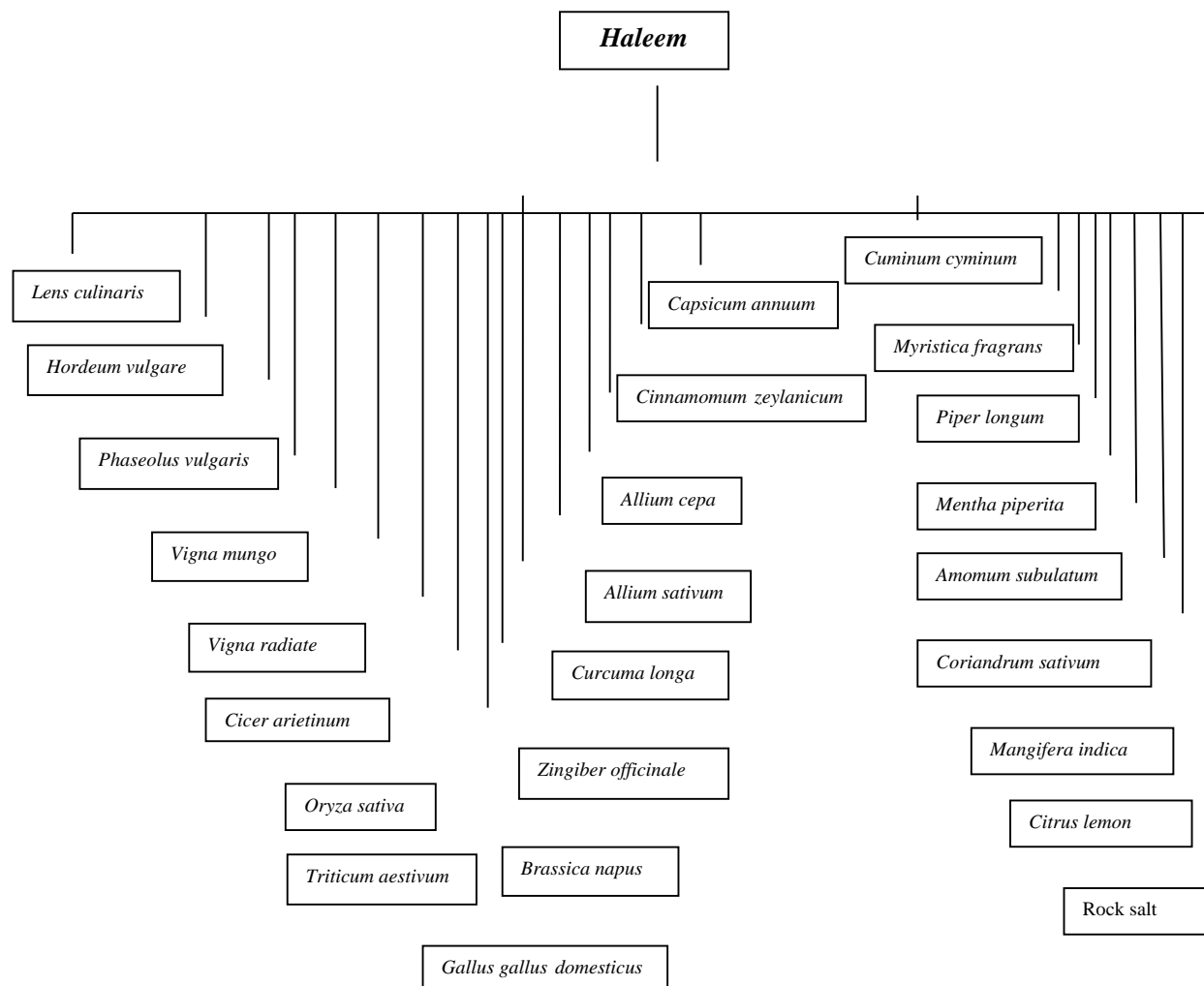
*P. longum* (Family Piperaceae) is known as black pepper (English) or kali mirch (Urdu). It consists of pipericyclobutanamides A and B, piperine, isopiperolein B, 1-peperoyl piperidine, phenolics, flavonoids, alkaloids, amides, steroids, lignans, neolignans, terpenes, chalcones, brachyamide B, dihydro-pipericide, (2E,4E)-N-eicosadienoyl-piperidine, N-trans-Feruloyltryamine, guineensine, N-formylpiperidine, pentadienoyl as piperidine, (2E,4E)- Nisobutyl- Idecadienamid, tricholein, isobutyl-eicosadienamide, trichostachine, isobutyl-eicosatrienamide, isobutyl-octadienamide, piperamide, piperamine, piperettine, pipericide, piperine, piperolein B, sarmentine, sarmentosine and retrofractamide (Li et al., 2005; Fujiwara et al., 2001).

### **Rock salt**

Rock salt from Khewra salt mine of Pakistan is composed of NaCl 93.6%, Ca 0.849%, Mg 0.438%, 1.300%, SO<sub>4</sub> 2.016%, silver 0.63 mg, aluminum 26.121 mg, boron 19.500 mg, barium 25.157 mg, bismuth 7.141 mg, cadmium 8.947 mg, cobalt 0.766 mg, chromium 3.769 mg, copper 1.984 mg, iron 49.844 mg, gallium 9.782 mg, indium 6.710 mg, lithium 3.82 mg, manganese 6.748 mg, nickel 6.096 mg, lead 9.714 mg, strontium 37.894 mg, tellurium 11.560 mg and zinc 17.548 mg in each 100 g (Titler, 2009).

### ***Triticum aestivum* L.**

*T. aestivum* (Family Gramineae) is called wheat (English) or Ghandum (Urdu). The nutritional value of wheat is extremely important as it is an important crop being widely grown as staple food. Wheat germ contains 11% oil, protein, threonine, methionine, lysine, raffinose, sucrose, thiamin, riboflavin, tocopherol, polyunsaturated fatty acid 45-60%, linolenic 11%, oleic acid 12-30%, saturated fatty acids 14-17%, palmitic acid, stearic acid 0.5-2.3% (Zarroug et al. 2015). Wheat is considered a good source of mineral such as zinc, iron, selenium and magnesium, vitamins such as thiamine, vitamin-B, pantothenic acid, pigments, enzymes, carbohydrate 78.10%, protein 14.70%, fat 2.10% (Kumar et al., 2010, 2011), antioxidants, phytochemicals and dietary fibers. It also contains lipids 8-13%, fats 1.5%, proteins 13% that



**Figure 1.** The tempting combination of haleem.

are albumins, globulins, glutenins, gliadins, minerals (ash) 0.5% and dietary fibers 1.5%, vitamin E, glutamine, proline, alanine, arginine, asparagine, glycine, lysine and threonine (Šramková and Šturdík, 2009).

### ***Vigna mungo* L.**

*V. mungo* (Family Leguminaceae) is commonly known as black gram (English) or urd (Urdu). It is an important pulse crop and provides major share of protein requirement for vegetarian population. It is used in the form of split pulse as well as whole pulse (Ajila, and Rao, 2009). Whole black gram is a rich source of protein, fiber, several vitamins, calcium, iron (Girish and Rao, 2012), fructose, non-reducing oligosaccharides, sucrose, raffinose, stachyose, verbascose, ajugose (Kotiguda and Mulimani, 2006; Suneja et al., 2011). *Vigna mungo*

contain moisture of 7.9%, ash 2.9%, fiber 3%, fat 1.01% and each 100 g contain energy 350 calories, carbohydrates 56.6%, proteins 26.2%, fat 1.2%, calcium 185 mg, iron 8.7 mg, phosphorus 345 mg, vitamin B1 0.42 mg, vitamin B2 0.37 mg and niacin 2 mg (Suneja et al., 2011).

### ***Vigna radiata* L.**

*V. radiata* (Family Leguminaceae) is called green gram (English) or mung bean (Urdu). It is an important pulse crops grown in Asia. *V. radiata* contain moisture content of 9.74%, ash 2.91%, fiber 3.1% and fat 1.35% (Shaheen et al., 2014), ascorbic acid, folic acid, protein, iron, calcium, fiber, alkaloid, flavonoid, tannins (Bhandurje et al., 2012), vitamin C, vitamin B complex, calcium, fiber, iron, potassium, magnesium and

**Table 1.** Pharmacological activities.

<b>Plant</b>	<b>Pharmacoligical activities</b>
<i>Allium sativum</i> L.	It has antithrombotic, antihypertensive, anti-hyperglycemic, anti hyperlipidemic, diuretic, inhibit sodium transporting epithelia and decrease ATPase activity, antimicrobial activity, anti-atherosclerotic, antidote for heavy metal poisoning, hepatoprotective, prevents cold and flu symptoms, anticancer chemopreventive and have antioxidant activity. It also have antiseptic, prevent anthrax in cattle, vermifuge and anti leishmaniasis activity (Lanzotti et al., 2015). It is used for treatment or prevention of type 2 diabetes and obesity (Schmidt et al., 2014; Ademiluyi et al., 2013).
<i>Allium cepa</i> L.	It reduces the risk of various chronic diseases such as cardiovascular, cancer, asthma, diabetes and have neuroprotective potential[88]. It have antibiotic, anti-diabetic, antioxidant, anti-atherogenic, anticancer activities, decrease plasma total cholesterol level (Singh and Goel, 2015), lower risk of breast cancer, provide protection against hepatotoxicity and nephrotoxicity. Onion has been known to have antimicrobial, antioxidant and/or anticancer effects. It is used for treatment or prevention of type 2 diabetes, obesity, hypercholesterolaemia, hypertension, coronary heart disease, cataracts, inhibit the tumor and microbial cells (Schmidt et al., 2014; Ademiluyi et al., 2013)
<i>Amomum subulatum</i> Roxb	It used in the treatment of gastrointestinal, digestive disorder, nausea, dyspepsia, cough, vomiting, itching, throat trouble, lung congestion, mouth infection, digestive disorders, cardiac tonic, expectorant, appetizer, diuretic (Jafri and Singh 2001; Verma et al., 2012).
<i>Brassica napus</i> L.	It is used as diuretic, anti-scurvy, anti-inflammatory of bladder and anti-goat. Also, the seeds were documented to use for treatment of hepatic and kidney colic. Colza seeds are also used in the Eastern folk medicine as bronchial cathartic (Zargari, 2001).
<i>Capsicum annuum</i> L.	It has antioxidant, antifungal, antiproliferative properties, seeds inhibited lipid accumulation, inhibit adipocytes differentiation, have potential against cancers, prevent gastric ulcer, stimulate the immune system, prevent the cardiovascular diseases, protect against age-related macular degeneration and cataract ((Barbero et al., 2015; Sung et al., 2015).
<i>Cicer arietinum</i> L.	Chicken pea is used as appetizer, anthelmintic, alleviates thirst and burning sensation, used for bronchitis, leprosy, skin diseases, inflammation of the ear, blood disorders and biliousness, liver and spleen diseases and is hypocholesteremic agent (Zia-UI-Haq et al., 2007). It is used as diuretic, antifungal, potent nutraceutical, complaints of chest, throat troubles, fever.
<i>Cinnamomum zeylanicum</i> L.	It is used in chronic bronchitis, impotence, frigidity, dyspnea, inflammation of the eye, leukorrhea, vaginitis, rheumatism, neuralgia, toothaches, hypoglycemic, cholesterol lowering agent, promotion of wound healing, antimicrobial agent, analgesic, anti-pyretic, immunomodulatory, anti-complementary, anti-arthritic activity, insecticidal, acaricidal, antityrosinase, antimutagenic, enteralgia, anti-nociceptive, antipyretic, anti-complementary and immunosuppressive activity (Barceloux 2009; Unlu et al., 2010).

Table 1. contd.

<i>Citrus lemon</i> L.	Lemon has antimicrobial, chemo-preventive, antispasmodic, anti-nociceptive, relief of heartburn, gastro esophageal reflux disorder, soothe sore throats, reduce high blood pressure, prevents kidney stones, treat flaky dandruff, washing agent for teeth, additive agent for flavoring foods, arthritis, rheumatism, headaches (Nasser and Hussain 2014; Mohanapriya et al., 2013)
<i>Coriandrum sativum</i> L.	Coriander has antibacterial, antidiabetic, anticancerous, antimutagenic, antioxidant, antiemetic, anti-inflammatory, antiseptic, emmenagogue, antihypertensive, lipolytic, myorelaxant, nerve-soothing and healing properties, used in anorexia, dyspepsia, flatulence, diarrhea, griping pain, vomiting, serves as tonic, diuretic and aphrodisiac (Darughe et al., 2012)
<i>Cuminum cyminum</i> L.	Cumin is used as anticarcinogenic, astringent, stimulant, carminative, remedy for indigestion, astringent, flatulence, diarrhea, hypoglycemic effect, aldose reductase (AR) and alpha glucosidase inhibitory activity, used for toothache, dyspepsia, epilepsy, jaundice, diuretic, carminative, antispasmodic, hoarseness, jaundice, antitumor, anti-inflammatory, antifungal, antibacterial, antioxidant, antispasmodic, stimulate breast milk production and jaundice (Kedia et al., 2014; Dhandapani et al., 2002; Kedia et al., 2015).
<i>Curcuma longa</i> L.	It is used as potential anti-cancer, antioxidant, anti-coagulative, anti-hepatotoxic, dissolve urinary calculus and control the diabetes (Selvam et al., 1995). In traditional medicines the rhizomes of <i>Curcuma longa</i> are used as carminative, stomachic, anthelmintic, laxative, in liver ailments, household remedy for anorexia, cough, rheumatism, intestinal disorder, headache, cold, chronic catarrh, migraine, inflammation, improve sex hormone, lower cholesterol, cytotoxins, have antioxidant (Ikpeama and Nwankwo, 2014). It's also used for the treatment of diseases that are associated with injury and inflammation, increase the capacity of learning and memory.
<i>Hordeum vulgare</i> L.	It is considered as an important nutraceutical grain due to its high dietary fiber and phenolics contents that reduce risk of cardiovascular diseases, cancer and used to treat bladder inflammation, cholera, dermatitis, diabetes and inflammations (Asima et al., 2015; Jebor et al., 2013)
<i>Lens culinaris</i> L.	It has antifungal, blood purifying property, remove old skin marks, used to treat kidney and gastric ailments while flour has bacteriostatic and/or antibiotic effect (Butu et al., 2014)
<i>Mangifera indica</i> L.	kernel are used as source for functional food ingredients, antimicrobial compounds, adsorbent, cosmetics and antioxidants (Kittiphoom, 2012)
<i>Mentha piperita</i> L.	Peppermint has an antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, emmenagogue properties. It is also used for colic in infants, flatulence, diarrhea, indigestion, nausea, vomiting, morning sickness, anorexia, and as a spasmolytic, treat the irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, liver complaints. Peppermint oil is used to relieve menstrual cramp, externally for neuralgia, myalgia, headaches,

Table 1. Cont'd.

	migraines and chicken pox, vapors used as an inhalant for respiratory congestion (Saharkhiz et al., 2012; Adel et al., 2015).
<i>Myristica fragrans</i> Houtt.	It has anti-carcinogenic, antioxidant, antifungal activity, carminative, astringent, hypolipidaemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac, and anti-inflammatory activities. It is also used for treatment of rheumatism, cholera, psychosis, stomach cramps, nausea, diarrhea, flatulence, anxiety and used as aphrodisiac and an abortifacient (Kwon et al., 2008).
<i>Oryza sativa</i> L.	It used for nourishment of skin and blood vessels, maintain internal water balance along with other nutrients. In ayurveda rice is considered as tonic, aphrodisiac, diuretic and useful in biliousness, increases appetite, cures indigestion, give tone to muscles, expel gas from the stomach and intestine, nourish the hormonal system, heal wounds and regulate blood pressure (Umadevi, et al., 2012; Saha, et al., 2014).
<i>Phaseolus vulgaris</i> L.	It has antiviral, antibacterial, antimutagenic, anticarcinogenic, antioxidant, antihypertensive activities, promotes the synthesis of C-globulin, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), induce mitosis, antiplatelet, antidiabetic, diuretic, antipyretic, carminative, diaphoretic, emmenagogue (Romero et al., 2013; Badari et al., 2015)
<i>Piper longum</i> L.	Black pepper is used in intermittent fevers, indigestion, diarrhea, flatulence, worm infestation, asthma, cough, heart troubles, diabetes, piles, epilepsy, elephantiasis, stimulate the pancreatic and intestinal enzyme. It is used as antihypertensive, anti platelets, antioxidant, antitumor, anti-asthmatics, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants, hepato-protective, immuno-modulatory, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, anti-spermatogenic, anti-colon toxin, insecticidal, larvicidal activities, enhance cognitive action (Chatterjee et al., 2007; Lee et al., 2008).
Rock salt	Rock salt aids in digestion, laxative, used for digestive disorders, improves appetite, removes gas, soothes heartburn, facilitates the cellular absorption of minerals, stabilizes blood pressure, aids in weight loss, strengthens the bones, connective tissue and give flavour to food (Titler, 2009).
<i>Triticum aestivum</i> L.	Prevent heart diseases, cancer, pyorrhea and diabetes, lower the risk of breast cancer and prostate cancers, enhance the heart and lung functions (Kumar et al., 2011), used in the treatment of cancer and in cute diarrhea, have antifungal and antioxidant activity (Kumar et al., 2010).
<i>Vigna mungo</i> L.	Black gram have cholesterol-reducing effect, hypolipidemic, hypoglycemic, protective effect against colon cancer, demulcent, aphrodisiac, used for diabetes, nervous disorders. It used for the treatment of rheumatism, affections of nervous system, diseases of the liver, in gastric catarrh, dysentery, diarrhea, cystitis, paralysis, piles, dropsy, diuretic (Verma et al., 2014; Pranshu et al., 2011).
	Mung bean have diuretic, hypotensive, hypolipidemic, hepatoprotective, antibacterial, antifungal, nematicidal,

Table 1, Cont'd.

<i>Vigna radiate</i> L.	antidiabetic activity, nervine tonic, improve learning and memory process (Bhandurge et al., 2012). It possesses detoxification activities, refresh brain, alleviate heat stroke, reduce swelling, regulate gastrointestinal upset, and moisturize the skin (Shaheen et al., 2014).
<i>Zingiber officinale</i> Roscoe	It is used for musculo-skeletal diseases, flatulence, spasmodic affections of the bowels, headache, affections of the throat, rheumatic disorders, carminative (Mustafa, 1989, 1992), atherosclerosis, high cholesterol, ulcers, depression, impotence, flu-like symptoms and intestinal lipase enzyme (Stoilova et al., 2007). Ginger is considered as rubefacient, antiasthmatic, stimulant to the gastrointestinal tract, antioxidant, antihypercholesterolem, inhibit LDL oxidation, lower blood glucose and urea, maintain the integrity of red blood cells (Morten, 2001; Penna et al., 2003).

manganese (Bhandurge, 2012).

### ***Zingiber officinale* Roscoe.**

*Z. officinale* (Family Zingiberaceae) is called ginger (English) or adrak (Urdu). Ginger contains zingiberene 35%, curcumene 18%, farnesene 10%, bisabolene, b-sesquiphellandrene, 1,8-cineole, linalool, borneol, neral and geraniol. Non-volatile pungent compounds gingerols, shogaols, paradols, zingerone, oleoresins, fats, waxes, carbohydrates, vitamins, minerals, potent proteolytic enzyme zingibain, poly phenolic compounds such as 6-gingerol and its derivative (Stoilova et al., 2007). Ginger contain essential oil, alpha zingiberene (Kelly et al., 2002), b-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol, a-zingiberene 30–70%, b-sesquiphellandrene 15–20%, b-bisabolene 10–15%, (E-E)-a-farnesene, arcurcumene, zingiberol. Ginger also contain gingerols, shogaols, 3-dihydroshogaols, paradols, dihydroparadols, acetyl derivatives of gingerols, gingerdiols, mono- and di-acetyl derivatives of gingerdiols, 1-dehydrogingerdiones, diarylheptanoids, gelicoidenol-2- $\alpha$

D-glicopyranoside (Penna et al., 2003). Each 100 g of ginger contain moisture 15.02%, insoluble fiber 23.5%, soluble fiber 25.5%, protein 5.087 g, fat 3.72 g, carbohydrates 38.35 g, vitamin C 9.33 mg, total carotenoids 79 mg, calcium 88.4 mg, phosphorous 174 mg, iron 8 mg, zinc 0.92 mg, copper 0.545 mg, manganese 9.13 mg and chromium 70  $\mu$ g (Prakash, 2010).

### **Conclusion**

From the above discussion, it is concluded that haleem can be used as a rich source of protein, carbohydrates, fatty acids, vitamins, minerals, alkaloid, glycosides, tannins, resins, flavonoids, pigments and source of energy. Due to these reasons, it is a good remedy for prevention and treatment of many diseases such as neurological problems, gastrointestinal problem and it is beneficial for growth of children and immunosuppressive patients.

### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of

interests.

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Review

# Traditional butter and ghee production, processing and handling in Ethiopia: A review

Alganesh Tola Gemechu<sup>1\*</sup> and Yetenayet Bekele Tola<sup>2</sup>

<sup>1</sup>Agriculture and Nutrition Research Laboratories, Holeta Agricultural Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 036, Ethiopia.

<sup>2</sup>Department of Post Harvest Management, Jimma University College of Agriculture and Veterinary Medicine, Jimma University, P. O. Box 307, Ethiopia.

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**In this review, traditional methods of processing, handling and indigenous preservation techniques of butter and ghee were assessed. In Ethiopia, butter and ghee processing are the responsibility of women. Traditional butter and ghee making in Ethiopia are based on indigenous knowledge using local materials and methods. Butter is made by churning naturally fermented milk. Butter is a raw material for ghee making. Salting, spicing, *nigur kibe* and traditional ghee making are major methods of butter preservation. Traditional ghee can be made from untreated butter, spiced butter, salted butter and *nigur kibe*. Butter and ghee are important components of Ethiopian traditional diets. Furthermore, butter is used for hair dressing and wound treatment. Ghee is commonly used for culinary, social functions and therapeutic purposes. There is scanty information on chemical and microbial quality of butter. Both butter and ghee are shelf stable dairy products but ghee is more shelf stable than butter. The chemical composition and microbial quality of butter is substandard. However, so far there is no such information on ghee quality. Hence, the quality and safety of traditional butter and ghee are subjects of further investigation.**

**Key words:** Traditional, butter, ghee, production, processing, handling, preservation.

## INTRODUCTION

Demand for dairy products has increased in the tropical areas including Ethiopia as people's income has been growing. Like other countries, Ethiopians have been using milk products such as butter and ghee as part of their diet since pre-historic times (Zelalem et al., 2011). Despite milk's contribution to gross domestic product

and value of butter as a food, sub Saharan Africa in self general and Ethiopia in particular have failed to attain sufficiency in dairy products. Butter fat is the second largest component of milk product and is of major commercial value. It serves as an energy source and supplies essential fatty acids. Such indigenous dairy

\*Corresponding author. E-mail: [alguto1999@gmail.com](mailto:alguto1999@gmail.com). Tel: 251912862017.

products made from different milk sources are traditionally produced and consumed in most of African countries including Ethiopia (Ashenafi, 2006; Almaz et al., 2001).

Worldwide, butter is made from a variety of animal milk including cow, goat, camel, buffalo and sheep (Curry, 2013). However, in Ethiopia, butter, locally named as '*dhadha/kibe*' is solely produced from cow milk. Rural producers make butter from the fat fraction of milk. In Ethiopia large amount of dairy products such as butter and ghee are produced on farm from sour milk through spontaneous fermentation (Alganesh and Fekadu, 2012; Fekadu, 1994; Getachew, 2003; Sintayehu et al., 2008). The vast majority of milk produced in the rural areas of the country is processed at household level into milk products such as butter using traditional technologies (O'Connor, 1994). In the rural areas of Ethiopia about 40% of the milk produced is converted to butter. At national level, 80% of butter is used as food ingredient (Getachew, 2003). Seventy percent of butter produced is used in rural and nearby urban areas (Getachew, 2003). *Dhadha /kibe* is the most shelf stable of all traditionally processed fermented milk products except for *niter kibe* (Yonad, 2009).

Butter, in addition to its dietary value, is also a major dairy product marketed in different parts of the country as an income source mainly for women (Fekadu, 1994; Zelalem and Inger, 2001a; Alganesh, 2002; Lemma et al., 2004; Eyassu and Asaminew, 2014). A significant portion of the community (rural women, retailers and assemblers of butter) acquire their household expenditure from butter sales.

In Ethiopia, ghee is used in different traditional diets and culinary purposes in different communities. Traditional butter or its derivative ghee is used as oil in cooking and flavoring purposes for different foods and snacks. The traditional materials and methods of dairy processing are inefficient. Hence, the quality of dairy products including butter is substandard (Zelalem, 2010). For instance, moisture content of *dhadha/ kibe* ranges from 20 to 43% as compared to the international commercial standard of 16% (Mekdes, 2008). Occurrence of spoilage when *dhadha/ kibe* is stored at room temperature for a long time is probably due to putrefactive microorganisms (Almaz et al., 2001; Wondu, 2007; Zelalem, 2010).

So far, in Ethiopia, no comprehensive review work was synthesized at national level to compile relevant endeavors on indigenous butter and traditional ghee production and handling practices. Previous reports indicated that there were some efforts made by post graduate students and very few researchers in the same area. However, comprehensive information on the existing indigenous technologies of butter and ghee handling is essential to plan effective intervention in the future. Hence, the aim of this paper is to combine and review existing information on traditional handling and

processing practices of butter and ghee from cow milk.

### **Milk collection, cleaning and fumigation of churns and traditional butter making**

In Ethiopia, smallholder butter making is based on naturally fermented sour (*ergo/ itittu*) milk (O'Connor, 1994). Souring milk has a number of advantages: it retards the growth of undesirable microorganisms such as pathogens and putrefactive bacteria and makes the milk easier to churn (O'Connor, 1994). Traditional butter is processed and sold by women in every community (Yonad, 2009). Milk for churning is accumulated over several days by adding fresh milk to the milk already accumulated in traditional spherical earthenware vessel or *wesso* or bottle gourds and allowed to sour into *itittu* or naturally fermented milk (Debela, 2016). To make butter, clay pot or bottle gourd (calabash) is used as a churner (Brannang and Persson, 1990; Abebe et al., 2013). Churners are smoked with chips of *Olea Africana* in Asela areas (Taye, 1998). Another report by Alganesh and Fekadu (2012) revealed that stems and leaves of *Ocimum hardiense* is used for cleaning milk vessels and churns in East Wollega. The same study showed that stems and leaves of *Ocimum urticifolium* and are also used for flavor impartation in to milk containers and churners. For the purpose of fumigation of churns most smallholders use chips of *Deinboll kilimandshorica* (*dabaqqaa*). While, some smallholders use *Gaarrii*, *Syzygium guineense* and *Olea Africana* (Alganesh and Fekadu, 2012). In southern Ethiopia, Mekdes (2008) reported *Gucha*, *Achyntes aspera* and *Eucalyptus globules* as the most important plants used for smoking churns and milk containers. Another report by Fikirneh et al. (2012) in mid rift valley revealed *Juniperous procera*, *Eruchstrum arabicum* and *Sida cuneifolia* being used for smoking milk vessels in addition to other trees. According to the local understanding, the practice of smoking vessels by burning wooden chips of specific trees and shrubs has an advantage of imparting special flavour and odour to the product, and to disinfect the vessels, thus reducing the numbers of micro-organisms and thereby extending the shelf life of the product.

The use of each tree and shrub species for the purpose of flavour impartation and disinfection depends on the geographical location and tradition of the smallholders. All the plant species used are believed to impart flavour and disinfect the vessels, but, the degree varies from plant to plant. The report of Ashenafi (2006) supported this assumption, as greater numbers and a faster development of aerobic mesospheric microorganisms occurred in milk kept in non-smoked as compared to smoked containers. Besides imparting a distinct flavor to the butter, this practice has a bacteriostatic effect, and may reduce processing time by heating the churn (O'Mahony and Ephraim, 1985). After smoking the churn, the curd is

broken either by hand or by agitation with a wooden stick and fermented milk is filled to about half of the capacity of the local churner or filled to a level depending on the availability of fermented milk. After filling, the churner is tightly closed with a plug, a false banana leaf, or piece of skin or leather (specifically made it for this purpose only) over the mouth of the churner. Maize grain outer cover and pieces of skin or hides or plastic materials can also be used. Finally, after the mouth of churn is securely tied agitation is performed for 3-4 h depending on environmental temperature, fat content, level of acidity of fermented milk and the speed at which the churning is done. Churning is exclusively done by women or children (Coppock, 1994; Alganesh and Fekadu, 2012).

Most smallholders perform churning when daily collections of about 3-8 liters of milk are achieved. Churning time usually takes 3-4 h using local churners. '*Ittitu*' /fermented milk made from accumulated milk for a week (20–25 liters) could yield approximately one kg of butter (O'Mahony and Ephraim, 1985; O'Connor, 1994; Zelalem, 1999; Ashenafi, 2006; Eyassu and Asaminew, 2014). In the traditional butter making, the equipments required for processing sour milk are simple and locally available. Local churners are made from clay, gourds and wood, and can be woven from fibre, such as the *gorfu* container used by the Borana pastoralists in Ethiopia (O'Connor, 1994). An on-farm report by Alganesh and Fekadu (2012) in East Wollega, Ethiopia revealed that 97.5% of smallholders use bottle gourd churn while 2.5% use clay pot churn. The smallholders preferred bottle gourd churner for milking and storage of different milk products including butter and ghee. The reason for the preference was that they believe that gourd churns are better in flavor impartation from wood smokes than other local churners.

Most of the traditional methods of milk processing are slow and inefficient. They give low yield of butter per unit of sour milk and require high labor input (O'Mahoney and Peters, 1987). It may take from 2-3 h depending on temperature, fat content, acidity and the milk volume to be churned. The time taken to make butter and to take it to market place is a considerable drain on the already limited time of women (O'Connor, 1990). Wooden paddle wheel internal agitator developed by the former International Livestock Center for Africa (ILCA) currently ILRI (International Livestock Research Institute) had reduced churning time from an average of 139 min to 61 min (O'Connor, 1993). A prototype of ILCA internal agitator was developed and used to introduce and verify the ILCA internal agitator around Bako for smallholder producers (Alganesh et al, 2001).

The efficiency of ILCA internal agitator was also compared with the traditional gourd churners. The result of the on-farm verification revealed an average of 128 min of churning time using the gourd churns, while the churning time for the prototype of ILCA internal agitator was only 23 min. The other advantage of using the

internal agitator was that every family member was able to perform churning, on the other hand, in the case of gourd churners; it is only women who performed churning. Besides, the internal agitator had 4% more butter fat recovery efficiency as compared to the gourd churners (Alganesh et al., 2001). According to another report by O'Connor (1993), the length of time required for churning was 65 and 139 min for the traditional churners in Debre Zeit and Debre Birhan areas, respectively. The author attributed the longer churning time in the latter area to the prevailing low ambient temperature.

Churning is the process whereby sour milk or cream is vigorously agitated in such a way that air is incorporated in the liquid (Berg, 1988). Different ways of shaking the churner include putting of the churn on the floor and rocking back and forth. This method is most common with the clay pot churner. In this method, the churner is placed on a mat consisting of a layer of grass, sheep skin or straw. The other option is to hang the churner on tripod or doorpost and swinging it to and fro until butter granules are formed. The third option is to rock the churner on the lap of women and shaking it with two hands. The latter option is only applicable to bottle gourd churners (O'Connor and Tripathi, 1992; Coppock, 1994). Besides, among some pastoral families, women carry sour milk in goat skin bags on their backs and agitate it with their elbows while walking or working (FAO, 1990).

The break point when butter starts to form can be detected by a change in the sound of the fermented milk up on agitation. A straw is inserted frequently through the vent into the churn; if small butter grains adhere to the straw surface, traditionally thought that the breakpoint has been reached (O'Connor, 1994). After a few minutes, the straw is again inserted through the vent. If it is clean from grains of butter, it indicates that the butter granules have coalesced into larger grains. After butter granules coalesce into large grains, the churn is slowly rotated anticlockwise on its base). This step enables to collect the grains in the centre and forms large mass of butter. The butter is then skimmed off, kneaded in cold water multiple times and washed to remove visible residual buttermilk (O'Mahoney and Ephraim, 1985; FAO, 1990; O'Connor, 1994).

## Butter

In Ethiopia, there are two types of butter, ripened/rancid and fresh locally called *besal kibe* and *lega kibe*, respectively (Mekedes, 2008). However, Abebe and others (2014) reported that there are three types of butter in Ethiopia, namely *lega*, *mekakelegna* and *besal*, which refer to fresh, semi-rancid and rancid butter, respectively, based on the degree of lipolysis of butter. Butter making and processing is solely done by women in every community in Ethiopia. *Dhadha* has an attractive appearance with a white to light yellowish color. Like

**Table 1.** Utilization of butter at regional level (2009/10) in Ethiopia.

Region	Utilization (%)				Total
	Household consumption	Marketed	Payment as a wage in kind	Other	
Oromia	60.38	36.28	0.11	3.23	100
Amhara	59.55	38.85	0.09	1.51	100
Tigray	91.8	1.34	0.42	6.43	100
Afar	74.35	20.48	0.13	5.04	100
Somali	64.61	33.39	0.28	1.73	100
Benshangul-Gumuz	51.55	46.16	0.01	2.28	100
SNNP	58.43	38.51	0.42	2.64	100
Gambella	73.1	24.53	0.41	1.96	100
Harari	66.3	-	-	33.7	100
Dire Dawa	95	-	-	5	100
Total	60.77	36.36	0.23	2.64	100

Source: Zelealem et al. (2011).

factory processed butter, locally produced butter is semi-solid at room temperature. It has a pleasant odor when fresh, but with an increase in storage time, changes will occur in odor and taste, unless refrigerated or further processed into traditional ghee (*dhadha baksaal nitir kibe*) by boiling with spices (Lola and Haile, 2015). *Dhadha* is the most stable product of all traditionally processed fermented milk products next to traditional ghee. It has relatively good keeping quality of 4-6 weeks at ambient temperature as compared to other dairy products such as cottage type cheese (Layne, 1994). The storage stability of butter gives it a distinct advantage over fresh milk in terms of more temporal flexibility for household use and marketing (Layne, 1994).

### Use of butter from traditional processing

Butter and some dairy products are called yellow fats which contains a number of products for spreading on bread or for indirect consumption as ingredients in other foods (Embaye, 2010). In Ethiopia, butter is exclusively used to make traditional ghee. Fresh/raw butter is used for hair dressing for women as hair cosmetics and as a skin cosmetic by both sexes. A report on the review of Ethiopian dairy sector (Zelealem et al., 2011) revealed that use of butter as hair oil is assumed to have dual functions: for hairdressing and to cure headaches. Butter is also used as ointment and for relief on wounds. Fresh butter is also used by children of weaning age and the elderly (Yonad, 2009). Traditionally, children of weaning age are fed on freshly made butter for different reasons which vary from society to society. In some societies it is believed to help the infants maintain body temperature during cold weather. In other societies, feeding freshly made butter is believed to help infants begin to speak some words earlier during childhood. Fresh butter is also fed to new born babies assuming that it lubricates and

facilitates bowel movement to assist ease of discharge of feces. In a study conducted in the Borena region of Ethiopia, butter was found to be an important source of energy as food for humans, and is used for cooking (Yonad, 2009). According to Zelealem et al. (2011) in different regions of Ethiopia, 60.77% is utilized for home consumption, and the remaining 36.36% is sold and used for household expenditure, 0.23% is paid as wage in kind for casual labor and 2.64% is meant for other purposes (Table 1).

### Traditional methods of butter preservation

In different communities, producers use various traditional preservatives and preservation techniques to increase shelf life of butter (Alganesh, 2002; Mekdes, 2008; Eyassu and Asaminew, 2014). The traditional preservatives and preservation techniques are used as a principle of acidification and moisture reduction and can give butter good storage stability (O'Mahony and Peters, 1987). Ghee has excellent storage stability. Where Ghee is not made, butter is occasionally spiced and heated for increasing its shelf life (O'Mahony, 1988).

### Spicing butter

In different rural areas of Ethiopia, spicing butter by thoroughly mixing with powders of spices is one of the traditional methods of butter preservation technique. To make spiced butter, preservatives such as *Curcuma domestica*, *Trachyspermum ammi*, *Trigonella foenum*, *Afruarum korerima*) are mixed with butter (Alganesh, 2002). Spiced butter can be used for household consumption or for sale. According to different reports butter preserved using such techniques can be kept with minimal spoilage at room temperature for 12 weeks

**Table 2.** Spices used in traditional butter preservation (spiced butter) in Ethiopia.

Vernacular name	Common Name	Scientific name	Plant part (s) used
Jinjibila	Ginger	<i>Zingiber officinale</i>	Tuber
Habasuuda adii	Bishop's weed	<i>Trachyspermum ammi</i>	Seeds
Habasuuda gurrachaa	Black cumin	<i>Nigella sativa</i>	Seeds
Mimmixa	Chillies	<i>Capsicum spp.</i>	Pods
Ogiyoo	Korerima	<i>Elettaria cardamoum</i>	Seeds
Qulubbiadi	Garlic	<i>Allium sativum</i>	Tuber
Siqaqibee	Basil	<i>Ocimum spp.</i>	Stems & leaves
Sunqoo	Fenugreek	<i>Trigonella foenum</i>	Seeds
Xoosinyii	Oregano	Satujera species	Leaves

Source: Alganesh and Fekadu, (2012) and Hailemariam and Lemma (2011).

**Table 3.** Traditional methods of butter preservation and their reported shelf life.

Methods of preservation	Shelf life (days)
Untreated butter (control)	19
Spiced butter	84-540
Salting butter	111
Melted butter/ <i>Nigur kibe</i>	111
Traditional ghee	333 -870

Source: Yitaye et al. (2009), Alganesh and Fekadu (2012), Eyassu and Asaminew (2014) and Debela (2016).

especially during Ethiopian Orthodox fasting period (Alganesh, 2002). Another study by Lemma et al. (2004) in east Shewa indicated that surplus butter produced during high production season was either sold at lower price or preserved and stored by mixing with spices for later use. According to Lemma et al. (2004), almost all the women in Adami Tulu and Arsi Negelle and 63.3% of the women in Lume District somehow used some form of preservatives to keep butter for longer period of time. The same study revealed that, according to the respondents, spiced butter can be kept for about 3 years if properly worked, kept clean and mixed with fresh butter from time to time. Another study in northwestern Ethiopia also reported that smallholders practice spicing of butter for preservation purpose (Eyassu and Asaminew, 2014). A former report by Alganesh (2002) also revealed that spiced butter can be kept for 2.80 months in eastern Wollega zone. However, in Northwestern Ethiopia, spiced butter can be kept for up to 11.40 months at an ambient temperature without deterioration (Eyassu and Asaminew, 2014). Table 2 shows different types of spices used to preserve butter in different communities in Ethiopia.

### Salted butter

Butter can be slightly salted by kneading in about 10 g of

salt/ kg of butter. Salting can also be done by sprinkling salt on the surface of butter. Similarly, salting butter and wrapping it in an air tight condition can partially prevent mould formation (O'Connor, 1994).

According to O'Connor (1994), butter should be salted at a rate of 16 g salt/kg or according to taste. The salt used should be dry and evenly ground and of the best quality available. Butter is highly stable against microbial spoilage after 2% salt addition, because of its high fat, low moisture and nitrogen ratios (Almaz et al., 2001; Wondu, 2007; Zelalem, 2010). The butter is worked mechanically both to disperse the salt and water, and to obtain the correct physical structure. This process greatly influences the microbiological stability of butter. If most of the water droplets present are less than 10  $\mu\text{m}$  in diameter, any microorganisms within the butter will not be able to grow and will gradually die off, owing to nutrient depletion and the inhibitory effect of salt. However, if larger water droplets are present in the butter, as a result of either over or under working, the compartmentalization effect is reduced and microbial survival and growth occurs.

A study conducted in Eastern Wollega by Alganesh (2002) showed that some smallholder farmers preserve butter by salting. According to the study, it is reported that salted butter can be kept for 3.70 months at room temperature. A report from northwestern Ethiopia (Yitaye et al., 2009) (Table 3) also revealed that smallholder producers use salting for preservation of butter. A recent report from west Shewa (Debela, 2016) revealed that a shelf life of 3.79 to 4.65 months for salted butter under smallholder storage condition.

Even though the smallholders use salting to increase shelf life of butter, they use ordinary type of coarse salt (iodine free) which may be inferior in terms of quality and hygiene. A study report by Debela (2016) on fresh butter samples treated with ordinary salt also showed high total bacterial count of (9.58 log cfu/g) compared to melted, spiced butter and traditional ghee. The author suggested that the salt used in butter treatment might be a source of contamination for high microbial load.

**Table 4.** Requirements of butter set by Ethiopian Standard Authority.

Characteristics	Milk fat/butter (%)	Test method
Milk fat, min,% by mass	99.6	ES 3476
Moisture, max,% by mass	16	ES ISO 5536
Acidity, max, % by mass as oleic acid	0.4	ES ISO 1740
Peroxide value, max, milli equivalent of oxygen/ 1kg fat	0.6	ES ISO 3976
Copper, max, ppm	0.05	ES ISO 5738
Iron, max, ppm	0.2	ES ISO 6732
Salt, NaCl, max, % by mass	2.5	ES ISO 1738 WD

Source: Ethiopian standard (ES), Milk fat products specification, 2008 and 2009.

### Melted butter/*Nigur kibe*

In Ethiopia, melting of butter /*mangor* or making *nigur kibe* is another traditional method of butter preservation technique. Butter made by the household or purchased from local market is put in a clay pot or saucepan and kept on a slow open fire or heat source. In some cases, during heating bishop's weed (*Trachyspermum ammi*) and cardamom (*Elettaria cardamoum*) are added to improve flavor and aroma of the butter. But in most cases, sole butter is melted and refined. Melted butter is kept for overnight in cool dry place to solidify. Impurities are decanted off from the re-solidified melted butter. All residues including residual butter milk and dirty materials that are settled at the bottom of the saucepan/pot are filtered out by making a hole from one side in the solidified butter. The melted butter is either kept by packing in plastic, gourds or wooden containers. The melted butter can also be directly processed into traditional ghee by further boiling and adding spices. Traditional ghee made from *nigur kibe* has longer shelf life and is cleaner due to the pre-removal of impurities. However, when traditional ghee is made directly from raw butter it can have some impurities after clarification and has less shelf life. Melted butter contains about 10% moisture (FAO, 1990). Alganesh and Fekadu (2012) reported that *nigur kibe* can be kept at room temperature for 6 months. But Debela (2016) reported that only a shelf life 87 - 93 days.

### Chemical composition and microbial quality of butter

According to Zelalem (1999), in the central high lands, traditionally made butter contains approximately 81.7% fat, 1.1% protein and 0.23% ash. However, a study conducted by Mekdes (2008), in Southern Ethiopia, showed that a moisture content of *kibe* as 20 to 43%, 84.82-86.86% total solids, 80.53-82.53% fat and 0.12 - 0.2% ash. The quantities of the main constituents of dairy products including butter can vary considerably depending on the individual animal, its breed, stage of lactation, age and health status. Herd management

practices and environmental conditions also influence dairy product composition. Besides, the moisture content of local butter can vary depending on the extent of kneading or working of butter (O'Connor, 1994). On the other hand, in the case of butter that is made using modern technology, the main constituents are standardized.

Alganesh (unpublished data) on butter samples collected from different local markets and butter shops in the central high lands of Ethiopia revealed that traditionally made butter has an average water activity of 0.974, titratable acidity of 1.482/mg KOH/g of butter samples, an acid value of 3.0 mg KOH/gram and with peroxide value of 10.1 mg equivalent peroxide/1000 grams of butter. According to O'Mahony and Ephraim (1985) older butter sold in Addis Ababa market had free fatty acids content of as high as 23%. The findings do not fulfill the requirements set by Ethiopian Standard Authority (Table 4). This might highly contribute to oxidative and hydrolytic rancidity in butter due to poor processing, handling and hygienic practices. Moreover, there is scanty information on chemical properties of Ethiopian butter and ghee.

### Microbial quality of traditionally produced butter

Butter samples collected from different parts of the country revealed the following microbiological quality. The average total bacterial count of butter samples collected from Selale and Sululta areas had 6.18 cfu/g and 7.25cfu/g, respectively (Zelalem, 2010). Another report by Wondu (2007) in Awassa, Southern, Ethiopia indicated that an average total bacterial count of 7.49 cfu/gram with high variation from different sources. Samples collected from southern Ethiopia from open local markets and rural producers had higher counts compared to dairy farms and urban producers. Besides, total bacterial count of fresh butter samples collected from Addis Ababa by ILCA (1992) showed a range of 4.7 log cfu/g to 8.27 cfu/g of butter samples. Report of Debela (2016) in west Shewa showed a mean aerobic mesophilic bacteria count of 8.71 log cfu/gram of fresh



**Table 5.** Microbiological limit specification for butter.

Micro organism	Maximum limit	Method of test
Total plate count	1,000,000/ml	ES ISO 6610
E. Coli	Absent / ml	WD 13540, WD 13541
Salmonella	Absent / 2.5 ml	ES ISO 6785
Molds and yeasts	10/ ml	WD 13539

Source: Ethiopian standard (ES), Milk fat products specification (2008).

butter samples. Un unpublished data of Alganesh also showed that a total bacterial count of 2.47 cfu/g of butter samples collected from open markets and whole sale shops in the central high lands of the country. These results are higher than the acceptable level of 6 log cfu/ml set by Ethiopian standard (Table 5) and world standards indicating that traditionally made butter in different areas of the country are substandard.

Studies conducted by different persons (Wondu, 2007; Zelalem et al., 2007; and Mekdes. 2008) showed coliform counts in butter samples ranged from 1.92 to 4.5 cfu/gram. While a Debela (2016) had reported a mean total coliform count of 5.62 log cfu/gram of fresh butter samples from west Shewa, Ethiopia. All the results were higher than the standard showing that butter is produced under unhygienic conditions. This might be attributable to the materials and methods of production, handling, hygiene of the producer and the animal from which the milk is obtained.

The yeast and mould counts in butter samples collected from southern Ethiopia (Mekdes, 2008) ranged from 5.52 to 5.74 cfu/g. Contrary to these findings, an average of 7.65 cfu/g of yeast and mold counts was reported in Awassa, Southern Ethiopia by Wondu (2007). A recent report of Debela (2016) revealed yeast and mould count of 6.7 cfu/g of fresh butter samples. Moulds are the primary spoilage factors in butter and their presence in butter indicates post production contamination from air or water.

### Traditional ghee

Ghee is a product that is made by indigenous methods in many countries around the world, largely in Asia, the Middle East and Africa (Afsaneh et al., 2016). In different parts of the world, products similar to ghee have been available probably since prehistoric times. They are known as “*Samna*” in Egypt (Aboudhonia and Elagamy, 1993), “*Meshho*” in ancient Assyrian empire (2400 BC to 612 BC) (Abdalla, 1994), “*Samin*” in Sudan (Hamid, 1993), “*Maslee*” or “*Samn*” in Middle East, “*Rogan*” in Iran (Urbach and Gordon, 1994), and “*Samuli*” in Uganda (Mohammed et al., 1998). In Ethiopia ghee is known by the name *dhadha baksal Neter Kibe*, which stands for

heated and clarified butter.

Some ambiguity in the definition of ghee occurs mainly due to regional deference and preferences for the product. The characteristic flavor and aroma of ghee is its major criterion for acceptance. Flavor is greatly influenced by the fermentation of the cream or butter and the heating processes (Mohammed et al., 1998). According to Illingworth et al. (2009) ghee can be defined as a pure clarified fat that is exclusively obtained from milk, cream or butter by application of heat for almost total removal of moisture and solid nonfat to give a product a unique flavor, physical structure and texture. A recent definition of ghee is stated as ‘a product exclusively obtained from milk, cream or butter by means of processes which result in almost total removal of water and non-fat solids, with an especially developed flavor and physical structure (Afsaneh et al., 2016). According to Mohammed et al. (1998) carbonyls, lactones and free fatty acids are reported to be the key ghee flavoring compounds. In the case of Ethiopian ghee, flavor determinants are fermentation time, type of fumigants used for milk vessels and churns and spices used in ghee making. Ethiopian traditional ghee *Nitir kibe* is made from butter made of sour milk. It has an attractive appearance, a grainy texture and a light yellow color. At room temperature it is semi-solid. It has a pleasant odor and good taste. Its good keeping quality allows storage for more than a year without significant deterioration (Almaz et al., 2001; Eyassu and Asaminew, 2014).

The western world standard specifies ghee to have 96% minimum milk fat, 0.3% maximum moisture, 0.3% maximum free fatty acids (FFA) (expressed as butyric acid), and a peroxide value (PV) less than 1.0. Its physical structure should consist of a mixture of higher softening point fats in crystalline form dispersed in the liquid lower softening point fats and this gives the ghee a somewhat granular appearance (Mohammed et al., 1998). However, there is no report on such parameters on the Ethiopian ghee. But the standard authority has set minimum requirements for the parameters as indicated in Table 6. Furthermore there is no information available on the effects of traditionally made ghee on human health.

Traditional ghee is a more convenient product than butter in the tropics, because of its better shelf life even under warm conditions (O’Connor and Tripathi 1992). In Ethiopia, traditional ghee is made exclusively for home consumption, not for market. Traditional ghee manufacture is based on individual experience and taste. Addition of combinations of spice powders, chopped tubers and or dry herbs, leaves and stems of green spices are used in traditional ghee making (Alganesh, 2002; Hailemariam and Lemma, 2011).

The main features of clarified butter manufacturing are identical in every country. However, some differences exist specially in the duration of heating and whether or not some clarifying agents are added to the boiling butter. According to survey reports (Alganesh and Fekadu,

**Table 6.** Requirements of ghee set by Ethiopian standard.

Characteristics	Value	Test method
Milk fat, min,% by mass	99.6	ES 3476
Moisture, max,% by mass	-	ES ISO 5536
Acidity, max, % by mass as oleic acid	0.4	ES ISO 1740
Peroxide value, max, milliequivalent of oxygen/ 1kg fat	0.6	ES ISO 3976
Copper, max, ppm	0.05	ES ISO 5738
Iron, max, ppm	0.2	ES ISO 6732

Source: Ethiopian Standard (ES), Milk fat products specification, 2009).

2012; Eyassu and Asaminew, 2014; Debela, 2016) traditional ghee making is the major option of preserving butter in Eastern Wollega, North Western and West Shewa of Ethiopia. According to the above authors' reports from different sites, traditional ghee can be kept at ambient temperature of between 20 to 30°C for 11.10, 19.20 and 7.3 to 7.7 months, respectively in East Wollega, North west and West Shewa, Ethiopia without significant change on quality. Another report from Dewachefo area of Amhara region showed that smallholders store ghee from 6 months up to 7 years and use such ghee for medicinal purpose to cure chronic coughs (Kefyalew et al., 2016). O'Mahony and Peters (1987) showed that salt is added to traditional ghee as a combination of preservative to prolong storage life. This view has been refuted on grounds that salt is not fat soluble and hence does not have a preservative effect against deteriorative reactions taking place in the fat.

### Procedure for traditional ghee making

According to Illingworth et al. (2009), worldwide, there are four methods for the production of ghee: the indigenous milk butter method, the direct cream method, the cream butter method and the pre-stratification method. In Ethiopia, traditional ghee is made from butter made of fermented milk, or butter preserved using different butter preservation methods. Hence, raw materials used for ghee making can be *nigur kibe*, spiced butter, salted butter, untreated rancid butter or fresh butter. Variety of herbs, powders and chopped tubers of spices are added during ghee making (Alganesh, 2002; Debela, 2016).

To make traditional ghee, butter is inspected and cleaned of any visible impurities and placed into a saucepan or clay pot and put on open fire or heat source (actual heating temperature not known) to melt. Heating and stirring continues until foam is formed and a clear liquid is obtained. Along heating the butter, combination of one or more spices are added to induce good aroma, increased shelf life and taste (Table 7). Heating of melted butter is continued until bubbling ceases and all moisture evaporates (assumed that foam and bubble are appearing due to water evaporation). When the non-fat

solid turns to brown and frothing stops, boiling is stopped (Kefyalew et al., 2016). Melted butter is then filtered through sieve or piece of cheese cloth to remove impurities and decanted into another vessel leaving the curd material in the pan. Well dried containers free from moisture with tight stopper are used to keep refined butter. Commonly ghee processed in such a step is stored in cool dark areas of the house. Small amount is daily removed and used in cooking and preparation of various traditional foods (Almaz, et al., 2001; Alganesh and Fekadu, 2012). Similar procedures have also been reported by Asamnew (2007) in Bahir Dar area. Some of the spices used for traditional-ghee making in different communities are indicated in Table 7.

### Use of traditional ghee

Ghee is the most important ingredient in food and it is rich source of dietary energy and contains high calorific value. Indian ghee contains approximately 0.5% moisture, 99% milk fat and other minor chemical components (Sukumar, 1980). Ghee contains certain acids which are very important and essential for the human beings. They are vehicle for the fat soluble vitamins (Jariwala, 2014). So far, there is no information on the chemical composition of Ethiopian ghee.

Traditional ghee is commonly used for culinary, social functions and therapeutic purposes (Mohammed et al., 1998). A major portion of ghee is utilized for culinary purposes such as a dressing for various foods, cooking and frying of different foods. In Ethiopia, traditional ghee is usually utilized for flavoring and as condiment for different types of pulse stews (lentils, beans and peas), chicken and meat stews and sauces from different species of domestic animals. Particularly no chicken stew is being thought with the absence of ghee, and commonly is an indication of well-being of a family who used ghee in their daily meal.

This is in agreement with a report of Zelalem et al. (2011) that stated that ghee is added to a variety of Ethiopian traditional dishes such as *Kitifo* (minced beef served raw or half cooked) and a variety of cereal, pulse and meat based sauces. It is also used in mixture with

**Table 7.** Spices used in traditional ghee making in Ethiopia.

Vernacular name	Common name	Scientific name	Plant parts used
Qullubii adii	Garlic	<i>Allium sativum</i>	Tuber
Jinjibila	Ginger	<i>Zingiber officinale</i>	Tuber
Irdii	Turmeric	<i>Curcuma domestica</i>	Tuber
Sunqoo	Fenugreek	<i>Trigonella foeniculum</i>	Seeds
Oogiyoo	Kororima	<i>Aframomum korarimao</i>	Seeds
Qurunfudii	Clove	<i>Syzygium aromatiun</i>	Seeds
Qarafaa	Cinnamon	<i>Cinnamomum verum</i>	Seeds
Habasuuda adii	Bishop's weed	<i>Trachyspermum ammi</i>	Seeds
Habasuuda gurraacha	Black cumin	<i>Nigella sativa</i>	Seeds
Kefoo	Basobila	<i>Ocimum urticifolium</i>	Seeds, stems and leaves
Qundabarbarree	Black pepper	<i>Piper nigrum</i>	Seeds
Siqaaqibee	Basil	<i>Ocimum spp</i>	Seeds, stems and leaves
Kusaayee		<i>Ocimum hardiense</i>	Stems and leaves
Mimmixa	Chillies	<i>Capsicum spp.</i>	Pods
Gaawwuzii		<i>Myristica fragrans</i>	Pods
Qullubbii dhiima	Onion	<i>Allium cepa</i>	Tuber
Cilaattama	Rue	<i>Ruta graveolence</i>	Stems and leaves
Dimbilaala	Cordiander	<i>Cordiandrum sativum</i>	Seeds, stems and leaves
Mekimeko	Spinach Rhubarb	<i>abyssinicus Rumex</i>	Stems and leaves
Xoosinyii	Oregano	<i>Satujera species</i>	Leaves

Source: Alganesh (2002), Hailemariam and Lemma (2011) and Bekele et al. (2015).

cottage type cheese and *kochkocha* and served with indigenous diets such as *chumbo* and *chororsa*. Traditional ghee is occasionally drunk with coffee. It is also used as input in cultural ceremonies for roasting coffee to make *buna kala* that is served during special occasions and holidays. Ghee is also used to prepare delicious indigenous snack foods such as '*chachabsa*, *chiko*, *anababiro*, *silcho* (traditional ghee and table salt mixed with roasted or boiled maize green cobs) and porridge (Yonad, 2009; Alganesh and Fekadu, 2012; Lola and Haile, 2015). According to study conducted, traditionally made ghee stored for more than a year is recommended for patients to treat chronic coughs (Alganesh and Fekadu, 2012). Ghee is also consumed with coffee and tea especially when important guests are received in a home and during major holidays.

### Packaging materials and methods for butter and ghee

Ghee is susceptible to deterioration from exposure to light, air and metal ions (Illingworth et al., 2009). Ghee is preserved by a combination of heat, which destroys enzymes and contaminating microorganisms and by removing water from the oil to prevent microorganisms growing during storage. According to ES (Ethiopian Standard Authority) (2009), butter shall be packed in containers that are proof to water and fat, non absorbent

and non-harmful to its composition, flavor and appearance. In smallholder farmers' case butter making, contamination can come from packing material, unclean surface, the butter maker, the wash water, cups and leaves. Moreover, traditional equipments are often porous and harbor dirt and microorganisms (O'Mahoney, 1988). Fellows (2008) recommended that, butter and ghee can have a longer shelf life if they are stored in cool place, using airtight, light-proof and moisture-proof containers to slow down the development of rancidity. Simon (2012) also stressed that the keeping quality of ghee can be affected by many factors, such as type of packaging material, permeability to oxygen and moisture, method of manufacture, presence of antioxidants, light and others.

A leaf of plant called *koba/inset* (false banana) is the most common material used for butter packaging in southern parts of Ethiopia. *Koba/inset* is believed to be important to keep butter fresh until marketed. However, some respondents indicated that the leaves may reduce the weight of the butter because when the cover is removed some butter remains stacked on the leaves (Mekdes, 2008). The same author recommended the need of further study on the effect of packaging butter with leaves on the quality and sensory characteristics. In other parts of Ethiopia, use of clay pot for storage or packaging of various dairy products including butter and ghee is common (O'Mahony and Ephirem, 1985; Zelalemand Inger, 2001b; Eyassu and Asaminew, 2014).

Use of gourd as a storage vessel or packaging material for *kibe* and *nitir kibe* and packaging *kibe in kobo* (Castor bean leaf) and other leaves during storage and while markets is common in east Wollega and Shashamane areas (Alganesh, 2002; Lemma et al., 2004).

## CONCLUSION AND RECOMMENDATION

Indigenous methods of production, processing and handling of butter and ghee were assessed. Butter is made from sour/naturally fermented whole milk. Butter and ghee are important component of Ethiopian traditional diets. Ghee can be made from untreated butter, spiced, salted butter and *nigur kibe*. During ghee making different types of spices are added for flavor impartation, acceptable taste and increased shelf life. There is scanty information available on chemical and microbial quality of butter. However, the available information on the quality and safety of butter shows that the products are in substandard state. Moreover, there is limited or no information on the quality and safety of ghee. The safety and quality aspect of butter and ghee and handling as well as processing practices still are subject of further investigation. Besides, there is a need to develop and optimize butter and ghee processing methods for better yield and quality for future commercialization.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## GLOSSARY OF TERMS

- Anababiro**- Ethiopian flat bread (*injera*) made from unleavened powder of red colored *Eragrostis tef* where two flat bread are merged together and freshly made traditional ghee and *Kochkocha* or spiced hot pepper powder mixture are rubbed on it. It is very delicious, it is served as snack or breakfast.
- Bala kobo** - Castor bean leaf.
- Buna qalal/buna kala**- Coffee bean roasted with freshly made traditional butter that is served on special occasions.
- Chachabsa** - Traditional pancake made from unleavened powder of red colored *Eragrostis tef*. The pan cake is chopped and mixed with freshly made traditional ghee and salt. It is served as breakfast and snack.
- Chiko**- A delicious traditional snack food made of roasted barley powder, barley powder is thoroughly mixed with fresh traditional ghee, salt and *Aframomum korarimao*.
- Chororsa**- Soft cottage cheese on which mixture of clarified butter and *kochkocha* served with tef.
- Chumbo**- Unleavened delicious Oromo cultural bread made of red seeded tef (*Eragrostis tef*) flour served with cottage cheese on which clarified butter mixed with *kochkocha* is sprinkled on it.
- Dhadha baksaal/ nitir kibe**- Traditional ghee made from local butter boiled with different spices and clarified.
- Dhaadha/kibe** -Local butter made by churning fermented milk.
- Ergo/ iittu**- Spontaneously fermented milk without defined starter culture.
- Gorfu**- Traditional milk vessel used by borana pastoralists.
- Kitifo**-minced beef served raw or half cooked.
- Kochkocha**- Finely chopped green hot pepper that is mixed with ginger, garlic, onions, and herbs of desirable aroma and salt and is served as sauce for different traditional foods.
- Mangor**- To make *nigur kibe*/the process of making *nigur kibe*/melting of butter keeping it to settle for overnight and decanting off impurities from the butter.
- Nigur kibe** - butter that is melted cooled over night and decanted off any impurities and residual butter milk settled at the bottom of pot/sauce pan.
- Silcho** - Roasted or boiled maize green cobs mixed with traditional ghee (made from freshly made butter) and table sprinkled on it and served hot and fresh.
- Spiced butter**- Homemade butter or butter purchased from local market that is meant for future use or sale is mixed with powders of some spices and wrapped in traditional containers.

Full Length Research Paper

## Fortification of hotcakes from edible flour of non-toxic Mexican *Jatropha curcas* L.

Jorge Martinez Herrera<sup>1\*</sup>, Elizabeth Arguello García<sup>2</sup>, Odilón Sanchez Sanchez<sup>3</sup> and Ofelia Andrea Valdés-Rodríguez<sup>4</sup>

<sup>1</sup>Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Huimanguillo, Km 1 Carretera Huimanguillo-Cárdenas. Huimanguillo, Tabasco C.P. 86400, Mexico.

<sup>2</sup>Universidad Popular de la Chontalpa. Km 2 Carretera Cárdenas-Huimanguillo Ranchería Paso y Playa. Cárdenas, Tabasco. C.P. 86500, Mexico.

<sup>3</sup>Centro de Investigaciones Tropicales (CITRO-UV) Ex-Hacienda Lucas Martín Priv. Araucarias s/n, Col. Periodistas. Xalapa, Veracruz. C.P. 91019, Mexico.

<sup>4</sup>El Colegio de Veracruz. Carrillo Puerto No. 26, Col Centro. Xalapa, Veracruz C.P. 91000, Mexico.

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The *Jatropha* flour is a promising source of protein in the fortification of various foodstuffs made from wheat and corn. The objective was to evaluate the use of non-toxic or edible *J. curcas* flour at different percentages (5, 10, 15 and 20%) in combination with wheat flour to make hotcakes. Whole and defatted *J. curcas* flour presented values of moisture between 4.5 and 6.8%, nutritional protein 23.5 and 54.0%, lipids 52.52 and 1.56%, ashes 5.29 and 11.4%. The protein content of a control sample of hotcakes was 8.4%, increasing to 16.5% when 20% *Jatropha* flour was added to wheat flour. The microbiological analysis of defatted flour recorded a count of aerobic mesophilic of 7100 UFC/g, fungus 200 UFC/g and yeasts 20 UFC/g. At last, it was determined that lead and cadmium were not detected in flour. Thus, the edible *Jatropha* flour has a potential for fortification in processed foods, but is very important to ensure the safety or low toxicity level of *Jatropha* seeds previously by chemical analysis (HPLC) to avoid problems of severe intoxication; therefore non-toxic plantations of *Jatropha* must be certified to avoid mixtures with other toxic seeds.

**Key words:** Physic nut, *Xuta*, flour, meals, fortification, microbiology, protein

### INTRODUCTION

*Jatropha curcas* is a perennial plant belonging to the Euphorbiaceae family and considered to be originating in

Mexico and Central America, growing in altitudes from 0 to 1700 msnm, and annual precipitations averages of

\*Corresponding author. E-mail: [martinez.jorge@inifap.gob.mx](mailto:martinez.jorge@inifap.gob.mx)

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300-2500 mm (Martinez et al., 2010). Traditionally it has been used as a hedge plant and for medicinal uses. In recent years, tropical countries like India, China, Malaysia, Indonesia, Mozambique, Brazil, and Mexico, among others, have explored the potential of the *Jatropha* plant as a feedstock for biofuel production, mainly biodiesel. The *Jatropha* tree has been proposed as a potential second-generation biofuel due to its many toxic components (phorbol esters mainly). These compounds have been reported in seeds from Central America, South America, African, and Asian continents (Makkar et al., 1997; Contran et al., 2013).

In Mexico, *J. curcas* is found in more than 17 states; among them are Veracruz, Morelos, Puebla, Hidalgo, Tamaulipas, Tabasco, Chiapas, Yucatán, Oaxaca, Guerrero, Michoacán, Colima, Jalisco, Nayarit, Sinaloa, Campeche and Sonora (Martinez et al., 2010). The plant is known by different names according to the region of Mexico as: pinion, hill pinion, piñoncillo, Aixte (Náhuatl language) Xuta or chuta (Totonaco language), Sikil Té (Maya language), Scu-Lu'ú. The diversity in Mexico is such that there are ecotypes with different levels of toxicity (Martinez et al., 2010). The main toxicity is due to the higher phorbol esters contents, which are co-carcinogenic compounds that attack the central nervous system, being capable of killing animals and humans (Wink et al., 1997). However, some provenances of *J. curcas* in Mexico have very low or zero phorbol ester contents. These provenances are considered edible or non-toxic because they are eaten by the natives of their regions (Martínez et al., 2006).

All the ecotypes have anti-nutritional compounds such as trypsin inhibitors, lectins, phytates, saponins and tannins. When defatted flour of *J. curcas* is cooked, non-nutritional compounds like trypsin inhibitors and lectins are partially or fully eliminated avoiding damage by ingestion (Martínez et al., 2006; Makkar and Becker, 2009; Martinez et al., 2010). Additionally, due to their physicochemical properties, *J. curcas* seeds provide a good source of oil that can be used as diesel substitute.

The chemical composition of the seeds, flour and nutritional quality of edible *J. curcas* have been reported and compared to toxic seeds. The oil content of the seeds and their fatty acid composition are similar in both edible and inedible ones. However, based on the agro-climatic conditions of the region they come from, the genotypes may have a major percentage of oleic acid than linoleic acid. The amino acid composition is not different in both, toxic and non-toxic seeds; their essential amino acid levels plus lysine are higher, according to the reference standard of FAO/WHO, and these are comparable to soybean flour (Martínez et al., 2006; Martínez et al., 2010; Azevedo et al., 2016). The edible Mexican *J. curcas* has high lipid (45-48%) and protein contents (18-30%). The defatted flour, also known as "press cake" or residual paste, has up to 55 to 60% of

protein.

The press cake is characterized by its high protein content. A whole seed press has 38-48% of protein with 10% of residual oil, and when it is extracted with organic dissolvent like hexane, the kernel has values higher than 55% of protein or more. Also, there are reports on protein efficiency (REP) of *Jatropha* flour supplemented with lysine of 1.77 in Wistar rats, which was higher than that reported for soybean (1.4). Therefore, it can be considered that non-toxic flour has high quality ingredients for animal nutrition (Makkar and Becker, 2009; Martinez et al., 2012; Richter, 2012).

*Jatropha* edible seed is used by the people of Papantla and the mountains of Puebla to prepare traditional dishes. These authors have used defatted flour to produce various industrial products to fortifying foods devoid of protein from wheat and corn. Thus, defatted flour of *Jatropha* with wheat flour was used to make breads, cookies, cakes, pancakes, pizza, etc. Also, it is permissible to make tortillas with corn flour (Arguello et al., 2016). Food fortification is an alternative for improving nutritional contents, through the addition of nutrients, such as vitamins, minerals, and amino acids (Figueroa et al., 2001). The aim of this study was to determine the fortification, quality flour and protein content in hot cakes added with *Jatropha* flour.

## MATERIALS AND METHODS

### Collection and conditioning of material

The *Jatropha* edible seed was recollected in August 2013 in Papantla, Veracruz, Mexico and were manually husked. The resulting kernel was ground in a mill Cyclotec™ Model 1093 (Höganäs, Sweden), for 16 h. The seeds were defatted with hexane P.A. with Soxhlet equipment at 68°C. Excess of hexane was eliminated in room temperature in exhaust hood for 12 h. The content of phorbol esters was determined in the flour to confirm its non-toxicity. Later, four mixtures of 0, 5, 10, 15 and 20% of *Jatropha*/wheat flour were prepared to cook the hotcakes as traditionally.

### Extraction and estimation of phorbol esters by HPLC

The phorbol esters were determined as described by Makkar et al. (2007) based on the method of Makkar et al. (1997). Ground seed kernels/defatted meal (2 g) was extracted with 1 mL solvent (99 percent methanol/1 percent THF) and centrifuged (Allegra Model X-15 R, USA) (10,000 × g) to collect methanolic supernatant. The residue was re-extracted (three times), centrifuged and supernatant was collected. The supernatant was concentrate together and condensed using pressurized air to get fraction. This fraction was re-dissolved in methanol (1 mL). A suitable aliquot was injected into a high-performance liquid chromatography (HPLC) fixed with a reverse-phase C18 LiChrospher100, 5 mm (250 mm × 4 mm id, from Merck (Darmstadt, Germany)) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23°C) using gradient elution (1.3 mL/min flow rate) (Makkar et al., 2007). The



**Figure 1.** Whole flour (A) and defatted *J. curcas* (B)

four phorbol ester peaks (containing 6 PEs) which appeared between 25 and 30 min were detected at 280 nm. The spectrum of each peak was taken using Merck-Hitachi L-7450 photodiode array detector. Phorbol-12-myristate 13-acetate (PMA) was used as an external standard which appeared between 31 and 32 min. The area of the four phorbol ester peaks was summed and the concentration was expressed as equivalent to PMA. The PEs in the meal was analyzed in triplicates.

#### Elaboration of hotcakes

Ingredients: 4.0 g Spring Chantilly® unsalted margarine, 500 g of flour for hot cakes, 2 eggs (107 g approximately), 500 ml of whole milk, *J. curcas* flour at 5, 10, 15 and 20%. Procedure: 1) all the ingredients were mixed until lumps dissolved completely, 2) over a hot skillet, a little margarine Primavera® was lightly spread, 3) the mixture was gently poured over the cookie sheet at a temperature of 50-55 °C, forming the circle of the desired size). Bubbles were formed and it was turned gently to cook the other side.

#### Proximal chemical analysis

Percentages of protein, lipids, ash and moisture, were quantified by triplicate in *J. curcas* flour (whole and defatted), and the fresh hotcakes (5, 10, 15 and 20%), according to AOAC methods (1995). Protein was estimated by the Kjeldahl method, using a digester (Digestive System 61007 digester) and a distilled (Kjeltec 1002 Distilling unit system Tecator) by manual operation. Lipids were obtained by extraction with petroleum ether in a Goldfish extractor. Ash was obtained by carbonization and subsequent samples calcined at 550°C to constant weight. Moisture was estimated by circulation oven drying air at 90° C to constant weight (AOAC, 2010).

#### Microbiological analysis of the defatted flour

The total mesophilic count, fungi and yeast were determined according to the following rules:

**Mesophilic aerobic count:** This assay was made with agar for standard count; it was incubated for 48 h at 35°C, in an Incubator 132000 3M, according to NOM-092-SSA1-1994.

**Fungi and yeasts:** Trials of fungi and yeasts were performed over potato dextrose agar acidification, incubated at 25°C ± 1°C for 5 days, as indicated in NOM -111 - SSA1-1994.

#### Heavy metals analysis

Lead and cadmium content were determined using the spectrophotometric atomic absorption equipment Shimadzu Model AA-7000, according the method indicated by the NOM-247-SSA-1-2008.

#### Statistical analysis

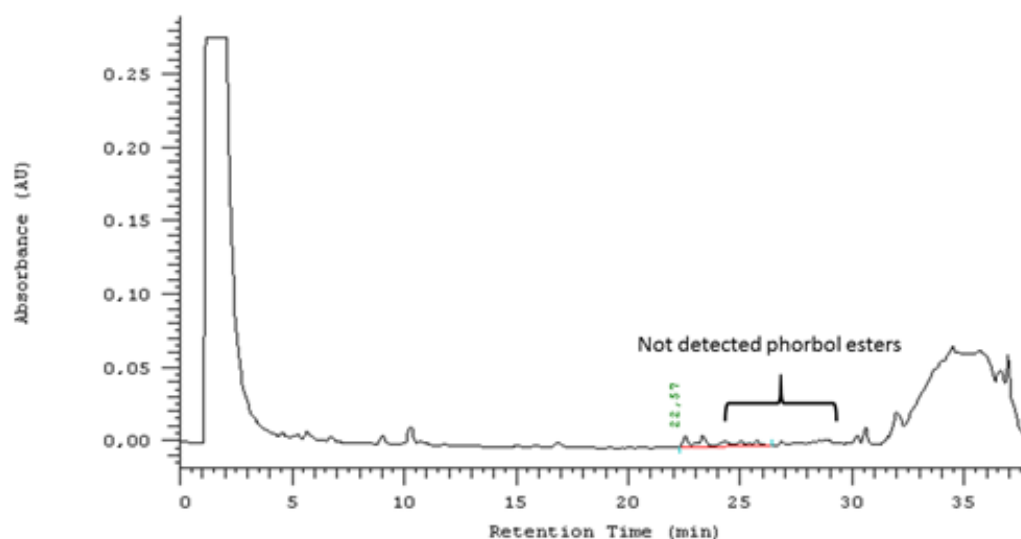
Data were processed with Statistical Analysis System software (version 9.0.; SAS Institute Inc., Cary, NC, USA), under the completely randomized model, and the means were compared with the Tukey test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

#### Flour yield

An analysis of whole flour of *J. curcas* had 57.17% oil and defatted flour has 42.83%. It will required 1.64 kg of whole seed to obtain 1.0 kg of whole flour; it takes 2.33 kg of whole flour to obtain 1.0 kg of defatted flour, which can be used in the fortification process of different foodstuff (Figure 1). These values can oscillate doubtlessly depending on the seed origin, which has different percentages of shell and kernel. Papanlla seed had 39.1% of shell and 60.9% of kernel; it has higher values when compared to other Mexican seeds of





**Figure 2.** High resolution liquid chromatography of *J. curcas* defatted flour.

**Table 1.** Chemical composition of whole flour and edible defatted *J. curcas*

Chemical composition	Whole flour	Defatted flour
Moisture (%)	4.5±0.2*	6.8±0.1*
Ashes (%)	5.2±0.4*	11.4±0.2*
Protein (%)	23.5±0.1*	54.0±0.3*
Lipids (%)	52.5±0.4*	1.56±0.2*

\*Average of three repetitions ± standard deviation.

different states (Makkar et al., 1997, 1998; Martínez et al., 2006, 2010).

### Estimation of phorbol esters by HPLC

In defatted *J. curcas* flour used for fortification of hot cakes, there was no toxicity found. The presence of phorbol esters (PE) was not detected by HPLC, which ensure their safety for human consumption (Figure 2). Since PE are the main toxic components in *J. curcas*, Martínez et al. (2010) recommend to corroborate that the flour has not PE or that only non-toxic seeds are used to prepare the flour. Toxic seeds have never been used to make food for human consumption, only edible from Mexico that do not have PE.

### Nutritional chemical composition

Table 1 shows the values of the whole and defatted *J. curcas* flour chemical composition, highlighting high

protein content of flour without oils, surpassing even oilseeds such as soybeans.

Hotcakes were made by mixing defatted *J. curcas* flour at concentrations of 5, 10, 15 and 20%. Table 2 shows that the proximal composition of the different flour mixtures contains more *Jatropha* flour. It is observed that in the control, the initial protein content of 8.45% increased to 16.49%, when 20% *Jatropha* flour was added. It is noteworthy that the addition of 20% flour of *J. curcas* led to obtain fortified traditional hotcakes. In every *Jatropha* meal and wheat mixture, there were meaningful differences in protein increase. Increasing the *Jatropha* flour concentration in the mixture has a direct relation with the protein content, without affecting its organoleptic quality (flavour, color, texture) (Arguello et al., 2016). The inclusion of *Jatropha* flour is nutritionally recommendable because it improves the end product. The ash increases of 1.57% to 2.54% are likely due to the minerals that *J. curcas* seeds contain (calcium, magnesium, potassium, among others) (Heller, 1996; Toral, 2008).

Nowadays, there are rheological results of *J. curcas* used to produce bread making products, even tortillas, giving a broad perspective of use. However, it is necessary to certify that *Jatropha* flour does not have phorbol esters with the use of liquids chromatography coupled to mass. There is certain reluctance in the large-scale utilization of flour by humans due to the possible presence of phorbol esters. Nevertheless, there are no scientific conclusive studies that could demonstrate that an accumulation can exist in the human body, due to long consumption of the different food based on *J. curcas* kernel.

In the results of the microbiological analyses, defatted flour of *J. curcas* was compared with other flours based

**Table 2.** Proximal chemical composition of mixtures of hotcakes flour with *J. curcas*

Chemical composition	Moisture (%)	Ashes (%)	Lipids (%)	Protein (%)
Control Hotcakes	0.580±0.21 <sup>a</sup>	1.57±0.26 <sup>a</sup>	0.3±0.28 <sup>a</sup>	8.45±0.07 <sup>a</sup>
5%	0.8 ± 0.13 <sup>b</sup>	1.87±0.03 <sup>b</sup>	0.1±0.00 <sup>b</sup>	9.95±0.14 <sup>b</sup>
10%	1.10 ± 0.11 <sup>c</sup>	1.96±0.06 <sup>c</sup>	0.13±0.03 <sup>b</sup>	11.57±0.1 <sup>c</sup>
15%	0.94 ± 0.03 <sup>d</sup>	2.24±0.03 <sup>d</sup>	0.2±0.18 <sup>c</sup>	13.25±0.03 <sup>d</sup>
20%	1.25 ± 0.05 <sup>e</sup>	2.54±0.46 <sup>e</sup>	0.1±0.01 <sup>b</sup>	16.49±0.04 <sup>e</sup>

Means with the same letter in each column are not statistically different (Tukey,  $p < 0.05$ ); \*Average of three repetitions ± standard deviation.

**Table 3.** Microbiologic analysis to defatted *J. curcas* flour compared to other flour source according to the official Mexican norm (NOM-247-SSA1-2008).

Flour source	Mesophilic aerobic (UFC/g)	Coliforms Totals (UFC/g)	Fungi (UFC/g)
Wheat flour, meal, or semolina	50,000	NA	300
<b>Defatted Jatropha flour</b>	<b>7,100</b>	<b>NA</b>	<b>200</b>
Corn flour	100,000	100	1000
Corn nixtmalizada flour	50,000	100	1000
Rye flour	100,000	100	200
Barley flour	100,000	100	200
Oats flour	50,000	50	100
Rice flour	100,000	100	200
Whole meal	500,000	500	500

UFC = colony forming units, NA = not applied

on the Official Mexican Norm for sanitary food regulations NOM-247-SSA1-2008 (Table 3). It clearly shows that the *Jatropha* flour has low aerobic Mesophilic bacteria compared to flours of wheat, maize, rice or oats. Even fungi are low in wheat and maize, similar to the values of rye, barley, and rice flours. These determinations are very important for the use of *Jatropha* flour in human consumption. In spite of its good properties, *J. curcas* flour is not a conventional food, and up to this moment it has not been considered for human consumption, as it lacks an official norm for its use. This is the first report showing the microbiological content of *J. curcas* defatted flour. Its moisture content is less than 6.8%, helping conservation and storage. Muhammad et al. (2003) concluded that wheat flour with less than 10% moisture content is suitable for extended shelf life and reduce the microorganism attack; in *Jatropha* flour moisture was 6.8%.

### Contents of lead and cadmium

Finally, lead and cadmium contents were determined to verify that these heavy metals are not found in *Jatropha* flour. The type of fertilizers used, soil pH and plant

irrigation with polluted water could be the cause for the presence of these metals (Kamran et al., 2014). *Jatropha* flour lacks these heavy metals. Some reports of Pb content in wheat found more than 1.627 mg/kg, while the Cd content in wheat was more than 0.344 mg/kg, most of times the bran will be exceeding feed safety standards, and cannot be used for feedstuff (Wei et al., 2016). For this reason, the *J. curcas* meal is an excellent nutritional source with good sanitary quality.

### Conclusion

According to the results obtained and due to lack of food with high protein in many flour based foods, it is proposed to use edible seed and *J. curcas* flour to make hotcakes and different foods for human consumption. With the addition of *Jatropha* flour, fortification of foods made with wheat flour or corn with lower protein is achieved. Besides this, with up to 20% addition of *Jatropha* flour, the rheological properties are not altered and the sanitary quality of this flour is higher than other conventional flours already used by the food industry.

We consider that *Jatropha* edible seeds can be used in other countries such as Africa or Asia, including Central

and South America as a source for the development and fortification of various food products or traditional foods.

It is noteworthy that to ensure the safety of *Jatropha* flour, one should test for the presence of phorbol esters that normally exist in toxic seeds; since an error in mixing the toxic seeds could cause a serious toxicological problem.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## Fortification of kununzaki drink with cocoa powder

Abidoye Olawumi Abidemi\*, Taiwo Kehinde and Adeniran Hezekiah

Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

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The aim of this work is to improve the nutritional properties of kununzaki drink with the addition of cocoa powder and also increasing the utilization of cocoa powder because of its antioxidants activities (anti-ageing). Kununzaki was prepared using sorghum as the base and supplemented with cocoa powder as varied levels. The most preferred sample (80/20%) was chosen following sensory evaluation for further analysis. The effect of cocoa powder on the proximate composition, physico-chemical properties, minerals, antioxidant and the sensory characteristics of the enriched drink were assessed. The added cocoa powder increased the protein, ash, fat and moisture content from 1.40 to 3.12%, 0.16 to 0.38%, 0.38 to 0.93% and 84.95 to 91.20%, respectively and decreased the carbohydrate and energy content from 12.26 to 5.75% and 61.10 to 39.11 kJ, respectively. The pH and titratable acidity had an inverse relationship and were within limit. The results also showed that enrichment of kununzaki with cocoa powder increased the mineral contents significantly ( $P>0.05$ ). The antioxidant activities in the drink increased from 40 to 50%. There were significant differences in the assessed sensory qualities. The addition of cocoa powder increased the nutritional properties of kununzaki drink thereby making it more suitable for all ages.

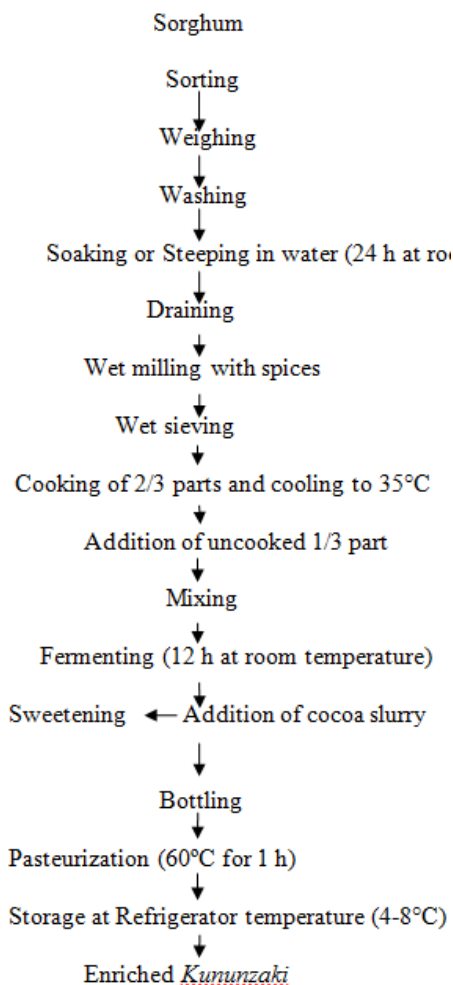
**Key words:** Kununzaki, cocoa powder, proximate, antioxidant, sensory.

### INTRODUCTION

Beverages are liquid foods that serve as a source of both fluids and nutrients that refresh and nourish the body (Ihekoronye and Ngoddy, 1985). They provide energy for daily manual work. There are different types of traditional beverages, alcoholic and non-alcoholic. Most beverages are made up of about 90% water, sugar, flavouring agents and sometimes preservatives (Onuorah et al., 1987). An alcoholic beverage is a drink containing ethanol (alcohol). Alcoholic beverages, such as wine, beer, and liquor have been part of human culture and development for more than 8,000 years (Wikipedia,

2016). A beverage such as kununzaki contains no alcohol and plays a very important role in the dietary pattern of people in developing countries like Nigeria. Kununzaki is a cereal based non-alcoholic drink. It is a locally produced beverage which of recent has become popular among the various non-alcoholic drinks in the northern part of Nigeria (Ayo, 1998; Gaffa, 2002). It is made from millet, sorghum and maize grains and flavoured with such spices as ginger, black pepper and tamarind for improvement in its taste and aroma, which also serve as purgative and cure for flatulent conditions

\* Corresponding author. E-mail: [olami\\_2k7@yahoo.com](mailto:olami_2k7@yahoo.com). Tel: +2348060221602.



**Figure 1.** Modified process flow chart for the production of enriched *Kununzaki* drink (Ayo et al., 2010).

(Omakwu, 1980). The variety of kununzaki drink made from sorghum is milky light brown colour, while that made from maize is whitish in colour (Adeyemi and Umar, 1994).

The component of kununzaki generally includes 85.0 to 87% moisture, 9.0 to 12.0% carbohydrate, 1.6 to 8.0% protein, 0.1% fat, and 0.6% ash (Ayo, 1998). It is a considerably cheap beverage drink because the ingredients used for the preparation are cheap and available anywhere in the market and stores. The basic ingredients of kununzaki are low in protein and some essential minerals and increasing prices of protein rich foods continue to force greater percentage of the populace, to eat food supplying less of the required dietary nutrient. This may have a negative effect on the nutritional status of the people who drink it, especially on the growth rate of infants who are given kununzaki as a weaning drink (Akintunde, 2005). Research work carried out on the improvement of the nutritional value of cereals, shows that the fortification of carbohydrate-rich foods with

protein-rich foods improves its nutritious values. Due to inadequate supplies of animal proteins, there has been a constant search for new protein sources, for use as both functional food ingredients and nutritional supplements (Obizoba and Atti, 1994). Cocoa is a plant rich in protein (19.60g) and in flavonoids (USDA Nutrient Database). The antioxidant capacity of fibre rich cocoa powder and its physico-chemical properties make it suitable to be used in the preparation of low-calorie, high-fibre food (Arts et al., 1999).

Cocoa powder has been demonstrated to exhibit high protein and good antioxidant properties. However, the use of cocoa powder in improving the quality of kununzaki is yet to be established, hence this study.

## MATERIALS AND METHODS

Sorghum (*Sorghum bicolor*), black pepper, red pepper (*Capsicum annum*), ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*), cocoa powder and granulated sugar were purchased from the Central Market, Ile-Ife.

### Preparation of enriched kununzaki drink

Figure 1 shows the flow chart of kununzaki production according to the method of Ayo et al. (2010). One kilogram of sorghum grains were cleaned and steeped in twice its volume of clean water (1:2 w/v) for 24 h in a covered plastic bucket at room temperature. The steeped sorghum grains were washed and wet milled with the spices (ginger, red pepper, black pepper, cloves) using a well cleaned disc attrition mill. The recipe is shown in Table 1. It was then wet sieved to remove the shafts after which the supernatant was decanted from the slurry. The slurry was divided into two unequal portions; two-third (75%) was added to boiling water, while stirring and cooled to a temperature of  $35 \pm 2^\circ\text{C}$  and subsequently added to the remaining one-quarter (25%) slurry. The mixture was thoroughly mixed and sweetened with 10% granulated sugar and was left for about 8 h to ferment. Twelve grams of cocoa powder was dissolved in 240 ml of water to produce cocoa slurry using the method of Crozier et al. (2011). Different percentages (100, 80, 70, 60, and 50) of the kununzaki were mixed with different percentages (0, 20, 30, 40, and 50) of cocoa slurry to obtain freshly processed enriched kununzaki drink. The most preferred sample (80/20%) was chosen after sensory evaluation for further analysis. The product (kununzaki) was packaged in plastic bottles, pasteurised at  $60^\circ\text{C}$  for 1 h. Analytical analyses were carried out on the drinks.

### Chemical analysis

#### Proximate composition

Proximate compositions of the freshly prepared enriched kununzaki samples were determined. The samples were analysed for crude protein, ash content, crude fat, and carbohydrate content based on the method of analysis of the Association of Official Analytical Chemists (AOAC, 2000).

#### Moisture content determination

The moisture content of the samples were determined by weighing about 5 ml of each sample into a porcelain dish of known weight

**Table 1.** Recipe for the production of enriched Kununzaki drink (Crozier et al., 2011; Obadina et al., 2008).

Ingredients	Kununzaki	Kununzaki+Cocoa
Sorghum (g)	500	500
Cloves (g)	0.5	0.5
Ginger (g)	6.5	6.5
Black pepper (g)	0.5	0.5
Red pepper (g)	2.5	2.5
Sugar (g)	50	50
Cocoa powder (g)	-	12

and heated in a Gallenkamp hot air oven at 105°C for 3 h. The samples were cooled in the desiccator and weighed. The samples were consequently heated, weighed and cooled until a constant weight was attained.

$$\text{Moisture content (\%)} = \frac{\text{loss in weight of sample}}{\text{original weight of sample}} \times 100$$

#### Ash content determination

Five grams of the samples (5 g) was weighed into already weighed ash crucible. The samples were then charred in an oven at 200°C. The crucibles were transferred into a muffle furnace chambers set at 700°C until the samples turn into white grey ash. The crucibles were thereafter removed, cooled in a desiccator and weighed. Ash content was expressed as the percentage of the original sample. The experiment was carried out in triplicate and the mean was calculated for each sample.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

#### Crude protein determination

The micro-Kjeldahl method was used in determining the protein content. The sample (2 g) was weighed into a kjeldahl digestion flask. Kjeldahl catalyst tablet was added to the flask with 10 ml of concentrated sulphuric acid and it was heated on preheated digester set at 420°C for about 30 min in a fume cupboard. It was then digested until a clear homogenous mixture was obtained. After digestion, the flask was removed from the heater, cooled and the content was diluted with about 50 ml of distilled water. The flask was placed in the micro-Kjedahl analyzer (Kjeltec-Auto 1030 Analyser, USA) distillation unit where 50 ml of 40% NaOH was dispensed automatically. The mixture was heated up to release ammonia which distilled into a conical flask containing 25 ml of 2% boric acid for about 4 min. During the distillation process, the ammonia combined with boric acid to form ammonium borate solution titrated against 0.1 M sulphuric acid until a purplish-grey end point was attained. The % nitrogen in the sample was calculated and the % protein was obtained by multiplying % Nitrogen by a factor of 6.25.

$$\text{Nitrogen (\%)} = \frac{0.28 \text{ gA}}{\text{Weight of sample in gram}}$$

Where A = volume (ml) of 0.1 M H<sub>2</sub>SO<sub>4</sub>.

#### Crude fat determination

Ether extraction method was used for the samples. Five grams of the sample was weighed into a fat extraction flask or tube of known weight. Sample was diluted with 7 ml water to make volume of 12 ml and shaken with slight warming until sample was completely dispersed. Two milliliters of NH<sub>4</sub>OH was added and mixed gently, 10 ml alcohol and 25 ml ether were added. The flasks were stoppered with cork unaffected by usual fat solvent and shake vigorously for 10 min. This was then cooled and 25 ml petroleum ether (30 to 50°C) was added and shaken vigorously again, then centrifuged at about 600 rpm. The ether solution was decanted into a suitable flask. After extraction, the solvent was evaporated completely on steam bath at a temperature that did not cause splattering. The fat was dried in an oven at 102°C, cooled in a desiccator and reweighed. Loss in weight = weight of fat. Weight of fat was corrected by blank determination on reagents used.

$$\% \text{ Crude fat} = \frac{\text{final weight of the flask} - \text{initial weight of flask}}{\text{Weight of sample}} \times 100$$

#### Carbohydrate determination

The carbohydrate content of each sample was calculated by difference. The total of all determined proximate compositions were deducted from 100.

$$\text{Carbohydrate (\%)} = 100 - (\text{Protein (\%)} + \text{Moisture (\%)} + \text{Ash (\%)} + \text{Fat (\%)})$$

#### Energy value determination

The energy value was obtained using the method of Osborne and Voogt (1978). This was calculated by multiplying the values of crude protein, fat and carbohydrate by factors of 4, 9 and 4, respectively. The sum of their product was expressed in kilojoules.

$$\text{Energy Value (kcal)} = (\text{Carbohydrate} \times 4) + (\text{Fat} \times 9) + (\text{Protein} \times 4)$$

#### Physicochemical properties of the Enriched kununzaki drinks

These analyses were carried out after production of the enriched kununzaki.

#### Determination of pH

The pH (hydrogen ion concentration) of the enriched kununzaki samples was determined using a standard pH meter (ATC, Model HI-8915). Sample was taken in a conical flask and electrode was directly inserted into the solution. After each reading, the electrode was wiped with distilled water and dried-up with tissue paper (AOAC, 2000).

#### Determination of total titratable acidity (TTA)

The production of lactic acid was determined by titrating 10 ml of the sample diluted with 10 ml distilled water against 0.1 N NaOH using 0.5 ml of phenolphthalein indicator (0.5% in 50% alcohol). The mixture was shaken thoroughly and titration was carried out until a faint pink colour was observed in each sample. The titratable acidity was calculated as percentage of lactic acid (v/v). Each

millilitre of 0.1 N NaOH is equivalent to 0.009 g of lactic acid (AOAC, 2000).

1 ml of 0.1 N NaOH = 0.009 g of lactic acid

$$\text{Lactic acid (mg/g)} = \frac{\text{mlNaOH} \times 0.09}{\text{ml of sample}}$$

#### Determination of total solids

Twenty five milliliters of the enriched kununzaki samples were evaporated to dryness on boiling water bath which was followed by drying until constant weight was obtained in an oven maintained at 130°C for 3 h (Makinde and Oyeleke, 2012).

$$\% \text{ Total solid} = \frac{\text{Dry weight}}{\text{Weight of sample}} \times 100\%$$

#### Determination of minerals

The analyses for essential mineral elements were investigated using Atomic Absorption Spectrophotometric method (Fashakin et al., 1991). The sample 0.5 ml was weighed into 75 ml digestion flask and 5 ml digestion mixture was added and left overnight in a hood. It was then digested for 2 h at 150°C, then left to cool for 10 min; thereafter, 3 ml of 6 M hydrochloric acid was added and digested for another 1½ h.

It was then cooled and 30 ml of distilled water was added. The tube was vigorously stirred. A sample aliquot was then transferred to the Autoanalyser (Technicon AAU model) for total mineral analysis at 420 nm. The digest was used to determine the elements (calcium, magnesium, iron and zinc) on the Atomic Absorption Spectrophotometer (Perkin Elmer, model 402) while potassium was determined by flame photometry.

#### Determination of antinutritional contents of the enriched kununzaki drink

The enriched kununzaki samples were examined for the following antinutritional components: Tanins, Oxalates and Saponin.

#### Determination of tannins

The modified vanillin-hydrochloric acid (MV-HCl) method of Price et al. (1978) was used.

#### Preparation of calibration curve

Various concentrations (0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of the catechin standard solution was pipette into clean dried test tubes in duplicate. To one set was added 5.0 ml of freshly prepared vanillin-HCl reagent prepared by mixing equal volume of 4% (w/v) vanillin/MeOH and 8% (v/v) HCl/MeOH and to the second set was added 5.0 ml of 4% (v/v) HCl/methanol to serve as blank. The solutions were left for 20 min before the absorbance was taken at 500 nm. The absorbance of the blank was subtracted from that of the standards. The difference was used to plot a standard curve of absorbance against concentration.

#### Procedure

Kununzaki was dried and flour was extracted separately with 10 ml of 1.0% (v/v) HCl-MeOH. The extraction time was 1 h with continuous shaking. The mixture was filtered and made up to 10 ml mark with extracting solvent. Filtrate (1 ml) was reacted with 5.0 ml vanillin-HCl reagent and another with 5.0 ml of 4% (v/v) HCl-MeOH solution to serve as blank. The mixture was left to stand for 20 min before the absorbance was taken at 500 nm.

#### Calculation

$$\text{Tannin (mg/g)} = \frac{x \text{ (mg/ml)} \times 10 \text{ ml}}{0.2 \text{ (g)}} = 50 \times \text{(mg/g)}$$

Where x is the value obtained from standard catechin graph.

#### Determination of oxalate

Oxalate was determined by the method of Oke (1966) with slight modification by Falade et al. (2005). About 4 g of the sample was weighed in quadruplicate into conical flasks and extracted with a 190 ml distilled water and 10 ml 6 M HCl. The suspension was placed in boiling water for 2 h and filtered and made up to 250 ml with water in a volumetric flask. To 50 ml aliquot was added 10 ml 6 M HCl and filtered and the precipitate washed with hot water. The filtrate and the wash water combined and titrated against conc. NH<sub>4</sub>OH until the salmon pink colour of the methyl red indicator changed to faint yellow. The solution was heated to 90°C and 10 ml 5% (w/v) CaCl<sub>2</sub> solution was added to precipitate the oxalate overnight. The precipitate was washed free of calcium with distilled water and then washed into 100 ml conical flask with 10 ml hot 25% (v/v) H<sub>2</sub>SO<sub>4</sub> and then with 15 ml distilled water. The final solution was heated to 90°C and titrated against a standard 0.05 M KmnO<sub>4</sub> until a faint purple solution persisted for 30 s. The oxalate was then calculated as the sodium oxalate equivalent.

1 ml of 0.05 M KMnO<sub>4</sub> = 2 mg sodium oxalate equivalent/g of sample

#### Determination of saponin

The spectrophotometric method of Brunner (1984) was used for saponin analysis. 1 g of finely ground sample was weighed into 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 2 h to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of magnesium carbonate was added and the mixture made up to 250 ml. The mixture obtained with saturated MgCO<sub>3</sub> was again filtered through a Whatman No. 1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipette into a 50 ml volumetric flask and 2 ml of 5% FeCl<sub>3</sub> solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0 to 10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solution was treated similarly with 5% of FeCl<sub>3</sub> solution as done for 1 ml of aforementioned sample. The absorbances of the sample as well as the standard solution were read after colour development in a Jenway V6300 Spectrophotometer at a wavelength of 380 nm.

$$\text{Saponin} = \frac{\text{absorbance of sample} \times \text{dil. factor} \times \text{gradient of standard graph}}{\text{sample weight} \times 10,000} \text{ (mg/g)}$$

## Determination of antioxidant properties of enriched Kununzaki

### Extraction of antioxidant

Extraction of antioxidant was carried out on dried kununzaki following the method of Yurttas et al. (2000) with minor modifications. About 5 g of each of the sample were separately mixed with 200 ml of 80% methanol (methanol:water, 80:20 v/v) in a conical flask and the extraction was done on a magnetic stirrer for 8 h at room temperature. The mixture was concentrated to dryness on a rotary evaporator. The crude concentrated extract was used for the analyses.

### DPPH assay

The radical scavenging ability of the extract was determined using the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) as described by Pownall et al. (2010). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm.

To 1 ml of different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml) of the sample or standard (vitamin C) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 min after which the absorbance was read at 517 nm against a DPPH control containing only 1 ml methanol in place of the extract.

The percentage of inhibition was calculated as shown in the equation:

$$\text{Inhibition (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

where  $A_{\text{control}}$  is the absorbance of the control reaction (containing all reagents except the test compound) and  $A_{\text{sample}}$  is the absorbance of the test compound. Sample concentration was calculated from the graph plotting inhibition percentage against extract concentration.

### Metal chelating ability assay

The metal-chelating assay was carried out according to the method of Singh and Rajini (2004) with some modifications. Solutions of 2 mM  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and 5 mM ferrozine were separately diluted 20 times. Briefly, an aliquot (1 ml) of different concentrations (6.25, 12.5, 25.0, 50.0 and 100.0 mg/ml) of the sample was mixed with 1 ml of diluted  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ . After 5 min incubation, the reaction was initiated by the addition of 1 ml of diluted ferrozine. The mixture was shaken vigorously and after a further 10 min incubation period, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- $\text{Fe}^{+2}$  complex formations was calculated as shown in the equation:

$$\text{Metal chelating activity (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where  $A_{\text{control}}$  = absorbance of control sample (the control contains mixture of  $\text{FeCl}_2$  and ferrozine) and  $A_{\text{sample}}$  = absorbance of a tested samples.

### Ferric reducing antioxidant power (FRAP)

The FRAP assay uses antioxidants as reductants in a redox-linked

colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method is based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous coloured form due to the donation of electron by antioxidant compounds.

**Procedure:** A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mmol/L  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  were mixed together in the ratio of 10:1:1, respectively, to give the working FRAP reagent. A 50  $\mu\text{l}$  aliquot of the sample at 1 mg/ml and 50  $\mu\text{l}$  of standard solutions of ascorbic acid (20, 40, 60, 80, and 100  $\mu\text{g}/\text{ml}$ ) were separately added to 1 ml of FRAP reagent. The mixture was well mixed and absorbance measurement at 593 nm against reagent blank (50  $\mu\text{l}$  of distilled water and 1 ml of FRAP reagent) after allowing reaction to complete at exactly 10 min. The reducing power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard. This was done by plotting the graph of the absorbance of ascorbic acid (standard) against concentration. The equation of the graph obtained was used to calculate the equivalent concentration based on the absorbance obtained for the extracts, and this is expressed as ascorbic acid equivalent per gram of the extract (AAE  $\mu\text{g}/\text{g}$  of the extract).

### Sensory evaluation

The 9-point hedonic scale assessment as described by Larmond (1977) was used. Panelists from the Department of Food Science and Technology were selected based on their familiarity with kununzaki beverage. The panelists scored the coded drinks in terms of degree of liking to taste, colour, texture and aroma. The 9-point hedonic scale used by the panelists for the evaluation ranged from 1 to 9 representing "extremely dislike" to "extremely like". The coded samples were served in clean, transparent cups at room temperature 25°C. Water was given to each panelist for oral rinsing in between tasting of the samples. The results obtained were analysed using statistical methods of analysis.

### Statistical analysis

The values obtained from each of the analyses were means of duplicate readings. The data obtained from physicochemical and sensory analysis were subjected to analysis of variance (ANOVA) and the mean were separated by Duncan multiple range test (SPSS, version 16). Significance was determined at 5% level.

## RESULTS AND DISCUSSION

### Proximate

The proximate composition of the enriched kununzaki drink is presented in Table 2. The moisture content of the enriched kununzaki samples ranged from 84.95 to 91.20% with sample KHC (100% Commercial kununzaki) having the highest value (91.20%) and sample KHN (100% kununzaki laboratory, kununzaki prepared from 100% sorghum) having the lowest value (84.95%). There was an increase in the moisture content of the kununzaki drink because of the added cocoa powder. This might be



**Table 2.** Proximate composition of enriched *Kununzaki* drink.

Composition	KHN	KEN	KHC
Moisture (%)	84.95±0.9 <sup>b</sup>	90.30±2.6 <sup>a</sup>	91.20±2.2 <sup>a</sup>
Protein (%)	2.16±0.04 <sup>a</sup>	2.64±0.02 <sup>a</sup>	1.40±0.0 <sup>c</sup>
Fat (%)	0.38±0.02 <sup>b</sup>	0.93±0.03 <sup>a</sup>	0.91±0.02 <sup>a</sup>
Ash (%)	0.25±0.06 <sup>b</sup>	0.38±0.04 <sup>a</sup>	0.16±0.04 <sup>c</sup>
Carbohydrate (%)	12.26±0.06 <sup>a</sup>	5.75±0.02 <sup>b</sup>	6.33±0.04 <sup>b</sup>
Energy (kJ)	61.10±2.00 <sup>a</sup>	41.93±1.99 <sup>b</sup>	39.11±3.10 <sup>c</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki/Cocoa*; KHC: 100% Commercial *Kununzaki*.

because the cocoa powder was diluted into solution before added to the kununzaki. The moisture content of any food is an index of its water activity. High moisture content makes beverage suitable as a refreshing and quench-thirsting product which is characteristic of a good beverage.

The protein content of the enriched kununzaki samples ranged between 1.40 and 2.64%. Sample with cocoa powder had the highest value (2.64%), while the commercial kununzaki had the least value (1.40%). There was an increase of 16.7% in protein with the addition of cocoa powder. This indicates that cocoa powder contributed to the increase in protein content of the drink. Kununzaki drink as it is known is not a good source of protein (Oluwalana and Adedeji, 2012).

The low protein value of each sample may be attributed to the low protein content in sorghum (10.4 g/100 g) as a result of its loss during processing in the removal of bran and germ (Hamad and Field, 1979; Ihekoronye and Ngoddy, 1985). The nutrient composition of sorghum is influenced by both environmental and genetic factors (Asiedu, 1989). Cocoa powder was noted to be a good source of protein (8.14 to 19.71%) (Elena et al., 2007; Ndife et al., 2013). The high protein content in cocoa powder gives it the potential of being used as a source of protein supplement in cereal based foods. This implies that addition of cocoa powder can be used to enhance the protein content of kununzaki, thus increasing or boosting its nutritional content.

The fat content of the enriched kununzaki increased significantly from 0.38 to 0.93% with addition of cocoa powder as stated in Table 2. Lower fat content (0.38%) was observed in 100% sorghum based kununzaki. Sample with cocoa powder and commercial kununzaki had similar values of fat content. The increase in fat was as a result of the cocoa powder added. Cocoa powder has fat content of 11.28% (Ndife et al., 2013). The increase in the fat content agrees with the reports of Olosunde et al. (2014) that inclusion of *moringa* seed powder increased the fat content of kununzaki.

Ash content of all the kununzaki samples ranged from 0.16 to 0.38%. There was increase in the ash content

with addition of cocoa powder but low ash content was observed in the commercial kununzaki (0.16%). This might be because of the cereal (millet) used in the preparation of sample KHC. Adedokun et al. (2012) reported that the ash content of kununzaki produced from millet (0.98%) is lower than that produced from sorghum (1.12%). The ash content of the kununzaki prepared from the samples confirms its high mineral content. Ash content is an index of inorganic mineral elements in the food (Onyeka, 2008).

This is in agreement with the finding that high amount of ash contained in plants, is an indication that the plant provides appreciable quantity of minerals essentially required by the body (Rao-Pu, 1996).

Carbohydrate content of the kununzaki samples ranged from 5.75 to 12.26%. Sample KHN had the highest carbohydrate content (12.31%). The addition of cocoa powder to the samples increased the protein content, while the carbohydrate contents were reduced. The result in this work agrees with the findings of Ayo et al. (2010) that the carbohydrate content of kununzaki enriched with beniseed is within the range of 7.23 to 10.21%. The result of this work also agrees with the findings of Sopade and Kassum (1992), that kununzaki contain 12.2% of carbohydrate.

It indicated that, the nitrified material (cocoa powder) which contains relatively lower carbohydrate could have affected the carbohydrate content in the kununzaki and increased the protein of the enriched kununzaki.

The energy values of all the kununzaki samples ranged from 39.11 to 61.10 kJ for freshly prepared samples, 38.98 to 57.96 kJ and 39.11 to 57.75 kJ. Sample KHN had the highest energy value (61.10 kJ), while sample KHC had the least value (39.11 kJ). The energy value decreased with addition of cocoa powder. The energy values were calculated from protein, fat and carbohydrate values.

The energy values follow the trend of carbohydrate for all samples. The result of this work agrees with the findings of Ayo et al. (2010) that the energy content of kununzaki enriched with beniseed is within the range of 7.23 to 10.21%.

**Table 3.** Physicochemical properties of enriched *Kununzaki*.

Properties	KHN	KEN	KHC
pH	4.2±0.04 <sup>a</sup>	4.5±0.04 <sup>a</sup>	3.7±0.02 <sup>b</sup>
TTA (%)	0.45±0.04 <sup>b</sup>	0.48±0.01 <sup>b</sup>	0.71±0.01 <sup>a</sup>
Total solids (%)	30±0.05 <sup>a</sup>	19.84±0.04 <sup>b</sup>	14±0.01 <sup>c</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki*/Cocoa; KHC: 100% Commercial *Kununzaki*.

**Table 4.** Mineral compositions (mg/100 mg) of enriched *Kununzaki* drink.

Mg/100 g	KHN	KEN	KHC
Calcium	4.1±0.02 <sup>c</sup>	5.8±0.00 <sup>a</sup>	5.2±2.2 <sup>b</sup>
Zinc	6.0±0.04 <sup>b</sup>	7.4±0.02 <sup>a</sup>	3.4±0.0 <sup>c</sup>
Magnesium	162±0.02 <sup>b</sup>	170±0.03 <sup>a</sup>	85±0.04 <sup>c</sup>
Iron	5.5±0.06 <sup>b</sup>	6.0±0.04 <sup>a</sup>	4.6±0.04 <sup>c</sup>
Potassium	129±0.03 <sup>c</sup>	155±0.02 <sup>b</sup>	199±0.04 <sup>a</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki*/Cocoa; KHC: 100% Commercial *Kununzaki*.

### Physicochemical properties of the enriched kununzaki drinks

The result of the variation in pH, titratable acidity and total solids values of the enriched kununzaki drinks were presented in Table 3. The pH values obtained for all the samples ranged between 3.7 and 4.5. *Kununzaki* with cocoa powder had the highest value (4.5) and the commercial kununzaki had the least value (3.7). There was an increase in the pH value with the addition of cocoa powder. Cocoa powder' addition affected the pH content of the kununzaki. This agreed with the submission of Ndife et al. (2013) that addition of cocoa powder decreased the pH of the kununzaki. The addition of cocoa powder reduced the acidity of the products and therefore could positively affect the storability of the kununzaki. The result of this work was comparable to pH 3.9 for kununzaki reported by Oshoma et al. (2009). The values obtained in this work were higher than (3.70 to 3.90) as reported by Makinde and Oyeleke (2012), when sesame seed was incorporated into kununzaki. However, result obtained was within the range of values (4.0 to 4.14) reported by Ayo et al. (2004) for kununzaki. The titratable acidity (TTA) found in the enriched kununzaki drinks are between 0.45 to 0.71%. There was a slight difference in the TTA of 100% kununzaki (0.45%) and kununzaki with cocoa powder (0.48%). Commercial kununzaki had a higher value (0.71%). This might be because of the cereal used and the method of preparation. The acidity of the samples can be attributed to the added species. It can also be traced to the presence of some bacteria like lactobacillus, acidophillus,

candida species and *Saccharomyces cerevisiae* which help in acid fermentation of kununzaki.

The total solids content of the enriched kununzaki samples ranged between 14 and 30%. Sample KHC (Commercial kununzaki) had the least total solid contents (14%), while sample KHN had the highest value (30%). This result showed that the total solid content of the sample decrease with the addition of cocoa powder. This might be because the cocoa powder was first diluted to solution before use. The result obtained in this study can be compared with the result 13.42% of kununzaki prepared from wet milled sorghum by Adejuyitan et al. (2008). Abel et al. (2011) reported 9.20 to 12.5% total solid content for kununzaki treated with chemicals. However, higher total solids content had effect on consumer acceptability as it imparts texture to the beverage (Adejuyitan et al., 2008).

### Minerals

The mineral composition of the enriched kununzaki samples when freshly prepared is shown in Table 4. The calcium content ranged from 4.1 to 5.8 mg/100 g. The calcium values obtained in this study are similar to the value 5.18 mg/100 g reported by Oluwalana and Adedeji (2012) for kununzaki. An increase of 41.46% was observed in the calcium content with the addition of cocoa powder. This is as a result of the high calcium content in cocoa powder as sorghum seed is low in calcium (Rooney and Serna-Saldivar, 1990). Calcium assists in teeth development (Brody, 1994). Calcium also

**Table 5.** Antinutrient content of enriched *Kununzaki* drink.

Samples (mg/100 g)	KHN	KEN	KHC
Oxalate	0.44±0.01 <sup>c</sup>	0.66±0.00 <sup>b</sup>	1.1±0.01 <sup>a</sup>
Tanin	3.66±0.01 <sup>c</sup>	3.87±0.01 <sup>b</sup>	3.95±0.01 <sup>a</sup>
Saponin	5.54×10 <sup>-5</sup> ±0.01 <sup>a</sup>	3.29×10 <sup>-5</sup> ±0.06 <sup>c</sup>	5.44×10 <sup>-5</sup> ±0.11 <sup>b</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki*/Cocoa; KHC: 100% Commercial *Kununzaki*.

helps in regulation of muscle contractions transmit nerve impulses and help in bone formation (Cataldo et al., 1999). Sample KHC made from millet is rich in calcium, as the value (5.2 mg/100 g) is higher than sample KHN (4.1 mg/100 g) made from sorghum.

Zinc content of the samples ranged from 3.4 to 7.4 mg/100 g in the prepared samples. Addition of cocoa powder increased the zinc contents by 23.33% in the samples. There was a significant difference ( $p>0.05$ ) in the zinc content of the samples. Sample KEN had the highest (7.4 mg/100 g) value and sample KHC, the least (3.4 mg/100 g) value of zinc content. This may be due to difference in the cereal (millet) used. Values obtained in this study (3.4 to 7.4 mg/100 g) were higher than the value 3.30 mg/100 g reported by Oluwalana and Adedeji (2012) for zinc in *kununzaki*. Zinc is a trace element which is needed in minute amount that enhances body functions.

Magnesium content ranged from 85 to 170 mg/100 g for freshly prepared samples. Sample KEN had the highest value (170 mg/100 g), while KHC had the least value (85 mg/100 g). Addition of cocoa powder increased the magnesium content by 5.0%. This result obtained in this study is higher than the findings (145.04 mg/100 g) of Oluwalana and Adedeji (2012). Magnesium helps in keeping the muscle relaxed and the formation of strong bones and teeth. It plays fundamental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acid and intestinal absorption while deficiency of magnesium in man is responsible for severe diarrhea, hypertension and stroke (Romani and Andrea, 2013).

Iron content of the enriched *kununzaki* drinks ranged from 4.6 to 6.0 mg/100 g for freshly prepared. There was an increase of 9.1% in the iron content of the sample with the addition of cocoa powder. Sample KEN (6.0 mg/100 g) had a higher value and sample KHC (commercial *kununzaki*) had the lowest iron value (4.6 mg/100 g), the low value of the iron is likely because sorghum and millet seeds have been reported to have small amount of iron (Shobha et al., 2008). The iron content of this work compares with results reported by Kayode (2006), 3 to 11 mg/100 g and Makinde and Oyeleke (2012), 2.7 to 4.2 mg/100 g to be the iron concentration of sorghum grains. Iron is an important element in the diet of pregnant women, nursing mothers, infants convulsing patients and

elderly to prevent anaemia and other related diseases (Oluyemi et al., 2006).

Potassium content of the enriched *kununzaki* drinks ranged from 129 to 199 mg/100 g for freshly prepared samples. There was an increase of 20.2% in potassium content with the addition of cocoa powder. Sample KHC had the highest (199 mg/100 g) potassium value and sample KHN the least value (129 mg/100 g). This may be because of the cereal (millet) used in the production of sample KHC. The result in this work agrees with the findings (134.27 mg/100 g) of Oluwalana and Adedeji (2012) for *kununzaki*. Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins (Malik and Scrivastava, 1982). It is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Fallon, 2001).

Generally, the addition of cocoa powder increased the mineral levels of *kununzaki* drinks.

#### Antinutrient contents in the enriched *kununzaki* drink

The result of the antinutrient contents (oxalate, tannin and saponin) of the enriched *kununzaki* samples is shown in Table 5. The oxalate content of the samples ranged from 0.44 to 1.1 mg/100 g. The oxalate content of the sample increased by 50% with the addition of cocoa powder. This might be because cocoa powder has high oxalate content of 650 to 783 mg/100 g (USDA, 2011). Sample KHC had the highest oxalate content (1.1 mg/100 g) and sample KHN had the least value (0.44 mg/100 g) oxalate content. Oxalate forms complexes with calcium thereby making it unavailable when consumed and more so high oxalate diets can increase the risk of renal calcium absorption (Osagie and Eka, 1998).

The total tannin contents of the samples ranged from 3.66 to 3.95 mg/100 g. The addition of cocoa powder increased the tannin content of the drink slightly. Tannins have been reported to affect nutritive value of food products by binding the metals such as iron and zinc and reduced the absorption of the nutrient and also form complex with protein thereby inhibiting their digestion and absorption (Obboh et al., 2003).

The total saponin contents of the samples ranged from  $3.29 \times 10^{-5}$  to  $5.54 \times 10^{-5}$  mg/100 g. Addition of

**Table 6.** DPPH inhibition (%) of Enriched *Kununzaki* drinks.

Samples/Concentrations (ml)	0.5	1.0	1.5	2.0	2.5
KHN	10.00±0.01 <sup>c</sup>	23.01±0.15 <sup>c</sup>	42.00±0.02 <sup>c</sup>	62.01±0.03 <sup>c</sup>	69.41±0.03 <sup>c</sup>
KEN	14.00±0.24 <sup>b</sup>	28.00±0.02 <sup>b</sup>	50.01±0.16 <sup>b</sup>	67.00±0.09 <sup>b</sup>	74.41±0.19 <sup>b</sup>
KHC	10.00±0.04 <sup>c</sup>	22.02±0.07 <sup>c</sup>	46.00±0.10 <sup>c</sup>	60.12±0.07 <sup>c</sup>	69.01±0.07 <sup>c</sup>
Ascorbic acid	20.29±0.01 <sup>a</sup>	45.83±0.01 <sup>a</sup>	62.09±0.01 <sup>a</sup>	82.62±0.01 <sup>a</sup>	95.34±0.01 <sup>a</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki*/Cocoa; KHC: 100% Commercial *Kununzaki*.

**Table 7.** Metal chelating ability (%) of enriched *Kununzaki* drinks.

Samples/concentrations (ml)	6.25	12.5	25	50	100
KHN	8.8±0.01 <sup>bc</sup>	15.50±0.11 <sup>bc</sup>	24.00±0.02 <sup>bc</sup>	50.01±0.03 <sup>bc</sup>	76.01±0.03 <sup>c</sup>
KEN	8.00±0.24 <sup>c</sup>	14.00±0.02 <sup>c</sup>	22.01±0.16 <sup>c</sup>	48.01±0.09 <sup>c</sup>	73.01±0.19 <sup>c</sup>
KHC	9.2±0.04 <sup>b</sup>	16.50±0.07 <sup>b</sup>	25.03±0.10 <sup>b</sup>	54.02±0.07 <sup>b</sup>	82.01±0.07 <sup>b</sup>
EDTA	16.00±0.01 <sup>a</sup>	20.72±0.02 <sup>a</sup>	30.34±0.01 <sup>a</sup>	50.67±0.01 <sup>a</sup>	96.30±0.01 <sup>a</sup>

Mean ± standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% *Kununzaki*; KEN: 80/20% *Kununzaki*/Cocoa; KHC: 100% Commercial *Kununzaki*; EDTA: Ethylene diamine tetra-acetate.

cocoa powder decreased the saponin content of the enriched kununzaki drinks. There was decrease in the saponin level with storage time. The results revealed that saponin contents of the freshly prepared samples were higher than the values in either ambient or refrigerated stored samples. Also, samples without cocoa powder (KHN and KHC) had higher saponin content than samples containing cocoa powder. The trend observed in this study agreed with the findings of Makinde and Oyeleke (2012) on the effect of sesame seeds on the anti-nutritional properties of kununzaki enriched with sesame seed flour. Antinutrients have potential in helping to reduce the risk of several deadly diseases in man if they are below the recommended or permitted level in the body (Fagbemi et al., 2005). Saponins have been found to cause haemolytic activity by reacting with sterols of erythrocyte membrane. All the antinutrients in the enriched kununzaki drink were low and within the permissible level for food.

### Antioxidant properties of the enriched kununzaki drink

#### DPPH radical scavenging activities

Table 6 shows the result of the DPPH free radical scavenging capabilities of the enriched kununzaki samples at different concentrations. The values ranged from 10 to 69.41 (KHN), 14 to 74.41 (KEN), 10 to 69.01% (KHC). The DPPH free radical scavenging activities of all the extracts were concentration dependent as shown in

Table 6. The free radical scavenging activities as measured by DPPH assay increased with increasing sample concentrations for all the samples from 0.5 to 2.5 mg/ml. There was an increase in the DPPH activity value with the addition of cocoa powder; this may be due to the antioxidant capacity of cocoa powder. Counnet et al. (2006) showed that cocoa exhibits a good antioxidant capacity and that cocoa powder is a potentially rich dietary source of flavonoids. Sample KEN exhibited the highest radical scavenging activity value (74.41% at 2.5 mg/ml) and sample KHC had the least radical scavenging activity value (69.01% at 2.5 mg/ml). DPPH radical scavenging activity of these extracts showed antioxidant potency when compared with ascorbic acid as shown in the Table 6.

#### Metal chelating activity

Table 7 shows the result of the metal chelating ability of the enriched kununzaki samples. At the different concentrations of the samples (6.25, 12.5, 25, 50, 100 mg / ml), the metal-chelating values for ranged from 8.8 to 76.01 (KHN), 8.0 to 73.01(KEN), and 9.2 to 82.01% (KHC).

The result showed the ability of the kununzaki drink enriched with cocoa powder to chelate and deactivate transition metals. The ferrous ion-chelating ability of all the samples increased as the concentrations of the samples increased from 6.25 to 100 µg/ml. The metal chelating ability is such that the sample without cocoa powder had higher values than sample with cocoa

**Table 8.** Ferric reducing antioxidant power (FRAP) (AAE  $\mu\text{g/g}$ ).

Sample	FRAP
KHN	60.2 $\pm$ 0.01 <sup>a</sup>
KEN	60.9 $\pm$ 0.11 <sup>a</sup>
KHC	58.2 $\pm$ 0.00 <sup>b</sup>

Mean  $\pm$  standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% Kununzaki; KEN: 80/20% Kununzaki/Cocoa; KHC: 100% Commercial Kununzaki; EDTA: Ethylene diamine tetra-acetate.

**Table 9.** Sensory score of preserved enriched *Kununzaki*.

Samples	KHN	KEN	KHC
Colour	1.4 $\pm$ 0.32 <sup>c</sup>	2.4 $\pm$ 0.04 <sup>b</sup>	5.0 $\pm$ 0.01 <sup>a</sup>
Taste	1.8 $\pm$ 0.01 <sup>b</sup>	3.3 $\pm$ 0.01 <sup>a</sup>	1.6 $\pm$ 0.01 <sup>b</sup>
Flavour	1.4 $\pm$ 0.04 <sup>b</sup>	3.1 $\pm$ 0.03 <sup>a</sup>	2.8 $\pm$ 0.01 <sup>a</sup>
Texture	1.8 $\pm$ 0.04 <sup>b</sup>	3.0 $\pm$ 0.10 <sup>a</sup>	2.0 $\pm$ 0.02 <sup>b</sup>
Acceptability	1.8 $\pm$ 0.02 <sup>b</sup>	3.4 $\pm$ 0.05 <sup>a</sup>	1.8 $\pm$ 0.01 <sup>b</sup>

Mean  $\pm$  standard deviation of triplicate determinations. Mean with the same superscripts in the same rows are not significantly different at 5% probability level. KHN: 100% Kununzaki; KEN: 80/20% Kununzaki/Cocoa; KHC: 100% Commercial Kununzaki; EDTA: Ethylene diamine tetra-acetate.

powder at all concentrations between 6.25 and 100  $\mu\text{g/ml}$ . At a concentration of 100  $\mu\text{g/ml}$ , sample KHC exhibited the highest ferrous ion-chelating ability value (82.01%) and sample KEN had the least value (73.01%). The chelating ability of the extract measures how effective the compounds in the sample can compete with ferrozine for ferrous ion.

### Ferric reducing activity power (FRAP)

The result of the ferric reducing ability power of all the samples is shown in Table 8. The values ranged from 58.2 to 60.9 AAE  $\mu\text{g/g}$ . Samples KHN and KEN are not significantly different from each other. They are higher than commercial sample. The ferric reducing ability of all the drinks increased by (1.2%) with the addition of cocoa powder. The result of this work is comparable with the results (72.32 AAE  $\mu\text{g/g}$ ) of Elena et al. (2007) on the ferric reducing abilities of fibre-rich product from cocoa. The sample containing cocoa powder had higher reducing abilities than the samples without cocoa powder.

### Sensory properties of enriched kununzaki drink

Table 9 shows the sensory scores of sample A (100% kununzaki), sample B (80% kununzaki/20% cocoa powder) and sample C (commercial kununzaki) as judged by the panelists.

There were significant differences ( $p > 0.05$ ) among the samples in colour, taste, flavour, texture and overall

acceptability with the addition of cocoa powder. Sample KHC (5.0) was mostly preferred in term of colour. This might be because the panelists were used to the whitish colour of kununzaki. The scores for the taste of the samples ranged from 1.6 to 3.3. The addition of cocoa powder to the drinks increased the likeness for the taste of the samples. Samples KHN and KHC are similar in taste because they do not contain cocoa powder.

The scores for the flavour of the samples ranged from 1.4 to 3.1. The addition of cocoa powder to the Kununzaki drink improved the flavour of the drinks. The scores were within the acceptable range. The scores for the texture of the samples ranged from 1.8 to 3.0. The addition of cocoa powder to the drink affected the texture of the samples with cocoa powder. This might be because the cocoa powder was first diluted to solution before use and this might have affected the texture. There might be poor solubility of the cocoa powder in dilution. Samples KHN and KHC have similar scores. The scores for the overall acceptability of the samples ranged from 1.8 to 3.4. Sample KEN was mostly preferred. Samples KHN and KHC (sample without cocoa powder) had similar scores.

### Conclusion

The study showed that enrichment of kununzaki with cocoa powder had significant effect on the nutritional and sensory properties of kununzaki drink. The addition of 20% cocoa powder resulted in kununzaki with high

nutritional value and it was highly acceptable by panelists over the ones with high percentages of cocoa powder. .

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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