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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Microbiological quality of ready-to-eat foods of Tehran province

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Recontamination of ready-to-eat (RTE) products during post-processing may be the cause of outbreaks of food-borne disease. In this study, a total of 150 RTE samples were obtained for bacteriological examination (*coliforms*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Bacillus cereus*, *Psychrotrophic bacteria* and *Psychrophilic* bacteria). Various types of RTE food products that contained frozen (cooked and semi-cooked) and refrigerated (cooked) poultry meat foods, were purchased randomly periodically in January and March, 2012. 65% of cooked samples and 62% of semi cooked samples contain more than 10^2 CFU/g coliform, while *S. aureus* was more than 10^2 CFU/g in 35 and 40% of samples, respectively. Also 28% of cooked samples and 44% of semi cooked samples contained *E. coli*. 14% of all samples were contaminated by *Salmonella*. The results for enumeration of *B. cereus*, psychrophilic and psychrotrophic microorganisms were: 2/96 ± 0/09 log CFU/g, 5/02 ± 1/77 log CFU/g and 3/05±0/04 log CFU/g, respectively.

Key words: Foodborne pathogens, coliforms, *Escherichia coli*, *Staphylococcus aureus, Salmonella, Bacillus cereus*, psychrotrophic bacteria, psychrophilic bacteria, cooked, semi-cooked.

INTRODUCTION

According to EC Regulation No. 2073/2005, “microbiological criterion is a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch” and “‘food safety criterion means a criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market”. Ready-to-eat (RTE) foods have become increasingly popular in the last two decades, particularly in metropolitan areas (Peck et al., 2008). In Tehran, Capital of Iran, there has been a marked increase in the sales of RTE food products in recent years. Familiarity taste, low-cost and convenience are some of the appealing factors that make RTE foods popular as food source. The RTE food products provide a source of readily available and nutritious meals for the consumer. However, questions

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have been raised about the safety and microbiological quality of these food products. The incidence of foodborne illness is increasing worldwide (Kaneko et al., 1996; Mead et al., 2009; Nguz, 2007). High counts of *Escherichia coli* and total coliform (TC) in foods usually indicates lack of hygiene in handling and production operations, inadequate storage and post-process contamination (De Sousa et al., 2002). Therefore, *E. coli* and TC enumeration are used as a food-quality parameter. *Bacillus cereus* is frequently isolated from both the natural environment (soil and growing plants) and foods, meat products, raw meat and meat product additives.

Psychrotrophic and psychrophilic bacteria are the main contributors to the spoilage of sea foods at refrigeration temperatures, in addition they are important in ready to eat food with chicken meat origin. *Salmonella* can frequently be isolated from raw foods of animal origin. Environmental contamination can also result in *Salmonella* being present in a wide variety of foods, although generally at lower numbers. Foods that are frequently implicated in Staphylococcal food poisoning include meat and meat products, poultry and egg products. Enterotoxin production of *Staphylococcus aureus* is also a public health concern owed to its ability to grow in environments of high salt concentration such as salami. Such foods can be important vehicles for infection by *Salmonella*, *Listeria monocytogenes* and *E. coli* O157 (Emberland et al., 2006; Swaminathan and Gerner-Smidt, 2007). The aim of this study was to identify and enumerate *Salmonella*, *Bacillus cereus*, psychrophilic and psychrotrophic microorganisms on frozen (cooked and semi-cooked) food for 104 samples and for 46 samples of refrigerated (cooked) poultry meat ready-to-eat food.

MATERIALS AND METHODS

Selection sampling

A total of 150 RTE samples were obtained for bacteriological examination. Various types of RTE food products were obtained from 23 brands that contained frozen (cooked and semi-cooked) and refrigerated (cooked) poultry meat foods. They were purchased randomly, periodically during January and March 2012. All samples were randomly purchased before their best before date, transported to the laboratory in their original package and kept 2 days at -18°C until their analysis.

Microbiological analysis

Twenty-five grams of each sample was added to a culture medium/diluent (1:10; homogenized for 2 min in a Stomacher), in agreement with specific standard methods for *Coliforms* (AFNOR/NF BIO 12/20-12/06), *E. coli* (ISO, 16649-2:2001) and the pathogenic bacteria *Salmonella* (ISO 6579:2002; AFNOR BIO 12/01-04/94 protocol) and *L. monocytogenes* (ISO, 11290-1:2004; AFNOR BIO-12/11-03/04 protocol) and meat foods standard of Iran.

Determination of coliforms

For investigation of coliforms, violet red bile agar (VRBA medium, Merck, Germany) were used after incubation at 30 ± 1°C for 24 h, as recommended by the manufacturer. Those positive tubes, which have formed a gas at the end of incubation period, were planted into the brilliant green bile (2%) broth (BGB), which has again contained a Durham tube and then they underwent the incubation process at 35°C for 48 h. Those tubes, that have formed a gas as a result of incubation process, were evaluated according to the MPN table and their total coliform counts were determined in this way. To defined *E.coli* by MPN method, gas positive BGB tubes were transferred to loop of each suspension and tubes were streaked to eosin methylene blue agar (EMB) and incubated at 37°C for 24 h. Ideally, *E. coli* should not be detected and as such a level of <3 per gram (the limit of the most probable number test) has been given as the satisfactory criteria for this organism.

Identification and numeration of *S. aureus*

Enrichment of 1 g sample in 10 mL cooked meat medium (Difco), streaking a loopful of the 24-h enrichment culture on Baird-Parker agar (BPA, Merck) containing egg yolk and potassium tellurite (Merck), and finally, incubation at 37°C for 48 h was done

Identification of *Salmonella*

For identification of Salmonella spp., 25 g of each food sample was pre-enriched in lactose broth (Merck) at 37°C for 18 h. Then, 1 mL was transferred into 10 mL selenite cysteine broth (Merck) for enrichment, incubated at 37°C for 24 h. Finally, Salmonella Shigella (SS) agar (Merck), bismuth sulfite agar (Merck) was used as selective media, triple sugar iron agar (Merck), lysine iron agar (Merck) as differential media and urease (Merck) as complement media.

Identification and numeration of *B. cereus*

Surface plate method on *B. cereus* selective agar (Merck) were used for identification of typical B. *cereus* colonies and incubated at 37°C for 24 h.

Determination of psychrotrophic microorganisms

69 g of Nutrient Agar powder was suspended in 3 L of distilled water. It was allowed to soak and brought to boil. They were distributed into suitable containers and sterilized in the autoclave at 121°C for 15 min.

Determination of psychrophilic microorganisms

114 g of king agar powder was suspended in 3 L of distilled water. It was allowed to soak and brought to boil. They were distributed into suitable containers and sterilized in the autoclave at 121°C for 15 min.

Statistical analysis

Probability value $p < 0.05$ was defined statistically significant. Data analysis was performed using SPSS 18 (IBM, PASW Statistics 18.0, USA).

Table 1. Mean±standard deviation in *B. cereus*, psychrotrophic, psychrophilic, coliform and *S. aureus* bacteria of cooked semi, cooked, refrigerated, frozen foods and total.

Condition of storage	Cooked	Semi cooked	Refrigerated	Frozen (cooked and semi cooked)	Total
N	65	39	46	104	150
B. cereus	2/98 ± 0/07	3/84 ± 0/05	4/44 ± 1/41	3/21 ± 0/06	2/96 ± 0/09
Psychrotrophic	3/63 ± 0/05	3/37 ± 0/09	3/96 ± 0/07	3/56 ± 0/08	3/05 ±0 /04
Psychrophilic	5/2 ± 0/08	5/73 ± 1/3	4/34 ± 0/04	5/34 ± 0/09	5/02 ± 1/77
Coliform	2/9 ± 0/09	3/15 ± 0/06	5/42 ± 1/53	4/47 ± 1/02	4/02 ± 0/07
E .coli	2/4±0/4	3/2 ±0/9	2/1 ±0/4	1/76±0/7	3/46±0/8
S. aureus	2/17 ± 0/08	2/04 ± 0/07	3/09 ± 0/09	3/42 ± 0/07	3/41 ± 0/09

N: Number of samples.

Table 2. Percentage of *B. cereus*, psychrotrophic, psychrophilic, *Salmonella* and *E. coli* bacteria in cooked, semi cooked, refrigerated and frozen foods.

Condition of storage	Frozen (cooked and semi cooked)		Refrigerated		Cooked		Semi cooked	
B. cereus	u	76/5	u	56/2	s	27/6	u	24/2
Psychrotrophic	u	76/5	u	43/8	u	22/3	u	54/7
Psychrophilic	u	97/3	u	87/5	u	41/5	u	35/8
Salmonella	u	14/7	u	12/5	u	4/3	u	7/4
E. coli	u	47/2	u	49	u	28	u	44
Coliform	u	71/2	u	78	u	65	u	65
S. aureus	s	26	u	33	u	35	u	40

s = Satisfactory, u = unsatisfactory.

RESULTS AND DISCUSSION

The results for enumeration of *B. cereus*, psychrophilic and psychrotrophic microorganisms were: 2/96 ± 0/09, 5/02±1/77 and 3/05±0/04 Log CFU/g, respectively. The fourteen percent of all samples were contaminated by *Salmonella*. Percentage of *Salmonella* contamination in cooked frozen samples were higher than semi-cooked ones, because of cross-contamination and inappropriate usage of time-temperature chain. The contamination percentage of *B. cereus* was higher in semi-cooked samples than cooked samples. Minimum and maximum and mean ± SD (standard deviation) of coliform and *Staphylococcus aureus* in frozen cooked samples are 5 (2/9±0/09) and 4 (2/17 ± 0/08), respectively. The number and mean ± SD of coliform and *S. aureus* in semi cooked samples are 12 (3/15 ± 0/06) and 14 (mean±SD, 2/4±0/7) respectively. 65% of cooked samples and 62% (62% of 39 samples contain more than 10^2 CFU/g coliform) of semi cooked samples contain more than 10^2 CFU/g coliform, while *S. aureus* was in more than 10^2 CFU/g in 35 and 40% of samples, respectively. Also, 28% of cooked samples, 44% of semi cooked samples, 47/2% of frozen samples and 49% refrigerator samples contain *E. coli*. Therefore, the level of contamination of cooked and semi cooked foods by these bacteria is high (Tables 1 and 2).

This study has shown that, *Salmonella*, *B. cereus*, *Coliforms*, *E. coli*, *S. aureus*, psychrophilic and psychrotrophic microorganisms can be isolated from many different ready-to-eat foods. Several investigations regarding the microbiological quality of various ready-to-use food products, such as vegetable salads (Albrecht et al., 1995; Garcı´a-Gimeno et al., 2005; Kaneko et al., 1999; Odumeru et al., 1997) cold and hot meals served by airlines (Hatakka, 1998a, b); cooked rice (Nichols et al., 1999), street-vended foods (King et al., 2000; Kubheka et al., 2001; Mosupye and von Holy, 2013), hot-held foods (Chiou et al., 1996), catering dishes (Alberghini et al., 2000), sliced meat and meat products (Gillespie et al., 2010; Soriano et al., 2000) and shrimp (Hatha et al., 1998; Valdimarsson et al., 2008), have been reported.

The Brazilian Food Sanitation Standard (Brazil. Agencies Nacional de Vigilance Sanitaria, 2001) used for the ‘‘ready-to-eat hot sandwich and finger food and cold sandwich categories were: Fecal coliforms 2 log MPN/g; *B. cereus* 3 log cfu/g (HS) and 3:7 log cfu/g (CS); coagulase positive *Staphylococcus* 3 cfu/g (HS) and 3:7 cfu/g (CS) and the present study found high count of *Salmonella*, *B. cereus*, psychrophilic and psychrotrophic microorganisms among frozen ready-to-eat food for a single sample. In the previously cited research carried out in Latin America, the incidence of *B. cereus* in counts

above the safe level ranged from 1.7 to 8.1% of street food samples, except in one country, where this number reached 32.2% (Almeida et al., 1996).

In South Africa, this frequency was 22% of the 51 street food samples, but the counts were below 1 log cfu/g (Mosupye and von Holy, 2013) and in this study, enumeration of *B. cereus* was 2/96 ± 0/09 Log10 cfu/g. In a study carried out in Zaria, Nigeria on street food contamination, *B. cereus* and *S. aureus* were observed in 26.3 and 15% of the samples, respectively (Umoh and Odoba, 2009). The detection of high levels (>103 cfu per gram) of *B. cereus* could result in an investigation of the food handling controls used by the food business. Levels of ≥104 cfu per gram are considered potentially hazardous as consumption of foods with this level of contamination may result in food borne illness. In the multicenter study of street foods in 13 towns, 41% of sandwich samples did not meet the bacteriological criteria. The proportion of unsuitable samples due to *E. coli* contamination ranged from 4.5 to 70.2%; the prevalence of *B. cereus* was between 0.4 and 3% and from 1.9 to 10.1% for *S. aureus* (Garin et al., 2002). *S. aureus* was found in only one sample (3 log cfu/g) (2.5%), suggesting that recontamination of food by this organism after cooking was not common. At the study area, contact of the consumers with the street foods was not observed, except in the case of industrialized product (chocolates, crackers, candies, etc). In the study carried out in South Africa, *S. aureus* was not detected in any of the street food samples (Mosupye and von Holy, 2013), whereas in Latin American cities, its occurrence ranged from 1.9 to 25.2% of the street food samples (in counts above 10^3 cfu/g) (Almeida et al., 1996).

In the study from Nigeria, none of the samples from mobile food vendors was contaminated with *S. aureus*, whereas those from stationary vendors, without shelter, had the highest frequency of contamination by *S. aureus* (22.9%) and *B. cereus* (32.9%) (Umoh and Odoba, 2009). In this study, 40% of cooked samples and 35% of semi cooked samples contain more than 10^2 cfu/g *S. aureus*. During the years 1986 to 1995, 104 outbreaks caused by *B. cereus* were reported in Taiwan, and this bacterium was noted to be the third most commonly implicated food-borne pathogen in this country (Pan et al., 1997). The increasing prevalence of precooked refrigerated food products could potentially exacerbate the problems associated with *B. cereus* (Choma et al., 2000; Nichols et al., 1999; Kaneko et al., 1996; Hatakka, 1998a, b).

Considering *Salmonella*, the results of Dom et al. (2014) study suggest a generally low prevalence of this microorganism in all analyzed products, with the exception of dried pork sausages. Previous studies of ice cream and cheese reported levels of less than 0.1% or no isolation (EFSA and ECDC, 2012; Ortolani et al., 2010). Salmonella in meat preparations, intended to be eaten without any additional treatment, were reported by Cabedo et al. (2008) with 2% in cooked ham and 11.1% in cured dried pork sausage. In this study, 14% of all samples were contaminated by *Salmonella*. All the tested ready-to-eat products in this study were of unsatisfactory quality according to coliforms (AFNOR/NF BIO 12/20-12/06), *E. coli* (ISO, 16649-2:2001) and the pathogenic bacteria *Salmonella* (ISO 6579:2002; AFNOR BIO 12/01-04/94 protocol) and *L. monocytogenes* (ISO, 11290-1:2004; AFNOR BIO-12/11-03/04 protocol).

Conclusions

This study shows that most ready-to-eat food samples (all types and brands) analyzed presented unsatisfactory microbiological quality according to the Iranian guidelines and they have high risk for consumer. Contaminated food is the usual source of human infections, and poultry products are considered the major infectious route for humans (Mead, 1999; Stern et al., 2001). Moreover, evidence exists that inadequate hygiene practices within food processing plants may result in the contamination of product with pathogens (Metaxopoulos et al., 2003) and therefore pose a subsequent risk in the product's safety. On the other hand, complete elimination of pathogens from raw materials (Eisel et al., 1997) and food processing environment (Tompkin, 2012) is difficult, particularly when many food pathogenic are known to be able to attach on food contact surfaces (Fonnesbech-Vogel et al., 2001; Jessen and Lammert, 2009; Deza et al., 2005).

Conflict of interests

The author(s) did not declare any conflict of interest.

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Experimental study of the kinetics and shrinkage of tomato slices in convective drying

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This work focuses on the convective drying of tomato slices with hot air at temperatures of 50 and 60°C and at velocities of 0.1, 0.25 and 0.5 m.s^{-1}. The kinetics and drying rates are determined according to the temperature and velocity of the drying air. The equation of "Gaussian", used for the first time and in this work, is more adaptable to the drying curves. Two methods have been used to determine the coefficient of effective diffusion (with and without the effect of shrinkage). The energy of activation is evaluated with the Arrhenius relationship. In this study, we are interested in the phenomenon of shrinkage, in particular, and the evolution of the relative thickness of the slice of tomato according to drying duration and moisture content. The study shows that on hot air drying, the influence of velocity is dominant as compared to temperature. We observed that for the same final moisture content, the final relative thickness of the product is not constant. It varies depending on the operating conditions.

Key words: Tomato drying, drying kinetics, drying speed, coefficient of effective diffusion, activation energy, shrinkage.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is a native plant of South America. It is the second vegetable crop after potatoes. The entire world production exceeded 161 million tons in 2012 (FAO, 2012). Tomatoes and tomato derived products are rich in nutrients and sanitary components, because they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, flavonoids and potassium (Alexandre et al., 2008).

In developing countries such as Burkina Faso, the tomato is a seasonal product. In addition, it is highly perishable and records huge losses during the period of maximum production (Manashi et al., 2011). In order to make it available on the market as long as possible after harvest, the products are most often subjected to the drying process. Drying also reduces the weight of the product. Considering the importance of drying, especially for developing countries, several studies have already been carried out on various products to optimize one or more parameters. We can quote for example the study of Bathiebo et al. (2009) on the drying of grains of maize in a vertical channel with a constant heat flux on walls. The importance of the tomato has led recently several authors to conduct various studies. Giuseppe et al. (2008) have

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examined the effects of partial dehydration of cherry tomatoes at different temperatures and showed that there is no direct relationship between the loss of carotenoid and changes in temperature and time. Heredia et al. (2009) studied the effect of some physical variables of the process (temperature, duration of processing and osmotic composition of the solution) on the colour and changes in carotenoids (lycopene and β-carotene) during the osmotic dehydration of cherry tomato slices. They show among other things that the colour changes were mainly due to the composition of the liquid phase and shrinkage. Cernîşev and Galina (2007) have studied the influence of the drying process on the quality and nutritional value of tomato, and have come to the conclusion that the sun-dried tomatoes are rich in antioxidants, vitamins and particular lycopene and β-carotene.

Drying is a complex phenomenon and we must repeat and diversify the study conditions in order to report more realistically the course of the drying process. The models of drying processes that describe the phenomenon of the drying of agricultural materials are grouped into three main categories, namely theoretical, semi-theoretical and empirical (Ebru, 2006).

During the drying process, water is transferred from the inside to the outside of the product, so there is water diffusion. Water diffusion is a process that has a very important effect on the phenomenon of drying of agricultural products. Knowledge of this phenomenon allows a better description of drying kinetics, a better interpretation of the results and better simulations. This is the way we estimate the coefficient of diffusion of tomato with different experimental conditions, through Fick's second law.

During the drying process, some products simultaneously undergo great changes in volume and surface (Lima et al., 2002). This shrinkage phenomenon particularly affects the diffusion rate of the material, which is one of the main parameters governing the drying process; it also has an influence on the drying rate (André et al., 2004). Najmur and Subodh (2006) reported in their work that the heat transfer coefficient increases with the shrinkage. Marcelo and Paulo (2011) showed in their work on potato that shrinkage must be taken into account in the modeling of the curve of drying kinetics. Another important consequence of shrinkage is the decrease in the rehydration capacity of the dry product (Mayor and Sereno, 2004). During drying, shrinkage occurs in all directions of the product, which justifies studies on the different sides. In fact, studies have focused on shrinkage along the diameter, length and thickness of the product.

Although, most of the works on the shrinkage of the products deal with volume shrinkage; the phenomenon of shrinkage is related to drying conditions such as temperature, velocity and the humidity of the drying air. The effect of temperature on shrinkage remains problematic as reported in the findings of various works. According to Alireza and Mehdi (2009), temperature increases the rate of cell shrinkage following an Arrhenius-type behavior. Mc Minn and Magee (1997) and Wang and Brennan (1995) in their work on potato, show that the increase in temperature reduces the phenomenon of shrinkage.

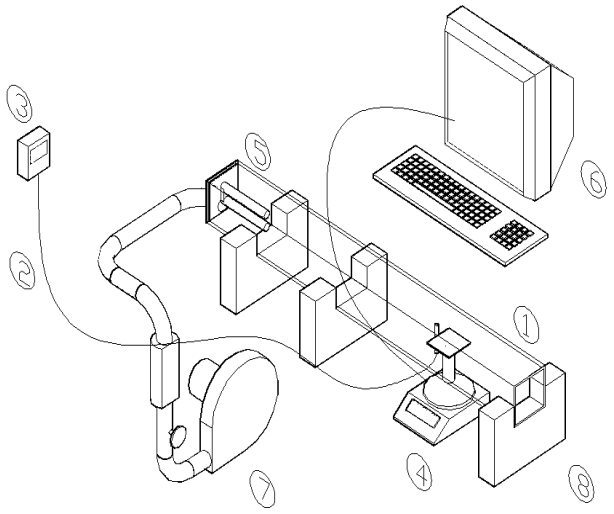

Figure 1. Diagram of the experimental setup. 1: tunnel dryer, 2: thermocouple cable, 3: temperature displayer, 4: scales, 5: heater resistance, 6: PC, 7: blowing device, 8: holder.

In the literature, there are few studies on the evolution of the thickness of tomato during its drying depending on the main physicochemical parameters governing the process.

Objective of this work

1. To determine experimentally within fixed aero-thermal parameters, the influences of temperature and velocity of drying air on the drying kinetics of the tomato slice and the advancement of shrinkage of tomato slices.
2. To determine experimentally the influence of shrinkage on the coefficient of effective diffusion.

MATERIALS AND METHODS

The tomatoes used were purchased from Brussels market in the month of October and November. Before each drying experiment, the tomatoes were washed, and cut into 1.9 x 1.8 x 0.7(cm) slices with a medium-sized, parallelepiped knife. The dryer used for our measurements was built at the TIPs laboratory, (Transfer Interface Process, Service) of the Université libre de Bruxelles. It is a tunnel dryer composed of a plexiglass parallelepiped-shaped tube of cross-sectional shape of 10.5 cm^2 (Figure 1). Inside the device is placed a holder with the sample of tomato to dry. The air is heated with two heating resistances located inside the dryer and its temperature is given by an adjustable digital display device. A blowing device, connected to the dryer is used for circulating air

inside the tunnel. The air flow is adjustable by means of a valve. The mass of the sample is measured every five minutes, using Sartorius brand electric scales with a precision of 0.01 g connected to a computer. Every five minutes, a camera provides photos of the tomato slice during drying. The photographs will allow us to follow the evolution of the thickness of the tomato during drying. The experimental setup is shown in Figure 1.

The initial water content was determined by measuring the mass of the sample before and after passage in an oven at 70°C for 24 h. The average water content determined is 17.5 kg water/kg of dry material or 94.6% in wet basis, which is in accordance with the data of literature (Ibrahim, 2007)

The experiments are carried put under several experimental conditions in order to highlight the effect of temperature and drying air velocity on the evolution of water content and the thickness of the slice of tomato during the drying process. The experimental temperatures are 50 and 60°C, to avoid the destruction of the vitamins, and experimental velocity are 0.1, 0.25and 0.5 $m.s^{-1}$ to approximate air velocity in natural convection.

Mathematical formulation

Moisture content and dry mass

The mass of the product after passing through the oven at 70°C constitutes its dry mass. When the initial water content is known, dry mass is determined by the following mathematical relationship:

$$m_s = \frac{m_0}{1 + X_0} \quad (1)$$

The water content in dry basis during drying at time "t" is determined by the following mathematical expression:

$$X(t) = \frac{m(t) - m_s}{m_s} \quad (2)$$

or

$$X(t) = \frac{m(t)(X_0 + 1) - m_0}{m_0} \quad (3)$$

The final water contents sought is that from which the product no longer deteriorates and keeps its nutritional and organoleptic qualities. This final water content is therefore a characteristic of the product, in the case of tomato; it is about 10%.

Drying velocity

The drying rate is determined by the following equation:

$$DR = -\frac{X^*_{t+dt} - X^*_t}{dt} \quad (4)$$

With X^*_t as the relative water content at the time "t", X^*_{t+dt} the relative water content at the time t+dt and t is the drying time.

Drying curve

The drying curve is generally represented by the water content reduced with time. The reduced water content is determined by the following expression:

$$X^* = \frac{X - X_e}{X_0 - X_e} \quad (5)$$

Where, X^* represents the content of reduced water, X represents the content of water at a time "t", X_e represents the water content at equilibrium.

This expression of the reduced water content can be approximated by the following expression:

$$X^* = \frac{X}{X_0} \quad (6)$$

The error is very small (Manashi et al., 2011).

The theoretical, empirical or semi-empirical expressions are used to account for the drying curve. These models generally derive from the simplification of the general solution of the set of Fick's second law except for the ''Gaussian equation'' used in this work which gives a better correlation. The correlation coefficient R^2 is used to evaluate the model, at the χ^2 statistical parameter and the square root of the mean systematic error RMSE. These parameters are given by the following relations:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(X^*_{pre,i} - X^*_{exp,i}\right)^2}{\sum_{i=1}^{N}\left(\overline{X^*_{pre}} - X^*_{exp,i}\right)^2} \quad \text{(Mortaza et al., 2009)} \quad (7)$$

$$\chi^2 = \frac{\sum_{1=1}^{N}\left(X^*_{exp,i} - X^*_{pre,i}\right)^2}{N - Z} \quad (8)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(X^*_{exp,i} - X^*_{pre,i}\right)^2} \quad (9)$$

The Gaussian equation is stipulated in the following expression:

$$X^* = a_1 \exp\left(-\left((-t - b_1)/c_1\right)^2\right) + \cdots + a_6 \exp\left(-\left((-t - b_6)/c_6\right)^2\right) \quad (10)$$

$X^*_{exp,i}$ is the experimental reduced water content at point i, $X^*_{pre,i}$ is the reduced water content predicted at point i. N represents the number of points, Z is the number of constants in the model.

The best model is the one that has the highest possible value of R^2 (close to 1), χ^2 and RMSE should be as small as possible (Ruiz Celma et al., 2012).

Shrinkage

Some scholars use volumetric ratio to express shrinkage ($\frac{V}{V_0}$). Shrinkage is also studied as a function of the relative variation of the thickness of the product. Various relationships are observed on the evolution of thickness depending on the water content, and based on experimental parameters. Indeed, some authors such as Lucia et al. (2007) for the 'chitosan' conclude linear relationship between variation in thickness and water content in the form:

$$\frac{L}{L_0} = a.X + b \quad (11)$$

Hashemi et al. (2009) for the bean, got the result:

$$\frac{L}{L_s} = 1 + \alpha . X \quad (12)$$

In these expressions, L_o, L, L_s respectively indicate the thickness of the sample at the initial time, at a time "t" and the thickness of the dry sample, a and b are constant, α is the coefficient of linear shrinkage. But some authors such as Kingsly et al. (2007) for the jujube, found a quadratic function to express shrinkage in thickness depending on the water content in the form:

$$\frac{\mathrm{L}}{\mathrm{L}_0} = \mathrm{a}.\mathrm{X}^2 + \mathrm{b}.\mathrm{X} + \mathrm{C} \quad (13)$$

In this study, we present the evolution of a slice of tomato flesh during its drying, depending on the experimental conditions and its water content.

Effective diffusion coefficient

For the determination of the diffusion coefficient, Fick's second law was used considering the unidirectional movement of water and the slice of tomato as an infinite plate. The equation of Fick's second law is as follows:

$$\frac{\partial \mathrm{X}}{\partial \mathrm{t}} = \mathrm{D}_{\mathrm{eff}} \frac{\partial^2 \mathrm{X}}{\partial \mathrm{x}^2} \quad (14)$$

Where, X is the water content, D_{eff} ($m^2.s^{-1}$) is the effective diffusion coefficient, t(s) is the drying time and x is the direction of water propagation. The solution proposed by Crank (1975) is that when assuming that initial water content is uniform, and the process isothermal, the solution is:

$$\mathrm{X}^* = \frac{8}{\pi^2} \sum_{\mathrm{n}=0}^{\infty} \frac{1}{(2\mathrm{n}+1)^2} \exp\left(\frac{-(2\mathrm{n}+1)^2 \pi^2 \mathrm{D}_{\mathrm{eff}} . \mathrm{t}}{4.\mathrm{L}^2}\right) \quad (15)$$

Where, X^*, shows the reduced water content, L (m) half the thickness of the sample and n ($n \in N$) the number of limits taken into account.

Equation (15) can be simplified at the first term of the series for long periods of drying. This gives:

$$\mathrm{X}^* = \frac{8}{\pi^2} \exp\left(\frac{-\pi^2 \mathrm{D}_{\mathrm{eff}} . \mathrm{t}}{4.\mathrm{L}^2}\right) \quad (16)$$

Taking the logarithm of Equation 16, we obtained Equation 17, whose representation in function of time is linear within a certain value of the water content.

$$\mathrm{Ln}(\mathrm{X}^*) = \mathrm{Ln}\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2 \mathrm{D}_{\mathrm{eff}} . \mathrm{t}}{4.\mathrm{L}^2}\right) \quad (17)$$

The slope of this line is:

$$\mathrm{K} = -\frac{\pi^2 \mathrm{D}_{\mathrm{eff}}}{4.\mathrm{L}^2} \quad (18)$$

Therefore:

$$\mathrm{D}_{\mathrm{eff}} = -\frac{4.\mathrm{k}.\mathrm{L}^2}{\pi^2} \quad (19)$$

Figure 4 shows the representation of $\ln(X^*)$ as a function of time. The values of the average effective diffusion coefficient for the various experiments are shown in Table 1, where we can see that the effective diffusion coefficient increases with both temperature and velocity of drying air.

In the present case of our study, the representation of $\ln(X^*)$ as a function of drying time is not quite linear as we really observe a curvature towards the end. Thus, Equation 17 considers that the diffusion coefficient is constant for a given temperature. Figure 5 illustrates the dependence of the effective diffusion coefficient as a function of the water content of the product. The Fourier number is used. It has already been proposed in the work of Ruiz Celma et al. (2012) to assess the effective diffusion coefficient as a function of water content.

If $F_0 = \frac{\mathrm{D}_{\mathrm{eff}} . t}{L^2}$ where, F_0 is the number of Fourier, Equation 16 becomes:

$$\mathrm{X}^* = \frac{8}{\pi^2} \exp\left(\frac{-\pi^2}{4} \mathrm{F}_0\right) \quad (20)$$

When taking the logarithm of Equation (20), we can express the number of Fourier with the following expression:

$$F_0 = \frac{4}{\pi^2} \mathrm{Ln}\left(\frac{8}{\pi^2}\right) - \frac{4}{\pi^2} \mathrm{Ln}(\mathrm{X}^*) \quad (21)$$

The effective diffusion coefficient is expressed by the equation:

$$\mathrm{D}_{\mathrm{eff}} = \frac{\mathrm{F}_0 \mathrm{L}^2}{\mathrm{t}} \quad (22)$$

RESULTS AND DISCUSSION

The drying process is stopped when the water content of the product has reached the equilibrium moisture content of the experimental conditions (8% <HR <10%) or a water content of dry basis range between 0.099 and 0.1 kg water/kg dry matter.

Determining the water content was made on a dry basis by using the formula of Equation 3 above. Relative water contents (X/X_0) are used to compare effectively the influence of aero-thermal parameters such as temperature and velocity of the drying air on the drying kinetics and the drying rate of the product. Thicknesses have also been reduced to relative thicknesses for the same reasons.

Effect of temperature and air velocity on the drying kinetic

For these experiments, we used the temperatures and velocities of drying air presented above. To determine the influence of temperature, the air velocity is fixed (V is

Table 1. Value of the activation energy depending on the experimental conditions.

Air velocity ($m.s^{-1}$)	Air temperature (°C)	Average effective diffusion coefficient (10^{-6}) [$m^2.s^{-1}$]	Activation energy Ea ($kJ.mol^{-1}$)
0.1	50	1.33	25.77
	60	1.77	
0.25	50	2.6	33.26
	60	3.79	
0.5	50	3.56	32.42
	60	5.11	

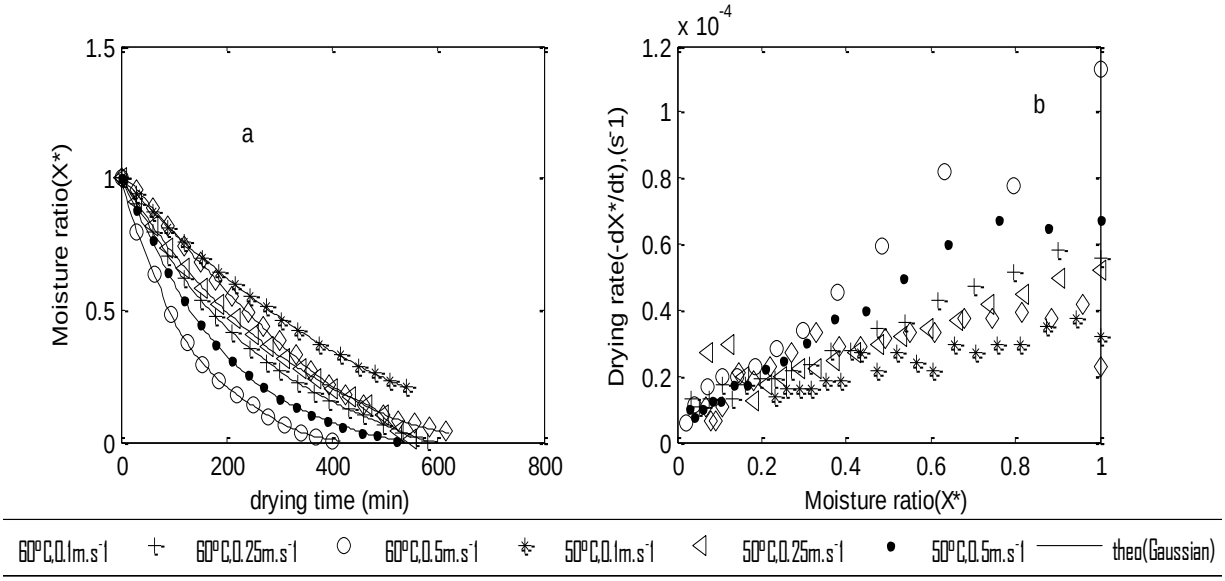

Figure 2. a) Evolution of water content as a function of time and experimental conditions; **b)** drying rate as a function of relative water content and experimental conditions.

constant), its temperature is varying, and the opposite is used for determining the influence of the air velocity.

At fixed air velocity, drying is naturally all the more fast as the temperature is high (Figure 2a). Figure 2a also allows us to observe the effect of air velocity on the evolution of water content during drying. We noticed that the effect of air velocity is dominant, as compared to that of temperature. Indeed we noted that at 60°C with a velocity of 0.25 $m.s^{-1}$, drying is less efficient than at 50°C under an air velocity of 0.5$m.s^{-1}$. The increase in velocity and temperature of the drying air accelerates the drying process of tomato.

Influence of temperature and air velocity on the drying rate

The drying rate in Figure 2b shows a single phase, the stage of drying with decreasing velocity which suggests that the drying of tomato is governed by a phenomenon of diffusion. Figure 2b shows that drying rate increases with air velocity. This is also observed by Salah et al. (2012) regarding the drying of apple. Drying rate also increases with air temperature according to Figure 2b. This is also observed by Abano et al. (2011), regarding the drying of tomato. But an analysis of the effect of these two parameters indicates that the effect of the air velocity is much greater.

Influence of temperature and air velocity on the phenomenon of shrinkage

Drying involves the water loss of the sample, leading to deformation of the skeleton of the sample. This phenomenon reduces the size of the sample. In the present work, the thickness of the slice of tomato was measured and reduced to relative thickness (e/e_0).

The resulting images (Figure 3) are processed using software called GIMP. The processing of images to

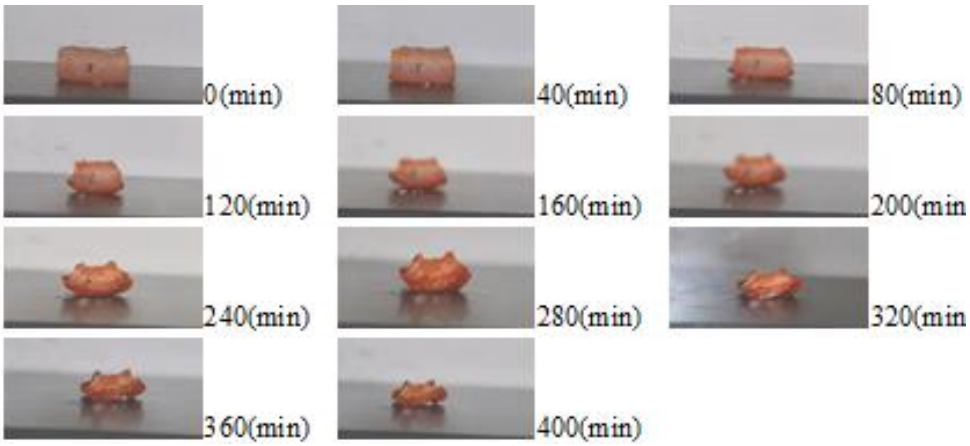

Figure 3. Evolution of the slice of tomato during drying.

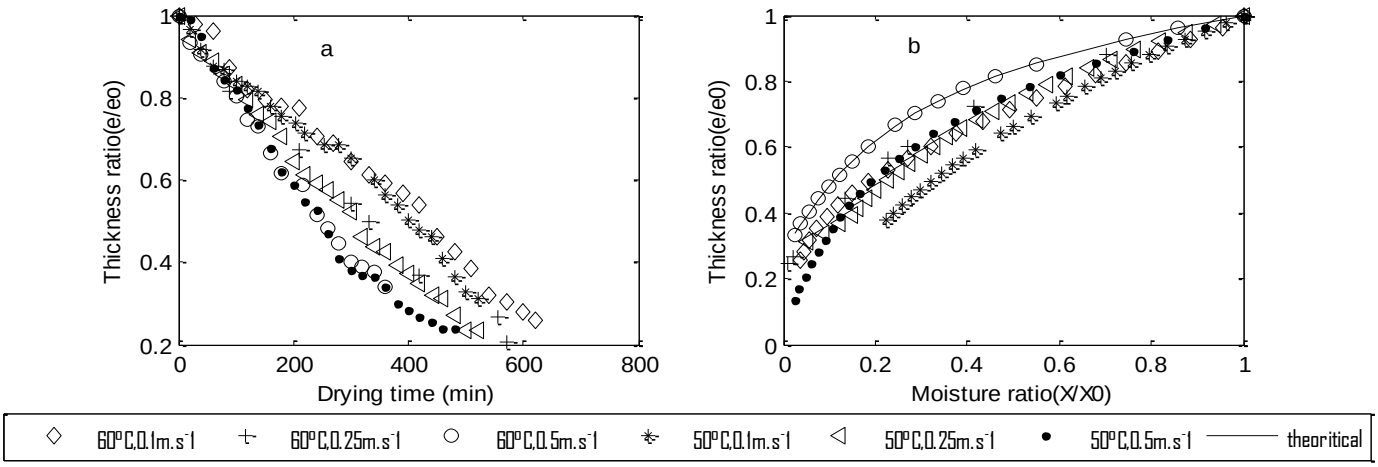

Figure 4. a) Evolution of the relative thickness as a function of drying time and experimental conditions; b) Shrinkage of the tomato slice according to relative water content.

assess shrinkage has already been used by other authors such as: Zhengyong et al. (2008) to measure changes in the size of pineapple, mango and banana during drying, Alireza and Mehdi (2009) to determine the effect of temperature and drying air on the shrinkage of the potato,
Lucia et al. (2007) to determine the evolution of the thickness of the 'chitosan' during its drying.

Figure 4a shows an almost linear decrease of the thickness of the slice of tomato with drying time.
It was observed that the air velocity had more pronounced effect on the shrinkage as compared to temperature. This constitutes another argument in favor of the dominant role of drying air velocity in this temperature range. The shrinkage is more pronounced as the air velocity is high.

Nevertheless, temperature (50 and 60°C) does not significantly influence the phenomenon of shrinking of the slice of tomato and it was also noted that the increase in the latter slightly disadvantages shrinkage. A typical result for the temperature was obtained by Mc Minn and Magee (1997) and Wang and Brennan (1995) on the potato.

Figure 4b shows that for relative water content fixed at 0.25, very different relative thicknesses are observed according to the selected operating conditions, including 0.41, 0.52, 0.55, 0.56, 0.58 and 0.67. Then, it is noticed that the final volume of the material is not only due to lossof water and it is likely that other physicochemical mechanisms related to the intrinsic nature of the material, determine the final shrinkage of the product.

Modelization of the curve of drying

The drying curves were adapted to the respective models, logarithmic (Henderson and Pabis, 1961; Verma et al., 1985; Aghbasho et al., 2009) the two terms, the

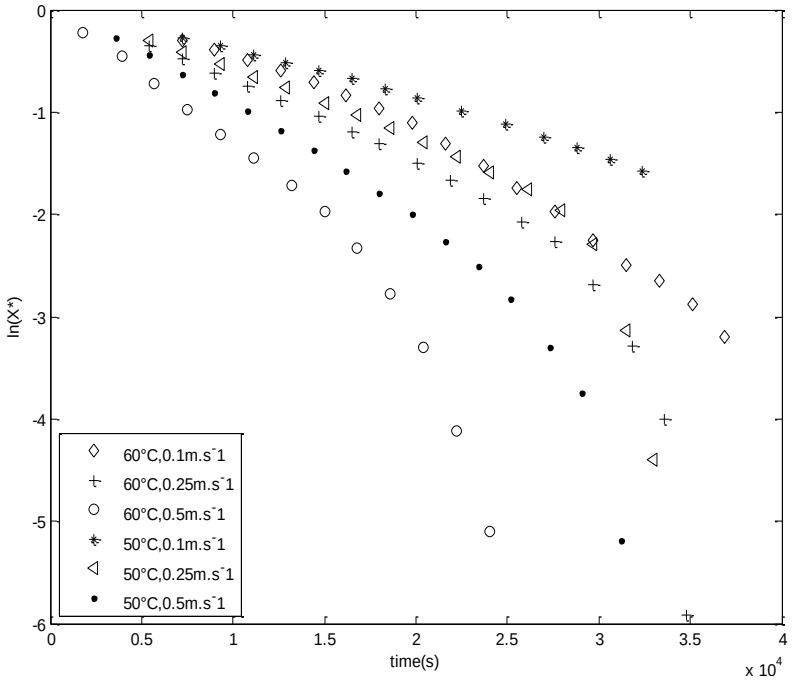

Figure 5. Representation of $\ln(X^*)$ as a function of time and experimental conditions.

modified (Henderson and Pabis, 1961; Wang and Singh, 1978) models, and to Gaussian equation (this work).

The parameters were evaluated through the Marquardt-Levenberg method of non-linear least squares algorithm. The best representation of the drying curve is obtained for the coefficient of the nearest possible correlation to 1, and the smallest possible values (close to zero) of χ2 and RMSE (Zhengfu et al., 2007). We can state that the Gaussian equation used in this work, represents better the drying kinetics of the samples of tomato flesh (Figure 2a).

Modelization of the curve of shrinkage according to water content

The best model obtained is a function of degree 5 (Figure 4b) represented as follows:

$$f(x) = a.x^5 + b.x^4 + c.x^3 + d.x^2 + g.x + h \quad (23)$$

Where, f represents e/e_0; a, b, c, d, e, f are constant and x represents X^*

Effective diffusion coefficient and activation energy

Effective diffusion coefficient

This effective diffusion coefficient of foodstuffs characterizes their intrinsic property of mass and moisture transport including molecular diffusion, liquid diffusion, vapor diffusion, hydrodynamic flow and other mechanisms Ruiz Celma et al. (2012)

Figure 6 shows that the value of the effective diffusion coefficient evolves in the same direction as temperature and air velocity and opposite direction with the water content. We also noted here that the curves of larger diffusion coefficients are those where the air velocity is higher despite the fact that temperatures are different. This shows the complexity of expressing this coefficient correctly.

By using Fick's equation and considering shrinkage, we noted that the effective diffusion coefficient presents a rather complex evolution (Figure 7). Indeed effective diffusion presents two phases: a first phase where it evolves in opposite direction with the water content until it reaches a maximum and a second phase where it evolves in the same direction as the water content.

This behavior of effective diffusion coefficient can be interpreted by the various mechanisms which control drying. Thus, in the first phase of drying, the diffusion of liquid water could be the principal mechanism of mass transfer. While drying progresses, the diffusion of the water vapor would be prevalent. This behavior was observed by several authors.

Activation energy

The Arrhenius expression enables us to determine the

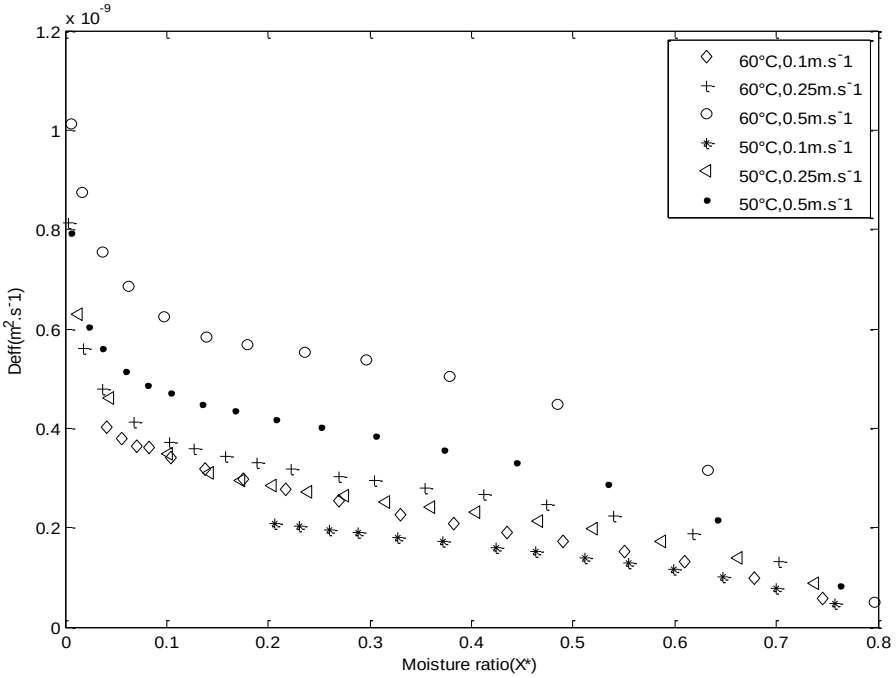

Figure 6. Representation of the effective diffusion coefficient D_{eff} without taking into account shrinkage as a function of the relative water content X* and experimental conditions.

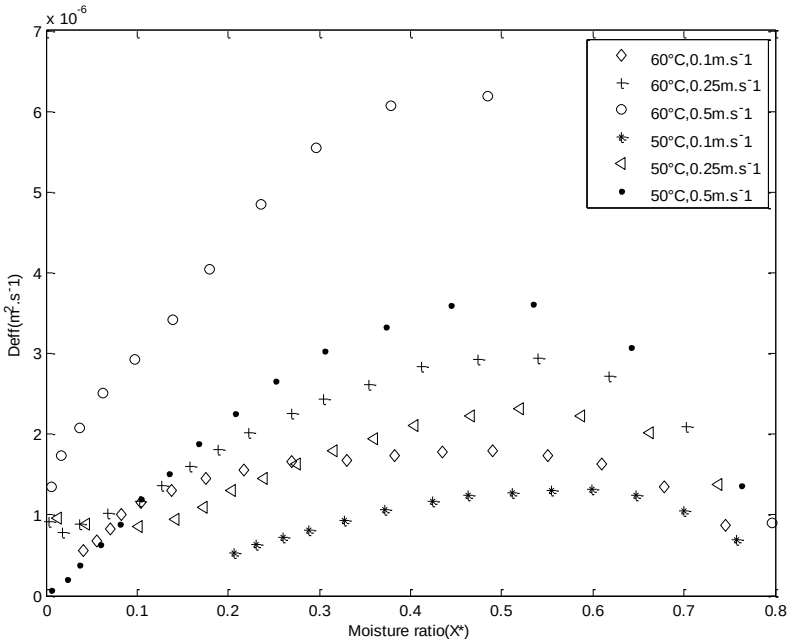

Figure 7. Representation of the effective diffusion coefficient D_{eff} as a function of the relative water content X* when shrinkage is included.

activation energy, which connects the latter to the diffusion coefficient through the following expression:

$$D_{eff,avg} = D_0 . \exp\left(-\frac{E_a}{R(T+273)}\right) \quad (24)$$

Where, D_0 is a constant of Arrhenius, E_a is the activation energy, T is temperature (°C) and R is the perfect gas constant.

It is necessary to express the average effective coefficient of diffusion according to the water content. The following relation is used:

$$D_{eff,avg} = \frac{\int_{X^*initial}^{X^*final} D(X^*)dX^*}{\int_{X^*initial}^{X^*final} d(X^*)} \quad (25)$$

Equation (24) is taken as a logarithm, which gives Equation 26 as follows:

$$Ln(D_{eff}) = Ln(D_0) - \frac{E_a}{R(T+273)} \quad (26)$$

The graphical representation of equation $\ln(D_{eff})$ as a function of the reverse of temperature gives a straight line whose slope is

$$P = -\frac{Ea}{R} \text{ where, } E_a = -P.R \quad (27)$$

The values of the activation energy found are shown in Table 1. The activation energy like the diffusion coefficient varies with experimental conditions.

Conclusion

An experimental study on the kinetics of shrinkage and evolution of thickness of a tomato slice during its drying was carried out. During this study, it was shown that Gaussian's equation used for the first time better adapts to the studied curves of experimental shrinkage.

The results confirmed the positive effect of temperature and air velocity on the kinetics of drying. We have also noticed a clear dependence of the effective diffusion coefficient on the water content of the product. The complexity of the effective diffusion coefficient has also been observed, which depicts that drying process is governed by several mechanisms. The obvious difference between the expressions of effective diffusion coefficient with or without taking into account the shrinkage of the product also shows the importance of taking shrinkage into account in the studies of simulation of drying kinetics.

Conflict of interests

The authors did not declare any conflict of interest.

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Safety of bread for human consumption in an urban community in Southwestern Nigeria

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Bread is an important staple food that does not require further processing before consumption. Despite the awareness created and efforts of government to ensure that quality bread are available for consumption of all, there are several routes through which bread is being contaminated. The aim of the study was to assess the hygiene involved from the point of production to sale of bread and document pathogens isolated from bread. This was a descriptive cross-sectional study with 10 out of the 30 registered bakeries spread across wards randomly selected and only six consenting to participate (60% response rate), and a total of 10 representative bread sellers were purposively selected with respect to their spread in Ile Ife. Observational checklist was used to assess environmental and personal hygiene while microbiological specimens taken from bread were assessed for pathogenic contamination. Only a third of bakeries assessed had good hygiene while hygiene of bread sellers was suboptimal. Bread samples from bakeries and bread sellers yielded *S. aureus* in 33 and 90%, respectively. Several points of contamination were identified and poor bread handling practices documented. Existing laws should be enforced and bread sellers educated on proper handling of bread to prevent outbreaks of food borne illnesses.

Key words: Bread, food safety, hygiene, food-borne.

INTRODUCTION

Globally, access to safe food is a basic human right. It is so important that this year the World Health Organization has made food safety the theme of the World Health Day 2015.The food sector is broad and diverse, with different regulations guiding practices of stakeholders from micro to macro sector players. Due to rapid rural urban migration, several urban dwellers depended on ready to eat food to satisfy their food requirements. In this regard, ready to eat food (RTE) refers to food that could be eaten as purchased and does not require further significant processing other than reheating or completion of a cooking process (Singh et al., 2014).

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One of the readily available RTE food is bread, which is a staple food that do not require further processing before consumption. It is produced in various forms and eaten in homes, restaurant and hotels in Nigeria (Emeje et al., 2010). In addition, the consumption of bread cut across socio-cultural and religious barriers and is a food of choice for both rich and poor in Nigeria (NAFDAC, 2010). The dough of bread is usually baked but in some eateries is steamed, fried or baked on an un-oiled platform. Bread is prized for its taste, aroma, quality, appearance and texture and, retaining its freshness is important to keeping it appetizing and appealing to consumers. Bread is made from low protein flour made from wheat or cassava and contains several ingredients that improve its quality. Some of the ingredients added to bread are table salt, sugar, flavour and at least an efficient oxidising additive to assist in the raising process and to produce a texture in the finished product that is appealing to the consumers (Emeje et al., 2010).

In Nigeria, commercial bread making is regulated by guidelines, through the National Agency for Food and Drug Administration and Control (NAFDAC), through the 4-point safety standards that bread bakers should comply with in Nigeria, as follows:

1. Consistent' production of flour fortified with Vitamins A&C and other '' safe enhancers in approved quantities to prevent bakers from adding dangerous chemicals like potassium bromate as flour/bread improvers,
2. Compliance with set standards of Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) guidelines and requirements of the Agency,
3. Stop the use of dangerous ingredients which are not on the list of substances generally regarded as "safe" and
4. Bakers should stop the habit of distributing loaves of bread to consumers without proper packaging and in unhygienic conditions (NAFDAC, 2010).

Nigeria has several food safety legislations, food is prepared in diverse socio and environmental settings and frequently contaminated with naturally occurring pathogenic microorganisms (Abdalla et al., 2008). In addition, concern on safety of bread have increased following the indiscriminate use of potassium bromate while the storage, handling process, the condition of the bread processing environment and health of the workers in the cottage industries and street hawkers are important considerations in ensuring the safety of consumers (Isong et al., 2013). Moreover, despite the awareness created and efforts to ensure that quality bread is available for citizens, there are several routes through which bread could be contaminated in the processing chain, especially during packaging, respectively at the factory and by vendors in containers to reduce dessication (HPA, 2009). Bread contamination and growth of pathogens alter its quality and a potential source of infection to consumers since improper handling and poor personal hygiene of food, and in particular during packaging is implicated in most food-borne illnesses through cross contamination and poor personal hygiene of bread handlers (Ehavald, 2009).

When food handlers do not practice safe personal hygiene, they may become vehicle for transmission of pathogens, through hands, mouth, skin, among others (HPA, 2009). Therefore, the aim of the study was to assess the hygiene involved from the point of production to sale of bread and document pathogens isolated from bread.

MATERIALS AND METHODS

The study was a descriptive, cross sectional design. The study was carried out in Ile-Ife, an urban city in Nigeria. The city is inhabited by 167,204 people, comprising 88,403 male and 78,801 females (NPC, 2009). Ile Ife has 11 wards and thirty registered bakeries.

Ten (10) out of the thirty registered bakeries spread across wards were randomly selected with only six consenting to participate (60% response rate) and all bakers on duty in each of the bakeries, totaling 40 were assessed for the study. Also, a total of 10 representative bread sellers were purposively selected with respect to their spread in Ile Ife from the following locations Mayfair, Campus gate, Sabo, General phase 1, Owode, Ilode, Arubiidi, Iyekere, Idi-omo and road 7, respectively.

Ten (10) different brands of bread produced by the bakeries were purchased from 10 market vendors at Ile Ife after being supplied and packaged. Observational checklist was used to assess hygiene in bakery houses and bread sellers while interviewer-administered questionnaires was employed to assess personal hygiene of bakery workers and swabs were taken from freshly baked breads, cutting tables, baking pans and mixing table while loaves of bread were collected as samples from the bakeries for laboratory analysis.

Microbiological assessment of bread samples

Swab samples obtained using sterile materials were collected from various baking surfaces, cultured into MacConkey and Chocolate agar and incubated at 37°C and 20% Carbon dioxide gas for 24 h. Also, 20 g of various bread samples were weighed and inoculated into sterile peptone (broth) and incubated for 24 h after which the growths were examined and isolates were identified by colony characteristics, Gram stain, catalase and coagulase tests as described by Monica Cheesbrough (1984).

Hygiene assessment of bakery houses in the study area

In assessing the level of hygiene in bakeries, a score of one (1) was assigned to correct hygienic practice and zero (0) for each wrong practice in each bakery which yielded a maximum score of 13. A composite score was graded as follows; less than 50% (<7), between 50-70% (7-9) and 70% (>9) and above signifies poor, fair and good level of hygiene, respectively in each of the six bakeries assessed.

Data analysis

Data were analysed using SPSS version 16 and presented using tables and charts as appropriate.

Informed consent

The informed consent of the bakery owners, workers and

Table 1. Socio-demographic characteristics of Respondents.

Variable	Characteristics	Bakery workers		Bread vendors	
		Frequency (N=40)	Percentage (%)	Frequency (N=10)	Percentage (%)
Age (years)	18-24	9	22.5	2	20
	25-31	17	42.5	5	40
	>31	14	35.0	3	30
Gender	Male	28	70	0	0
	Female	12	30	10	100
Educational status	Primary	9	22.5	3	30
	Secondary	27	67.5	7	70
	Tertiary	4	10.0	0	0

Table 2. Environmental hygiene characteristics of selected bakeries in Ile-Ife.

Variable	A	B	C	D	E	F
Is the bakery close to a residential area	1	1	1	1	1	1
Is the bakery close to any refuse dumping ground	1	0	0	0	0	0
Are there any unsanitary drainages around the bakery	1	1	1	0	0	1
Does the bakery have a proper refuse disposal system	0	1	0	0	1	0
Does the bakery have adequate space for storage of raw materials	0	1	1	1	1	0
Is the bakery surrounding bushy	0	0	0	0	0	0
Does the bakery have a toilet	0	1	1	1	1	1
If yes is the toilet separated from the processing area	0	1	1	1	1	1
Does the bakery have adequate supply of potable water	0	1	0	1	1	0
Are the doors and windows of the storage room protected against insects/pests	0	1	0	0	1	0
Does the bakery have handwashing facilities	0	0	0	0	1	0
Are the equipment cleaned regularly	0	0	1	1	1	0
Is the bakery premises cleaned regularly	0	1	0	1	1	1
Total score (%)	3 (27)	9 (70)	6 (46)	7 (55)	10 (75)	5 (40)

Key: 0=No; 1=Yes.

commercial bread vendors were obtained while ethical approval was given by the ethics committee of the Institute of Public Health.

RESULTS

The socio demographic information of 40 bakery workers and 10 randomly selected bread vendors that participated in the study is presented in Table 1. The result revealed that 65% of the bakery workers age ranges between 18-31 years with 24 years as the median age. 70% of them were males and majority of the bakers (77.5%) completed at least secondary schools education whilst, 70% of the bread vendors had ages ranging between 18 and 31 years with a median age of 26 years. Interestingly, all the bread sellers were females and most of them (70%) had educational attainment up to secondary school level.

The level of hygiene in the bakeries assessed was suboptimal with only two of the six facilities assessed having good level of hygiene with 1 having fair hygiene while three had poor hygiene (Table 2).

Though the use of head gear by bakery workers is mandatory, the study shows that 6% of the respondents had never use head gear while only 30% always wear it and only 10% use face mask. About two-thirds (65%) of bakery workers wear aprons regularly while only 45% wear hand gloves regularly. Hand washing before

Table 3. Personal Hygiene of Bakery workers and commercial bread sellers.

Hygiene practice	Bakery workers			Commercial bread sellers	
	Always N (%)	Sometimes N (%)	Never N (%)	Yes N (%)	No N (%)
Use of head gear	12(30)	4 (10)	24 (60)	10 (100)	0 (0)
Use of face mask	4 (10)	0 (0)	36 (90)	-	-
Use of foam to clean bread	-	-	-	10 (100)	0 (0)
Use of apron	26 (65)	8 (20)	6 (15)	2 (20)	8 (80)
Washing of hands	38 (95)	0(0)	2 (5)	4 (40)	6 (60)
Use of hand gloves	18 (45)	4 (10)	18 (45)	-	-
Adequate covering of bread	-	-	-	10 (100)	0 (0)
Cutting of finger nails	38 (95)	2 (5)	0(0)	10 (100)	0 (0)

Table 4. Microbiological isolates from bakery surfaces and bread samples.

Surface	A	B	C	D	E	F
Finishing table	+§	-	+§	-	-	-
Baking pan	-	-	-	-	-	+§
Cutting table	+§	+§	-	-	+§	+§
Mixing table	-	+§	-	-	-	-
Bread sample	+*	+§	+§	+§	+§	+* +

* -pathogenic organism (*Staphylococcus aureus*)
§-nonpathogenic organism (aerobic spores).

processing bread was almost universally reported by bakery workers.

Among the commercial bread sellers, use of head gear was universally practiced. Only 60% reported washing their hands before handling or packaging bread with use of apron practiced by 20% of bread sellers. The study further revealed that all bread sellers use" foam" to clean bread before packaging (Table 3).

The study further assessed the microbial contamination of various surfaces in use in bakeries, namely the finishing table, baking pan, cutting table and mixing table of the six bakeries assessed. The analysis of the swab samples collected from each of the surfaces revealed that the cutting tables in most of the bakeries (66.7%) developed growth of non-pathogenic aerobic spores of bacilli while a significant 17.6% of the swab samples from bakeries finishing table, mixing table and baking pan respectively revealed growth of non-pathogenic aerobic microbes. A third of bread samples collected from the bakeries grew pathogenic organism (*S. aureus*) (Table 4).

The widespread handling of bread from point of transportation to point of sale is depicted in Figures 1 to 3. Figure 1 showed the supplier handling bread with bare hands while Figure 2 showed the bread sellers handling bread with bare hands and "cleaning" the bread with foam and Figure 3 shows the bread uncovered whilst

Figure 1. Handling practice of bread supplier and seller.

Figure 2. Bread seller using foam to clean bread.

the seller was "cleaning" the bread.

Similarly, the study further revealed that most bread

Figure 3. Bred seller cleaning bread with foam while bread left exposed.

Table 5. Microbiological analysis of bread samples from commercial bread vendors.

Bakery	Gram reaction	Isolated Micro-organism
C1	Gram +ve Cocci	*Staphyloccus spp.*
D1	Gram +ve Bacilli	*Aerobic spore(NP)*
E.	Gram +ve Cocci	*Staphyloccus aureus*
F	Gram +ve Cocci	*Staphyloccus aureus*
A1	Gram +ve Cocci	*Staphyloccus aureus*
B1	Gram +ve Cocci	*Staphyloccus spp.*
A2	Gram +ve Cocci	*Staphyloccus aureus*
B2	Gram +ve Cocci	*Staphyloccus aureus.*
C2	Gram +ve Cocci	*Staphyloccus aureus*
D2	Gram +ve Cocci	*Staphyloccus aureus.*

samples collected from vendors (80%) were contaminated by pathogenic *S. aureas* while 20% showed growth of non-pathogenic aerobic spore (Table 5).

DISCUSSION

Living organisms in this environment are always looking for food to stay alive, therefore food safety is very important to ensure wholesomeness of food for human consumption. Studies have shown that poor handling practice tend to cause food borne illness (Clayton et al., 2002). Bread being a meal that is usually eaten without further processing makes it a good source of food borne illness if improperly handled.

This study reveals that over 60% of bread sellers do not wash their hand before packaging bread, which is contrary to ideal hygiene practice as stated by Ehavald (2009). This finding is much higher than reported findings by Ifeadike et al. (2014), Isara et al (2013), Altekruse et al. (1996), Yang et al. (1998) and Shiferaw et al. (2000). This finding may be attributed to the fact that respondents may not necessarily refer to bread as raw food and thus little risk of contamination. Placing this finding in perspective of the frequent handling of bread by sellers it is imperative to note that whilst only a third of the bread sampled from the bakery were contaminated by *S. aureus*, almost all the bread sampled from the bread sellers were contaminated with *S. aureus*. This buttresses the assertion from studies on Hazard analysis and critical control points (HACCP) that shows that most foods are contaminated along the processing chain (Daniyan and Nwokwu, 2011, Adesetan et al., 2013).

The unwholesome practice of cleaning bread with foam has never been reported in literature and is a major mechanism for contamination of bread as it provides ample opportunity to expose the bread to handling with hands that are rarely ever washed before handling bread; the hands that are used to receive money in between transactions which has been reported in a study by Nurudeen et al. (2014).

Food hygiene is the set of basic principles employed in the systematic control of the environmental conditions during production, packaging, delivery/transportation, storage, processing, preparation, selling and serving of food in such a manner as to ensure that food is safe to consume and is of good keeping quality. Hygiene of the bread sellers was assessed and in this study it was discovered that use of head gear among bread sellers is universally practiced perhaps due to the fact that they were majorly female and it is culturally and religiously inappropriate for most women to leave their head uncovered.

This finding is however at variance with less than half (47%) reported by Nurudeen et al. (2014). Use of apron was reported by none of the bread sellers and by 65% of bakery workers which is similar to reports from study by Nurudeen et al. (2014).

The bread sellers often left their bread exposed granting access to flies which are common in a humid, hot environment. These flies have been shown by various studies to transmit food borne pathogens which can cause food borne illnesses such as Cholera, Campylobacteriosis, *E. coli* gastroenteritis, Salmonellosis, Shigellosis, Typhoid and paratyphoid fevers, Brucellosis, Amoebiasis, and Poliomyelitis (De Jesus et al., 2004; Keiding 1986; WHO, 1997).

Hygiene of bakeries assessed was poor as only a third of bakeries assessed had good hygiene which is similar to findings by Huq et al. (2013) in Bangladesh. This seems to be a common occurrence in developing countries where quality of bread for human consumption is doubtful as premised by the fact that not much is published in literature from developed countries.

Among the bakery workers, 45% wears hands gloves regularly while 15% uses it occasionally and 45% do not see any reason why they should use it. This is also similar

to findings by Chukuezi (2010) where 47% of bakers uses bare hands while working in bakeries, against the standard practice but much higher than the 16.7% reported by Huq et al. (2013). However, the use of gloves should protect the hands of workers during packaging and sealing the nylon and is expected to minimize direct hands contact with finished bread. At Ile Ife, most of the bakeries utilize manual processes of feed, to slicing and packaging. Therefore, non-use of hand gloves compromises bread quality safety and health of people who eat them since bread post production is sold to the residents and travelers having stopovers at Ile Ife.

Conclusions

The hygiene conditions in bakeries, handling of bakeries processes by bakers and attitude of bread vendors is suboptimal and predisposes bread to contamination by pathogenic and non-pathogenic microorganisms. Therefore, regulatory agencies, States ministries of health and environmental health units of local government areas should ensure compliance and adherence of bakeries and bread sellers to the regulations and public health ordinance guiding the approval and monitoring of bakeries as a regulated premise.

There is need for re-orientation of bakeries on the basic ideal practice which has to be enforced to sound the seriousness of government to entrench sound bakery operations and practice in Nigeria. Therefore, all bakery workers should receive training in food hygiene and handling. This should be supported by country-wide health education, training and registration of formal bread vendors to promote compliance with best practices globally.

Conflict of interests

The authors did not declare any conflict of interest.

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Nutritional potential of yam chips (*Dioscorea cayenensis* and *Dioscorea rotundata* Poir) obtained using two methods of production in Togo

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Yam is one of the most staple foods in West African countries and provides an important part of the energetic for people. This study examines chemical composition of *Dioscorea cayenensis* and *Dioscorea rotundata* Poir species dried yam chips obtained using two methods of production in Togo. Three local varieties of yams (*Koukou, Kéki* and *Laboco)* were processed in chips and were sun dried (at 28-30°C) or oven-dried (at 50°C). Nutritive components (carbohydrates, protein fats, mineral salt, vitamin C and anti-nutritional factors) of yam chips were assessed and compared with those of fresh yam. Sugar is a major component of yam chips followed by proteins, vitamin, fats, mineral salt and anti-nutritionals factors. For the same variety of yam, the nutritional quality depends on the method of production followed and the drying methods.

Key words: Yam chips, *Dioscorea cayenensis, Dioscorea rotundata*, nutritional potential.

INTRODUCTION

Yam serves as an important source of carbohydrate and a major source of income in countries where they are cultivated. In 2007, 96% of the worldwide production of yam (52 million tons) was from Africa while 94% of the yam was from West Africa with Nigeria alone producing 71% (http://www.iita.org/yam). Yams are usually processed into dry-yam tubers/slices and flour in West African countries such as Ghana, Benin and Nigeria (Bricas et al., 1997).

Yam tubers are consumed in forms of chunks, flour, chips, fufu and slices, which are obtained from any of the processes of boiling, frying, drying, fermentation, milling, pounding, roasting and steaming (Iwuoha, 2004). The genus *Dioscorea* contains a wide range of yam species used as food. There are many varieties of yam species widespread throughout the humid tropics, the most economically important species which are grown are white yam (*Dioscorea rotundata)*, yellow yam (*Dioscorea*

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cayensis), water yam (*Dioscorea alata),* Chinese yam (*Dioscorea esculenta)* aerial *yam (Dioscorea bulbifera)* and trifolate yam (*Dioscorea dumentorum*) (Ike and Inoni, 2006). White yam (*D. rotundata)* originated from Africa and is the most widely grown and preferred yam species. The tuber is roughly cylindrical in shape; the skin is smooth and brown and has a white firm flesh. A large number of white yams exist with difference in the production and post-harvest characteristics. Yellow yam (*D. cayensis*) derives its common name from its yellow flesh, which is caused by the presence of carotenoids. It is also native to West Africa, the yellow yam has a longer period of vegetation and a shorter dormancy than white yam. In the past, yellow yam and white yam were considered as two separate species but most taxonomists now regard them as the same species, there are over 200 cultivated varieties between them. The ‘’*Kokoro”* variety is important in making dried yam chips*. D. rotundata* which is referred to as dried yam contains a large amount of energy, starch, iron, the smallest amount of dietary fiber, Sn, Ca, P, Mg, Ca and Mn. The protein content is low.

Yam is a good root crop with high energy, acceptable protein and iron content but lowest in Ca and Zn. There is a high concentration of protein and minerals in the peels, which forms about 19% of the tubers. Dried yam is lower than water yam in ash content but high in soluble carbohydrate. Yam also provides protein three times more superior than the one of cassava and sweet potato. Apart from food, yams are also sources of pharmaceutical compounds like saponins and sapogenins, which are precursors of cortisone used medically in the treatment of arthritis and some allergies (Ezeocha and Ojimelukwe, 2012).

The perishability nature of yam due to its high moisture content suggests the need to process it into less perishable products such as yam chip through drying process (Karim et al., 2013). Up till today, this age-old traditional method is still being used for the processing of yam, to dry yam (yam chips) and yam flour. The quality of the chips and flour varies from processor to processor and from location to location (Ojokoh and Gabriel, 2010; Adewale et al., 2014). In Togo, this practice follows two methods: one method is according to Bassar region, the northern of part Togo and Ghana. The second method is according to Est-Mono region, Benin and Nigeria. The objective of the present study was to explore the effects of the two processing methods used in Togo on yam chips qualities, including proximate composition and anti-nutritionals factors content.

MATERIALS AND METHODS

Sampling

Yam tubers of varieties “*Koukou*, *Laboko*, *kéki*” of *Dioscorea cayenensis and Dioscorea rotundata* where collected in April 2008 at Est–Mono and Bassar and dried to yam chips.

Processing of yam to dry-yam

Yam tubers were processed in to chips since May 2008 in the Microbiology Laboratory of Food stuff and Quality Control in the University of Lomé. Two methods described during the survey with farmers were adopted. Three varieties of yam were used (*Laboco*, *Kéki* and *Koukou*).

Method A

This process was used in Bassar region and in northern Togo. Yam tubers were peeled and cut into slices (0.5 to 1 cm of diameter and 5 to 10 cm of length). After washing, slices were divided into two lots. The first one was sun-dried at 28-32°C and the second was oven-dried at 50°C.
Samples process in method A (method of Bassar region):

KoBSRS: Chips of ‘’*koukou”* variety processed following method of Bassar with sun drying
KoBSRE: Chips of ‘’*koukou*’’ variety processed following method of Bassar with oven drying
KeBSRS: Chips of ‘’*kéki*’’ variety processed following method of Bassar with sun drying
KeBSRE: Chips of ‘’*kéki*’’ variety processed following method of Bassar with oven drying
LaBSRS : Chips of ‘’*Laboco*’’ variety processed following method of Bassar with sun drying
LaBSRE: Chips of ‘’*Laboco*’’ variety processed following method of Bassar with oven drying.

Method B

This process was generally used in the Est-Mono region. Yam tubers were first peeled and cut into slices (0.5 to 1 cm of diameter and 5 to 10 cm of length) and cooked at 60-70°C. Cooked slices were divided into two lots. The first was oven dried at 50°C and the second were sun-dried at the ambient air (28-32°C).
Samples process in method B (method of Est-Mono region):

KoEMNS: Chips of ‘’*koukou”* variety processed following method of Est-Mono with sun drying
KoEMNE: Chips of *‘’koukou”* variety processed following method of Est-Mono with oven drying
KeEMNS: Chips of *‘’kéki”* variety processed following method of Est-Mono with sun drying
KeEMNE: Chips of ‘’*kéki*’’ variety processed following method of Est-Mono with oven drying
LaEMNS: Chips of *‘’Laboco”* variety processed following method of Est-Mono with sun drying
LaEMNE: Chips of ‘’*Laboco”* variety processed following method of Est-Mono with oven drying.

Carbohydrate content determination

Total sugar

One gram of yam flour was dissolved in 10 mL of dimethylsulfoxyde (DMSO: (C_2H_6OS) 25% v/v). After 15 min of incubation in bain-marie (90-100°C), 0.1 mL of the mixture was diluted into 9.9 mL of distilled water, 0.5 mL of the last mixture were added to 0.5 mL of phenol (5%). And then, 2 mL of H_2SO_4 (75%) were added. The absorbance was read at 492 nm (Fox and Robyt, 1991).

Starch

0.1 g of yam chip flour was dissolved in 5 ml of KOH 1 N. After

homogenization, the unit was neutralized by 5 ml of HCl 1 N. The mixture thus obtained was allowed to boil for 15 min in a water-bath. After filtration, volume was adjusted to 10 mL. 0.05 mL were diluted to 5 mL with 4.85 mL of distilled water and 0.1 mL of reagent (I_2/KI) and incubated for 10 min. The absorbance of samples was carried out at 580 and 720 nm (Jarvis and Walker, 1993).

Fats content determination

Lipid content was determined using a Soxhlet apparatus and hexane reagent. 10 g of yam chip flour were weighed and were putted in a balloon. Vacuum was given before introducing 175 mL of hexane. The weight of lipids extracted was obtained by difference between the final weight P1 after evaporation of solvent and drying of the balloon and the initial weight of the empty balloon (PO).

Proteins content determination

The protein content was determined by the Kjeldahl method (AOAC, 2000) according to the following protocol:

A mixture of two catalyst tablets, 12 mL of concentrated sulfuric acid, pumice stone and 1 g of yam chips was placed in a fume hood digestion unit at 420°C for 3 h and a clear liquid was obtained. This product was diluted with 50 mL of distilled water and 75 ml of sodium hydroxyde solution 38% and then distilled in 25 mL of another mixture consisting of 4% boric acid, 25 mL (w/v) of 1-methyl red (w/v) of 1% bromocresol green (w/v) and sodium hydroxide 4% (W/V). The product obtained was titrated with 0.1 N hydrochloric acid solution until the color changes from blue to pale pink. The protein content wass expressed as a percentage:

$$\text{Proteins (\%)} = \frac{1.401 \times 6.25 \times (Ve-Vb) \times T}{\text{Weight of sample in grams}}$$

T = Concentration of HCl solution; Ve = volume of hydrochloric acid used to titrate the sample (containing chips); Vb = volume of hydrochloric acid used to titrate the blank containing no chips to titrate the blank; 6.25 = the nitrogen conversion factor protein; 1.401 = constant.

Mineral content determination

Ten grams of yam chips were incinerated in a furnace (Prolabo Volca V50) at 550°C. After incineration, ash was dissolved into 100 mL of distilled water and shaked for 30 min. The solution obtained was filtered on Whatman paper No. 1. Mineral content was obtained using ionic chromatography and molecular spectro-photometry of absorption.

Ascorbic acid content determination

The ascorbic acid content of samples was determined by the volumetric method using Tillmans reagent (Sawadogo, 1993). 2.5 g of yam chip flour were dissolved in 50 mL mixture of acid solution (metaphosphoric 5%/acid acetic 10%). After 1 h of agitation, the mixture obtained was filtered. The proportioning of the vitamin in the material was carried out by using 2,6-Dichloro indol phenol (2,6-DIP) solution (0.5 mg/mL). Then, 25 mL of the filtered solution were titrated by the 2,6-DIP until the red color at the point of equivalence persists for 5 s (dryness). The quantity of vitamin was given using the reference established by the proportioning of 10 mL of pure ascorbic acid.

Determination of anti-nutritional factors (ANF)

Total oxalate

The total oxalate was determined by the method of Day and Underwood (1986). 1 g of yam chip flour was dissolved in 75 mL of sulfuric acid 15 N. The mixture was homogenized for one hour and filtered on Whatman No.1 paper. 25 mL of the filtrated solution were titrated with 0.1 N of permanganate of potassium.

Phytic acid

Phytic acid was determined according to the method of Reddy and Love (1999). This method consists of adding 4 g of flour of yam ships to 100 mL of hydrochloric acid 2% under magnetic agitation for 5 h. After filtration, 25 mL were added to 5 mL of ammonium thiocyanate 0.3%. The mixture was titrated with ferric chloride until the yellow color brown was obtained, persisting for 5 min.

Tannins

Tannins are proportioned by the method of Trease and Evans (1978). 1 mL of methanolic extract was treated with 5 mL reagent of Folin-Dennis in basic medium. The absorbance of the mixture was read at 760 nm. The content of tannins was given using a curve standard built starting from a range of concentration of gallic acid.

Saponins

Saponins was carried out according to the method of Birk et al. (1968) modified by Hudson and El Difrawi (1979). According to this method, 1 g of flour of sample was dissolved in 20 ml of the ethanol 20% (ethanol-water) under the magnetic agitation for 12 h at 55°C. The solution was filtered on Whatman No.1 and adjusted with 40 ml then 20 mL of the filtered solution was added to diethyl ether followed by a vigorous manual agitation. The aqueous phase was adjusted to pH 4.5 with a hydrochloric acid solution 0.2 N. 60 mL of the solution of N butanol were added to this phase and the unit was washed with 10 mL of NaCl 5%. The organic phase was then evaporated to have the content of saponins. The saponin was determined by the difference between the weight of the container after evaporation and the tare weight of the same container.

Statistical analysis

All analyses were carried out in triplicates and the data were subjected to analysis of Epi Info 3.5.1 for windows which was the statistical software used.

RESULTS AND DISCUSSION

Proximate composition of yam chip

The proximate compositions of yam chips manufactured by Bassar and Est-Mono processing, sun and oven dried are presented in Table 1 for carbohydrate, fat, proteins, vitamin C content, in Table 2 for anti-nutritional factors content and Table 3 for mineral content

Carbohydrate content of the chips of ‘’*Koukou”* variety resulting from method of Bassar was higher to a significant degree ($P<0.05$) than those produced with

Table 1. Proximate composition of yam chips product from *''koukou'','' kéki*''and ''*Laboco''* (g/100 g).

Yam varieties	Samples processed	Protein (g/100)	Carbohydrate (g/100)	Fat	Vitamin C (mg/100)
''Koukou''	KoBSRS	3.47±0.2	62.61±0.05	0.11±0.02	1.19±0.39
	KoBSRE	5.71±1.23	86.57±2.72	0.14±0.02	0.71±0.07
	KoEMNS	4.05±0.07	66.40±0.07	0.19±0.01	0.84±0.21
	KoEMNE	4.34±0.45	67.79±0.04	0.1±0.09	0.73±0.07
''Kéki''	KeBSRS	4.68±0.06	44.99±0.08	0.11±0.01	0.94±0.01
	KeBSRE	4.89±0.55	55.05±0.04	0.1±0.02	0.83±0.07
	KeEMNS	3.74±0.13	37.75±0.11	0.1±0.01	0.65±0.05
	KeEMNE	4.68±0.07	43.45±0.11	0.11±0.02	0.61±0.10
''Laboco''	LaBSRS	5.58±0.16	44.21±0.24	0.09±0.05	0.05±0.00
	LaBSRE	5.43±0.23	42.40±0.06	0.13±0.02	0.2±0.02
	LaEMNS	03.6±0.28	48.85±0.11	0.13±0.01	0,3±0.01
	LaEMNE	3.92±0.52	46.62±0.09	0.13±0.06	0,1±0.03

Table 2. Anti-nutritionals factors content of yam chips (mg/100).

Yam varieties	Treatment	Oxalate	Phytic	Saponin	Tannins
''Koukou''	KoBSRS	3,3±0.01	0.15±0.01	0.7±0.02	0.38±0.01
	KoBSRE	1.4±0.02	0.16±0.03	0.2±0.01	0.32±0.03
	KoEMNS	1.65±0.05	0.26±0.01	0.57±0.01	0.31±0.01
	KoEMNE	0.82±0.01	0.19±0.05	0.77±0.1	0.3±0.06
''Kéki''	KeBSRS	0.93±0.05	0.22±0.08	0.52±0.02	0.21±0.01
	KeBSRE	0.88±0.03	0.24±0.01	0.44±0.02	0.08±0.03
	KeEMNS	0.88±0.01	0.13±0.05	0.31±0.01	0.02±0.01
	KeEMNE	0.77±0.03	0.13±0.01	1.03±0.06	0.87±0.02
''Laboco''	LaBSRS	0.79±0.05	0.53±0.01	0.8±0.02	0.13±0.03
	LaBSRE	0.56±0.02	0.22±0.03	0.42±0.01	0.12±0.03
	LaEMNS	0.66±0.07	0.2±0.05	0.46±0.01	0.13±0.03
	LaEME	0.65±0.05	0.41±0.01	0.72±0.02	0.13±0.01

the method of Est-Mono, Moreover, oven drying results to less loss in carbohydrate-than sun drying (Table 1).

With regards to *''keki''* variety, the chips resulting from method of Bassar associated with oven drying causes significant degree less loss in carbohydrate than those obtained from method of Est-Mono (Table 1)

For the ''*Laboco*'' variety, the chips resulting from method of Est- Mono associated with oven drying had less loss in carbohydrate content than the other (Table 1).

It emerges from these results that the carbohydrate content of the chips were influenced by varietal factor and transformation. In view of these results, to avoid lost of carbohydrate during the transformation of yam into chips, it is proper to choose Bassar method for *koukou* and *''Kéki''* yam varieties and Est-Mono method for *''Laboco''* variety.

In general, it is desirable to use the oven drying because it is done quickly without allowing microorganisms to grow and alter nutrients. These results are in agreement with earlier reports of Okigbo and Nwakammah (2005) and Adewale et al. (2014). However, carbohydrate content were contrary to those obtained by Ojokoh and Gabriel (2010) and Adewale et al. (2014) with regards to *''Laboco''* and *''kéki''* varieties.

Proteins content

According to proteins content**,** the results indicate that:

Table 3. Mineral content of yam chips product from ‘’*koukou”, ” keki”* and ‘’*Laboco”* (mg/100 g dry basis).

Yam variety	Treatment	Fe	PO_4	Na	K	Mg	Ca	Cu	Zn
‘’Koukou”	KoBSRS	0.37±0.01	1859.53±4.62	105.7± 2.41	7845.2±51.03	145.82±0.97	16.69±0.11	13.53±0.02	15.48±00
	KoBSRE	0.34±0.1	2695.4±59.22	38.85±1.56	8840.8±41.238	142.1±2.49	13.6±0.29	4.2±1.69	13.19±2.19
	KoEMNS	0.5±0.01	2661.16±75.5	180.32±9.63	7314.37±10.54	15.88±1.42	20.09±0.18	12.21± 2.46	90.14±4.93
	KoEMNE	0.3±0.10	2024±1.16	440.29±8.78	7763.85±29.04	139.98±0.53	17.71±0.29	3.76±0.01	5.64±0.25
‘’*Kéki”*	KeBSRS	0.37±0.01	65.11±2.40	61±0.91	7679.46±27,48	315.66±0,36	23.57±0.43	6.82±3.21	28.02±7.45
	KeBSRE	0.23±0.01	36.63±0.25	26.83±3.46	6050.42±13.13	857.15±1.02	11.70±0.08	2.53±0.01	17.71±3.57
	KeEMNS	0.43±0.01	306.41±2.48	232.29±4.01	7222.56±72.96	131.09±1.45	17.34±0.07	4.81±2.26	4.81±2.26
	KeEMNE	0.32±0.01	32.98±0.33	560.05±4.01	6519.37±47.63	123.01±1.68	17,47±0.01	7.44±00	16.74±2.74
‘’Laboco”	LaBSRS	0.56±01	1678±0.81	56.28±1.24	6008.14±31.04	272.31±1.61	17.97±0.18	2.53±00	57.51±9.03
	LaBSRE	0.36±01	1834.71±1.22	136.71±2.11	7266.33±15.83	246.97±5.30	17.34±0.07	4.26±00	30.9±7.28
	LaEMNS	0.58±0.,03	1494.67±3.19	67.67±0.06	5488.95±35.21	260.75±13.68	25.1±1.13	6.01±2.84	24.06±2.25
	LaEMNE	0.47±0.01	1415.33±2.56	107.06±0.90	6683.2±39.22	427.16±2.67	30.55±0.58	9.3±2.25	22.9±3.23

1. Regarding “*koukou*” variety, the method of Bassar combined with oven drying would preserve the content of proteins better with significant degree ($P<0.05$) than the method of Est-Mono.
2. For ‘’*kéki”* variety, method of Bassar combined with oven drying caused no significant degree less loss of proteins. With ‘’*kéki”* variety, no influence of method production and drying method on the protein content was seen.
3. *‘’Laboco”* variety gave the same observations like ‘’*koukou*” variety.

The protein contents obtained were contrary to those obtained by Oyeyiola et al. (2014), Adewale et al. (2014) and Ojokoh and Gabriel (2010) who obtained lower protein content of yam chips.

Fats content

The results reveal that the content of fat is very low and which is about 0.1, varietal influence and transformation process are not significant (Table 1). This content were in agreement with earlier reports (Oyeyiola et al., 2014) but were contrary to those reported by Adewale et al. (2014) and Ojokoh and Gabriel (2010) who obtained higher content of fat.

Vitamin C content

The results indicated that vitamin C content varies from 0.71 to 1.19 mg for ‘’*koukou”* chips, 0.6 to 0.94 mg for chips of ‘’*kéki”* and 0.05 to 0.3 mg for ‘’*Laboco”* chips. Process and variety did not influence significantly vitamin C content (Table 1). Vitamin C content was contrary to those obtained by Gbolagade et al. (2011) who reported higher content of ascorbic acid (4.44 -6.46 mg/100 g).

Anti-nutritionals factors content of yam chips

The edible, matured yam does not contain any anti-nutritional factors however, bitter components tends to accumulate in immature tuber tissues of *D. rotundata* and *D. cayenesis*. They may be attributed to polyphenols or tannin-like compounds. Some workers (Asuzu and Undie, 1986; Okeola and Machuka, 2001; Ajibade et al., 2005) have identified the presence of some anti-nutritional factors (ANF) such as alkaloids, flavonoids, saponins, lectin, trypsin inhibitors, phytate and oxalate in the African yam. List of the anti-nutritional factors in the African yam bean includes trypsin inhibitor, haemagglutinating, tannic acid (tannins), phytic acid, oxalate (Apata and Ologhobo, 1997). In addition to the above list of ANF are α-galactosides (stachyose) and lectin (Oboh et al., 1998).

For ‘’*Koukou”* variety, the oxalates deteriorate more with method A with sun drying, whereas the content of phytate does not vary to a significant degree according to the yam chips methods of production used ($P > 0.05$). The method of Est-Mono (MN) associated with oven drying and those of Bassar (BSR) with sun drying caused more loss of total phenols. The content of tannins and

saponins varies less with the method of Est-Mono associated with sun drying.

As compared to ‘’*keki”* variety, the oxalates content decreases with method of Est-Mono associated with sun and oven drying (50°C) and the drying oven with 50°C. The phytate content remains constant. Total phenols and saponins content deteriorated by the bleaching with oven drying. The content of tannins varies less with the method of Bassar (BSR) associated with sun and oven drying, whereas the method of Est-Mono (EMN) with sun and oven drying caused more deterioration in yam ships

For ‘’*Laboco”* variety, the phytate and oxalate content of yam ships decreases less with the method of Bassar (BSR) associated with sun drying, whereas it decreases much more with method of Est-Mono (EMN) with sun and oven drying (50°C). The tannins and saponins contents remain constant with the other experimental conditions. However, these variations observed between the various treatments are not significant ($P > 0.05$).

The anti-nutritionals factors are substances of reserves of plants which complex some nutrients like rock salt, proteins and reduce their biodisponibility during digestion. It is the case of oxalates, the phytate, saponins, tannins and total phenols, etc. These results of content of anti-nutritional factors were lower than those reported by other authors on the yam and these studies relate to only the fresh tubers. Thus, Jau-Tien et al. (2009) found saponin values in the yam from 247 to 619 µg/g. FAO (1991) found 637 mg/100 g for the phytate.

Rock salt content of yam ships

The samples of yam ships contain a broad rock salt range the cations (Mg, Na, K and Ca), anions (Cl, SO_4, PO_4, Br, NO_2 and NO_3) and oligo elements (Zn, Cu and Fe). Only the principal ones are illustrated here.

For ‘’*Koukou”* variety, the most representative oligo elements were zinc with contents between 5.64 and more than 90 mg per 100 g of dry matter, followed by copper with 3.76 to 13.53 mg. The content of iron is very weak and varies from 0.3 to 0.5 mg per 100 g.

Among the anions, phosphorus is most significant with contents from 1859 to 2695 mg per 100 g whereas among the cations, potassium is most significant with values ranging from 7314 to 8840 mg per 100g. According to ‘’*Kéki”* variety, potassium and phosphorus are the principal biogenic salts with contents respectively from 6050 to 7679 mg and 1625 to 2634 mg/100 g. Oligo significant elements were Zn with content varying from 8 to 28 mg/100 g followed by copper whose values were from 2.53 to 7.44 mg/100 g. Concerning ‘’*Laboco”* variety, potassium and phosphorus are the principal biogenic salts with contents respectively from 5488 to 7266 mg and 1415 to 1834 mg/100 g. The Mg, Na and Ca were slightly represented in all the samples of the variety. Oligo significant elements were Zn whose content varies from 22.9 to 5.51 mg /100 g followed by the copper whose values went from 2.53 to 9.3 mg/100g. Iron is the oligo element most slightly represented in the studied yam chips.

This study revealed that yam chips contain majorly potassium and phosphorus. FAO (1970) showed mineral salt dominating in yam is potassium thus, yam could cover a substantial part of the requirements of manganese and phosphorus for the adults. Our results were contrary to those obtained by Agbor-Egbé and Streche (1995) which found 65-125 mg/100 g of phosphorus; 8-36 mg for calcium; 590-1740 mg for potassium; 19-72 mg for magnesium.

Conclusion

The proximate compositions of yam chips for each yam variety, for drying and processing methods showed that these products have an important nutritional potential. Outside carbohydrates, protein, fat, vitamin C, minerals, the chips also contains anti-nutritional factors. There are no significant effects of processing ($P>0.05$) on lipid, vitamin C, mineral and anti-nutritional factors. In contrast, yam variety, drying and processing methods have significant effects on carbohydrates and proteins content. This can be exploited to provide food for diabetics, as an ideal food source of carbohydrates without affecting their disease.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Microbiological quality of food sold by street vendors in Kisangani, Democratic Republic of Congo

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Food sold by roadside vendors was compared with French Standards (AFNOR, 1996) in order to determine the microbiological quality of cooked meals. Forty-two samples of fresh and smoked fish and bushmeat were collected between March and May 2013 in Kisangani (The Democratic Republic of Congo), and analysed. Analysis of variance (ANOVA) and post-hoc Tukey tests were used to analyse the level of contamination according to the category of cooked food. Results were significant at the 0.05 threshold. For all three categories of dishes, the average bacterial counts (total aerobic plate count) were above the critical threshold: bushmeat (6.70 ± 0.15 log cfu g^{-1}), smoked fish (6.44 ± 0.09 log cfu g^{-1}) and fresh fish (5.97 ± 0.33 log cfu g^{-1}). The difference in levels of contamination between groups was statistically significant ($p < 0.05$, ANOVA test). Bushmeat was the most contaminated category ($p < 0.05$, Tukey test). Most of the 42 samples were of unsatisfactory microbiological quality: 38 (90.5%) due to total aerobic plate count; 24 (57.1%) to *Salmonella* sp. and 21 (50%) to *Staphylococcus aureus*. The application of hygienic practices during the preparation and sale of street food could reduce the microbial risk. Such training is highly recommended for roadside food vendors.

Key words: Microbiological quality, street food, food contamination, bacteriological count, Kisangani, Democratic Republic of Congo.

INTRODUCTION

Consumption of food prepared and sold by street vendors is growing rapidly in developing countries. Street food, particularly beverages and cooked snacks or meals, are sold in public places such as roadsides, markets and similar locations (Muyanja et al., 2011). They represent an important part of the daily diet for millions of low- and middle-income consumers in urban areas (FAO, 2003).

Street food plays an important socio-economic role: it provides a regular source of income for millions of low- or unskilled men and women in developing countries (FAO, 2010). In Indonesia, street food also contributes to local economic growth. This informal activity generates an

annual estimated income of US$ 1 million in taxes (FAO, 2010; Cohen, 1986).

In the Democratic Republic of Congo (DRC), meals sold by the street vendors (called *Malewa* in Lingala, a local dialect) are very popular and are primarily consumed in Kisangani. The menu usually consists of a meal cooked in sauce, either based on beans (*madesu* in Lingala), meat (smoked or fresh) or fish (fresh or smoked). These stews are often accompanied by cassava-based starches (*fufu*), mashed plantains (*lituma* in Swahili) or rice (*loso* in Lingala). These street meals are sold in small restaurants next to public places (markets, schools, hospitals), along main roads or on the ground in the street.

Salmonella sp. and *Staphylococcus aureus* are the most common foodborne pathogens and are responsible for food poisoning and food-related infections (Akbar and Anal, 2013). Manguiat and Fang (2013) showed that contamination of street food in the Philippines was mainly due to *S. aureus*, *Salmonella* sp. and *Vibrio cholerae*, while *Salmonella* sp. was isolated in 15% of samples from grilled pork and chicken.

According to a study carried out in Egypt by Moustafa El-Shenawy et al. (2011), 24% of street food was infected with *Listeria* sp. This bacterium is responsible for listeriosis and is manifested by septicaemia, meningitis and intrauterine infections, leading to spontaneous abortion in pregnant women. In Nigeria (Omemu and Aderoju, 2008), Kenya (Muinde and Kuria, 2005) and South Africa (Holy et al., 2006) researchers have shown that the overall hygiene of pre-prepared street food is poor.

One strategy to reduce microbial risk in the consumption of street food is the World Health Organisation resolution AFR/RC53/R5/2003 (WHO, 2003) that recommends capacity strengthening of health authorities in signatory countries to control the hygienic quality of street food, which must comply with international standards.

However, relation to hygiene at the locations where food is sold (rubbish in the streets, blocked drains, the hygiene of cooked street food in Kisangani (D. R. Congo) remains questionable and addition to problems asked) and quality checked by Health Inspectors (and other health services) are often irregular.

Although, the phenomenon of the sale of street food is a major public health problem (Rane, 2011), unlike other African countries (Muyanja et al., 2011; Barro et al., 2006; Diouf, 1992) there are very few international studies of the situation in the DRC. A rare example is a study by the Food and Agriculture Association (FAO, 1996) in Kinshasa, which identified the following variables: consumer typology, the role of female sellers, daily revenue earned through the sale of street food and the support of United Nations in this sector. However, there was no evaluation of the hygiene of cooked street food.

The objective of this study was therefore to evaluate the microbiological quality of meals cooked in sauce (bushmeat, smoked and fresh fish) sold in the surroundings of the central market in Kisangani, DRC. Total aerobic plate count at 30°C (as indicator of hygienic quality of cooked meals), *S. aureus* and *Salmonella* sp. (as pathogens) were investigated. The contamination level of the different categories of meals was also assessed in order to identify the dishes with high microbial risk.

MATERIALS AND METHODS

Sample collection

Cooked meals sold by street vendors were collected by a simple random sampling method from around the central market in Kisangani (DRC). The sampling period (three months) spanned from March to May 2013. A total of 42 sites (restaurants) were investigated. The number of consumers per day ($n \geq 30$) was a determining factor in the choice of sampling sites. Thus, the samples (n=42) were randomly collected at noon for microbiological analysis. Samples of meals cooked in sauce consisted of bushmeat (n=16); smoked fish (n=18) and fresh fish (n=8). The samples (weighing approximately 100 g) were collected in sterile borosilicate glass bottles which were then placed in a cooler. Each sample was given an alpha-numeric code that represented the sample number and the meal category. Samples were immediately brought to the laboratory for microbiological analyses.

Microbiological analysis

Microbiological analyses were performed according to the protocol described by Manguiat and Fang (2013). A 25 g sample was diluted in 225 ml of peptone water (Merck KGaA, Germany). The mixture was macerated in a sterile Stomacher bag for two minutes. The supernatant representing the stock solution was then diluted up to 10^{-6} using sterile physiological water and the corresponding plate agar media were seeded. Counts (colony-forming units per gram) were expressed as a logarithm (log cfu g^{-1}).

Total aerobic plate count at 30°C

Total aerobic plate count (TAPC) was determined by the pour plate method using Nutrient Agar (Liofilchem, Italy). Then, one millilitre of the 10^{-3} dilution was introduced aseptically into the Petri dish and was incubated at 30°C for 72 h. After incubation, the colonies were counted. The number of colony-forming units (cfu) obtained was multiplied by the reciprocal of the dilution.

Staphylococcus aureus

S. aureus plate count was determined by the pour plate method using Mannitol Salt Agar (Biomérieux, France). After thoroughly

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Table 1. Hygienic quality of street food cooked in sauce (Central Market, Kisangani, the DRC).

Hygienic quality	Critical threshold AFNOR (1996) $log_{10}cfu\ g^{-1}$	Fish				Meat		Total (n = 42)	
		Fresh (n = 8)		Smoked (n = 18)		Bushmeat (n = 16)			
		n	%	n	%	n	%	n	%
TAPC at 30°C									
Satisfactory	<5.5	1	12.5	0	0	0	0	1	2.4
Acceptable	5.5–6.0	3	37.5	0	0	0	0	3	7.1
Unacceptable	> 6.0	4	50	18	100	16	100	38	90.5
***Salmonella* sp.**									
Satisfactory	0	2	25	9	50	7	43.75	18	42.9
Unacceptable	> 0	6	75	9	50	9	56.25	24	57.1
***S.aureus* (coagulase+)**									
Satisfactory	< 2	1	12.5	8	44.4	0	0	9	21.4
Acceptable	2–3	7	87.5	4	22.2	1	6.25	12	28.6
Unacceptable	> 3	0	0	6	33.3	15	93.75	21	50

TAPC = Total aerobic plate count at 30°C; *S. aureus* coagulase + = *S. aureus* coagulase-positive; $log_{10}cfu\ g^{-1}$ = colony-forming units per gram of food (logarithm).

mixing the food sample (25 g) in peptone water (225 mL) in 1: 10 dilution, the inoculum (1 ml) was introduced aseptically into the Petri dish. Incubation lasted for 24 h at 37°C. Mannitol positive colonies, surrounded by a yellow halo were counted. Coagulase tests on *S. aureus* strains were carried out using conventional techniques (AFNOR, 2004).

Salmonella sp.

The sample was enriched by incubating the parent suspension for 18-24 h at 37°C. Then, one millilitre of the (enriched) parent sample was added to 9 ml of Selenite broth (Merck KGaA, Germany). This was incubated at 37°C for 24 h. Positive samples (cloudy broth) were cultivated in Salmonella Shigella Agar (Biomérieux, France). The sample taken from the positive Selenite broth was cultured by the streaking method on the Salmonella Shigella agar medium, after which it was incubated at 37°C for 24 h. Translucent *Salmonella* sp. colonies with a black centre appeared and were counted.

Interpretation and statistical analysis

Logarithmic bacterial counts ($log_{10}cfu\ g^{-1}$) were compared against French standards in order to determine the hygienic quality of the cooked dishes (Table 1). An analysis of variance (Anova) compared the level of contamination between categories of dishes. In cases where a significant difference was found between cooked food categories, the Tukey test (post-Anova test) was used to determine the most contaminated food categories (Walpole et al., 2002). Microsoft Excel (2007 edition) and R (version 2.15) software was used for statistical analyses. The *p*-value was considered to be significant at the 0.05 threshold.

RESULTS

The hygiene of dishes cooked in sauce was evaluated (satisfactory, acceptable and unacceptable) according to French standards (AFNOR, 1996) by an assessment of the TAPC, *S. aureus* and *Salmonella* sp. (Table 1).

Food quality: TAP count at 30°C

Out of the total of 42 meals analysed, 38 (90.5%) were considered to be of unsatisfactory quality following the TAPC bacterial analysis (Table 1). The difference in level of contamination was statistically significant according to the meal category ($p<0.05$, ANOVA test) (Table 2). Bushmeat was the most contaminated ($p<0.05$, Tukey's post-ANOVA test) (Table 2).

Food quality: *Salmonella* sp.

Out of a total of 42 samples, 24 (57.1%) were non-compliant with French standards (Table 1) following the bacterial analysis. Fresh fish samples (n=8) were the most contaminated category of food with *Salmonella* sp. (2.4 ± 1.5 log cfu g^{-1}, Table 3). A comparison of bacterial counts of *Salmonella* sp. in the three categories of dishes showed no statistically significant difference ($p> 0.05$, ANOVA test, Table 3).

Food quality: *S. aureus*

Out of the 42 dishes street analysed, 21 (50%) were declared unsatisfactory with respect to French standards for cooked foods (Table 1) and unfit for consumption

Table 2. TAPC bacterial count (log cfu g^{-1}) according to meal category.

Meal category	TAPC at 30°C(log cfu g^{-1})		Anova			Tukey
	$\bar{X}$	SD	df	f	p^1	p^2
Fresh fish (n = 8)	5.96	0.33	2	45.03	<0.05*	<0.05*
Smoked fish (n = 18)	6.44	0.09				
Bushmeat (n = 16)	6.71	0.15				
Average	6.37	0.19				

$\bar{X}$ = mean bacterial count; SD = standard deviation; n = number of dishes analysed; * = significant at p = 0.05 (difference in contamination levels between categories); p^1 = p calculated using the ANOVA Test; p^2 = p calculated using Tukey's post-ANOVA test; f = F-test of the equality of two variances.

Table 3. *Salmonella* sp. count (log cfu g^{-1}) according to meal category.

Meal category	*Salmonella* sp. (log cfu g^{-1})		Anova test		
	$\bar{X}$	SD	df	f	p
Fresh fish (n = 8)	2.4	1.5	2	0.915	> 0.05
Smoked fish (n = 18)	1.5	1.5			
Bushmeat (n = 16)	1.6	1.5			
Average	1.8	1.5			

$\bar{X}$ = mean bacterial count; SD = standard deviation; df = degrees of freedom; f = F-test of the equality of two variances; p = non-significant.

Table 4. *S. aureus* count (log cfu g^{-1}) according to meal category.

Meal category	*S. aureus* (log cfu g^{-1})		Anova			Tukey
	$\bar{X}$	SD	df	f	p^1	p^2
Fresh fish (n = 8)	0.4	1.0	2	13.92	<0.05*	<0.05*
Smoked fish (n = 18)	1.7	1.5				
Bushmeat (n = 16)	3.3	0.8				
Average	1.8	1.1				

$\bar{X}$= mean bacterial count; SD = standard deviation; df = degree of freedom; * = value is significant (difference in contamination between cooked food categories); p^1 = p-value calculated from the ANOVA test; p^2 = p-value calculated from Tukey's post-ANOVA test.

following the analysis of *S. aureus*. A comparison of *S. aureus* bacterial counts in the three categories of meals was statistically significant (p<0.05, ANOVA test, Table 4). Bushmeat was the category most affected by *S. aureus*, demonstrated by the assessment of the bacterial count (3.3 ± 0.8 log cfu g^{-1}, Table 4) using the post-Anova Tukey Test (p< 0.05).

DISCUSSION

The study demonstrated that the popularly sold street foods (cooked meals) were been contamined by TAPC at 30°C, a microbiological indicator of hygien, demonstrated that the popularly sold street food (cooked meals) were contamined. Food pathogens (*S.aureus and Salmonella sp.)* were also found. This situation puts the health of the population at risk. According to Rane (2011) and Ghosh et al. (2007), street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature.

Food quality: TAPC at 30°C

AFNOR Standards (AFNOR, 1996) are used in the DRC

for microbiological food evaluation and provide the criteria for the microbiological evaluation of samples. Studies carried out in the Philippines on grilled pork (Manguiat and Fang, 2013), in South Korea on grilled meat (Cho et al., 2011) and in Nigeria on roasted chicken (Ologhobo et al., 2010) showed an average TAPC of ≤ 6.0 log cfug^{-1}. This count is similar to that observed in our study (6.37±0.19 log cfu g^{-1}, Table 2) and suggests non-compliance with good hygiene practices during the handling, cooking and storage of street food in Kisangani, DRC.

Food quality: *Salmonella* sp.

The prevalence of *Salmonella* sp. (57.1%, Table 1) in Kisangani was very high as compared to that of, for example, in Mexico (5%) (Estrada-Garcia et al., 2004). According to Rane (2011), the contamination of street food due to *Salmonella* sp. can be explained by the use of dirty dishwater (from dirty dishes) or lack of good hygiene practices of vendors when handling street food.

Food quality: *S. aureus*

In Mexico, Diaz-Lopez et al. (2011) detected *S. aureus* in 4 out of 43 (9.3%) street dishes. According to Guven et al. (2010) and Harakeh et al. (2005) meals prepared in the street provide a suitable culture medium for the emergence of *S. aureus* strains that are resistant to multiple antibiotics. These antibiotic-resistant strains are thus transmitted to humans through eating contaminated street food.

Conclusions

This study on the hygienic quality of cooked meals sold in the main public places in Kisangani (DRC) showed that these foods are mostly unfit for consumption and present a significant risk of food poisoning to consumers.

With the proliferation of street restaurants in Kisangani, this situation puts the health of the population at risk. This should be a wake-up call for those responsible for health services to carry out regular quality checks and ensure compliance with best hygiene practices for the safety of cooked food sold in the streets of Kisangani.

The application of these measures could reduce morbidity from diarrheal diseases related to contaminated cooked meals sold by street vendors. A training programme in good hygiene practices (food handling and sale) is highly recommended for these vendors.

Conflict of interests

The authors did not declare any conflict of interest.

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Effect of solar drying methods on total phenolic contents and antioxidant activity of commonly consumed fruits and vegetable (mango, banana, pineapple and tomato) in Tanzania

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The effects of solar drying methods [Cabinet direct (CDD), cabinet mixed mode (CMD) and tunnel (TD) drying] on total phenolic content (TPC) and antioxidant capacities of commonly consumed fruits and vegetable in Tanzania were investigated. The TPC and FRAP in mangoes (*Mangifera indica* cv. *Dodo*, *Viringe* and *Kent*), bananas (*Musa acuminata*, cv. *Kisukari*, *Kimalindi* and *Mtwike*), pineapples (*Ananas comosuss* cv *Smooth cayenne*) and tomatoes (*Lycopersicum esculentum* cv. *Tanya*, *Cal J* and *Onyx*) were evaluated using Folin-Ciocalteu reagent and ferric reducing antioxidant power (FRAP) methods, respectively. There were significant ($p<0.05$) variations in TPC (mg GAE/100 g DM) and FRAP (µmol/100 g DM) among the fresh fruit and vegetable samples. The highest TPC was in tomato (476.6±8.6 - 538.9± 1.4) and lowest in banana (139.3±2.3 - 189.2±2.7). Drying methods had significant ($p<0.05$) effect on TPC and antioxidant values of the samples. All fresh samples had higher TPC levels but declined significantly in dried samples with the exception of tunnel dried tomatoes. Among the dried samples, the tunnel dried samples had less TPC loss (6-16%) than the cabinet dried samples (17-42%). The cabinet direct and mixed mode samples were not statistically different ($p>0.05$) from each other. However, tunnel dried samples had less FRAP loss (6-13%) which were statistically different ($p<0.05$) from the cabinet dried samples (14-56%). Percentage TPC and FRAP recoveries (%) differed significantly ($P<0.05$) between the varieties within the fruits/vegetable for both drying methods. A strong correlations between TPC and FRAP in both fresh ($R^2= 0.970$) and dried samples ($R^2=0.8636$) suggests that solar drying methods have significant effects on total phenolic contents and antioxidant activities of fruits and vegetables with tunnel drying method giving significantly less effects.

Key words: Mango, banana, pineapple, tomato, solar drying, total phenolic content (TPC), antioxidant activity, FRAP.

INTRODUCTION

Fruit and vegetables are both major food products and key ingredients in many processed foods (Jongen, 2007). They contain many essential vitamins, minerals, fibre and phytochemicals such as phenolic compounds and

caretonoids, many of which are antioxidants (Yahia and Berrera, 2009). Various epidemiological studies have demonstrated a strong correlation between adequate consumption of fruits and vegetables with reduced risk of some major diseases such as cardiovascular, diabetes, hypertension, certain types of cancer and some of the degenerative diseases (Segura-Carretero et al., 2010). It has been reported that, up to 2.7 million lives could potentially be saved each year with sufficient intake of fruits and vegetables (WHO/FAO, 2003). The protective role against mutagenicity and cytotoxicity provided by fruits and vegetable has been attributed to the presence of phytochemicals, mainly the phenolic compounds linked with their antioxidant capacity (Rodríguez-Medina et al., 2009; Bennet et al., 2011). The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical and damage to biological molecules like lipids and DNA may be one of their protective mechanisms (Prior et al., 2004). These free radicals and reactive oxygen species (ROS) are generated endogenously through anaerobic respiration and are potent genotoxins, causing mutation, oxidative damage to DNA, protein and lipids *in vitro* and *in vivo* (Perry et al., 2007). Phytochemicals are also known to protect and regenerate other dietary antioxidant and chelate pro-oxidant metal ions (Segura-Carretero et al., 2010). Therefore, adequate daily consumption of fruits and vegetable is an important health-protecting factor (Wijngaard et al., 2009).

However, despite their nutritional and health benefits, many fruits and vegetables are highly seasonal and perishable resulting into huge postharvest losses (Idah and Aderibigbe, 2007). Post-harvest loss of 30-40% is estimated in developing countries, like Tanzania (Karim and Hawlader, 2005) mainly due to poverty, inadequate postharvest handling techniques, improper processing technology and storage facilities, poor infrastructure as well as poor marketing systems (Perumal, 2007). Drying of fruits and vegetables remains an important method of food preservation. It reduces the moisture content of food to a level, which allows safe storage over an extended period, and prevents the growth of mould and fungi and thus minimizing microbial degradation (Chong and Law, 2010; Doymaz, 2011). Furthermore, it brings about substantial reduction in weight and volume, and in packaging, storage and transportation costs (Chan et al., 2012).

Among all the drying methods, sun drying is a well-known method for drying agricultural commodities in tropical and sub-tropical. However, it has many disadvantages such as long drying time, exposure to contamination from dust, soil, sand particles and insects (Folaranmi, 2008). Consequently the quality of sun dried products may adversely be affected, failing to meet the required local and international standards (Ivanova and Andonov, 2001). To overcome these problems, it is necessary to use alternative drying methods. Solar energy is one of the most promising renewable energy sources in the world because of its abundance, inexhaustible and non-pollutant in nature compared with higher prices and shortage of fossil fuels (Basunai and Abe, 2001). Condori et al. (2001) reported that, attractiveness of solar dryers is further enhanced by its ability to dry the product rapidly, uniformly and hygienically to meet national and international standards with zero energy costs.

Recently, harnessing of solar energy for fruits and vegetable preservation as an alternative to open sun drying and unaffordable mechanical dryers in Tanzania is increasing (Ringo, 2008). Dried fruits and vegetables represent a relatively concentrated form of fresh fruits with higher total energy, nutrient density, fibre content and often significantly greater antioxidant activity (Bennett et al., 2011). According to Vinson et al. (2005), the plasma antioxidant activity may be significantly elevated by consumption of dried fruits, demonstrating bio-availability of antioxidant species. However, drying has been reported to affect the antioxidant activity of fruits and vegetables diversely (Chantaro et al., 2008; Kuljarachanan et al., 2009). Enzymatic and non-enzymatic processes that may occur during drying of fresh plant tissues may lead to significant changes in the composition of phytochemicals (Capecka et al., 2005). Enzymatic browning occurs when the fruit and vegetables polyphenoloxidase (PPO) comes into contact with the endogenous phenolic compounds during drying (Aydemir, 2004). Despite adequate literature review, information on the effect of solar drying methods on total phenolic and antioxidant activities of dried mango, banana, pineapple, and tomatoes is limited. This study therefore, carried out to study the effect of solar drying methods on total phenolic compound and antioxidant capacity of selected fruits and vegetable from Tanzania.

MATERIALS AND METHODS

Study areas

This study was carried out at Sokoine University of Agriculture (SUA), Morogoro, Tanzania and Norwegian University of Life Sciences (NMBU), Aas Norway. Drying activities were conducted at SUA while chemical analyses were carried out at NMBU.

Plant materials

Three common consumed fruits: mango (cv. *Dodo*, *Viringe* and *Kent*), banana (cv. *Kisukari*, *Kimalindi* and *Mtwike*) and pineapple (cv. *Smooth cayenne*) and one vegetable; tomato (cv. *Tanya*, *Cal J*

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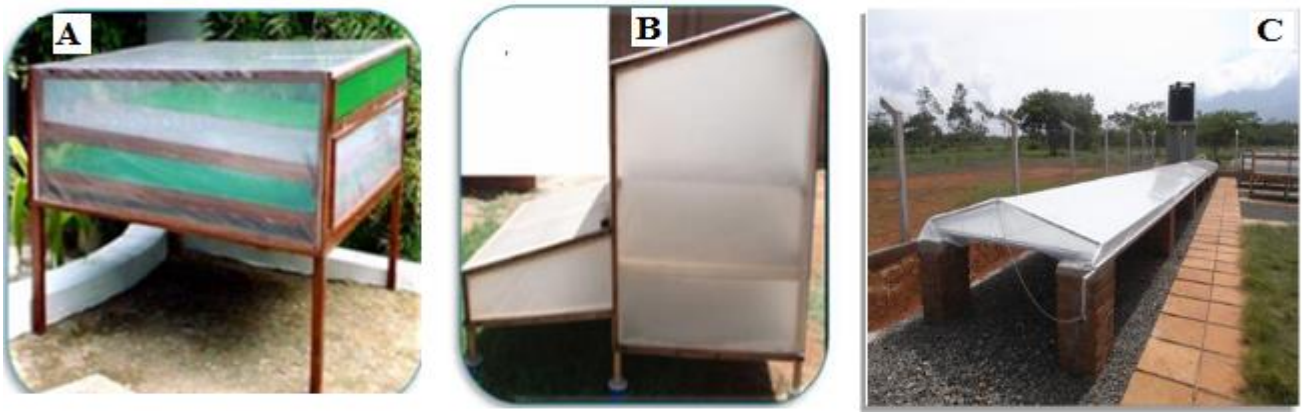

Plate 1. Different solar dryers used in the study: (A) Cabinet direct dryer-CDD, (B) Cabinet mixed mode dryer-CMD and (C) Hoeinheim Tunnel dryer-TD.

and *Onyx*) were procured at physiological maturity and ripeness from selected farmers in Morogoro and Pwani regions, Tanzania.

Drying equipment

Two solar cabinet dryers: direct and mixed modes were locally fabricated and one Hoenheim solar tunnel dryer (Innotech, German) was imported and installed in the study area. The dryers consisted of two parts namely collector and a drying unit/tunnel. In addition, the tunnel dryers consists small fans to provide the required air flow over the products to be dried. The CDD had collector dimension of (1.17 × 2.35 m) and drying section of 0.67 × 1.44 × 2.29 m, respectively while the d CMD had collector dimension of 1.03 × 1.16 plus 90 × 1.16 m for extension and drying section of 1.13 × 1.19 × 1.23 plus 0.99 × 1.23 m for extended part. The tunnel dryer had dimension of 7.1 × 2 m and 10 × 2 m for collector and drying chamber respectively (Plate 1). Both collector and the drying units were covered with UV stabilized visqueen sheets and food grade black paint was used as an absorber in the collectors. The products to be dried were placed in trays in cabinet dryers and a single layer on a wire mesh in the tunnel dryer.

Chemicals

Methanol, acetonitrile, acetic acids, $FeCl_3.6H_2O$, $FeSO_4.7H_2O$, anhydrous sodium carbonate, were obtained from Merck KGaA (Darmstadt, Germany), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2pyridyl)-s-triazine (TPTZ) were obtained from Fluka Chemie GMBH (Buchs, Switzeland). Folin-Ciocalteu phenol reagent (2.0, N), 3, 4, 5,-Trihydroxybenzoic acid (Gallic acid) were bought from Sigma-Aldrich (St Louis, MO, USA). Liquid nitrogen was supplied by Hydro Gas and Chemicals AS (Oslo, Norway). All chemical and gases were of analytical grade.

Research design

Completely randomized design (CRD) was used in the study and principal factor was solar drying method (Local cabinet direct dryer (CDD), cabinet mixed mode (CMD) and Tunnel dryer (TD). The samples were analyzed for dry matter, total phenolic content and antioxidant. The effect of the principal factor on these parameters was determined. The mathematical expression is:

$$y_{ij} = \mu i + \tau_i + \varepsilon ij \quad (1)$$

i=1,2,..., t, j=1,2,...,ni

Where μis the overall mean, τ*i* is ith treatment effect and ε*ij* is the random effect due to jth replication receiving ith treatment.

Drying process

The drying to assess performance of the dryers in retaining phytochemicals was done following methods described by Leon et al. (2002). Fresh mature ripe fruit and vegetable samples were washed, peeled and sliced to 5 mm thick and each sample divided into three portions that were subjected in equal loading density of 2.91 kg of fresh produce/m^2 of solar aperture to either cabinet direct dryer (CDD) with temperature ranging from 30-55°C for about 3 days, cabinet mixed dryer (CMD) with temperature ranging from 25-49° C for about three days and tunnel dryer (TD) with temperature ranging from 60-73°C, for about two days. Since solar drying solely depends on weather conditions, these temperatures were not preset but obtained during drying process and samples were offloaded from dryers after predetermined duration. The dried products were packed in polyethylene bags and stored at -4°C prior to laboratory analysis.

Determination of dry matter

Dry matter contents of fresh and dried products were determined in triplicate according to the standard methods of AOAC (1995). About 5 g of samples were put in pre-weighed crucibles and oven dried at 105°C for 24 h until constant weight was achieved.

Sample extraction and preparation for phytochemical analyses

Three grammes of each sample was diluted in 30 ml of methanol and sonicated at 0°C for 15 min in an ultrasonic bath (Model 2510, Branson Ultrasonics Corp, USA). The sample was then flushed with nitrogen in order to prevent oxidation and stored frozen at -20°C prior to analysis. During analysis, the homogenate was centrifuged at 31,000 g for 10 min at 4°C using a Beckman J2-21M/E centrifuge (GMI Inc., Ramsey, MIN, USA). The supernatant was decanted and subjected to analysis of total phenols and antioxidant power. All samples were extracted in duplicate and analyzed in triplicate.

Table 1. Dry matter content (%) of fresh and dried fruits and vegetable varieties of three solar drying methods.

Fruit/veg.	Variety	Drying method			
		Fresh DM (%)	CDD DM (%)	CMD DM (%)	TD DM (%)
Mango	*Dodo*	21.0±0.0^{a}	83.5±0.01^{b}	84.0±0.02^{b}	86.0±0.49^{c}
	Viringe	20.9±0.3^{a}	83.6±0.01^{b}	83.8±0.02^{b}	85.9±0.06^{c}
	Kent	19.1±0.29^{a}	82.2±0.0^{b}	82.51± 0.01^{b}	85.0±0.13^{c}
Banana	*Kisukari*	29.2±0.5^{a}	83.4±0.17^{b}	83.3±0.16^{b}	86.1±1.5^{c}
	Kimalindi	28.3±0.46^{a}	82.2±0.09^{b}	82.2±0.24^{b}	86.0±2.3^{c}
	Mtwike	28.6±0.62^{a}	82.3±0.10^{b}	82.9±0.03^{b}	84.7±2.12^{c}
Pineapple	*Smooth cayenne*	19.4±0.31^{a}	81.4±0.01^{b}	81.2±0.00^{b}	84.8±0.28^{c}
Tomatoes	*Tanya*	7.8±0.13^{a}	85.5±0.01^{b}	85.8±0.02^{b}	88.9±0.00^{c}
	Cal J	7.7±0.00^{a}	85.6±0.00^{b}	85.4±0.00^{b}	88.3±0.01^{c}
	Onyx	8.0± 0.00^{a}	85.7±0.00^{b}	85.1±0.14^{b}	88.9± 0.01^{c}

Data presented as arithmetic means ± SD (n = 3). Means in row with different small letter are significantly different ($p<0.05$) between drying methods for the same variety.

Determination of total phenolic contents (TPC)

Total phenolic content was determined using a Konelab 30i (Thermo Electron Corp., Vantaa, Finland) clinical chemical analyser. The procedure was based on using the Folin-Ciocalteu reagent (FCR), as described by Singleton et al. (1999). A 20 µl sample were added to 100 µl FCR (diluted 1:10 with distilled water), mixed and incubated at 37°C for 60 s prior to addition of 80 µl 7.5% (w/v) sodium bicarbonate solution. The samples were again mixed and incubated at 37°C for 15 min prior to absorbance reading at 765 nm. TPC were assessed against a calibration curve of gallic acid, and the results presented as mg gallic acid equivalents (GAE) per 100 g dry weight (DW).

Determination of ferric reducing antioxidant power (FRAP)

Antioxidant activity in the samples was measured using the ferric reducing ability of plasma (FRAP) assay described by Benzie and Strain (1996) using the KoneLab 30i (Kone Instruments Corp, Espoo, Finland). Briefly, 200 µl of the FRAP reagents (3.0 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3.6H_2O$, ratio 10:1:1) were automatically pipette separately and mixed in the cuvettes; 8 µl of sample were added and mixed and left to incubate at 37°C for 10 min and absorbance read at 595 nm. Trolox (Vitamin E analogue) was used as a control. The antioxidant activity in the samples was calculated as mmol Fe^{2+} per 100 g dry matter.

Statistical analysis

Data obtained was analyzed in triplicates using analysis of variance by R statistical software ((R Development Core Team, Version 3.0.0 Vienna, Austria). One way analysis of variance (ANOVA) was done to determine significant differences between factors. Means were separated by Turkey Honest Significant Difference (THSD) at $p<0.05$. Pearson correlation coefficient was done to determine the relationship between TPC and FRAP.

RESULTS AND DISCUSSION

Dry matter content

Contents of dry matter in fresh and dried samples are shown in Table 1. The results showed significant variations in dry matter contents between fresh and dried samples and within the drying methods. Dry matter contents of all fresh samples increased significantly ($p<0.05$) and the increase was more pronounced in tunnel dried samples for all fruits and vegetables. Cabinet direct and mixed mode dryers were not significantly different in terms of performance for both food products ($p>0.05$). Drying significantly reduces moisture contents of food materials and causes changes in dry matter contents. Lefsrud (2008), reported that the moisture content within biological samples changes during drying and can result in the release of organic compounds, volatile organic compounds (VOCs), destruction of pigments, and changes in chemical composition. The higher dry matter content in tunnel dryer than in cabinet dryers could be associated with its high drying temperature, which caused more moisture release in addition to the release of other of organic compounds.

Total phenols

The mean total phenolic compounds (TPC) of fresh and dried fruit and vegetable varieties are shown in Table 2. Significant differences ($p<0.05$) in TPC among the fresh fruits/ vegetable were observed. The highest TPC contents (g/100 g DM) were found in tomato cv. *Onyx*

Table 2. Total phenolic contents (mgGAE/100g DM) of fresh and dried fruits and vegetable varieties of three solar drying methods.

Fruit/Veg	Variety	Drying method			
		FR	CDD	CMD	TD
		Mean (%)	Mean (%)	Mean (%)	Mean (%)
Mango	*Dodo*	315.3±5.4 (100)[a]	261.3 ± 6.7 (83)[b]	263.4 ± 3.1 (84)[b]	291.8 ± 5.4 (93)[c]
	Viringe	311.4±1.5 (100)[a]	261.6 ± 1.3 (84)[b]	259.2 ± 3.8 (83)[b]	292.9 ± 0.6 (94)[c]
	Kent	239.4±7.9 (100)[a]	184.3 ± 1.8 (77)[b]	181.1 ± 0.8 (76)[b]	201.5 ± 4.4 (84)[c]
Banana	*Kisukari*	139.3±2.3 (100)[a]	81.2 ± 0.5 (58)[b]	83.0 ± 0.8 (59)[b]	105.96 ± 2.1 (76)[c]
	Kimalindi	189.2±2.7 (100)[a]	116.9 ± 0.8 (62)[b]	118.1 ± 1.5 (62)[b]	145.90 ± 6.4 (77)[c]
	Mtwike	173.6±4.2 (100)[a]	98.5 ± 0.4 (57)[b]	100.3 ± 1.8 (58)[b]	133.70 ± 4.4 (77)[c]
Pineapple	*Smooth cayenne*	282.9±4.2 (100)[a]	226.7 ± 3.1 (80)[b]	232.8 ± 4.6 (82)[b]	262.5 ± 4.5 (92)[c]
Tomato	*Tanya*	476.6±8.6 (100)[a]	448.2 ± 0.8 (94)[b]	454.6 ± 3.1 (95)[b]	587.2 ± 1.3 (123)[c]
	Cal J	448.2±5.8 (100)[a]	418.1 ± 4.8 (79)[b]	415.7 ± 2.8 (79)[b]	588.1 ± 5.8 (112)[c]
	Onyx	538.9±1.4 (100)[a]	512.9 ± 0.9 (95)[b]	511.6 ± 1.7 (95)[b]	675.5 ± 1.5 (125)[c]

Data presented as arithmetic means ± SD (n = 3). Data in parentheses represent percent recovery relative to untreated. Means within fruit/ vegetable in row with different superscript letters are significantly different ($p<0.05$).

followed by mango cv. *Dodo* pineapple cv. *Smooth cayenne* and the lowest in banana cv. *Kisukari*. The level of polyphenolic compounds present in fruits and vegetable depends on cultivar, growth condition (soil, fertilizer, temperature, and cultivation techniques), storage and transport conditions and processing technology (Bennett et al., 2010). The effect of drying methods on TPC was significant ($p<0.05$) with all fresh samples having higher TPC levels but declined significantly in dried samples with exception of tunnel dried tomatoes. No significant differences were found between the cabinet direct and mixed modes of drying ($p>0.05$). These findings suggest that drying has variable effects on TPC contents of plant samples. It could result in little or no change, significant declines or enhancement of the TPC (Hamroun-Sellami et al., 2012). Chan et al. (2009) found that, all methods of thermal drying (microwave, oven and sun drying) resulted in sharp decline in TPC in dried leaf vegetables. The decline is attributed to degradation of phenols during drying (Suvarnakuta et al., 2011). Bennett et al., (2010) explained that, the phenolics present in fresh fruit and vegetables are susceptible to oxidative degradation by polyphenol oxidase (PPO) during drying, which leads to intermolecular condensation reactions and their level decreased. Similar decline in polyphenolic content after drying has been reported in prune (Caro et al., 2004), persimmons (Park et al., 2006), mulberry leaves (Katsube et al., 2009), apricots (Madrau et al., 2009), olive mill waste (Obied et al., 2008) and ginger leaves (Chan et al., 2009). Among the dried samples, the tunnel dried samples had less loss (6-16%) than cabinet dried samples (17-42%). This difference might be ascribed to greater enzymatic degradation by PPO as direct and mixed mode dryers took comparatively longer time for drying compared to tunnel drier resulting to additional enzymatic reactions (Chan et al., 2009).

The higher TPC contents in tunnel dried tomato than fresh samples and generally lower decline in TPC for other tunnel dried samples could be attributed to the release of more bound phenolic compounds from breakdown of cellular constituents due to high drying temperature (Randhir et al 2007; Boateng et al., 2008; Vega-Galves et al., 2009; Marshall et al., 2000). Along with that, the application of temperature in the 70-90°C range associated with faster dehydration reduces the opportunity for PPO oxidation process that accompanies drying (Uhlig and Walker, 1996). This is consistent with the findings of this study.Similar increase in polyphenolic contents after drying has been reported in sweet potatoes (Mao et al., 2010), prune (Caro et al., 2004) tomatoes (Dewanto et al. 2002; Chang et al., 2006) and Shitake (*Lentinus edodes*) mushroom (Choi et al., 2006). In general, the significant effect of different drying methods on total phenolic compound of fruits, vegetables and herbs has widely been reported (Hamrouni-Sellami et al., 2012; Zhang et al., 2012).

The effect of varieties in percentage TPC recovery of dried products in each drying methods are shown in Figure 1. There were significant differences ($p<0.05$) in recoveries between the fruits and vegetable products and between the varieties within the species in each dryer. The highest recoveries between the species were found in mango varieties (77-94.06%) and the lowest in banana varieties (56.8-77.1%). The highest recoveries between the varieties within the species for all drying methods were found in *Dodo* and *Viringe* for mango (83.2-94.06%), *Kimalindi* for banana (61.8-77.10%) and *Onyx*

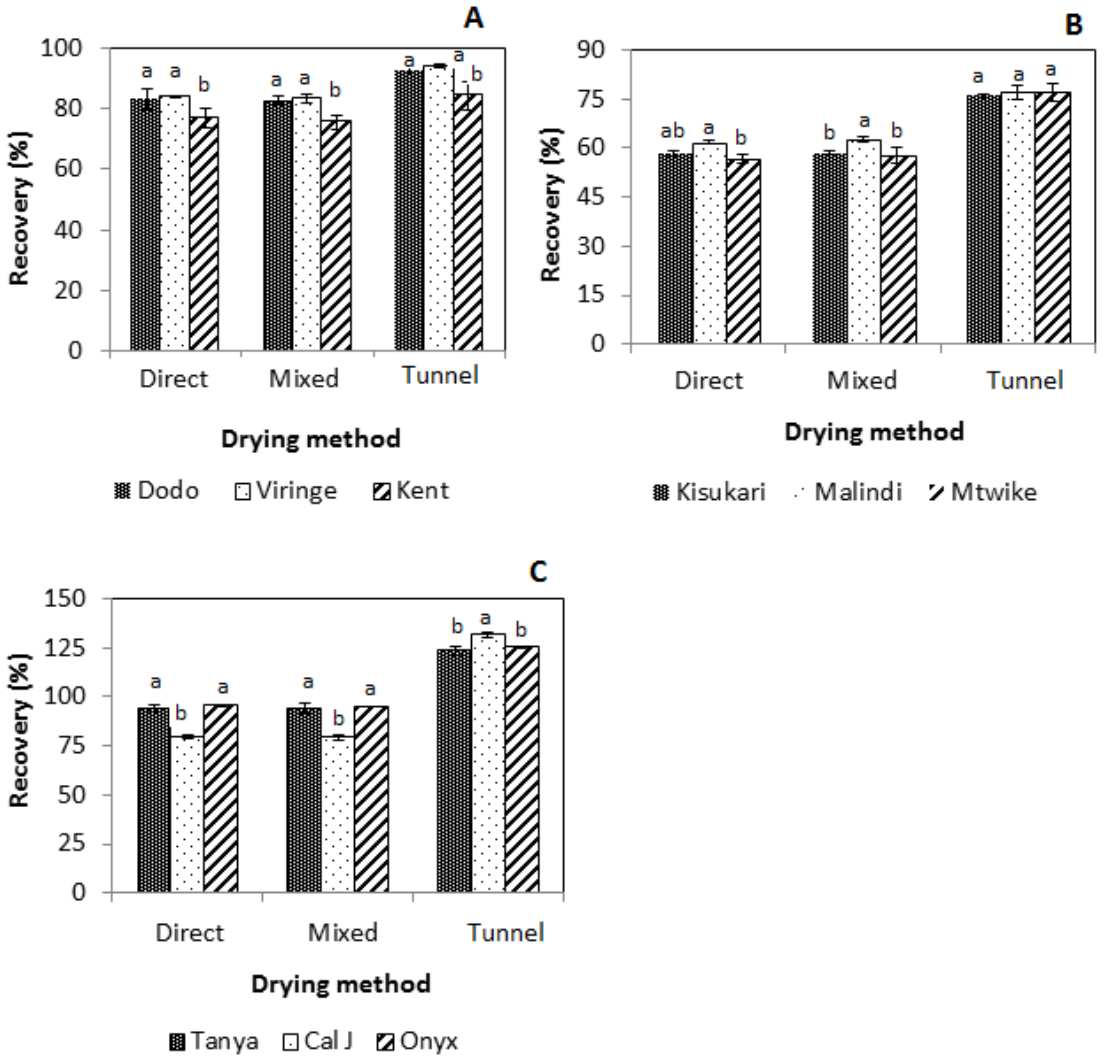

Figure 1. Recoveries of TPC in varieties of Mango (A), Banana (B) and Tomato (C) in three drying methods (mean±SD, n=3). Bars with different letter indicates means are significantly different at p<0.05 for varieties in each drying method.

and *Tanya* for tomato (94-125%). *Kent*, *Kisukari*and *Cal J* varieties of mango, banana and tomato had the lowest percentage recoveries in all drying methods. These findings suggest that, amongst other factors, such as maturity stage and light exposure, phenolic composition varies with cultivars (Segura-Carretero et al., 2010). Similar variation in TPC between varieties of the fruits were reported in dried apricot (Madrau et al., 2009), palm (Piga et al., 2005) and mango (Ribeiro, et al., 2007).

Ferric reducing antioxidant power (FRAP)

The mean ferric reducing antioxidant power (FRAP) of fresh and dried fruits and vegetables varieties are shown in Table 3. Significant differences (p<0.05) in FRAP between fresh fruits/vegetable were observed. The highest FRAP contents (µmol/100 g DM) were found in tomato cv. *Tanya* followed by mango cv. *Viringe* pineapple cv. *Smooth cayenne* (and the lowest in banana cv. *Kisukari*). The differences in the antioxidant activities among the fruits and vegetables samples could be attributed to their polyphenol contents and composition and to other non-phenolic antioxidants present in samples such as vitamin C, vitamin E, Mallard reaction products, β-carotene and lycopene (Hassanien, 2008; Ali et al., 2010). Fresh samples had higher FRAP levels than dried samples. However, these decreased significantly in dried samples for all drying methods. Tunnel dried samples had significantly less FRAP loss (6-13%) than cabinet dried samples (14-56%), confirming the finding that the two drying methods were statistically different (p<0.05). However, the direct and mixed dried samples were statistically similar (p>0.05) in antioxidant activity.

Table 3. Ferric Reducing Antioxidant Power (FRAP) (µmol/100 g DM) of fresh and dried fruits and vegetable varieties of three solar drying methods.

Fruit	Variety	Drying method			
		Fresh	Direct	Mixed	Tunnel
		Mean (%)	Mean (%)	Mean (%)	Mean (%)
Mango	*Dodo*	27.3 ± 0.3 (100)^{a}	21.3 ± 0.2^{b} (79)	21.6 ± 0.1^{b} (80)	25.1 ± 0.4^{c} (93)
	Viringe	28.5 ± 0.4 (100)^{a}	24.2 ± 0.5^{b}(86)	24.1 ± 0.1^{b} (86)	26.9 ±0.5^{c} (96)
	Kent	23.1 ± 0.4 (100)^{a}	15.1 ± 0.2^{b} (65)	14.9 ± 0.2^{b} (64)	20.3 ± 0.2^{c} (88)
Banana	*Kisukari*	10.8±0.1 (100)^{a}	5.7 ± 0.1^{b} (53)	6.0 ± 0.2^{b} (55)	8.5 ± 0.2^{c} (78)
	Kimalindi	15.8±0.2 (100)^{a}	8.6 ± 0.0^{b} (55)	8.9 ± 0.0^{b} (57)	12.6 ± 0.5^{c} (80)
	Mtwike	14.5±0.2 (100)^{a}	6.4 ± 0.0^{b} (44)	6.7±0.0^{b} (46)	13.1 ± 0.3^{c} (90)
Pineapple	*Smooth cayenne*	24.8±0.5 (100)^{a}	18.4 ± 0.2^{b} (74)	18.2 ± 0.1^{b} (73)	23.1 ± 0.3^{c} (93)
Tomato	*Tanya*	46.8±0.5 (100)^{a}	27.9±0.3^{b} (60)	28.3±0.4^{b} (60)	43.0±0.4^{c} (92)
	Cal J	44.6±1.6 (100)^{a}	23.8±0.5^{b} (53)	24.4±0.3^{b} (55)	39.2±0.4^{c} (88)
	Onyx	44.6± 0.3 (100)^{a}	26.5±0.2 ^{b}(59)	25.7±0.6^{b} (58)	38.6± 0.3^{c} (87)

Data presented as arithmetic means ± SD (n = 3). Data in parentheses represent percent recovery relative to untreated. Means within fruit/vegetable in row with different superscript letter are significantly different ($p<0.05$).

Drying affects the antioxidant activity of fruits and vegetables differently (Kuljarachanan et al., 2009; Chantaro et al., 2008; Choi et al., 2006). Chemical and enzymatic processes during drying and/or storage can lead to either loss of phenolic-related antioxidant capacity or may generate chemical derivatives with little or no change, significant declines or enhancement in antioxidant capacity (Bennet et al., 2011).Nevertheless, the best drying method leads to the least alteration in phenolic content and enhances antioxidant activity of the sample. Madrau et al. (2009) found that, high drying temperature gave a product with better polyphenol content with enhanced antioxidant activity. Similar effect of drying on antioxidant capacity of fruits and vegetable has been reported in apple (Anwar et al., 2012), sage (Hamrouni-Sellami et al., 2012) and *Enicostemma littorale (Blume*) (Sathishkumar et al., 2009).

The influence of varieties in percentage FRAP recoveries within each fruit/vegetable samples in each drying method was significant ($p<0.05$) as indicated in Figure 2. The highest recoveries were found in *Viringe* for mango (85.6-95.8%), *Kimalindi* and *Mtwike* for banana (54.6-56.5 and 89.9% respectively) and for tomato (81.88-90.62%). The influence of varieties in antioxidant capacity of dried fruits has also been reported in apricot (Madrau et al., 2009).

Correlation analysis between total phenolic contents and FRAP

The correlation analysis between total phenolic and antioxidant activity of the fresh and dried fruits and vegetable are shown in Figure 3. There was a strong positive correlation between both the TPC and antioxidant activities in fresh (R^2=0.9709) and dried samples (R^2= 0.8636). This finding implies that, the antioxidant activity of plants materials including fruits and vegetables is strongly correlated to the TPC contents (Anwar et al., 2012; Zhang et al., 2012). Similar correlation between total phenols and antioxidant activity in plants have been reported (Ichuen et al., 2010; Sreeramulu et al, 2010; Mao et al., 2010).

Conclusion and recommendations

Solar drying has significant effect on total phenolic contents and antioxidants activities of dried mango, banana, pineapple and tomato which varies depending on the method used. Tunnel dried samples have lower decline in TPC and antioxidant activities than cabinet dried samples due to higher drying temperature and shorter drying rate. Moreover, the percentage recoveries of total phenols and antioxidant capacities of dried fruits and vegetables differ according to varieties. Finally, the antioxidant capacities of plants materials including fruits and vegetables are strongly depend on the total phenolic compounds present. Based on the better performance of the tunnel dryer over local ones in retaining and enhancing TPC associated with higher antioxidant activities, then its use in drying and extending shelf life of agricultural produces is highly recommended and should be advocated in Tanzania and in developing countries at large.

Conflict of interests

The authors did not declare any conflict of interest.

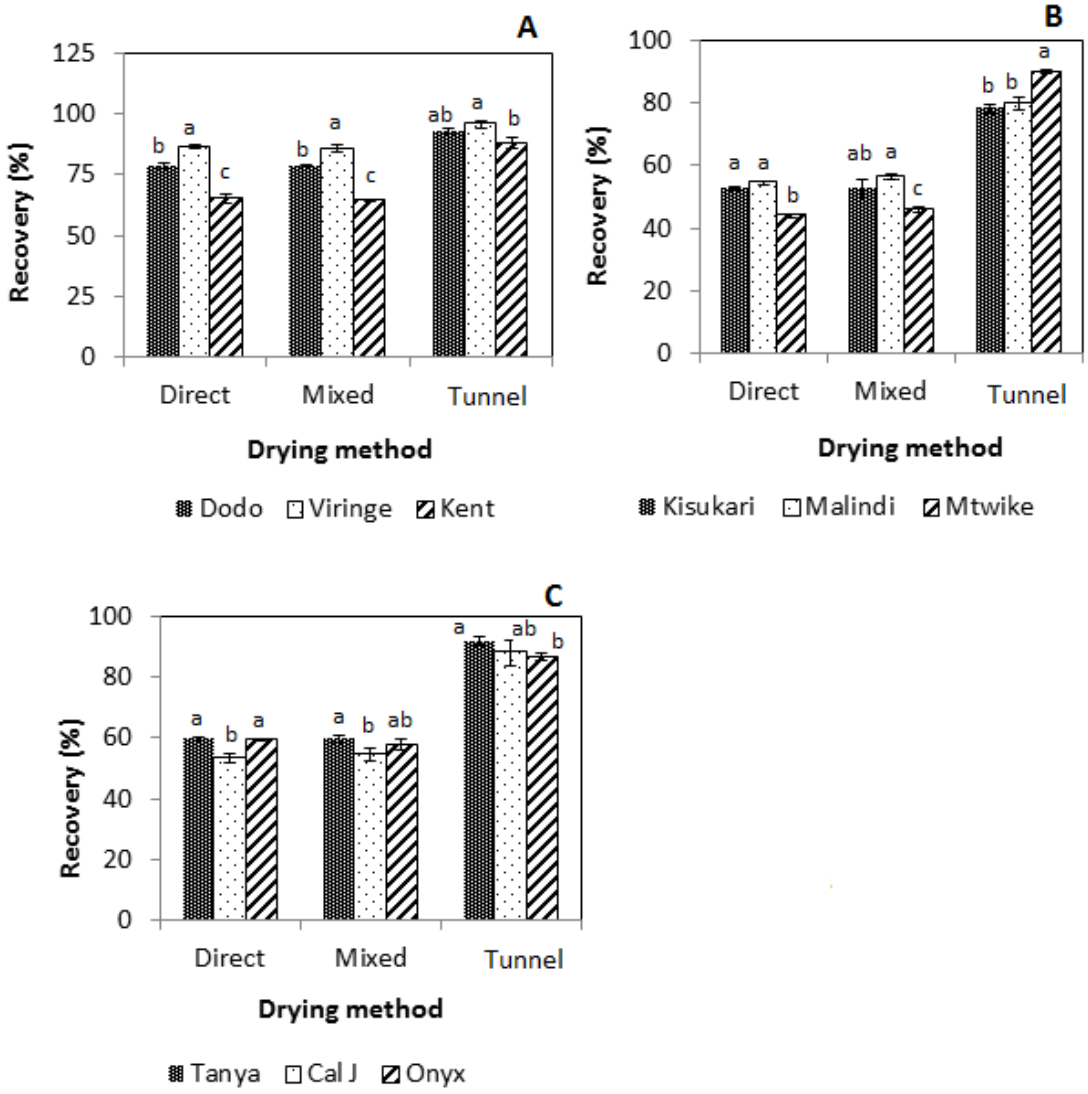

Figure 2. Recoveries of FRAP in three varieties of Mango (A), Banana (B) and Tomato (C) in three drying methods (mean±SEM, n=3). Bar means with different letter are significantly different at $p<0.05$ for varieties in each drying method.

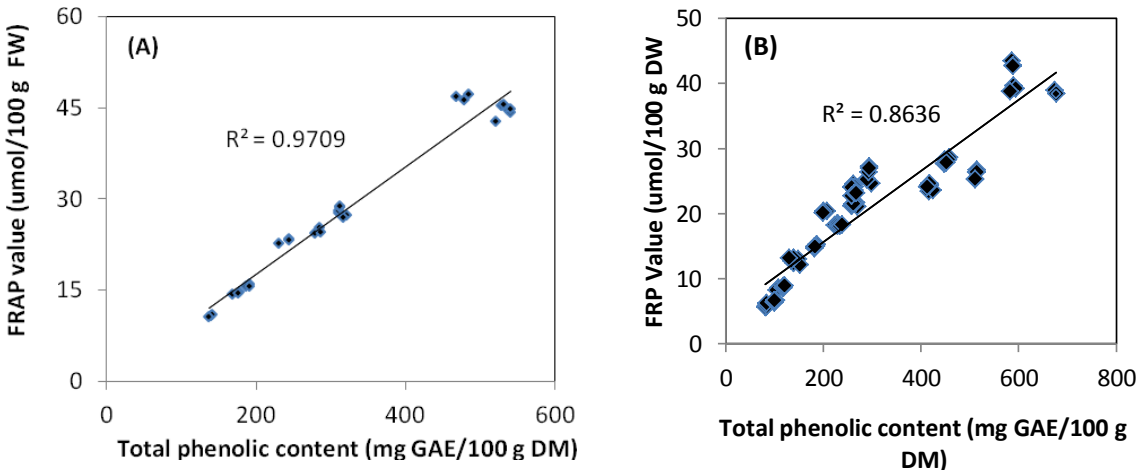

Figure 3. Correlation between total phenolic contents and FRAP in Fresh (A) and Dried (B) Fruits and vegetable.

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Quality of beef, chevon and mutton at Hawassa, Southern Ethiopia

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The study was conducted to study meat quality of beef, chevon and mutton consumed at Hawassa city in Southern Ethiopia. Purposive sampling technique was used to collect information from butcheries. From each sub cities, 15 butcheries were selected randomly for the purpose thus, a total of 45 sample butchers were used. From longissimus dorsi muscle, sample of beef, chevon and mutton were taken and analyzed for the study. The average pH value of beef, chevon and mutton was 5.6, 5.8 and 5.5, respectively. The average water holding capacity (WHC) was 23, 29 and 32%, respectively, for beef, chevon and mutton. The average cook loss of beef, chevon and mutton was 33.8, 32.5 and 29.9%, respectively. Protein content of raw, boiled and roasted beef was 16.1, 23 and 31.2% DM, respectively, while the average fat of raw, boiled and roasted beef were 5.4, 7.2 and 10% DM, respectively. Ash content of 1.2, 1.8 and 2.7% DM was found for raw, roasted and cooked beef, respectively and the average moisture of raw, boiled and roasted beef was reported as 72.7, 63.2 and 51.8%, respectively. The average value of raw, boiled and roasted protein and fat chevon was 20, 29.8, 34; 5.3, 8 and 11.4%, respectively. On the other hand, ash content was 0.9, 2 and 3. 6% DM; moisture 74.2, 60.6 and 48.2%; were found for raw, roasted and cooked chevon, respectively. For raw, roasted and cooked mutton, protein content of 19, 28.2 and 32% DM; fat 6.4, 8.1 and 11.6% DM; ash 1.1, 2.7 and 3.7% DM and moisture 72.7, 59.4 and 44.8%, respectively, were found. The results indicate that the moisture, ash, protein, fat, cooking loss and water holding capacity of the beef, chevon and mutton were almost in comparable with the results reported by various researchers in Africa.

Key words: Quality of meat, beef, cheven, mutton, Hawassa city, Ethiopia.

INTRODUCTION

Meat is one of the most nutritious foods that humans can consume, particularly in terms of supplying high-quality protein (essential amino acids), minerals (especially iron) and essential vitamins. Meat is defined as all animal tissues suitable as food for human consumption. This includes all processed or manufactured products prepared from animal tissues (Amaha, 2006; Soniran and Okunbanjo, 2002).

Consumers often tend to evaluate meat quality on the basis of organoleptic evaluation parameters such as,

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tenderness, juiciness, flavor, palatability, color, and neatness (Beriain et al., 2001). However, the best method of determining of meat quality are assessing pH, water holding capacity, chemical composition of meat (Fakolade and Omojola, 2008; Abd El-aal and Suliman, 2007; Gustavson et al., 2011).

Meat pH level value, in normal circumstances, decreases during post-mortem due to formation of lactic acid from glycogen. The low pH-value is favorable for keeping quality and flavor (FAO, 2004). Determining of meat water-holding capacity is important because it can affect on both the yield and the quality of meat, and is often described as drip loss. This parameter can also indicate the whole performance condition of the live animal at the time of harvest, or the entire system of live animal production and handling history (Andrzej, 2010).

Many scientific studies also indicate that the most valuable components of meat from the nutritional and processing point of view are water, fat, protein and minerals (FAO, 2004; Adam et al., 2010). However, values of chemical composition from raw and cooked meat are not the same. The values from raw meat enable to predict the management situation of animal till slaughtering (Sainsbury, 2009). On the other hand, values from cooking of meat are used to achieve a palatable and safe product (Tornberg, 2005). Cooking may also affect nutritive value and consumer preference of flavour and tenderness of meat (Pietrasik et al., 1995). Cooking loss is an indicator of meat quality; the lower the cooking loss, the better the juiciness of the meat (Ameha, 2006). Therefore, the type of cooking may have effects on nutritive values, organoleptic attributes and acceptability of meat from ruminants (Wood et al., 2003; Olfaz et al., 2005).

Therefore, the study was conducted to evaluate pH value, cook loss, water holding capacity, and chemical composition of meat beef, chevon and mutton consumed in the Hawassa city of Ethiopia. The study was concerned of three meat types: beef, chevon and mutton because these meat types are dominantly available and consumed by the people in the study area.

Therefore, evaluation of meat quality on the basis of pH value, water holding capacity, chemical composition (water, fat, protein and minerals), cooking loss of meat (Gustavson et al., 2011) is important. These days, consumers demand to know the nutrient quality of the food they consume because they are more conscious of their health and are increasingly focusing on their feeding habits (Sainsbury, 2009). It is also important to improve livestock production sector through designing appropriate livestock development strategies and policies. Meat quality has a direct relationship to the whole management (feeding, watering, caring, handling, transporting, marketing, slaughtering) of livestock produc-tion. However, there is no documented information on pH, water holding capacity, chemical composition of meat in the study area so far. Therefore, the study was focused on determining the above mentioned quality parameters. The study included both raw and cooked meat. The quality of the raw meat and that of the cooked meat affects its attributes. Accordingly, the study was focused to study with objectives on pH value, water holding capacity, chemical composition of meat from meat ruminant (cattle, goat and sheep).

MATERIALS AND METHODS

Description of the study area

The study was carried out from December 2012 to June 2013 in Hawassa city, which is the capital city of the Southern regional state of Ethiopia. It is located 270 km south of Addis Ababa via Debre Zeit, between 7.05° N to 7°3′N latitude and 38°28′ E to 38.467° E longitude (CSA, 2007). Hawassa city had a total population of 183,027 residents, of whom 94,366 were men and 88,661 women (CSA, 2007). The city has an area of 157.21 square kilometers which of course has increased since 2007. In the year 2007, the Hawassa city had 45,823 households, with an average of 4.22 persons per households, which also increased over time.

Sample collection

Purposive and random sampling methods were used for the study. The study city has eight administrative sub-cities of which five sub-cities which had more butchers were purposively selected. From the selected sub-cities, 15 butcheries were selected randomly for each beef, chevon and mutton. Thus, a total of 45 butchers were selected. From these selected butchers, meat sample of beef, chevon and mutton were purchased from the *Longissormus dorsii* muscle.

Laboratory analysis

The following parameters were determined: fat, protein, ash and moisture content, pH level, water holding capacity as well as loss on cooking. The study was carried out at the Animal Nutrition Laboratory of Agriculture College, Hawassa University.

Sampling and analytical procedures

The meat samples were collected in aseptic containers labeled and transported in an ice box from the selected butchers. The sample muscle considered for the study was the *Longissormus dorsii*. After bringing the sample to the laboratory, it was stored in a refrigerator at 4°C until required for analysis. The pH of the muscle was estimated within 48 hof its collection using a digital pH- meter (Basic 20, Crison Instrument, Spain). The muscle sample was divided into two parts; one for estimation of raw muscle quality while second was for cooking. There were two types of cooking, roasting and boiling. On average the meat was roasted for about 12 min, or boiled for about 25 minon a stove where the temperature was maintained at 180°C.

The contents of moisture, protein, fat and ash were determined according to the AOAC (1990) and the pH of the samples was measured using pH-meter, Basic 20, Crison Instrument, Spain.

Determination of water holding capacity of meat

Water-holding capacity of meat was measured using the method

Table 1. Quality parameters of beef, chevon and mutton meats in Hawassa city (mean ± SD).

Parameter	Category	Meat type		
		Beef (mean±SD)	Chevon (mean±SD)	Mutton (mean±SD)
pH level	Raw	5.6±0.1	5.8±0.14	5.5±0.09
WHC (%)	Raw	23±1.92	29±1.58	32±0.40
Cook loss (%)	Raw	33.8±3	32.5±2.2	29.9±1.3
Moisture (%)	Raw	72.7±0.5	74.2±0.8	72.7±0.9
	Boil	63.2±1.3	60.6±1.1	59.4±2.3
	Roast	51.8±1.1	48.2±2	44.8±2.8
Protein (% DM)	Raw	16.1±2.1	20±1.4	19±1.9
	Boil	23±2.2	29.8±1.8	28.2±2.6
	Roast	31.2±2.3	34±1.5	32±2.6
Fat (% DM)	Raw	5.4±0.8	5.3±0.6	6.4±1.5
	Boil	7.2±0.6	8±0.4	8.1±1.6
	Roast	10±0.7	11.4±1	11.6±1
Ash (% DM)	Raw	1.2±0.26	0.9±0.06	1.1±0.06
	Boil	1.8±0.4	2. ±0.18	2.7±0.35
	Roast	2.7±0.68	3.6±0.3	3.7±0.45

SD=Standard deviation; WHC=water holding capacity; N=15 for each beef, chevon and mutton.

suggested by Kauffman et al. (1986) and Trout (1998). A 0.5 g of meat sample was weighed and placed between two filter papers. This in turn was placed between two glass sheets. Over it, a weight of 4.015 kg weight was placed while the glass sheet weighed 0.8278 kg, giving a total compression weight of 4.8428 kg for 5 min. The water from the meat was then absorbed on the filter paper and the filter paper was dried. Then after the area of the filter paper marked with and meat was later determined using a compensatory planimeter. Taking differences from the resulting areas of the sample from a marked borderline on the filter paper (moisture) and meat and a ratio area marked borderline was expressed as water holding capacity of the meat (WHC):

WHC % = (Area marked borderline-area meat)*100 / Area marked borderline.

Determination of cooking loss of meat

Cooking loss of meat was determined by using procedure described by Bouton et al. (1971). Three replicates of 0.5 g of each of the meat sample were freshly cut and represented by individual slices. The meat samples were then placed in three test tubes. They were then placed in a boiling water bath for 5 min and was removed then cooled. Cook loss of meat was obtained by taking difference of initial and final weight.

Cook loss%= Initial weight of the sample (before cooking) - final weight of the sample (after cooking)*100/ initial weight of the sample.

Data analyses

The data was analyzed statistically using SPSS V 17 [2007] for Windows, using linear regression analysis.

RESULTS

The quality parameters of meat in Hawassa city, Ethiopia are presented in Table 1. As shown in Table 1, average pH values of beef, chevon and mutton were 5.6, 5.8 and 5.5, respectively, while water holding capacity was 23, 29 and 32%, respectively. In this study, average cook loss was 33.8 for beef, 32.5 for chevon and 29.9% for mutton.

For beef meat, protein content of raw was lower (16.1% DM) than that of boiled (23.0% DM) and roasted (31.2% DM); in similar manner, the average fat of raw (5.4% DM) was lower than boiled (7.2% DM) and roasted (10.0% DM). However, in ash content, both raw (1.2% DM) and roasted (1.8% DM) had lower content than cooked (2.7 % DM). On the other hand, due to the effect of cooking, the average moisture of beef from raw (72.7%) through boiled (63.2%) to roasted (51.8%) was reduced.

As shown in Table 1, for chevon meat, the protein content (%, DM) was 20.0 (raw), 29.8 (cooked) and 34 (roasted); fat content (%, DM) 5.3 (raw), 8.0 (cooked) and 11.4 (roasted); ash content (%, DM) 0.9 (raw), 2.0 (cooked) and 3.6 (roasted) and moisture content (%) 74.2, 60.6 and 48.2 for raw, roasted and cooked, respectively. For raw, roasted and cooked mutton meat, protein content (%, DM) of 19.0, 28.2 and 32.0; fat content (%, DM) 6.4, 8.1 and 11.6; ash (%, DM) 1.1, 2.7 and 3.7 and moisture content (%) 72.7, 59.4 and 44.

DISCUSSION

The pH of beef and chevon was lower than the values

observed by Fakolade and Omojola (2008) and Maiti and Ahlawat (2011). However, the values as obtained for mutton was similar to those reported by Abd El-aal and Suliman (2007) who found that the average pH- value of lamb fed on ration containing different levels of leucena leaves to be similar to those observed in this study. The low values of pH as observed in the study may be attributed to high lactic acid content in the muscle which can be a fall out of several factors, like poor pre-slaughter handling and which sometimes leads to spread of infection during transportation and in overcrowded lair ages, as well as to loss of weight, long distance travelled by the animal just prior to slaughter and also inadequate rest between the travelling and slaughtering period, absence of stunning facilities in the slaughter houses (Amha, 2006; Daniel, 2008; Elias et al., 2007; Gary et al., 2004; Yacob, 2002). Another factor which can attribute to low pH is the long period between slaughtering time and the time the meat is actually sold. Absence of chilling facilities within the butcheries and hence that the cold chain is not maintained also leads to changes in pH values. The washing of the carcass may also be carried out with contaminated water and sanitation within the slaughtering facility itself may not be favorable. All the above mentioned parameters to some extent or the other lead to the development of low pH in the muscle fibers which of course affects the organoleptic quality of the meat to a greater or lesser extent (Abbey, 2004; Amha, 2006; Yacob, 2002).

Water-holding capacity of the meat refers to its ability to retain inherent water and its value is influenced by both the pH of the tissue and by the amount of space in the muscle cell, particularly the myofibril that exists for water to reside. The current result of all types of meat (beef, chevon and mutton) showed that the values were lower than that reported by Abd El-aal and Suliman (2007) from lamb reared on a ration containing of leucaena hay (leucaena leucocephala) as forage was 43.61-48.26%. This might be attributed by the live animal performance condition at the time of harvest; the muscle in the live animal can have a strong influence on the amount of moisture that is lost from the resulting meat products (Andrzej, 2010). As revealed in this study, the low water-holding capacity of the meat muscle may be due to the effect of low pH-value of the meat muscle (being more acidic) and such types should not be used for processing as the product developed from it is usually dry and tasteless. On the contrary, the present study was almost in agreement to Maiti and Ahlawat (2011) found from heart muscle of goat 29.19%.

Meat loss during cooking measures the decrease in edible meat mass for human consumption (Gustavson et al., 2011). The average values of cook loss of beef was higher than those reported by Jama et al. (2008) for Nguni, Bonsmara and Angus cattle breeds. The values were also higher than those reported by Nikmaram et al. (2011) while the cook loss values for chevon as assessed in this study was lower than the values reported by Amha (2006) and Maiti and Ahlawat (2011). But it was higher than that of cook loss value reported by Adam et al. (2010). Similarly, the cook loss value for mutton as observed in this study was lower than the values reported by Abd El-aal and Suliman (2007). The differences as observed in this study may be attributed to the sex, breed, age besides both ant mortem and postmortem of animals and the carcass (Amha, 2007). In general, the lower the cooking loss, the better the juiciness of the meat. This is another valuable quality trait observed in some Ethiopian indigenous sheep and goats useful in market promotion efforts.

Determining proximate composition of both cooked and raw meat is necessary for assessing nutritive value of meat. The nutrient value of cooked meat is more useful than raw as the cooked meat show actually consumed meat (Ono et al., 1984). However, the raw value was used to evaluate the effect of husbandry practices, production and marketing on the nutrient composition of the muscles (Sainsbury, 2009).

For crude protein, the average value in beef was comparable to those observed by Fernanda et al. (2003), Williams (2007), Fakolade and Omojola (2008) and Nikmaram et al. (2011). Similarly, the average value for protein in chevon and mutton as observed in this study were similar to the values reported by Schonfeldt (1989) and Williams (2007). The values were also similar to the results observed by Ghita et al. (2009) and Maiti and Ahlawat (2011).

The values pertaining to the average fat% of beef as observed are similar to those of Fakolade and Omojola (2008) from dried beef (in Nigeria); Williams (2007) from red beef. However, the results of mutton and chevon fat (%) are lower than Maiti and Ahlawat (2011) who found 21.63% of chevon fat values of cooked while Ghita et al. (2009) found 35.07% from Carabash lamb. On the other hand, the results of average mutton and chevon fat value are higher than that of the findings of Schonfeldt (1989) of lamb (4.7%), angora (4.7%) and boar goat chevon (4.4%) from the M semi membranous muscle. The discrepancy might be due to the age and breed of the animals in the various studies. However, the relatively higher mean carcass fat in some Ethiopian goat breeds would be useful in reducing chilling losses and improving quality (Amha, 2007).

For ash content, the present result in beef meat is similar to the results published by Fernanda et al. (2003) and Nikmaram et al. (2011). Also, the results for ash % of chevon and mutton were similar to those obtained by Schonfeldt (1989) for lamb (1.06%), angora (1.07%) and boar goat chevon (1.08%) from M LongissimusThoracis and lumborum.

For moisture content, the results in beef were almost in similar range to those reported by Nikmaram et al. (2011) who found that 73.45% for raw, 34.8% microwave, 42.55% roasting and 38.19% braising beef and Williams

(2007) reported as 35.09 and 73.17% from dried and lean red beef, respectively. The results for chevon and mutton as assessed in this study finds consonance with the observations of Adam et al. (2010) from Nilotic male kid (fresh meat) on feed lot trial sorghum and molasses-based diets and Maiti and Ahlawat (2011).

Finally, the results also indicate that the raw meat samples (beef, chevon and mutton) had higher moisture (%) when compared to the cooked meat (beef, chevon and mutton) while protein, fat and ash nutrient components (%) showed an increase after cooking. This may be because that there is coagulation of meat protein thereby hardening of the muscle fibers which leads to expulsion of water from the muscles which resulted in lower moisture content of the cooked meat. The result is in accordance with the observations of Jamora and Rhee (1998), Aaslyng et al. (2003) and Sainsbury, (2009).

Conclusion

These study indicate the overview about the quality parameters of beef, chevon and mutton meats at Hawassa city in Ethiopia.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Synergistic fermentative nutritional quality of *Lactobacillus delbrueckii* and *Bacillus pumilus* on date fruits (*Phoenix dactylifera*)

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The work examined the effect of mixed cultural fermentation on the nutritional quality of date palm fruits (*Phoenix dactylifera*). This was done by isolating microorganism from samples subjected to natural fermentation. The isolated organisms were thereafter used as starter culture in a five day controlled fermentation. *Lactobacillus delbrueckii* and *Bacillus pumilus* were identified as the predominant microorganisms and were used as starter culture. pH, titratable acidity, proximate, amino acid and mineral composition of the fermented samples were determined. The sample fermented with mixed culture gave best nutritional value of 15.3% protein as compared to the 9.62% and 11.61% yield in *B. pumilus* and *L. delbrueckii*, respectively, on the fifth day of fermentation. Arginine was found to be on the high side among the amino acids discovered and the mineral composition of the products gave an appreciable amount of sodium, potassium and calcium.

Key words: Fermentation, Nutritional quality date palm fruits, *Lactobacillus delbrueckii*, *Bacillus pumilus.*

INTRODUCTION

Fermentation is a metabolic process that converts sugar to acids, gases and/or alcohol. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium. Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide and hydrogen gas (H_2) (El-Sohaimy and Hafez, 2010). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Lactic acid fermentation is commonly used in many parts of the world as a method for preservation of plant materials as well as importing desirable sensory and nutritional properties to the product (Cooke et al., 1987). Fermentation has been reported to improve the nutritional values, taste and aroma (Adegbehingbe et al., 2014).

Date fruit (*Phoenix dactylifera* L.) is a palm in the genus *Phoenix* belonging to the *Arecaceae* family, cultivated for its edible sweet fruit. Dates, is identified by its clusters of oval, dark, reddish-brown drupe with the skin of the dried fruit wrinkled and covered in a sticky waxy film. The date palm (*P. dactylifera* L.) is one of mankind's oldest

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cultivated plants and has been used as food for about 6000 years (Sahari et al., 2007). It is an important food crop in Middle East and is considered to be one of the most important fruit tree particularly in North African, the Middle Eastern and Asian countries. The fruit contributes to the economy and social life within these regions (Bastway et al., 2008) and it is considered as a vital component of their diet (Vayalil, 2002). Date fruits are well known as a staple nutritious food and source of wealth for many years (Khan et al., 2008). Due to its high nutritional value, great yields and its long life, the date palm has been mentioned as the "tree of life" (Augstburger et al., 2002). Date fruits are considered as major source of carbohydrate which include simple sugars like glucose and fructose (Ahmed and Ahmed, 1995; Al-Hooti et al., 1997; Myhera et al., 1999) and sucrose (Guizani et al., 2010). They are good sources of dietary fiber and some important minerals which include iron, potassium, selenium, calcium and vitamins and it also contains vitamin C, B_1, B_2, A, riboflavin and niacin but it is low in fat and protein contents (Myhera et al., 1999; Guizani et al., 2010; Sawaya et al., 1983).

Al-Shahib and Marshall (2003) reported that the fruits (dates) of the date palm contain a high percentage of carbohydrate (total sugars, 44-88%), fat (0.2-0.5%), 15 salts and minerals, protein (2.3-5.6%), vitamins and a high percentage of dietary fibre (6.4-11.5%). There are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g date depending on the type of mineral. Additionally, the seeds contain aluminum, cadmium, chloride lead and sulphur in various proportions. The protein in dates contains 23 types of amino acids. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 thiamine, B_2 riboflavin, nicotinic acid (niacin) and vitamin. Date fruit is served mainly as desserts, these date fruits are generally chopped into tiny slices and scattered across cakes and puddings to ameliorate their flavor. Some people mix date paste with yogurt, milk, bread, or butter to enhance their flavor. Dates are also deseeded and stuffed with several sweet fillings like apricot. Besides the versatility of date preparation and consumption, these dry fruits also feature various nutritional values.

Dates are opulent in vitamins, minerals, natural fiber and are also cholesterol-free. Dates may be considered as an almost ideal food, providing a wide range of essential nutrients and potential health benefits (Al-Shahib and Marshall, 2003). In Tunisia, the date palm sap from date palm is directly consumed as a fresh drink called "Legmi" or used as an alcoholic beverage after natural fermentation. The fresh sap is purgative, sweet, clear, translucent and rapidly fermented (Ben Thabet et al., 2007). Composition analysis of fresh sap from date palm revealed that sugars are the major components (92-95% dry matter basis) with the dominance of sucrose. It contains also 2.7-5% of proteins and 2.3-2.6% of minerals (Ben Thabet et al., 2007, 2009). Palm sap is rapidly fermented by autochthones microflora composed essentially of yeasts, lactic acid bacteria and acetic acid bacteria. This is why date fruit benefits are widely known across the globe. Over the years, several approaches have been adopted in order minimize production cost, make available varieties of fermented drinks in the market and also to explore the arrays of underutilized nutritional fruits. Development of new beverage from date fruits using indigenous microorganisms may be a promising idea to the beverage industries. The aim of this research was therefore to study the quality of the date fruit upon fermentation by indigenous organisms.

MATERIALS AND METHODS

Collection of date palm fruits

Date palm fruits were purchased from a main market of Akure, Nigeria and transported to the Microbiological Laboratory of Federal University of Technology, Akure, Nigeria. The fruits were sorted and the apparently healthy fruits were washed, subjected to natural fermentation at 25±2°C for five days and samples were examined at each day of fermentation. Colonies were isolated and subjected to biochemical identification (Erdoğrul and Erbilir, 2006; Gueimonde et al., 2004). New set of apparently healthy ones were subjected to controlled fermentation using the single culture and mixed culture of the predominant cultures obtained from the fruits in a modified bioreactor at 25°C for 5 days (Adegbehingbe et al., 2014). Samples were aseptically withdrawn from the solution throughout the fermentation period and subjected to chemical, nutritional and microbiological analyses. Samples were also filtered and presented for sensory evaluation.

Determination of pH

The pH of the sample was determined at each day of treatment for five consecutive days using Jenway pH meter (Jenway 3010; Jenway Ltd., Essex, UK) which had been standardized with buffer solution of 7.

Titratable acidity

This was determined using the method of Valverde et al. (2005) by titrating 20 ml of fermented sample with 0.1 M NaOH to an end point of permanent pink colour using phenolphthalein as indicator.

Proximate composition

This was analyzed using standard procedure to determine the moisture, protein, fat, fiber, ash and carbohydrate (AOAC, 2003 and 1984).

Processing of the fermented samples

Both fermented samples were processed on the fifth day of fermentation for sensory evaluation. The flow sheet showing the process used to obtain fermented samples is given in Figure 1.

Amino acid analysis

The amino acid composition of the fermented sample was

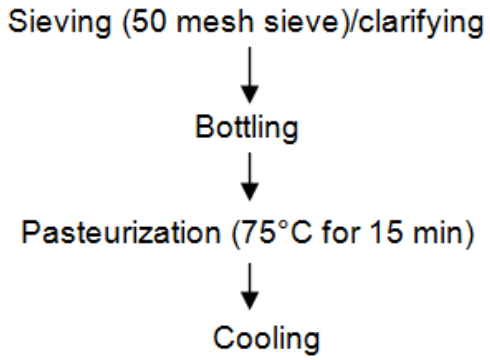

Figure 1. The flow sheet showing the process used to obtain fermented samples.

Table 1. Occurrence of organism during the fermentation periods.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B. pumilus	X	X	√	√	X
L. delbrueckii	X	√	√	√	√
B. pumilus + *L. delbrueckii*	X	√	√	√	√

determined by reverse phase HPLC by Kang-Lyung (2001).

Sensory evaluation

Sensory characteristic of the fermented processed drinks were assessed by 5 untrained panelists for their colour, texture, odour, taste and overall acceptability. The panelists recorded quality characteristics of each sample on an 8- point hedonic scale (8 = like extremely, 1=dislike extremely).

Statistical analysis

All analyses were carried out in triplicates. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used for comparison of means SPSS software (version 16.0 for Windows, SPSS Inc., Chicago). Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

This study revealed that *B. pumilus* and *L. delbrueckii* (Table 1) are the predominant fermentative microorganisms of date fruits with the occurrence even after 24 h of fermentation. This observation is in line with the findings of Okafor (2007) and On-Ong et al. (2012) who reported similar organisms as part of organisms that are associated with the fermentation of the fruit. The absence of *B. pumilus* on the fifth day of fermentation may be an indication that the environmental condition of the fermenting medium is not conducive for its metabolic activities. This observation may be supported by the result of the total acidity (Table 3) of the medium. *B. pumilus* is an alkaline active organism so the acidity produced by the fermentation of the date fruits makes the substrate unfavorable for its reproducibility. The use of starter cultures is in line with the research of Kimaryo et al. (2000) as an appropriate approach for control and optimization of the fermentation process in order to alleviate problems of variations in organoleptic quality and microbial stability observed in African fermented foods.

The observed increase in protein content could be due to the activity of *B. pumilus* which is mostly used for alkaline protease production. The reduction in the carbohydrate content can be attributed to the utilization of the sugars present in the fruits by the utilizing microorganisms (Akande et al., 2010). The decline in the carbohydrate content during the fermentation period may be connected to the ability of fermenting microorganisms to metabolize carbohydrate as carbon source in order to synthesize cell biomass (Vadivel and Janardhanan, 2005), thereby producing alcohol or acids. Ogunbanwo et al. (2004) reported the effect of fiber in food as an aid to digestion. The insignificant increase in the crude fiber may also be an indication that the product will have easy digestion when consumed. Fermentation using the mixed culture of *L. delbrueckii* and *B.pumilus* gave the best nutritional value in all the days of fermentation (Figures 2 to 4). This finding agrees with the work of Achi and Akubor (2000) who stated that the use of mixed cultures improve the nutrition of fermented foods while working on the effect of bacteriocinogenic *Lactobacillus* spp. on the shelf life of fufu, a traditional fermented cassava product.

The observed decrease in the pH of all the samples (Table 2) and subsequent increase in the titratable acidity justifies the claim that the fermentation process by the organisms is not only alcohol based but also acidic based. In food safety, fast reduction in pH is desirable in order to inhibit the growth of microorganisms especially Gram negative and acid sensitive food borne pathogens (Orji et al., 2003). The observed increase in total titratable acidity of the fermented sample during the fermentation period could also be linked to the ability of the organisms to secrete acid while utilizing the available nutrient for their metabolic activities (Holzapfel, 2002). The results of the sensory evaluation (Table 4) from this study has shown that product from combined starter cultures of *B. pumilus* and *L. delbrueckii* had higher acceptability as compared to the spontaneously fermented product. The observed amino acids content of the product is an indication that the product can be recommended or even used as fortifier in nutrient food deficient in amino acids (Table 6). The observed high value of one of the essential amino acid arginine may also make the product recommendable for patient having low erection as arginine has been reported as amino acid used by the

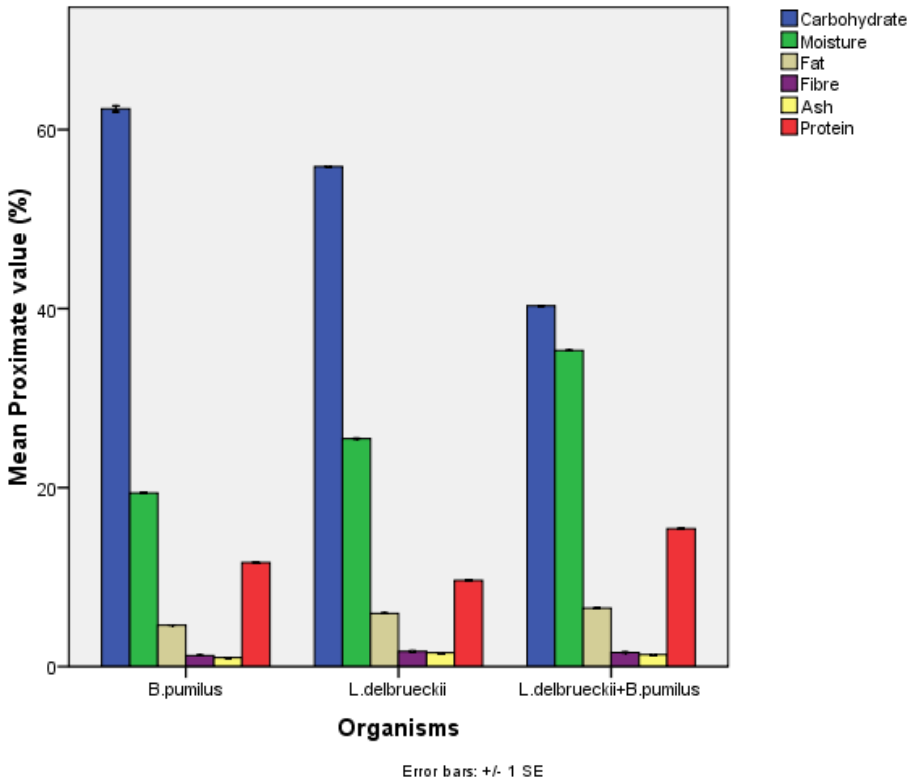

Figure 2. Proximate composition of fermented samples at the end of fermentation period (5th day).

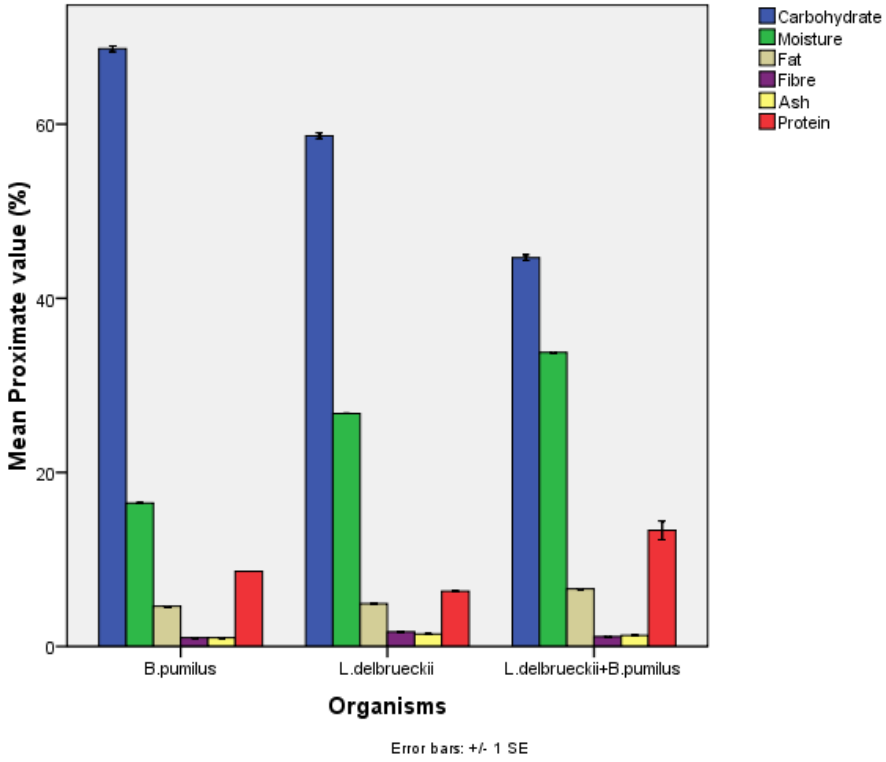

Figure 3. Proximate composition of fermented samples at the 3rd day of fermentation.

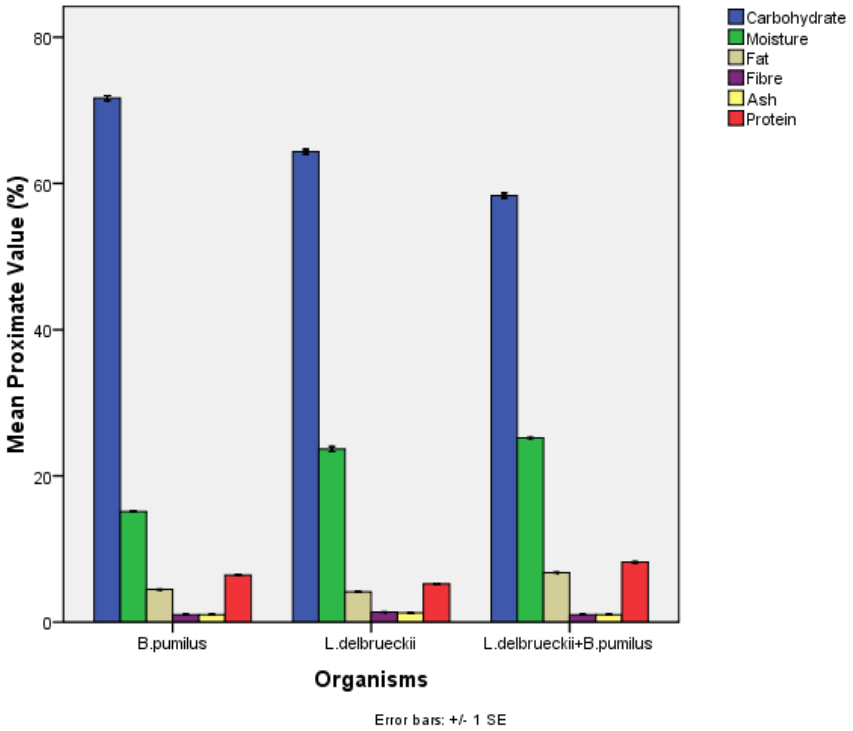

Figure 4. Proximate composition of fermented samples at the 1st day of fermentation.

Table 2. pH during fermentation period.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B.pumilus	7.2±0.0^{b}	6.7±0.06^{b}	6.8±0.05^{b}	6.4±0.1^{c}	6.5±0.0^{c}
L.delbrueckii	7.0±0.0^{b}	6.7±0.06^{a}	6.4±0.05^{a}	6.0±0^{b}	5.7±0.1^{b}
B.pumilus+L. delbrueckii	6.8±0.17^{a}	6.5±0.14^{a}	6.3±0.05^{a}	5.4±0.0^{a}	4.7±0.1^{a}

Samples with the same superscripts down the column are not significantly different.

Table 3. Total titratable acidity during fermentation period.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B.pumilus	1.1±0.0^{a}	1.3±0.0^{a}	1.33±0.1^{a}	1.7±0.0^{a}	1.7±0.0^{a}
L.delbrueckii	1.2±0.1^{b}	1.5±0.0^{c}	1.7±0.0^{b}	1.9±0.0^{b}	2.1±0.1^{a}
B.pumilus+ L.delbrueckii	1.2±0.0^{ab}	1.37±0.1^{b}	1.7±0.0^{b}	2.03±0.1^{c}	3.27±1.3^{a}

Samples with the same superscripts down the column are not significantly different.

Table 4. Sensory evaluation of the processed samples.

Fermented drinks	Appearance	Sourness	Odour	Taste	Acceptability
B. pumilus fermented drink	4.0±0.0^{a}	5±0^{a}	6.8±0.89^{a}	4±0.0^{a}	4±0.0^{a}
L. delbrueckii fermented drink	5.2±0.45^{b}	6.2±0.45^{b}	4.5±0^{a}	5.4±0.54^{b}	6.4±0.55^{b}
B. pumilus+ L. delbrueckii fermented drink	5.6±0.89^{b}	6.2±0.45^{b}	5.2±0.44^{b}	6.2±0.44^{c}	7±0.0^{c}

Samples with the same superscripts down the column are not significantly different.

Table 5. Mineral composition of the processed samples (µg/ml).

Fermented drinks	Mineral concentration (µg/ml)		
	Na	K	Ca
B. pumilus fermented drink	113.4±0.61[b]	354.1 ± 0.61[a]	118.7±0.58[a]
L.delbrueckii fermented drink	110.3±0.21[a]	355.0 ± 0.51[a]	118.5±0.58[a]
B. pumilus+ *L. delbrueckii*	114.8±0.5[c]	364.0 ± 0.72[b]	124.7±0.58[b]

Samples with the same superscripts down the column are not significantly different.

Table 6. Amino acid composition of the processed drink.

Amino acid	Concentration (µml/ml)
Alanin	0.78
Arginine	346.45
Aspartic	126.24
Cystine	3.47
Glutamin	87.31
Methionine	7.42
Proline	286.43
Threoine	43.21
Valine	76.32
Glycine	101.32
Histidine	178.90
Isoleucine	43.29
Leucine	30.63
Lysine	30.18
Phenylalanine	26.74
Serine	13.93
Tyrosine	15.42

body to make nitric oxide (NO), which plays a role in the development of erection by enhancing the circulation of blood (Onibon et al., 2007). Fermentation of date fruit gave an appreciable mineral content (Table 5). The high potassium content observed in all the fermented products conforms with the work of Beaumont (2002) while working on nutritional and anti-nutritional composition of some Nigerian fruits. The high odour reported by the panel in the *B. pumilus* fermented product may be due to their ability to secrete ammonia a bye product during the utilization of the amino acids present in the product.

Conclusion

The result of this investigation showed that a new beverage with acceptable organoleptic properties can be produced from date palm fruits using co-cultural fermentation of *B. pumilus* and *L. delbrueckii*. This fruits can be adopted by beverage industries to introduce new product with high nutritional quality into the market.

Conflict of interests

The authors did not declare any conflict of interest.

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Genetic variation of biochemical characteristics of selected sorghum varieties from East Africa

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A total of 30 sorghum varieties from East Africa were analysed for their biochemical characteristics. The objective was to ascertain the extent of the genetic diversity underlying their biochemical and physiological characteristics that included starch (%), amylose (%), amylopectin (%), proteins (%), tannins (mg/100 ml), yield (Kg/ha) and height (cm). The principal component analysis (PCA) showed that the first two contributed to the 69.66% of the variability among the sorghum varieties. Cluster analysis of these parameters resulted into four distinct groups with a genetic distance ranging from 0.74 - 6.42. The open pollinated and the hybrids showed the greatest genetic distances while the hybrids exhibited relatively low genetic distances. The biochemical content is a useful tool for measuring the genetic divergence among sorghum varieties to identify possible donors for future sorghum quality enhancement/breeding.

Key words: Biochemical characteristics, principal component analysis, genetic distance, cluster analysis, sorghum breeding.

INTRODUCTION

Sorghum is one of the world's most important cereals, with over 500 million people in the hot dry tropics dependent on it. Approximately 35% of sorghum is utilised as a food grain and the balance is used primarily in alcohol production, animal feed and industrial products (Dicko et al., 2006; Mehmood et al., 2008). Sorghum requires less moisture than other cereal crops and is more tolerant to extreme environment, making production easier in most agro-ecological zones subject to limited rainfall areas which are unfavorable for most cereals (Maunder, 2002).

Evaluating genetic diversity of germplasm can assist in differentiating varieties with the greatest novelty which as a result is most desirable for incorporation into crop improvement programmes. Given the diversity of sorghum, studying genetic diversity (Ayana, 2001) and

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biochemical composition of sorghum germplasm from East Africa is very important for numerous reasons. Sorghum breeding programmes have offered a wide range of new varieties with interesting traits that improved production and productivity (FAO and ICRISAT, 1996). Screening and selection of improved varieties for specific local food and industrial requirements from the broad sorghum biodiversity is extremely important for food security and poverty alleviation (Akintayo and Sedgo, 2001; Dicko et al., 2006).

The variation of heritable characteristics present among alleles of genes in different individuals of populations of species serves as an important role in evolution that allows a species to adapt to a new environment is termed as genetic diversity (IPGRI, 1993; Weir, 1996; Kremer et al., 1998). Genetic distance estimates determined by phenotypic and molecular markers help identify suitable germplasm for incorporation into future plant breeding programmes. Hence, assessment of genetic diversity in sorghum germplasm and determination of sorghum phenotypic and biochemical activities would help to determine the breeding potential of the accessions in East Africa.

Quantitative assessment of genetic diversity is important in determining the extent of genetic difference between and within crop species (Adugna, 2002). Genetic distance is a measure of the average genetic divergence between two sequences, species or between populations within a species or taxa (Souza and Sorrells, 1991). Genetic similarity is the converse of genetic distances, that is, the extent of sequence similarities among cultivars.

The measure of distance or similarity among cultivars is the covariance of allele frequencies summed for all characters (Smith, 1984). Genetically diverse parents produce high heterotic effects and yield desirable segregates. The pattern of genetic relationships between accessions can be shown by multivariate analyses. Cluster analysis is a useful statistical tool for studying the relationships among closely related accessions.

The quality of grain sorghum is determined by nutritional quality and anti-nutritional factors, such as tannins, processing characteristics, cooking quality and consumer acceptability (Hulse et al., 1980). Hence, it is important to assess genetic diversity based on quantitative and qualitative traits and identify promising accessions for different traits that could be utilised in breeding programmes.

Thus the objective of the study was to ascertain the extent of the genetic diversity underlying their biochemical and physiological characteristics that included starch (%), amylose (%), amylopectin (%), proteins (%), tannins (mg/100 ml), yield (Kg/ha) and height (cm). These traits are important because they have a direct effect on the use of sorghum varieties for different industries like baking, brewing, animal and poultry feed and other industries.

MATERIALS AND METHODS

Thirty sorghum varieties collected from the East African region were provided by ICRISAT Kenya (Table 1). The mid lowland sorghum was grown in Kampi Ya Moto (035° 56' E and 00° 05' S) at an altitude of 1660 m while the highland sorghum was grown at Egerton University altitude 2,250 m. The sorghum materials was grown in a randomized complete block design (RCBD) and replicated three times during the April to August season. The grain from two middle rows in each four row of the experimental unit was harvested, dried, threshed and used for subsequent laboratory and industrial tests.

Determination of protein content

Total nitrogen and protein was determined using Kjeldahl method (AOAC, 1999). Sorghum grain was finely milled and 0.1 g was transferred into a digestion tube. Selenium catalyst mixture weighing 1g was mixed with the sample and 5 ml of sulphuric acid (96%) was added into the tube. The tubes were then heated slowly in the digestion apparatus, until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into a 100 ml graduated flask. A boric acid indicator solution (5 ml) was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. The clear supernatant (10 ml) was then transferred into the apparatus and 10 ml of 46% sodium hydroxide was added and then rinsed again with distilled water. Colour changed from pink to green when the first distillation drops mixed with the boric acid indicator solution. A total of 150 ml of the distillate was collected and titrated with 0.0174N sulphuric acids until the colour changed from green to pink. The titer volume was then read. Total nitrogen (N) was then determined as follows:

$$\% N = \left\{ \frac{a \times N \times M_w \times 100}{b \times c} \right\} \times 100\%$$

Where, a = ml of sulphuric acid used for titration of the sample,
N = Normality of sulphuric acid (0.0174),
a = Titer volume (10 ml),
M_w = Molecular weight of N_2 (0.014),
c = ml digest taken for distillation (10 ml),
b = g sample taken for analysis (0.1 g),
% crude protein = 6.25 × % N.

Determination of starch content

Percent starch content was estimated by the Anthrone method (Hodge and Hofreiter, 1962). A powdered sample (0.25 g) was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. Of this supernatant, 0.1 ml was pipetted out and made up to 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working solution and the volume made up to 1 ml in each tube with water. To these standards, 4 ml of anthrone reagent was added to each tube and the sample heated for eight minutes in a boiling water bath. The sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to determine starch content.

Table 1. A list of the 30 selected sorghum varieties with high and low tannin, starch, protein, yield and height amounts used in this study.

Code	Variety	Code	Variety	Code	Variety
1	Ainamoi #1	11	IS 25547	21	ICSA 276 X ICSR 162
2	Siaya # 24-2	12	SDSH 90003	22	1S 25546
3	Kipkelion # 2	13	UasinGishu #1	23	IESH 22012
4	Nyiragikori	14	1S 25561	24	ICSA 12 X WAHI
5	Kipkelion # 1	15	UasinGishu #2	25	SDSA 29 X KARI MTAMA 1
6	MB 27	16	IESH 22006	26	IESV 92033 SH
7	Kabamba	17	Gadam Hamam	27	IESV 91104 DL
8	Nyangezi	18	ICSA 276 X ICSR 38	28	ICSA 371 X ICSR 108
9	BM 32	19	Kisanana	29	Busia # 21
10	1S 11162	20	Siaya # 2-3	30	IESV 92043 DL

Determination of Amylose content

Amylose was determined using the Mc Cready, (1950) method where a sample (0.1 g) of the powdered flour was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. To a 2.5 ml extract, 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Dropwise HCl 0.1N was then added until the pink colour disappeared. To this solution, 1 ml iodine reagent was added till the volume was 50 ml and the colour read at 590 nm using a spectrophotometer. Standard amylose working solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the colour developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the drawn standard graph. The blank was obtained by diluting 1 ml of iodine reagent to 50 ml with distilled water. Amylose content was obtained thus:

$$\% \text{ amylose } = \left[\frac{x}{2.5}\right] \times 100 \text{ mg amylose}$$

Where *x* is the absorbance obtained.
The amylopectin content was obtained thus: % Starch - % Amylose.

Determination of tannin content

A sample of 0.5 g of the milled flour was weighed and transferred to a 250 ml conical flask, and then 75 ml of water added. The flask was heated gently and boiled for 30 min, then centrifuged at 2000 rpm for 20 min. The supernatant was collected in a 100 ml volumetric flask. A measure of 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. Five ml of folin reagent and 10 ml of 35% sodium carbonate solution were added, and then diluted to 100 ml with water. The sample was shaken and the absorbance read at 700 nm after 30 min. A graph was prepared using 0 - 100 mg tannic acid, where 1 ml contained 100 mg tannic acid. The tannin content of the sample was calculated as tannic acid equivalent from the standard curve. Tannins content was determined by the Folin-Denis method (Schanderl, 1970).

Determination of height and yielding ability

The height (cm) of the mature sorghum varieties was determined by measuring the average of three plants in each plot of each variety in every treatment, then the average of each plot in the three treatments.

The yielding ability (Kg/Ha) of sorghum varieties was determined in grams by getting the average yielding ability of three plants in each plot of each variety in every treatment, then the average yield of each plot in the three treatments.

Data analysis

Multivariate analysis was undertaken using JMP statistical software, version 10. Principal component analysis (PCA) was used to correlate the standardized data from biochemical composition analysis with genetic background and physiological characteristics. The determination of genetic dissimilarity was done using Euclidean distance and the hierarchial agglomerative clustering methods (Shergo et al., 2013). Euclidean measure of distance was used for the estimation of Genetic Distance (GD) among varieties.

RESULTS AND DISCUSSIONS

Principal component analysis

The biochemical composition of 30 genetically diverse sorghum varieties was measured in order to identify varieties for breeding programs. Components with an eigenvalue of less than 1 were eliminated because they were not significant (Chatfield and Collins, 1980), while those with eigenvalue greater than one, and component loadings greater than ±0.3 were regarded meaningful and significant, as reported by Hair et al. (1998). Therefore, from this study, only the first two eigenvectors, which had eigenvalues greater than 1, and cumulatively explained 69.6% of the total variation among the seven biochemical compositions describing the varieties, were considered (Table 2). Thus the principal component 1 (PC1) had an eigenvalue of 3.39 and accounted for 48.4% of the variation. This represents an equivalent of 6 variables and showed that starch, amylopectin, protein, tannins, yield, and height significantly contributed to the variation among varieties. The ones with high PC1 scores therefore contain the high levels of this biochemical

Table 2. Principal component analysis of starch, amylose, amylopectin, proteins, tannins, yield and height in sorghum varieties showing eigenvectors, eigenvalue and their percentage contribution to the total variation in the first two principal component axes.

PC	Total variance			Eigen vectors (loading) for						
	Eigenvalue	Individual %	Cumulative %	Starch	Amylose	Amylopectin	Protein	Tannins	Yield	Height
1	3.39	48.45	48.45	0.854	-0.28	0.89	-0.78	0.60	0.45	0.76
2	1.48	21.21	69.66	0.34	0.58	0.21	-0.01	0.52	-0.72	-0.41

parameters. PC2 had an eigenvalue of 1.48 contributing 21.2% of the variation that represents five variables (starch, amylose, tannins, yield and height), which contributed to the variation among the varieties. Shergo et al. (2013) found a genetic variation in 30 sorghum land races from Ethiopia using multivariate analysis. They discovered that multivariate analysis was useful measure of genetic variability among landrace accessions to single out potential donors or parental lines for future sorghum quality improvement.

The biplot (Figure 2) classifies the varieties with biochemical characteristics explained by the first two dimensions PC1 and PC2. As a result a breeder can easily pinpoint distances between the varieties and make decisions based on the principal component simultaneously. In the score plots, varieties close to each other are similar while the ones found near the origin are distinct and the ones further out are extremes. PCA analysis classifies the varieties into groups over the four quadrants based on the concentrations of these seven parameters. The varieties were distributed throughout the quadrant demonstrating large genetic variability in their biochemical contents. The ones on the top left quadrant were related in their amylose contents while, the right top quadrant contained varieties related in their tannin, starch and amylopectin contents. The left bottom quadrant and the right bottom contain varieties related in their protein, yield and height, respectively. The distance between the locations of any two varieties on the score plot is directly proportional to the degree of similarity/difference between them as per their biochemical components (Shergo et al., 2013). The score plot showed that varieties *Kisanana (#U), Ainamoi #1 (#D), IS11162 (#A), IS25546 (#B), IESV 92043 DL (#P), ICSA 371 X ICSR 108 (#K)* were the most divergent from the major group, which in the PCA as concentrated on the center demonstrating some similarity in their biochemical values. Varieties which overlapped in the principal component axes had similar biochemical contents. Thus *Kisanana (#U)* and *IS25546 (#B)* showed similar relationship in the first principal component axis.

The loading plot (Figure 1) shows the correlation among biochemical compositions. The elements with small loading located near the origin have little influence on data structure while those with high loadings represent the greatest influence on data structure on clustering and separation of sorghum varieties. Thus, all the parameters in this study had significant influence of the data structure with starch, amylopectin and tannins having influence on most varieties.

Genetic distance and cluster analysis

The genetic distance estimates matrix based on the biochemical characteristics for all pair wise combinations of (30×29)/2 = 435 of the 30 sorghum varieties is shown in Table 3. Genetic distances from 0.74 - 6.42 was observed in the pair wise combinations demonstrating that the varieties were diverse in the measured traits as their biochemical composition. The minimum genetic distances of 0.74 and 0.91 was observed between varieties *IESV 92043 DL* and *IESV 91104 DL* and between *ICSA 276 X ICSR 162* and *IESH 22006*, respectively. The highest recorded genetic distance 6.42 was between *ICSA 371 X ICSR 108* (Hybrid) and *Kabamba* (OPV) varieties, 6.30 between *SDSA 29 X KARI MTAMA 1*(Hybrid) and *Kabamba* (OPV) and 6.00 between *ICSA 371 X ICSR 108* (Hybrid) and *Ainamoi #1* (OPV) demonstrating their high genetic diversity between the varieties as a result of their biochemical contents. The wide genetic distance in this data set gives sufficient evidence of the presence of genetic diversity for the determined biochemical characteristics with little relatedness. Therefore varieties that possess with the highest genetic distance between them can be used in breeding programs crossing blocks.

Cluster analysis of biochemical composition (Figure 3) revealed a clear separation between the varieties. It showed the differences between clusters by summarizing cluster means for the seven biochemical traits. The highest cluster mean (Table 4) were recorded in yield (4153.25 Kg/ha) while the lowest were in proteins (8.9%) and amylose (17.7%). The existence of maximum genetic divergence among varieties was observed in clusters 2 and 3 due to their high cluster means. The varieties in this study were divided into four main clusters in the dendrogram. The first cluster contained varieties with the lowest starch, tannins and height as compared to the other clusters. Most varieties had a lower amylopectin to amylose ratios as compared to the other clusters. This cluster registered highest proteins and were mostly the hybrids while the yields of the varieties varied. It is useful when identifying the varieties that are favorable for

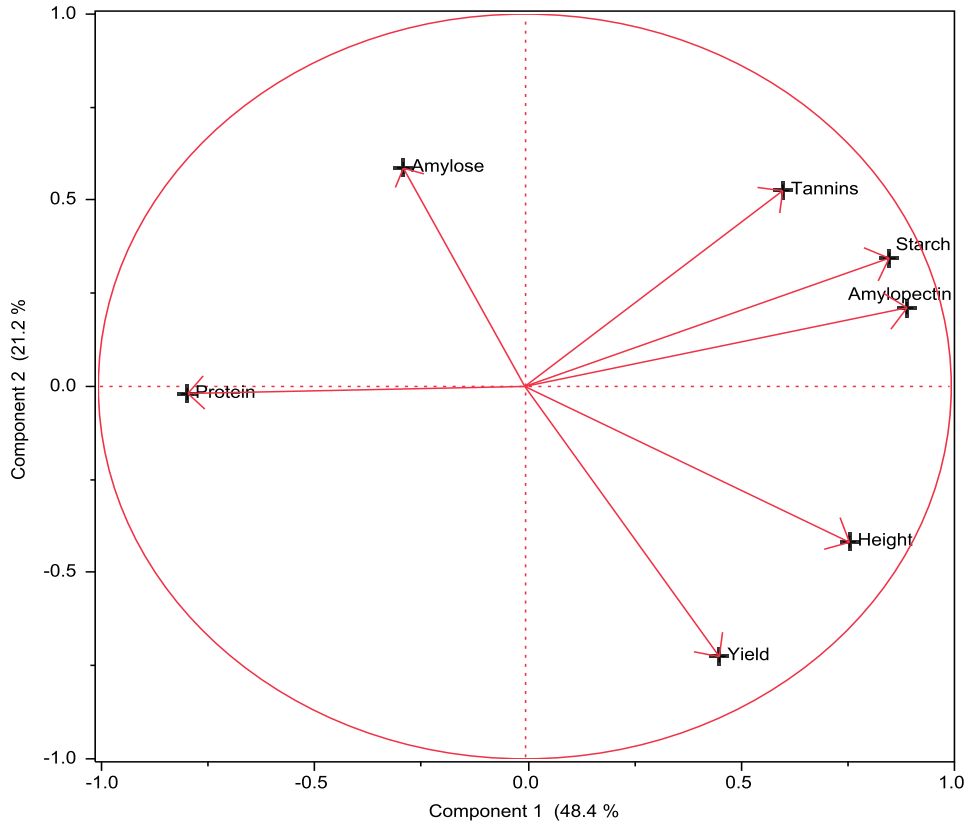

Figure 1. Principal Component Analysis loading plot for seven biochemical traits of 30 the sorghum varieties.

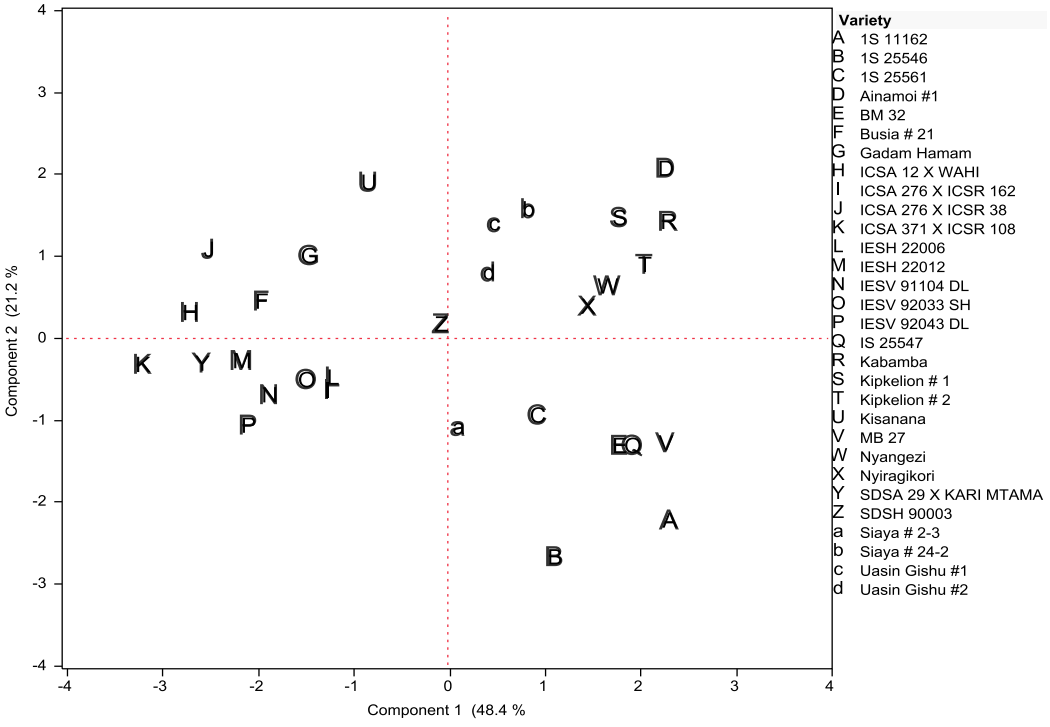

Figure 2. Principal component score plot of PC1 and PC2 describing the overall variation among sorghum varieties estimated using biochemical data.

Table 3. Estimates of genetic distance based on biochemical characteristics of 30 selected sorghum varieties from East Africa.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ainamoi #1	1																													
Siaya # 24-2	2.39	0.00																												
Kipkelion # 2	1.98	1.75	0.00																											
Nyiragikori	2.68	2.22	1.89	0.00																										
Kipkelion # 1	1.62	2.13	1.38	1.93	0.00																									
MB 27	4.05	3.42	2.67	2.84	3.31	0.00																								
Kabamba	3.21	3.53	2.27	3.72	2.56	4.27	0.00																							
Nyangezi	2.12	2.83	1.70	2.23	1.54	3.45	2.31	0.00																						
BM 32	3.45	3.64	2.96	2.72	3.26	2.38	4.53	2.69	0.00																					
1S 11162	4.33	4.44	3.38	3.36	3.97	2.48	4.50	3.11	1.49	0.00																				
IS 25547	3.52	3.81	2.79	2.78	2.95	2.24	3.94	2.29	**1.11**	1.45	0.00																			
SDSH 90003	3.53	2.02	2.66	2.71	2.81	2.84	4.24	3.14	3.02	3.90	3.10	0.00																		
UasinGishu #1	2.61	2.58	2.72	3.17	1.99	3.83	3.63	2.53	3.48	4.50	3.25	2.16	0.00																	
1S 25561	3.51	3.50	2.72	2.64	3.01	3.23	3.60	1.82	1.91	2.05	1.71	3.02	3.33	0.00																
UasinGishu #2	2.75	2.71	2.41	3.09	2.14	3.77	2.90	1.76	3.14	3.83	2.79	2.31	1.44	2.32	0.00															
IESH 22006	4.47	3.00	3.70	3.36	3.94	3.85	5.10	3.75	3.37	4.11	3.65	1.67	3.26	2.95	2.93	0.00														
GadamHamam	4.54	2.94	3.64	3.63	3.54	4.66	4.23	3.72	4.82	5.37	4.61	2.41	3.05	3.82	2.79	2.48	0.00													
ICSA 276 X ICSR 38	4.96	4.01	5.10	4.54	4.76	5.91	6.30	4.78	4.95	6.03	5.27	3.32	3.69	4.49	3.80	2.54	3.23	0.00												
Kisanana	3.90	3.98	4.55	4.66	3.76	5.52	5.35	4.06	4.65	5.86	4.64	3.36	2.00	4.52	2.78	3.81	3.86	2.97	0.00											
Siaya # 2-3	4.52	3.61	3.25	3.16	3.28	2.74	4.07	3.13	3.13	3.38	2.56	2.19	2.97	2.63	2.62	2.70	2.84	4.52	4.32	0.00										
ICSA 276 X ICSR 162	4.53	3.33	3.81	3.27	3.80	3.89	5.07	3.57	3.30	4.04	3.40	1.85	3.07	2.75	2.77	**0.91**	2.38	2.52	3.58	2.25	0.00									
1S 25546	5.08	4.96	4.24	4.28	4.53	3.05	5.14	3.76	2.11	1.96	1.84	3.66	4.22	2.50	3.63	3.73	5.13	5.58	5.21	2.80	3.49	0.00								
IESH 22012	5.32	3.78	4.42	4.12	4.64	5.02	5.22	4.27	4.65	5.07	4.70	2.95	4.15	3.53	3.49	1.76	2.01	2.90	4.60	3.29	1.87	4.75	0.00							
ICSA 12 X WAHI	5.55	4.16	4.90	4.45	4.91	5.79	5.61	4.60	5.25	5.77	5.30	3.55	4.40	4.06	3.84	2.43	2.18	2.57	4.56	3.94	2.38	5.49	**1.04**	0.00						
SDSA 29 X KARI MTAMA 1	5.44	4.40	5.17	4.53	5.09	5.64	**6.30**	4.71	4.55	5.35	4.87	3.42	4.25	3.86	3.90	2.00	3.31	1.61	3.95	4.10	1.92	4.84	2.07	1.94	0.00					
IESV 92033 SH	4.85	3.70	4.09	3.66	3.88	4.11	5.07	3.80	3.82	4.51	3.66	2.07	2.93	3.22	2.78	1.74	2.13	2.84	3.42	1.92	**1.02**	3.72	2.23	2.61	2.50	0.00				
IESV 91104 DL	5.54	4.23	4.46	4.19	4.42	4.67	4.96	4.16	4.64	4.95	4.32	2.96	3.83	3.54	3.29	2.46	1.89	3.70	4.50	2.20	1.98	4.29	1.84	2.25	3.06	1.48	0.00			
ICSA 371 X ICSR 108	**6.00**	4.88	5.59	5.08	5.39	5.99	**6.42**	5.08	5.17	5.88	5.25	3.66	4.32	4.31	4.00	2.45	3.02	2.05	3.94	3.94	2.12	5.06	2.06	1.83	1.29	2.15	2.45	0.00		
Busia # 21	5.19	4.43	4.73	5.10	4.53	5.51	4.85	4.18	4.98	5.55	4.70	3.33	3.19	3.95	2.60	3.07	2.64	3.40	3.18	3.37	2.84	4.60	2.85	3.05	3.29	2.47	2.52	2.66	0.00	
IESV 92043 DL	5.93	4.69	4.86	4.73	4.86	4.89	5.26	4.50	4.82	5.07	4.47	3.25	4.07	3.75	3.48	2.70	2.41	3.96	4.62	2.36	2.24	4.15	2.16	2.65	3.23	1.70	**0.74**	2.49	2.31	0.00

different end uses such as for the brewing and baking industry. Cluster 2 contained varieties with medium starch, height, average protein contents and high amylopectin compared to their respective amylose amounts which are lower than cluster 4 and 3. They possessed the highest yields and height compared to the rest of the clusters. Variety *MB 27* is separated from this cluster due to its low protein and tannin contents

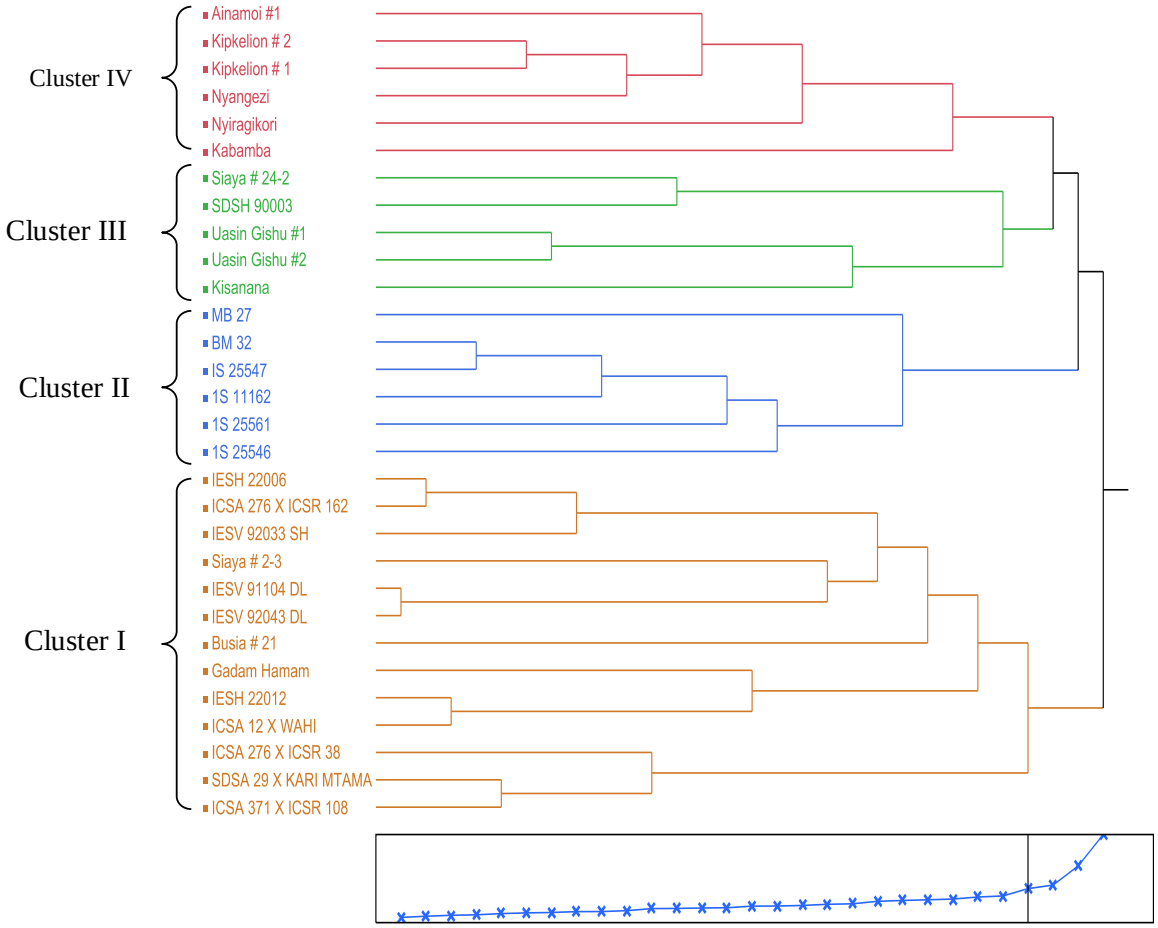

Figure 3. Dendrogram showing clusters 1 - 4 of 30 biochemical and morphological characteristics of sorghum varieties colored with red, green, blue and orange respectively.

Table 4. Summary of cluster means of 30 sorghum varieties biochemical characteristics.

Parameter	Cluster means				
	I	II	III	IV	Mean
Starch %	73.4	50.4	57.9	37.8	54.8
Amylose %	17.8	19.8	15.2	18.1	17.7
Amylopectin %	55.6	30.6	42.7	19.7	37.1
Proteins %	7.7	6.8	7.5	13.6	8.9
Tannins Mg/100 ml	61	50.3	35.1	14.9	40.3
Yield Kg/Ha	2877.2	3750.2	7058	2927.6	4153.2
Height cm	226.6	172.4	298.5	142.7	210.05
Mean	474.2	582.9	1073.6	453.5	646.0

unlike others varieties in this cluster. Cluster 3 varieties had medium starch, height and good protein amounts. They exhibited high amylopectin compared to their respective amylose, high yields and tannins. Cluster 4 varieties had the highest starch and tannins content and also had high amylopectin compared to their respective amylose amounts. They also had medium height, yield and good protein amounts. In the dendrogram *Kabamba* variety in this cluster was separated because it had the highest tannin compared to the rest. Varieties in clusters 2,

3 and 4 were predominantly open pollinated varieties with the exception of *SDSH 90003* in cluster 3.

The dendrogram showed that the varieties in this study could be valuable sources of genetic variability in sorghum breeding programmes. Multivariate analysis can therefore be a good tool for classifying biochemical characteristics. This is because, higher heterotic groups in sorghum breeding programmes in East Africa would be attained by selection and crossing of sorghum varieties in these different clusters. Grouping of these varieties according to their similarities and differences via multivariate analysis allows the breeder to better understand the data. Aremu et al. (2007) and Shergo et al. (2013) reported considerable genetic divergence among sorghum varieties on the basis of their chemical contents and phenotypic characteristics. Similar to the approach taken in the present study, Shergo (2010) also showed the grouping of accessions by multivariate method of analysis based on their divergences and similarity among sorghum varieties. The results of the present study could similarly be valuable for sorghum breeders because the most vital accessions in different clusters may be crossed with other groups to enhance the trait of interest.

Conclusion

Based on the observed variation of both quantitative and qualitative traits, it was concluded that characterizing the phenotypic diversity of sorghum varieties is important to categorize the genetic potential of varieties and could be used to increase the efficiency of sorghum breeding programmes. High genetic distances were observed among some varieties, especially between the hybrids and open pollinated varieties. The multivariate analysis successfully estimated genetic diversity among the tested varieties based on their biochemical characteristics.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Production and evaluation of organoleptic characteristics of fruit juice and low-sugar pulp of Behbahan variety dates of Kasi and Kabkab

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The purpose of this study was the production of high-value date syrup and low sugar powdered pulp and evaluation of its organoleptic characteristics. In this study, first, 100 g date were weighted and after disinfection, cleaning and removing of wastes and crushing, were mixed in 300 mL of boiling water for 20 min and then dates syrup were obtained by filter. The low sugar pulps were weighted. Next, the organoleptic characteristics of date fruit juices such as taste, color and aroma by panelists were evaluated by 5 people. The samples were analyzed by Hedonic preference test. The results showed there is no significant difference between the scores of the color or but there is a significant difference for aroma ($p < 0.05$). Average values of pulp obtained were equal to 50 g and there is no significant difference between the results of ten samples. Pulp material obtained after drying was converted to powder.

Key words: Kabkab and Kasi dates varieties, organoleptic properties, panelists, date syrup, low sugar pulp.

INTRODUCTION

Dates fruits of Kabkab and Kasi, are widely cultivated in semi-humid areas of Behbahan, in southern Iran. Because each year significant amounts from produced dates, is wasted and disposed, so it is better for them to be processed. Date fruits are well known as a staple nutritious food and source of wealth for many years (Vayalil, 2002). Due to its high nutritional value, great yields and its long life, the date palm has been mentioned as the “tree of life” (Khan, et al. 2008). The fruits of the date palms are consumed throughout the world. Dates are being consumed in modern cultures for their pleasant flavor, odor and their biting texture in addition to their use for flavoring foods, beverages and medication (Augstburger et al., 2002). Date fruits are considered as major source of carbohydrate which include simple sugars like glucose and fructose (Myhera et al., 1999) and sucrose. They are good sources of dietary fibre and some important minerals which include iron, potassium, selenium, calcium and vitamins and it also contains vitamin C, B1, B2, A, riboflavin and niacin but it is low in fat and protein contents (Guizani et al., 2010).

In addition to their significance as an ideal high-energy food, in the folklore, the dates are believed to have many medicinal properties such as to provide strength, fitness,

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and relief against a number of ailments and pains including fever, stomach disorders, memory disturbances, nervous disorders, as well as aphrodisiac and to boost the immunity. They are also considered to protect against many chronic diseases including cancer and heart diseases (Vyawahare et al., 2009) as they have been shown to contain antioxidant and antimutagenic properties (Al-Farsi et al., 2005; Vayalil, 2002; Allaith, 2008). Aqueous extracts of dates have also been shown to inhibit the lipid peroxidation and protein oxidation as well as exhibit a potent superoxide and hydroxyl radical scavenging activity (Allaith, 2005). Panahi and Asadi (2009) reported that extract of date fruit were useful in controlling the blood cholesterol levels and also protected the CA1 neurons against oxidative injury. Methanolic and aqueous extracts of date flesh and seeds have also been shown to exhibit anti-inflammatory properties and suppressed the swelling in the foot and adjuvant arthritis (Mohammed and Al-Okbi, 2004).

Date fruit is known as a source of food rich in carbohydrates and calories. Dates and its products are used in the food and pharmaceutical industries, such as ethanol, citric acid, yeast bread and high fructose date syrup (HFDS) (Botes and Zaid, 2002). World production of dates in 2002 was 4.5 million tons, and increased. 65 percentage of world production is produced in the Persian Gulf. Iran produces 18 percentage of the world's date and is one of the main producing countries (Al-Bazzaz, 2004; Iranian Date and its Market in the World, 2001; Iran Nap Co., 2001). Sucrose is the main sweetener in the world, but after 1967, other sweeteners such as high fructose syrup, with sucrose has been replaced.

Today, syrup contains high fructose concentration are produced and used as a sweetener in many countries such as the United States of America, Canada, Europe Union, Japan, South Korea and Argentina (Forristal, 2001; U.S. Department of Agriculture and Economic, 1991). Dates constitute the main part of the diet and an important source of income for the majority of the population in rural areas. They are also important for the environment, so that this product is compatible to environmental stresses such as temperature, salinity and drought (Benyamin, 1993). Low quality date is cultivated in about 60 percent of the total. This date with low size and taste, is suitable for consumption, which usually is sold as animal feed.

Despite the high sugar content of such low quality date, making them suitable for industrial use, and thus the range of by-products can be produced from these dates. Dates syrup that is probably the most common product is produced in especial way, including the extraction and boiling the syrup, in industry half or full-scale, which include the extraction process, refining and then the dates syrup is concentrated (Barreveld, 1993; Dowson, 1994; Al-Rawi et al., 1997). Aim of this study was preparation and organoleptic evaluation of syrup of two varieties date and low-sugar pulp from Behbahan, Khuzestan, Iran.

MATERIALS AND METHODS

Plant material

Two date palm cultivars grown in Behbahan, Iran were used for this study. The dates in “Tamr” stage (maturity stage) are obtained from the farmers. The samples were collected during 2014 season. 4 kg of each variety were used for experimentation. Each sample was cleaned and placed in polyethylene bags with labels, and stored in refrigerator until analysis.

Extraction of palm syrup

Certain varieties of dates (Kasi and Kabkab) from the local market in Behbahan, Khuzestan, Iran were purchased. Grinding system (EMS, MTK20, Saarbrucken, Germany) used to fine the date to thickness 0.5-1 cm was used, and after the dates with an equal amount of water were mixed in a container vapor (Model 241, Benham, London, UK), then were stirred for 20 min at 60 °C. The produced liquid was passed through the fabric, and the remain pulp was mixed with water and was extracted again.

Refining method of date syrup

In order to improve the quality of the extracted syrup, filtration treatment was used. Filtration using a filter press (Model 13039; William Boulton, Burslem, UK) was performed in two stages, large (50-mesh filter paper) and small (3 mesh filter paper).

Evaporation

Refined syrup under the same conditions using the evaporator BCH (30-L, Rochdale, UK) was evaporated (70 C ° under a vacuum of 500-600 mm Hg) to 70 ° Brix syrup was produced. Then, 5 person panelists, organoleptic characteristics of date fruit juice such as flavor, color and aroma were evaluated. The samples were analyzed by Hedonic preference test. The samples with the evaluation forms to evaluate the sensory characteristics were presented to panelists. Panelists were provided the points on each of the sensory characteristics based on scoring on any of the sensory properties. Here, assessment was carried out as a seven-point test (+3 to -3) that was very good to very bad. After scoring on the acceptability of each product quality properties in accordance with the scoring form, the results were evaluated and analyzed. The results of the samples were evaluated through a randomized complete block design.

100 g of dates from the two varieties with 300 mL of water carefully was mixed by mixer and was heated to boil. Each of the samples for 10 minutes after boiling, were heated. We control the mixture boiling time by chronometer. The most nutrients of date by the heat were extracted into the water. The obtained mixture was filtered separately to the date syrup and pulp. The obtained pulp, carefully was weighed.

Statistical analysis

The results obtained from the analyses of the samples were

Table 1. Panelists scoring in each of evaluated sensory characteristics based on a seven-point test.

Organoleptic characteristics	Taste	Color	Aroma	Result
Excellent	-	-	-	Negative
Good	+	+	-	+2
A little good	-	-	+	+1
Neither good nor bad	-	-	-	Negative
A little bad	-	-	-	Negative
Bad	-	-	-	Negative
Devilish	-	-	-	Negative

Mark (-) means rejection and mark (+) means accepting the sample sensory characteristic. Each mark shows mean of 5 times panelists assessment. Samples were obtained from varieties of dates grade 2 and 3.

Table 2. Mean scores given by panelists to each of the evaluated sensory characteristics of samples.

Organoleptic characteristics	Taste	Color	Aroma
Treatment 1	32	33	24
Treatment 2	34	36	27
Treatment 3	35	34	22
Treatment 4	33	32	20
Treatment 5	31	33	21

Each data means 5 replicates comments 5 panelists. Maximum scores given of taste, color and smell, were 40, 30 and 30 respectively. Maximum given scores to each treatment was 100. Samples obtained from varieties of dates grade 2 and 3.

computed using one-way analysis of variance (ANOVA) by SPSS program.

Sensory evaluation

The sensory panel includes ten (10) individuals (5 women and 5 men, aged 25-50 years) having already consumed dates fruit syrup. Fruit quality was assessed by comparing aroma, color and flavor. For visual evaluation or flavor, the samples order was randomized. During flavor evaluation panelists rinsed their mouth with water at room temperature after intake of each variety of product.

RESULTS AND DISCUSSION

The obtained results from the first stage project showed there was no significant differences between the scores of the color and taste of the product but there was significant differences in smell ($P < 0.05$). Thus it can be inferred that the panelists evaluated, date syrup taste and color with good score but smell with little good score degree (Table 1). It is observed that the date juice acceptability by consumers were at desirable and good level. Because the nutritional value of dates that are rich in minerals and vitamins and sugar, it can be as one kind of good juice is proposed for people consumption. The 5 ppanelists, from a maximum of 40 scores date syrup taste, presented 35 scores. All panelists were provided maximum scores to color characteristic and one person panelist was provided from maximum of 30 scores, 27 scores to smell characteristic. Overall 3 panelists were provided from total of 100 scores, 97, 91 and 89 scores, which is acceptable for product acceptance by consumers (Table 2). There were no significant differences between the scores given by panelists for the taste and color, and there was significant difference for smell characteristic. The obtained results from second stage can be concluded that the wet pulp and the dried powder contents obtained from certain variety of Kabkab date was more than Kasi variety (Table 3). Although, since the amount of sugar and sweetness percentage of certain variety of date Kasi was more than variety of Kabkab date, and Kabkab date usually fresh is consuming and usually this kind of date not have any in grades 2 and 3, but Kasi date variety have necessary conditions for extraction of syrup and dried powder. So it seems that it is appropriate for preparation of the pulp from grades 2 and 3. This results are in agreement with Acourene et al. (2001) and Al-Abdoulhadi et al. 2011.

Sensory evaluation

To the consumer, the most important attributes of a food are its sensory characteristics such as flavor, aroma,

Table 3. The average yield pulp obtained and weighed (in 100 gram sample) of two varieties of dates.

Varieties	Yield										Average
	1	2	3	4	5	6	7	8	9	10	
Kabkab	51	53	49	47	52	54	48	46	51	49	50
Kasi	48	46	50	44	49	45	47	48	46	47	47

color, texture and overall acceptability. Thus, sensory evaluation was performed for this product in order to determine the most acceptable sample by the panelists. The panelists prepared were palatability tested in terms of taste, after-taste, color, aroma and overall acceptability by panelists. With regard to the same data, there were no significant differences among all the studied products in their aroma, taste and color.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Nutritional suitability of bred sorghum (*Sorghum bicolor*) accessions from East Africa

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In many semi-arid and tropical areas of the world especially in sub Saharan Africa, sorghum is a staple food grain and has great potential to be used in various industries. Thirty sorghum highland and open pollinated varieties were analysed for their biochemical and physiological characteristic to determine their industrial suitability and breeding impacts. The results show that sorghum varieties have the capability to be used for different industries and can be good alternatives to other cereal varieties. Majority of the varieties like *Ainamoi #1, Siaya # 24-2, Kipkelion # 2, Kipkelion # 1, Nyangezi, Uasin Gishu #1* and *Uasin Gishu #2* with high starch and amylopectin contents also recorded high tannin contents and vice versa. Hybrids are bred to give low tannins, but unfortunately this also affects their starch amounts and in the long run, decreases the suitability of sorghum for industrial and domestic use. In addition, there was a significant correlation between yielding ability and plant height.

Key words: Breeding, industrial suitability, biochemical characteristics.

INTRODUCTION

Sorghum, *Sorghum bicolour,* is the fifth most important cereal crop after rice, wheat, maize and barley (Smith and Frederiksen, 2000; FAO, 2005), and it contributes significantly to the protein and energy requirements of millions of people, especially the poor in Africa and Asia (Elkhier and Hamid, 2008). Sorghum has the potential to drive economic development in Africa. In developing countries, the commercial processing of these locally grown sorghum grains into value-added food and beverage products is an important driver for economic development (Taylor, 2004). Subsistence farmers in Africa cultivate sorghum widely as a staple food for home consumption. In particular, sorghum is mainly important for people in sub-Saharan Africa as a food crop because it is adapted to a wide range of ecological conditions and can tolerate adverse conditions such as hot, dry, wet and water logged conditions. It can also adapt to poor fertility and high salinity soils (FAO, 2012).

Sorghum grain is an important staple food in developing countries of the semi-arid tropics and is also used as an animal feed in both developed and developing countries. The use of sorghum not only

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provides farmers with incomes after marketing their products but also saves foreign exchange, which would otherwise be required to import cereals. More than 35% of sorghum is grown primarily for human consumption, while the rest is used for feeding animals, brewing alcohol among other uses (Amir et al., 2009). Cereal grains like sorghum can constitute major energy sources and starch as raw materials for several end uses such as in baking, brewing, poultry and livestock industries. Despite this, maize, wheat and barley still remains the primary energy source in these industries. Ajaja et al. (2002) stated that maize is still the main energy source in compounded diets and constitutes about 50% of poultry ration. Like other cereals, starch is the principal storage form of carbohydrate and sorghum has an average starch content of 69.5% (Jambunathan and Subramanian, 1987). High fiber content and poor digestibility of nutrients are characteristic features of sorghum grains, which severely influences its consumer acceptability. Proteins form the second major component of sorghum grains. The protein content of sorghum is affected by both genetic and environmental factors (Arun et al., 2009).

Phenolic compounds in cereal grains encompass a diverse group of secondary plant metabolites. They can be conveniently divided broadly into phenolic acids, flavanols, polymeric flavanols and condensed tannins. Tannins bind proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents. Sorghum varieties rich in tannins are recommended for obese individuals and diabetic patients. Animal studies observed a 50% weight loss when fed with sorghum containing high tannin levels (Ambula et al., 2001). This is because they have a longer emptying time in the stomach (Awika and Rooney, 2004). The low digestibility of high tannin sorghums is due to the inhibition of hydrolytic enzymes and their potent antioxidant activities (Dicko et al., 2005) may be interesting from a nutritional standpoint for obese persons.

Anthocyanins are nowadays regarded as an important nutraceuticals mainly due to their possible antioxidant effects. They have a potential therapeutic role related to some cardiovascular diseases, cancer treatment, inhibition of certain types of virus including human immunodeficiency virus type 1 (HIV-1) and improvement of visual acuity (Stintzing et al., 2002; Sandvik, 2004; Talavera et al., 2006; Beattie et al., 2005). Mild levels of reactive oxygen species (ROS) in food have been shown to induce proliferation of cancer cells (Arora et al., 1999; Del Bello et al., 1999). Therefore, foods rich in antioxidant phytochemicals are important for the prevention of diseases related to oxidative stress such as heart disease and cancer.

Sorghum hybrids have contributed significantly to increased grain and forage yields in several countries. A large number of hybrids have been developed and released for commercial cultivation in East Africa. Achievements in sorghum breeding in Africa have mainly been in the development and release of improved varieties based on higher grain yield and resistance to diseases, insect pests and Striga (Obilana, 2004). Little focus has been put on improving the nutritional aspects of cereal grains.

In plant breeding traits such as yielding ability and quality should be the fundamental objectives. However before a new cultivar is released to farmers, it is important that its nutritional quality is properly evaluated for their biochemical characteristics. Therefore, to screen varieties for consumer acceptability, simplified laboratory tests, applicable to a very large number of samples are required. The objective of this study was to determine biochemical and physiological characteristics of sorghum grain that lead to food and nutritional security.

MATERIALS AND METHODS

Sample preparation

Thirty (30) sorghum materials used in the study were obtained from the East African region. The mid lowland sorghum were grown in Kampi Ya Moto (035°. 56' E and 00°. 05' S) at an altitude of 1660 m a s l while the highland sorghum were grown at Egerton University at an altitude of 2,250 m a s l . Both sorghum materials were sown in a randomized complete blocking design and replicated 3 times during the April – August season 2013. The grain from two middle rows in each experimental unit was harvested, dried, threshed and used for subsequent laboratory and industrial tests (Table 1).

Determination of protein content

Total nitrogen and protein was determined using Kjeldahl method (AOAC, 1999). Sorghum grain was finely milled after which 0.1 g was weighed and transferred into a digestion tube. Selenium catalyst mixture weighing 1 g was mixed with the sample and 5 ml of sulphuric acid (96%) was added into the tube. The tubes were then heated cautiously in the digestion apparatus, at the fume cupboard until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into 100 ml graduated flask upto the mark. Boric acid indicator solution of 5 ml was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. 10 ml of the clear supernatant was then transferred into the apparatus; 10 ml of 46% sodium hydroxide was added and then rinsed again with distilled water. Colour changed from pink to green when the first distillation drops reached the boric acid indicator solution. A total of 150 ml of the distillate was collected and was titrated with 0.0174 N sulphuric acids. Total nitrogen (N) was then determined as follows:

$$N\ (\%) = \left\{ \frac{a \times N \times M_w \times 100}{b \times c} \right\} \times 100\%$$

Where a = ml of sulphuric acid used for titration of the sample; N = normality of sulphuric acid (0.0174); a = titer volume (10 ml); Mw = molecular weight of N_2 (0.014); c = ml digest taken for distillation (10 ml); b = g sample taken for analysis (0.1 g); % crude protein = 6.25 × % N.

Table 1. A list of the selected sorghum varieties used in the study.

Code	Variety	Code	Variety	Code	Variety
1	Ainamoi #1	11	IS 25547	21	ICSA 276 X ICSR 162
2	Siaya # 24-2	12	SDSH 90003	22	1S 25546
3	Kipkelion # 2	13	Uasin Gishu #1	23	IESH 22012
4	Nyiragikori	14	1S 25561	24	ICSA 12 X WAHI
5	Kipkelion # 1	15	Uasin Gishu #2	25	SDSA 29 X KARI MTAMA 1
6	MB 27	16	IESH 22006	26	IESV 92033 SH
7	Kabamba	17	Gadam Hamam	27	IESV 91104 DL
8	Nyangezi	18	ICSA 276 X ICSR 38	28	ICSA 371 X ICSR 108
9	BM 32	19	Kisanana	29	Busia # 21
10	1S 11162	20	Siaya # 2-3	30	IESV 92043 DL

Determination of starch content

Percent starch content was estimated by the Anthrone method (Hodge and Hofreiter, 1962). A powdered sample (0.25 g) was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and retained. The residue was dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. Of this supernatant, 0.1 ml was pipetted out and topped to the 1 ml mark with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and the volume made up to 1 ml in each tube with water. To this, 4 ml of anthrone reagent was then added to each tube and sample heated for eight minutes in a boiling water bath. Sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to arrive at the starch content.

Determination of amylose content

Amylose was determined using the Mc Cready et al., (1950) method where a sample (0.1 g) of the powdered flour was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. To a 2.5 ml extract, 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Dropwise HCl 0.1 N was then added until the pink colour disappeared. To this solution, 1 ml iodine reagent was added till the volume was 50 ml and the colour read at 590 nm using a spectrophotometer. Standard amylose solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the colour developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the standard graph. The blank was obtained by diluting 1 ml of iodine reagent to 50 ml with distilled water. Amylose content was obtained thus:

$$\text{Amylose (\%)} = \left(\frac{x}{2.5} \right) \times 100 \text{ mg amylose}$$

Where, x is the absorbance obtained. The amylopectin content was obtained thus: Starch (%) - amylose (%).

Determination of tannin content

A sample of (0.5 g) of the powdered flour was weighed and transferred to a 250 ml conical flask, and then 75 ml of water added. The flask was heated gently and boiled for 30 min, then centrifuged at 2000 rpm for 20 min. The supernatant was collected in a 100 ml volumetric flask. A measure of 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water.

5 ml of folin regent, 10 ml of 35% sodium carbonate solution were added, and then diluted to 100 ml with water. The sample was shaken and the absorbance read at 700 nm after 30 min. A graph was prepared using 0 - 100 mg tannic acid, where 1 ml contained 100 mg tannic acid. The tannin content of the sample was calculated as tannic acid equivalent from the standard curve. Tannins content was determined by the Folin-Denis method (Schanderl, 1970).

Determination of height and yielding ability

The height of the mature sorghum varieties was determined in centimeters by getting the average of three plants in each plot of each variety in every treatment, then the average of each plot in the three treatments.

The yielding ability of sorghum varieties was determined in grams by getting the average yielding ability of three plants in each plot of each variety in every treatment, then the average yield of each plot in the three treatments.

Data analysis

Data obtained from this study was statistically analyzed using one way analysis of variance (ANOVA) with JMP 10.0.0 Software, 2012 SAS Institute Inc. Turkey's test was used to find the difference among the means. Pearson's correlation analysis was also carried out to determine the relationship between biochemical parameters at $p \leq 0.05$ and 0.001

RESULTS

The starch content of dry seed weight of the genotypes ranged from 25.5 to 81.1% with a mean of 52.2% while amylose and amylopectin ranged from was 12.3 to 26.7%

and 6.6 to 59.8% with means of 12.3 and 6.7%, respectively. Proteins ranged from 3.1 to 18.1% with a mean of 9.8%. The tannin content ranged from 2.5 to 100.0 mg/100 g with a mean of 35.5 mg/100 g. Yield values were 8570 to 863.6 kg/ha with a mean of 3879.0 kg/ha while height ranged from 341.5 to 97.0 cm with a mean of 198.4 cm.

There was a statistically significant correlation between starch and amylopectin (0.97, $p \leq 0.001$) while starch and protein had a negative significant correlation (-0.55, $p \leq 0.001$). The correlation between starch and tannins was 0.49, $p \leq 0.05$, whilst starch and height had a correlation of 0.47, $p \leq 0.05$. Other significant correlation includes amylopectin and proteins (- 0.58, $p \leq 0.001$), tannins and height (0.47, $p \leq 0.05$); 0.52, $p \leq 0.05$. Protein and tannin contents had a negative correlation of – 0.52, $p \leq 0.05$ whereas protein and height had a correlation of –0.46, $p \leq 0.05$. The yielding ability and height has a positive significant correlation of 0.64, ≤ 0.001.

Hierarchical clustering (method = ward)

Cluster 4 varieties had high starch and high tannins contents and also had high amylopectin as compared to their respective amylose amounts (Figure 2). They also had medium height, yield and good protein amounts. On the other hand, cluster 3 varieties had medium starch, height and good protein amounts. They exhibited high amylopectin as compared to their respective amylose high yields and tannins.

Cluster 2 varieties had high amylopectin as compared to their respective amylose amounts, medium starch, height and average protein contents lower than cluster 1 and 2. They possess the highest yields and height as compared to the rest of the clusters. Cluster 1 varieties had the lowest starch, tannins and height as compared to other clusters. Most varieties with lower amylopectin to amylose ratio are in this cluster. They registered highest proteins and are mostly the hybrids while the yields of the varieties varied. These clusters are useful when identifying the varieties that are favourable for different end uses.

DISCUSSION

The varieties used in this study were both hybrids and open pollinated and they portrayed a wide range of nutritive contents (Table 2). Starch is the main component of sorghum grains (BSTID-NRC, 1996) as evident, thus this makes it favorable to be explored for different industries. This study has demonstrated that sorghum varieties possess biochemical characteristics that can match up to other cereals thus should be considered as alternative to these industries. Sorghum with starch as high as 70% *Ainamoi, kipkelion, Siaya* 24-2 (Figure 1 and Figure 2, Cluster 4) is a reliable source of energy that can be explored by many industries. In terms of nutritive value, cost and availability sorghum grains are probably the next best alternative to maize in poultry feed as reported by Lepleaideur (2004). As per this study, most of the varieties with good starch contents are open pollinated while the hybrids showed moderate to low levels of starch (Table 2). Normally, hybrids are improved varieties thus expected to have good starch amounts but this was not the case as shown in Table 2. More needs to be done to improve the starch contents of these hybrids if they are to be accepted in these industries.

Apart from the high starch content, the ratio of amylose to amylopectin is vital for the baking and brewing industries because it affects the quality of the end products. Generally, the amylopectin are higher than their respective amylose contents in sorghum. This study demonstrated some exceptions (Table 2 and Figure 2, Cluster 1) like *IESV 92033 SH, SDSA 29 X KARI MTAMA 1, ICSR 276 X ICSR 38* and *Kisanana*. These exceptions are favourable for the baking industry because high amylose gives stabilization to bread quality as stated by Taylor et al. (2006). Sorghum alone cannot be used for baking due to its lack of gluten, but addition of 20 to 50% sorghum flour to wheat flour results to good quality bread (Hugo et al., 2003). Sorghum is a good candidate for the brewing industry and has been adopted by the East African industry to some extent. This is because of lack of specific varieties that are tailored for this industry. The amylose to amylopectin ratio in this study was 1:2, and this is lower than the 1:3 ratio reported by Taylor et al. (2006). On the other hand, varieties favourable for brewing ought to have more amylopectin than amylose contents because Sharma et al. (2008) reported that high amylose affects starch digestibility. As per this study, most varieties suitable for brewing were mostly the hybrids.

The protein contents of sorghum varieties ranged from 3 - 18% and this was similar to 6 – 18% reported by Lasztity (1999). Varieties *ICSA 12 X WAHI* and *SDSA 29 X KARI MTAMA 1* had notably high protein contents (Table 2). These varieties would be good for animal and human foods, thus boost food security. The wide variety of sorghum protein gives it an advantage to be utilized for different uses depending on the protein requirements of that industry. The brewing and baking industry needs varieties with moderate proteins while animal and human feeds needs maximum proteins, and sorghum offer a wide choice. All the hybrids recorded high protein value unlike the open pollinated varieties. This can be attributed to sorghum breeding efforts as originally sorghum was more subsistence than industrial. Sorghum protein is gluten free and this is safe to be used by celiac patients (Ciacci et al., 2007) and high proteins means high food quality. This gives a good alternative to wheat flour due to their neutral flavor and the hybrids with white pericarp (Normell et al., 2010).

Table 2. ANOVA output of biochemical and physiological traits of sorghum accessions.

Genotype	Starch (%)	Amylose (%)	Amylopectin (%)	Protein (%)	Tannins mg/100 (g)	Yielding ability (kg/ha)	Height (cm)
Ainamoi #1	81.1^{a}	22.2^{abc}	58.9^{a}	7.8^{efgh}	79.8^{b}	3362.2^{cdef}	241.5^{de}
Siaya # 24-2	76.4^{ab}	19.4^{bcde}	56.9^{ab}	9.5^{def}	41.7^{g}	3245.5^{cdef}	125.0^{jk}
Kipkelion # 2	76.0^{ab}	16.2^{cdef}	59.8^{a}	7.4^{fgh}	58.7^{f}	3617.9^{bcdef}	209.2^{efg}
Nyiragikori	75.2^{ab}	18.2^{cdef}	56.9^{ab}	8.2^{efgh}	15.5^{kl}	3014.5^{def}	260.4^{cd}
Kipkelion # 1	71.6^{abc}	19.1^{bcdef}	52.5^{abc}	5.2^{hi}	59.9^{ef}	2263.1^{ef}	233.5^{de}
MB 27	68.9^{abcd}	14.0^{def}	54.9^{abc}	3.1^{i}	18.9^{kl}	6975.4^{abcd}	218.0^{def}
Kabamba	68.9^{abcd}	12.3^{f}	56.5^{ab}	6.9^{fgh}	100.0^{a}	1591.3^{f}	226.2^{de}
Nyangezi	64.4^{bdce}	17.1^{cdef}	47.2^{abcde}	8.7^{defg}	69.1^{cd}	3045.5^{def}	290.1^{bc}
BM 32	62.7^{bcdef}	18.1^{cdef}	44.5^{abcdef}	8.1^{efgh}	33.1^{h}	7460.9^{abc}	306.7^{abc}
1S 11162	61.7^{bcdefg}	13.4^{ef}	48.2^{abcd}	8.6^{defg}	35.1^{h}	7909.5^{ab}	341.5^{a}
IS 25547	57.4^{cdefgh}	16.1^{cdef}	41.3^{bcdefg}	5.9^{ghi}	42.3^{g}	6459.3^{abcde}	317.7^{ab}
SDSH 90003 (H)	57.0^{cdefgh}	18.6^{cdef}	38.3^{cdefgh}	7.9^{efgh}	24.4^{ij}	4777.3^{abcdef}	124.8^{jk}
Uasin Gishu #1	55.2^{defghi}	21.5^{abc}	33.6^{defghi}	5.8^{hi}	65.1^{de}	3253.8^{cdef}	173.1^{fghi}
1S 25561	54.5^{defghij}	15.4^{cdef}	39.0^{cdefgh}	11.8^{cd}	44.6^{g}	4972.6^{abcdef}	305.8^{abc}
Uasin Gishu #2	51.6^{efghijk}	18.3^{cdef}	33.2^{defghi}	8.7^{defg}	73.7^{g}	3382.1^{cdef}	204.3^{efg}
IESH 22006 (H)	48.7^{fghijk}	18.4^{cdef}	30.2^{efghi}	13.4^{bc}	9.4^{mno}	5020.7^{abcdef}	139.0^{hijk}
Gadam Hamam	47.4^{ghijk}	16.5^{cdef}	30.9^{efghi}	10.9^{cde}	26.2^{i}	863.6^{f}	97.0^{k}
ICSA 276 X ICSR 38 (H)	44.6^{hijkl}	25.5^{ab}	19.0^{ijkl}	15.6^{ab}	13.8^{lmn}	2872.3^{def}	131.2^{hijk}
Kisanana	44.5^{hijkl}	26.7^{a}	17.7^{ijkl}	7.2^{fgh}	68.3^{d}	3186.2^{cdef}	155.6^{hij}
Siaya # 2-3	43.4^{hijkl}	13.8^{def}	29.6^{fghi}	5.1^{hi}	19.5^{jk}	4151.4^{bcdef}	203.8^{efg}
ICSA 276 X ICSR 162 (H)	43.4^{hijkl}	18.5^{cdef}	24.8^{ghijk}	11.8^{cd}	6.3^{op}	4323.2^{abcdef}	176.6^{fgh}
1S 25546	41.9^{ijklm}	14.2^{def}	27.6^{fghij}	7.1^{fgh}	36.0^{h}	8570.1^{a}	300.7^{abc}
IESH 22012 (H)	41.6^{ijklm}	15.7^{cdef}	25.9^{ghijk}	16.8^{a}	9.7^{mno}	2738.1^{def}	127.4^{ijk}
ICSA 12 X WAHI (H)	40.1^{jklmn}	17.6^{cdef}	22.4^{hijkl}	18.1^{a}	7.7^{op}	1245.4^{f}	134.7^{hijk}
SDSA 29 X KARI MTAMA 1(H)	38.4^{klmn}	21.7^{abc}	16.6^{ijkl}	18.0^{a}	2.5^{p}	3888.2^{bcdef}	173.6^{fghi}
IESV 92033 SH	36.8^{klmn}	18.0^{cdef}	18.7^{ijkl}	9.1d^{efg}	8.6^{no}	3448.2^{cdef}	163.4^{ghij}
IESV 91104 DL	32.1^{lmn}	13.9^{def}	18.2^{ijkl}	10.8^{cde}	9.1^{no}	2095.3^{ef}	154.0^{hij}
ICSA 371 X ICSR 108 (H)	27.0^{mn}	20.4^{abcd}	6.6^{l}	16.0^{ab}	4.5^{op}	2915.8^{def}	147.2^{hij}
Busia # 21	26.9^{mn}	17.6^{cdef}	9.3^{jkl}	11.8^{cd}	66.2^{d}	2869.8^{def}	121.0^{jk}
IESV 92043 DL	25.5^{n}	13.3^{ef}	12.1^{jkl}	10.8^{cde}	14.6^{klm}	2849.5^{def}	146.5^{hij}
Standard Error (SE)	2.69	1.22	3.08	0.58	0.96	785.5	8.42

Means with the same letter are not significantly different, H– hybrids.

Sorghum grains are characterized by their high content of condensed tannins which limits their suitability for several end users. Tannins are considered undesirable due to their capacity to bind proteins making them less digestive, and also producing astringent taste (Ambula et al., 2003). Sorghum genotypes with low tannin contents have been produced due to plant breeding efforts and this is evident in the hybrids varieties *ICSA 12 X WAHI, SDSA 29 X KARI MTAMA 1*, *ICSV 92033 SH* and *IESV 91104 DL* (Table 2 and Figure 2, Cluster 1). The open pollinated varieties on the other hand had considerably high tannin contents (Figure 2, Cluster 4). Low tannin content sorghum especially the hybrids are favorable for poultry feeding due to their proteins and carbohydrate contents. This is because high tannic acid varieties are not good for monogastrics feed as reported by Smithhard (2002). He also observed that when monogastrics were fed with high tannic sorghum based diet, there was a general poor performance due to the binding effect of tannins to digestive enzymes. However, when used in conjunction with maize, these effects were minimized. Thus moderate to low tannin sorghum can be used to supplement the poultry industry in an effort to reduce overdependence on maize.

Legumes like lucerne and clovers contain saponins and on fermentation release vast quantities of soluble proteins this is because these legumes lack tannins and this result to bloat in livestock (Jansman, 1993). According to Jones et al. (1973), this can be minimized by using high tannin sorghum that have been shown to posses the ability to control bloat. It has also been reported that after the digestion of tannin foods, the

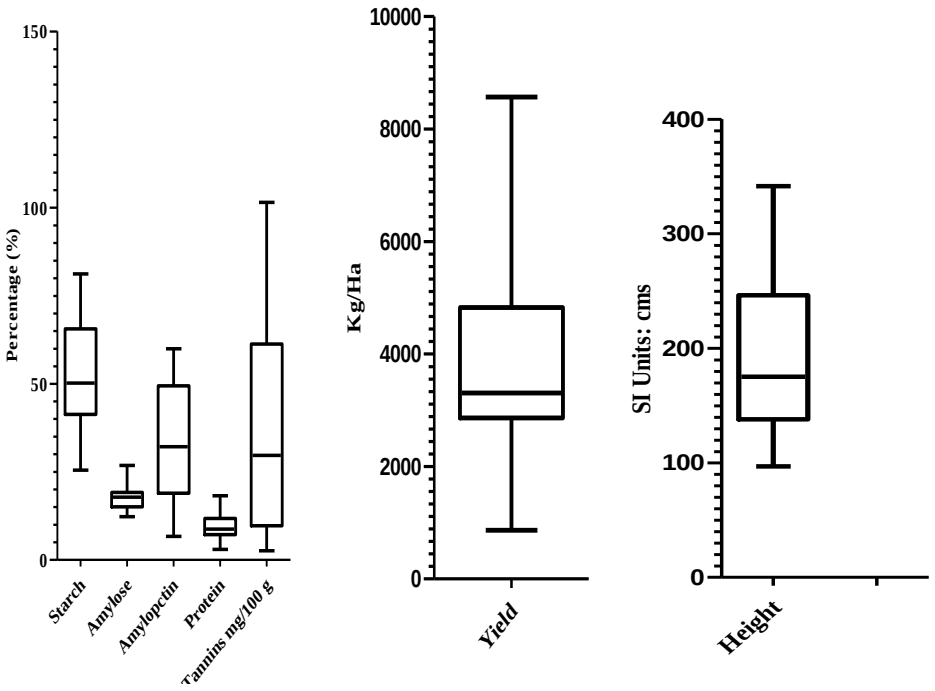

Figure 1. Biochemical and physiological traits of 30 sorghum varieties showing their ranges and means.

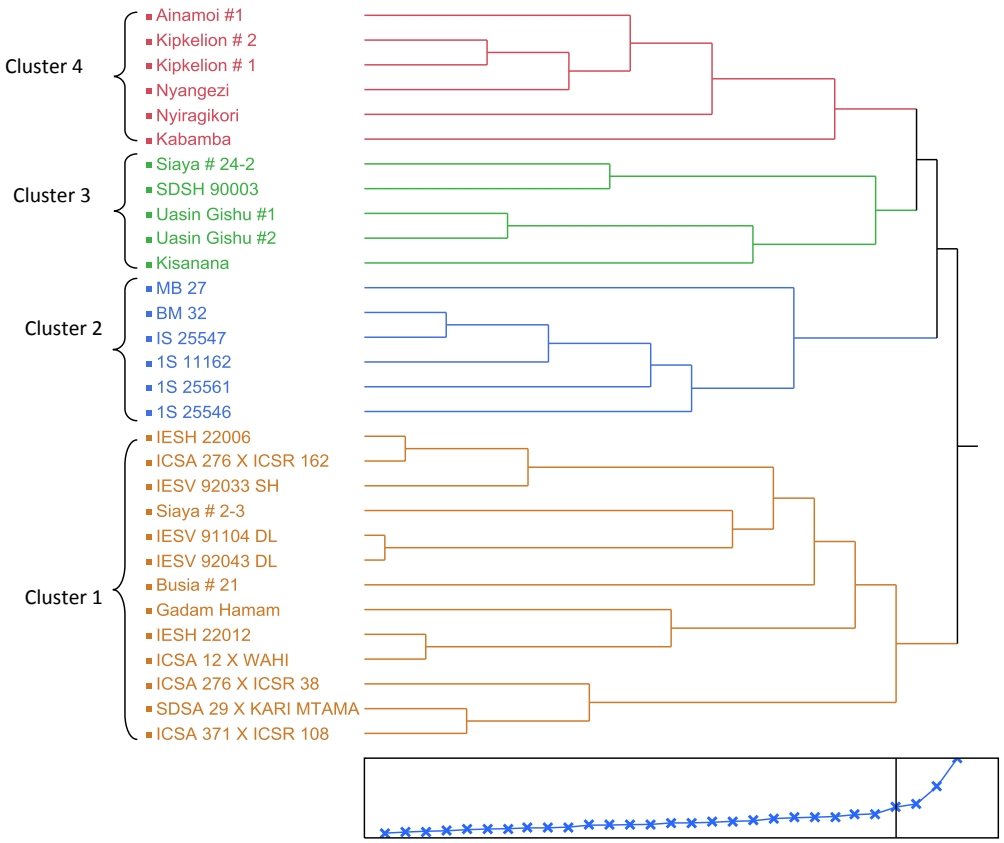

Figure 2. Dendrogram of 30 sorghum varieties shown by ward cluster analysis based on biochemical and morphological data set.

Table 3. Pearson's correlation coefficient results for 30 sorghum accessions.

N = 30;	Starch	Amylose	Amylopectin	Protein	Tannins	Yield	Height
Starch	1.00	0.003 ns	0.97***	- 0.55***	0.49**	0.16 ns	0.47**
Amylose		1.00	- 0.21ns	0.22 ns	0.05 ns	-02.3 ns	- 0.31
Amylopectin			1.00	- 0.58***	0.47**	0.20 ns	0.52**
Protein				1.00	- 0.52**	- 0.34 ns	- 0.46**
Tannins					1.00	- 0.10 ns	0.31ns
Yield						1.00	0.64***
Height							1.00

N = 30; Significant at**- P ≤ 0.05, ***-0.001; NS – non significant at P ≤ 0.05 and 0.001.

tannin-protein complex formed reacts with gut wall proteins and stimulates growth hormones that in turn increases lipid turnover and nitrogen retention (Muir et al., 1983). The significance is that it protects plant protein degradation in the rumen and this is the advantage of sorghum grain in animal feed. Despite sorghum being undesirable in many industries, animal feed industry can take advantage of the availability of sorghum tannins. The varieties that can be explored as per this study include *Ainamoi, Kipkelion # 1, Nyiragikori, Kipkelion # 2, Kabamba* and *Nyaangezi* (Figure 2, Cluster 4).

Sorghum varieties rich in tannins can be recommended for obese individuals and diabetic patients. According to Ambula et al. (2001) animals fed with sorghum containing high level tannins demonstrated 50% weight loss. This was attributed to their long emptying time of the stomach and their low digestibility through the inhibition of hydrolytic enzymes together with their antioxidant activities (Dicko et al., 2005).

In agronomical point of view, tannins are desirable due to their protection against bird, insect and disease damage (Waniska et al., 2001). According to Bullard and Elvis (1980) the astringent taste of tannins is due to tannin-saliva protein complex in the mucous epithelium of the oral cavity thus decreasing its palatability. This largely contributes among other factors to the high yield in tannin sorghum (Table 2).

Yielding ability is a vital parameter when selecting varieties for different end uses because high yielding varieties are economical both to farmers and the industry. The varieties in this study demonstrated a wide range in yielding ability as shown in Figure 1. This study demonstrated yielding ability as high as 8570 kg/ha with some varieties such as *1S 25546* and 7909.5 kg/ha as in *1S 11162*. Industries that focus greatly on yielding ability can adopt these particular varieties. This is vital because due to climate change wheat and maize production have been greatly affected but sorghum is known to be resilient to harsh climatic conditions and give good yield (Dicko et al., 2006). Plant height is also an important parameter especially for large scale farmers because short varieties reduce the cost of production due to the possibility of mechanization. As shown (Table 2), the hybrid varieties were shorter than the open pollinated varieties with less than 176 cm in length while the open pollinated had height as high as 317 cm *1S 25546* and 341 cm *1S 11162* (Figure 2, Cluster 2). These are the same varieties that registered high yields. Thus, it is important to note that yielding ability positively, significantly correlated with plant height in this study. The consequence is that in the event of trying to reduce height via breeding, might lead to reduction of yielding ability.

Correlation analysis

Most of the varieties with high starch and amylopectin contents also recorded high tannin contents with a few exceptions (Table 2). These correlations suggest a genetic association between the parameters both in the hybrids and the open pollinated varieties. The positive significant correlation between tannins and starch of these varieties is a bad characteristic for breeding since starch is desirable in most end uses unlike tannins (Table 2). On the other hand, the negative correlation between tannins and proteins is a good selection indicator for varieties in sorghum breeding (Table 3). The protein was negatively correlated with starch and amylopectin content which is expected, as protein and starch make the major component in grains. This is evident in Table 2 where *SDSA 29 X KARI MTAMA 1* and *ICSA 12 X WAHI* are hybrids with high protein and low starch and tannin amounts. On the other hand, *kabamba, ainamoi # 1, kipkelion* are open pollinated varieties with high tannins and starch but low protein contents (Table 2). Hybrids are bred to give low tannins but unfortunately this also affects their starch content and in the long run decrease the suitability of sorghum for some industrial uses. There was a significant correlation between yielding ability and plant height, as similar to results of Alhassan et al. (2008). This demonstrates the correlation between plant height and yielding ability and it might be attributed to genotypes with good plant height being able to receive more sunlight for photosynthesis, which translates to more photosynthates resulting to increased yield. Plant height also

showed a significant positive correlation with starch and amylopectin contents. This is due to increased photosynthesis due to height which positively affects the yields.

Cluster analysis

Cluster 4 varieties had high starch and high tannins contents and also had high amylopectin as compared to their respective amylose content (Figure 2). They also had medium height, yielding ability and good protein content. On the other hand, cluster 3 varieties have medium starch, height and good protein content. They exhibited high amylopectin as compared to their respective amylose, high yields and tannins. Cluster 2 varieties had high amylopectin as compared to their respective amylose amounts, medium starch, height and average protein contents lower than cluster 1 and 2. They possess the highest yields and height as compared to the rest of the clusters. Cluster 1 varieties had the lowest starch, tannins and height as compared to other clusters. Most varieties with lower amylopectin to amylose ratio are in this cluster. They registered highest proteins and are mostly the hybrids while the yields of the varieties varied. These clusters are useful when identifying the varieties that are favourable for different end uses.

Conclusion

Success of any food grain or its product depends on acceptance by the consumers. In the process of achieving food production targets, agricultural scientists concentrate their efforts on developing high-yielding varieties giving little importance to other quality characteristics. From the study, we find that proteins and tannins were in high contents among the hybrids and the open pollinated varieties (OPVs) respectively. Most of the hybrids had greater amylose than amylopectin and the vice versa was true for the OPVs. It is clear that breeding programmes that aim to reduce tannin contents also result to lower starch as evident in the hybrid varieties. Plant breeders should put into consideration not only agronomical characteristics but also biochemical characteristics. There is need for further research through biotechnological techniques to identify genes responsible for specific biochemical traits if sorghum and other cereals are to be completely adopted by the huge African market.

Conflict of interests

The authors did not declare any conflict of interest.

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Effect of paddy storage and processing parameters on quality of *Ofada* rice in the production of ready to eat flakes

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Effect of paddy storage duration and processing parameters (soaking time, parboiling temperature and drying temperature) were optimized using response surface methodology for the production of ready to eat *Ofada* rice flakes which serves as alternative mean of harnessing its potential. There exist significance differences at p<0.05 for all the parameters except carbohydrate and metabolizable energy. The quadratic models were fit for prediction of effect of storage and processing parameters on quality of flakes produced from ofada paddy. The R^2 ranged from 0.9662 to 0.7318 which confirmed the fitness of the model. The predicted and validated values were related. The optimum storage duration and processing parameters for treatment of *Ofada* paddy in the production of ready to eat flake include storage of paddy for 9 months, soaking for 4 days and 17 h, parboiling at 106°C and drying at 30°C to yield optimum quality of *Ofada* rice flakes. The sensory assessment showed significant acceptability of colour, crispiness, aroma, taste and overall acceptability.

Key words: Ofada, processing parameters, response surface methodology, ready-to-eat flakes, optimization.

INTRODUCTION

Rice (Oryza sativa L) is enjoyed by many people as staple food especially varieties with distinctive aroma and flavor (Bryant and McChung, 2011). One of the popular indigenous rice varieties in Nigeria is *Ofada* rice. "*Ofada*" is a generic name used to describe all rice produced and processed in South-West, Nigeria. One of the early cultivated variety is OS6 and it is relished because of is aroma (NCRI and WARDA, 2007). Rice produced in Nigeria is consumed mostly in the form of boiled rice and as mashed porridge rolled into round balls both eaten with soup.

Nowadays, ready-to-eat foods are gaining much importance as they are convenient to use, easy to handle compared with ready-to-cook foods (Itasi et al., 2012). There is few or no utilisation of Nigeria rice flour in pastry production, however, production of breakfast cereals from maize, sorghum, millet is common. The use of rice for preparation of breakfast cereal such as noodles, flakes is

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Table 1. Independent variables and levels.

Variables	Levels		
Storage duration (months)	1	5	9
Soaking time (days)	1	3	5
Parboiling temperature (°C)	80	100	120
Drying temperature (°C)	30	50	70

common in United States and Europe, therefore, utilization of Nigeria local rice for ready-to-eat flakes is another way of harnessing the potential of local rice. The aromatic characteristic, taste and immense nutritive value of *ofada* rice (OS6) compare to other local varieties are potentials that worth to be investigated in the production of high quality *ofada* ready-to-eat flakes.

According to Fred (2007), processing conditions of grain in flakes production could affect the quality of flakes. The major traditional unit operations in paddy processing include soaking, parboiling, and drying. The effects of storage history and ageing on rice were also documented (Zhou et al., 2001; Daniel et al., 1998). Therefore, the storage duration and processing conditions of paddy into rice flour for the production flake may have effect on quality of flake. Response surface methodology (RSM) is important in designing experiment, formulating, developing and analysing new and existing scientific studies cutting the cost, and measures several effects by objective test (Akinoso and Adeyanju, 2010; Montgomery, 2005). The main objective of the study was to optimize paddy storage duration and processing parameters (soaking time, parboiling temperature and drying temperature) of ofada paddy using response surface methodology for the production of quality ready-to-eat ofada rice flakes. The results of this study will make relevant technical data available for the present and prospective investors that would have relied on “trial and error” method in processing *ofada* rice and its utilisation for flake production. This will help to make the local rice sufficiently more competitive thereby increasing its demand.

MATERIALS AND METHODS

Ofada paddy (OS 6) was purchased at farm gate in Mokolokin-Ofada, a renowned area for *Ofada* rice production. The processing unit operations (soaking, parboiling, drying) used as treatment was adopted from the methods of processing of paddy adopted by the rice farmers in the area. D- Optimal response surface methodology was used for the design of the experiment. The independent and levels of variables is shown in Table 1. The rice stalk was threshed manually and cleaned to obtain paddy within 12 h, mixed thoroughly and stored in a dry cool place for processing at 1, 5 and 9 months as described in the experimental design.

Paddy (4kg) was soaked in cold water at ambient temperature (28±2°C) for typically 1, 3 and 5 day(s) to hydrate the kernels. The soaked paddy were parboiled at varied temperatures (80, 100, and 120°C) at constant pressure using digital autoclave for 15 min. The parboiled paddy were tempered for 30 min to cool and air dried in oven at 30, 50 and 70°C. The rice samples were milled (hulling and debranning) in grantex cono disc milling machine. The rice obtained were subsequently ground in a hammer mill, sieved (200 micron size) and analysed.

Production of *Ofada* rice flakes

The laboratory production of ready-to-eat flakes was carried out as described by Lu and Walker (1988). The milled rice was cleaned and ground into flour. The flour was sifted with sieve (60-mesh testing sieve) and the coarse residues were discarded. Flour (400 g) was mixed with 300 ml of water, 25 g of sugar, and 4 g of salt in a kitchen Kenwood mixer for 5 min. The dough was placed in a pasta extruder attachment and forced through a die with 5-mm hole. After the extrusion, it was cut 0.5 cm long pellets, and steamed using a pressure cooker for 15 min. After cooking, the pellets were tempered and pressed through a heavily spring roller. The resulting flakes were toasted on pans at 200°C for 20 min, cooled, and packaged in plastic.

Analysis of rice flakes

The analysis carried out on flakes include moisture, crude protein, crude fat, and ash contents, using AOAC,(2000); carbohydrates by difference and metabolizable energy by FAO (2002); phytic acid was carried out as described by Garcia-Estepa et al. (1999) and water absorption capacity as described by Walker et al. (1988).

RESULTS AND DISCUSSION

The result of chemical and functional qualities of ready-to-eat *ofada* rice flakes as affected by storage and processing parameters of *ofada* rice paddy is presented (Table 2).

Moisture composition of ready to eat *ofada* rice flakes

There were significant differences in the result of moisture contents obtained at $p < 0.05$. The minimum and maximum moisture content of the rice flakes were 3.10 and 6.89 respectively (Table 2). The least percentage moisture content was obtained from flake produced from *Ofada* paddy stored for 9 months and subjected to processing operations which involved 5 days of soaking, 80°C parboiling, and 30°C drying temperature while the maximum moisture in the flake was obtained from the paddy stored for 1 month, soaked for 1 day, parboiled at 120°C, and dried at 30°C temperature.

The R^2, adjusted R^2, and adequate precision are presented in Table 3 and coefficient of the model depicting effect of processing conditions of paddy and storage duration in the production of rice flour for the manufacturing of flakes is presented in Table 4. Positive coefficient of drying temperature showed that drying process of paddy has a major influence on moisture content of flake. The movement of water out of the flake during drying and into the flake during food processing (water absorption) has implications on quality (Fred, 2007).

Table 2. Result of chemical and functional composition of Ofada rice flakes.

Number	Soaking time (day)	Parboiling temp. (°C)	Drying Temp. (°C)	Storage Duration	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)	Carbohydrate (%)	Phytate (%)	Water absorption ratio	Metabolisable Energy (kcal/100 g)
1	5	120	70	1	5.9	8.5	0.89	2.93	81.78	0.8	2.37	368.46
2	1	80	70	1	5.6	8.81	1.49	2.4	81.7	1.51	3.43	374.81
3	1	80	30	5	6.25	9.73	0.3	1.28	82.44	1.47	4.34	382.63
4	5	80	30	1	5.6	8.77	0.49	1.57	83.57	0.69	2.65	373.03
5	5	80	30	9	5.65	12.84	0.49	1.59	79.43	1.15	4.29	383.53
6	3	80	50	5	5.15	10.26	0.52	0.95	83.12	1.46	4.77	389.33
7	5	120	30	5	5.4	9.64	0.4	0.2	84.36	0.39	4.51	391.11
8	1	80	70	1	5.69	8.9	0.97	2.43	82.01	1.36	3.41	371.67
9	5	120	70	9	5.75	12.21	0.51	1.68	79.85	0.51	4.41	383.09
10	3	120	50	5	5.85	10.5	0.37	1.13	82.15	0.62	4.17	384.95
11	3	120	30	9	3.7	13.1	0.66	1.6	80.94	0.92	4.55	392.28
12	5	100	50	5	5.25	10.19	0.31	1.03	83.22	0.64	4.69	387.72
13	1	120	70	5	5.1	9.76	0.24	1.03	83.87	1.01	4.88	388.19
14	3	100	50	9	3.73	13.01	0.76	1.72	80.76	1.09	4.74	392.02
15	1	120	50	9	5.6	12.81	0.32	1.52	79.75	1.14	4.32	383.37
16	5	120	70	1	5.24	8.62	0.86	2.94	82.34	0.72	2.38	370.89
17	1	100	50	5	5.3	9.96	0.46	1.12	83.16	1.36	4.59	387.84
18	3	100	50	5	4.05	6.99	0.46	2.63	86.87	0.89	2.95	378.99
19	5	80	30	1	5.01	8.81	0.56	1.65	83.97	0.71	2.67	375.42
20	1	120	30	1	6.89	9.36	0.63	1.42	85.79	1.42	3.22	385.49
21	1	100	30	9	3.33	12.76	0.44	1.74	81.73	1.53	4.52	392.42
22	5	80	70	5	6.85	9.94	0.43	1.01	81.77	1.42	4.66	381.73
23	1	80	70	9	3.8	12.93	0.52	2.05	80.7	1.67	4.21	389.46
24	5	80	30	9	3.1	12.61	0.38	1.65	82.26	1.22	3.92	393.55
25	1	120	30	1	6.82	9.28	0.67	1.41	81.82	1.25	3.24	369.66

Flake thickness influences the rate of diffusion of water during drying. However, Machado et al. (1998) reported that range of thickness did not correlate with water absorption.

Oeding (1996) has profound that higher steam temperature and great moisture during steaming resulted in increased water absorption, and also suggested that this was related to changes in pasting behaviour. The report justified the result of this experiment with rice parboiled at highest temperature measuring highest percentage of moisture while this decreased with reduction in parboiled temperature.

Protein composition of ready-to-eat *ofada* rice flakes

The results of the protein content of rice flakes ranged between 6.99 to 13.10 % (Table 2). Some of the values obtained in this study were comparable to protein content of flake made from wheat (USDA, 2013). There exist significant differences in the result for protein at $p < 0.05$ and values of R^2, adjusted R^2, and adequate precision are presented in Table 3. The closeness of R^2 and Adjusted R^2 to 1 and extent of greatness of adequate precision above 4 were indicators of the

Table 3. ANOVA of regression of chemical and functional qualities of Ofada rice flakes.

Parameters	p-value	R^2	Adjusted R^2	Adequate precision
Moisture (%)	0.0207	0.84	0.6161	7.088
Protein (%)	0.0019	0.9084	0.7802	7.58
Ether extract (%)	0.0029	0.8991	0.7579	9.795
Ash (%)	0.0267	0.8297	0.5914	7.966
Carbohydrate (%)	0.146	0.7318	0.3562	5.317
Phytate (%)	<0.0001	0.9662	0.919	16.673
Water absorption ratio	0.0229	0.8361	0.6066	5.835
Metabolisable energy (kcal/100 g)	0.0627	0.7882	0.4917	5.013

Table 4. Coefficient of the quadratic model for the effect of storage and processing parameters on ofada rice quality in production of ready to eat flakes.

Parameter	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)	CHO (%)	Phytate (%)	Water absorbtion	M.E (kcal/100 g)
Intercept	36.41	23.04	-0.76	-4.27	38.55	2.77	3.56	296.21
S	-2.31	-0.65	-0.02	0.42	2.9	-0.12	-0.45	3.72
P	-0.66	-0.37	0.03	0.16	1.01	-0.03	-0.03	1.44
D	0.21	0.19	0.03	-0.06	-0.39	0.01	0.04	-0.21
T	-0.27	-0.09	-0.24	-0.34	0.79	0.18	0.48	5.86
S^2	0.28	0.12	-0.03	-0.12	-0.3	$6.30X10^{-3}$	0.08	-0.27
P^2	$3.33x10^{-3}$	$2.02x10^{-3}$	$-1.44x10^{-4}$	$-9.17x10^{-4}$	$-4.89x10^{-3}$	$2.17x10^{-4}$	$2.19x10^{-4}$	$-5.92x10^{-3}$
D^2	$-2.30x10^{-3}$	$-1.45X10^{-3}$	$-1.55X10^{-5}$	$3.42X10^{-4}$	$3.68X10^{-3}$	$2.97X10^{-5}$	$-3.32X10^{-6}$	$5.65X10^{-3}$
T^2	-0.03	0.07	0.02	0.06	-0.11	$3.38X10^{-3}$	-0.05	-0.32
SP	$-2.63X10^{-3}$	$-1.63X10^{-3}$	$1.87X10^{-3}$	$2.62X10^{-3}$	$-3.99X10^{-3}$	$-2.11X10^{-3}$	$-1.75X10^{-3}$	$-1.44X10^{-3}$
SP	6.01	$2.12X10^{-3}$	$-1.55X10^{-4}$	$1.19X10^{-3}$	-0.01	$3.40X10^{-3}$	$7.94X10^{-4}$	-0.04
ST	0.04	$-6.07X10^{-3}$	$8.27X10^{-3}$	$-8.16X10^{-3}$	$-7.57X10^{-3}$	$-1.30X10^{-3}$	0.02	$5.19X10^{-3}$
PD	$-3.18X10^{-4}$	$-5.50X10^{-4}$	$-1.47X10^{-4}$	$4.73X10^{-4}$	$2.29X10^{-4}$	$-2.03X10^{-4}$	$-3.98X10^{-4}$	$-3.26X10^{-3}$
PT	$1.98X10^{-3}$	$-5.35X10^{-4}$	$2.42X10^{-4}$	$-1.50X10^{-3}$	$-3.28X10^{-3}$	$-1.43X10^{-3}$	$1.95X10^{-3}$	-0.01
DT	$1.81X10^{-3}$	$-9.5X10^{-4}$	$-1.06X10^{-3}$	$-2.08X10^{-3}$	$5.53X10^{-3}$	$-9.09X10^{-4}$	$-3.65X10^{-4}$	$3.95X10^{-3}$

S,soaking time; P, parboiling temp.; D,drying temp.; T, storage duration.

degree of fitness of the model. The coefficients of the model are presented in Table 4. Maximum protein content in rice flake was obtained from paddy stored for 9 months, and subjected to 3 days soaking, 100°C parboiling temperature, and 50°C drying temperature. It was revealed that ready-to-eat *Ofada* rice flakes produced from paddy stored for longer duration relatively shown improvement in protein content (Table 2).

The protein composition recorded in this experiment was higher than the result as reported by Nazni and Bhuvaneswari, (2011), and also higher than the nutritional protein composition of the popular 'Kelloggs' products.

Fat composition of ready to eat *ofada* rice flakes

The results of crude fat were significant at $p < 0.05$ and ranged from 0.24 to 1.49% with mean value of 0.57%. Generally, the crude fat contents were low (<1.5 %) with the maximum extract recorded from the flake produced from paddy stored for 1 month and treated under 1 day soaking, 80°C parboiling and 70°C drying temperatures respectively while the minimum value was from rice stored for 5 months, and processed by soaking for 1 day, parboiling at 120°C and drying temperature at 70°C. The coefficient of the model was presented (Table 4). The result obtained from this experiment is comparable to the general fat content recorded for most commercial rice flakes (Fatsecret, 2013). High fat content can easily cause rancidity of the package foods, however, maximum of 3% fat has been found in most commercial rice flakes. Gupta et al. (2012), recorded fat content range of 0.76 – 5.91 % in the production of rice flakes mix using dehydrated herbs.

Ash contents of ready to eat rice flakes

The ash content is the total mineral composition of rice

flakes and it ranged from 0.20 to 2.94 %. Maximum ash content was from rice flake produced from the paddy stored for 1 month followed by soaking of the rough rice for 5 days, parboiling at 120°C, and drying at 70°C while minimum value was obtained from rice stored for 5 months, soaked for 5 months, parboiled for 120°C and dried at 30°C drying temperature. It was observed that the effect of storage duration and drying temperature could have been responsible for the variation. The coefficient of the model revealed that soaking time and parboiling temperature treatment were major indicators of the ash contents of the flakes (Table 4). The milling operation might affect ash content of the flakes due to the presence of bran.

Carbohydrate contents of ready to eat *ofada* rice flakes

There was no significant difference ($p>0.05$) in the data obtained for carbohydrate. The carbohydrate contents ranged from 79.43 – 86.87% and "Lack of Fit test" is not significant which revealed that such model can still be used in prediction. The values of R^2, Adjusted R^2, and adequate precision of the model were presented in Table 3. The report has shown that storage duration of paddy and processing conditions of ofada rice does not have significant influence on the carbohydrate content of ready to eat rice flakes.

Water absorption of ready-to-eat *ofada* rice flakes

Water absorption is one of the major factors in determining the quality of flakes. The results obtained ranged from 2.37-4.88 and there were significant differences in the water absorption ratio values of the rice flakes ($p<0.05$). Observation of the data revealed that the rate of water absorption majorly varied with the storage duration of paddy before processing. However, the highest water absorption ratio was obtained in rice flakes processed from rice subjected to 1 day soaking, 120^0C parboiling temperature, 70^0C drying after storage for 5 months duration. However, values of R^2, Adjusted R^2, and adequate precision were presented (Table 3). The coefficient of the model showed a positive influence of drying temperature (D) and storage duration (T) on raw material processing (paddy) in manufacturing of flakes (Table 4).

Phytate composition of ready-to-eat *ofada* flakes

Rice contains some important anti-nutritional factors, most which are concentrated in the bran. All anti nutritional factors in rice except phytate are proteins and denatured by heat. The phytate levels from the experiment ranged from 0.39-1.67%. Variation in respect of phytate content was found to be significant ($p < 0.05$). The highest amount of phytate was recorded in the flake produced from rice stored for 9 months, processed by soaking for 1 day, 80°C parboiling temperature, and 70°C drying temperature while the least value of phytate was at 5 days soaking, 120°C parboiling, 30°C drying and 5 months storage duration of paddy. According to Noreen et al. (2009), soaking and boiling processes caused significant decrease in phytic acid in rice. This report may be related to the reason why soaking at longest day and highest degree of parboiling temperature resulted to the least value of phytate while the opposite of the processing conditions in terms of soaking and parboiling gave the highest phytate value in the rice flake. Humans have limited ability to absorb and hydrolyse phytate (Pawar and Ingle, 1988). Binding of minerals with phytic acid decrease bio availability of calcium, iron, phosphorus, zinc and other trace elements to human and other monogastric animals. This may lead to severe nutritional and consequently health problems in the consuming population (Thompson (1987).

Metabolization energy of ready to eat *ofada* rice flakes

The metabolizable energy in the flakes was between 368.46-393.55 kcal/100 g. There exist no significant difference ($P > 0.05$) in the metabolizable energy obtained from the rice flakes as affected by the processing condition and storage duration of paddy before processing. The R^2 and Adjusted R^2 were 0.7882 and 0.4917 respectively (Table 3).

Optimisation and validation of chemical quality of ready-to-eat *ofada* rice flakes

The optimisation of the ready to eat *ofada* rice flake was based on quality indices and level of desirability expected from high profile ready-to-eat flakes. These indices include maximum protein for good nutrition, minimum fat to prevent rancidity, minimum moisture to increase its shelf life and to prevent microbial activities, maximum ash represents total mineral composition which is also vital in nutrition because of their various functions, carbohydrate was not specified since the product have high carbohydrate value, minimum phytate (antinutritional factor usually found in rice), maximum metabolizable energy and maximum water absorption.

There were ten solutions suggested with desirability range of 0.745 to 0.783 with the first predicted solution having the highest desirability (Table 5). This involved storage of paddy for 9 months, soaking for 4 days and 17 h, parboiling at 106°C and drying at 30°C before milling to flour of desired particle size for the production of ready to

Table 5. Result of optimisation of quality of Ofada rice flakes.

S/N	Soaking Time (day)	Parboiling temp.	Drying Temp.	Storage Duration	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)	Phytate %	Water absorption ratio	Metabolisable Energy (kcal/100 g)	Desirebility
1	4.70	106.16	30.00	9.00	3.1	12.164	0.67	1.621	0.665	4.4035	393.192	0.7832
2	4.75	105.32	30.00	8.99	3.099	12.148	0.663	1.619	0.67	4.40266	393.191	0.7831
3	4.62	107.24	30.07	9.00	3.099	12.178	0.678	1.63	0.664	4.40116	393.166	0.7829
4	4.79	103.94	30.13	9.00	3.102	12.144	0.654	1.63	0.685	4.39364	393.112	0.7824
5	4.90	99.66	30.01	9.00	3.099	12.123	0.623	1.651	0.743	4.36695	392.962	0.7787
6	1.94	114.80	70.00	9.00	3.1	11.818	0.466	2.183	0.881	4.21874	388.686	0.7774
7	2.06	114.92	70.00	9.00	3.1	11.793	0.479	2.207	0.866	4.20355	388.584	0.7773
8	1.92	114.76	70.00	9.00	3.099	11.816	0.463	2.178	0.883	4.22206	388.71	0.7773
9	1.91	114.54	70.00	8.93	3.099	11.745	0.458	2.178	0.887	4.23492	388.817	0.7773
10	3.64	113.71	30.00	8.95	3.1	12.269	0.698	2.178	0.786	4.37523	392.861	0.7773

Table 6. Result of sensory evaluation of *ofada* rice flakes.

Ofada rice flakes	Colour	Taste	Aroma	Crispiness	Overall acceptability
First solution	6.7a	7.1a	6.9a	6a	7.30a
Sixth solution	6.3a	7.5a	7.2a	6.3a	6.4b
Tenth solution	7.8b	7.20a	6.80a	6.2a	6.6a

eat flakes to yield 3.10% moisture, 12.16% protein, 0.67% ether extract, 1.62% ash, 393.19 kcal/100 g, 0.67% phytate, and 4.40 water absorption ratio. However, the first three solutions as presented (Table 5) have similar desirability with high closeness of processing conditions and the same storage duration of paddy. Observations of the yields were also closely related.

The result of the validation of the best predicted solution showed yields of 4.08% moisture, 11.93% protein, 0.86% ether extract, 1.92% ash, 81.21%, 390.60 kcal/kg, 4.57 water absorption and 0.32% phytate. However, the results of the validation are closer to the result predicted by the response surface.

Sensory evaluation of ready-to-eat *ofada* rice flakes

The first, sixth and tenth predicted solutions were chosen for sensory evaluation based on variation of the levels of processing conditions. The ready to eat Ofada rice flakes manufactured were subjected to sensory analysis (colour, crispiness, taste, aroma, and overall acceptability) based on a nine point hedonic scale, where 1 is dislike extremely and 9 is like extremely. The result of the sensory analysis is presented in Table 6.

There exist no significant differences in crispiness, taste, and aroma, however, significant difference was shown in colour attribute with flakes produced from tenth predicted solution been rated better than the two. For the overall acceptability, the first solution was most accepted.

Conclusion

The effect of paddy storage duration and processing parameters (soaking time, parboiling temperature and drying temperature) were optimized for the production of ready to eat of ofada rice flakes. The coefficient of the model shown that soaking time of paddy has positive influence on ash, carbo-hydrate and metabolisable energy of rice flakes; parboiling temperature was positive on ether extract,

ash, carbohydrate and metabolisable energy; drying temperature of paddy gave positive influence on moisture, protein, ether extract, phytate, metabolisable aenergy and water absorption; storage duration of ofada paddy also influence carbohydrate, phytate, water absorption, and metabolisable energy. The R^2 ranged from 0.9662 to 0.7318 which confirmed the fitness of the model. The optimum storage duration and processing parameters for treatment of ofada paddy for the production of ready to eat flake include storage of paddy for 9 months, soaking for 4 days and 17 h, parboiling at 106°C and drying at 30°C to yield optimum quality of ofada rice flakes. The sensory assessment showed good acceptability, colour, crispiness, aroma and taste.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Full Length Research Paper

Microstructure formation and rheological properties of bread containing medium-chain triacylglycerols (MCT) and its comparison with long-chain triacylglycerols (LCT) and butter containing bread

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The microstructure formation and rheological properties of bread containing medium-chain triacylglycerols (MCT) were investigated in comparison with long-chain triacylglycerols (LCT) and butter. Baked breads absorbed moisture in storage, and consequently water activity (a_W) decreased, reaching an equilibrium after eight days; the a_W of LCT bread was 0.61. In contrast, the MCT and butter breads showed a_W of 0.76 and 0.72, respectively. Assessment of the rheological properties of LCT bread showed greater hardness than breads containing MCT or butter; a significant difference ($p<0.05$) was observed between the MCT and LCT breads. In contrast, no significant differences in the adhesion of breads were observed. With the addition of various lipids, changes were observed in the bread microstructure. With respect to the fine surface structure, a smooth surface was formed in the case of the MCT bread, as compared to that with LCT and butter. This suggests that the properties of MCT make it applicable to bread making.

Key words: Medium-Chain, triacylglycerols (MCT), long-chain triacylglycerols (LCT), microstructure formation, rheological properties, water activity.

INTRODUCTION

Medium-chain triacylglycerols (MCT) composed exclusively of medium chain fatty acids (C8 and C10) were first used in the 1950s for the dietary treatment of malabsorption syndromes caused by the inadequate absorption of nutrients. Since then, these diseases have been widely studied. Moreover, although a large number of reports have been published, the majority have focused on the issue from a clinical nutrition or biochemical standpoint (Leveilie et al., 1967; Chanez et al., 1991; Kris-Etherton and Yu, 1997; Ecelbarger et al., 1991; Papamandjaris et al., 1998). In contrast, very few studies have been conducted from a food science standpoint.

Wheat gluten proteins are mainly comprised of gliadin and glutenin (Schofield, 1986). During dough mixing gluten

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proteins are hydrated and form a three-dimensional network, which is responsible for the unique visco-plasto-elastic property of bread. Gluten contents are involved large as a factor that determines the quality of the bread, and as a factor of the other, involvement of lipids is considered. However, detailed studies as at present, are few. We previously reported (Toyosaki et al., 2008, 2010, 2013; Toyosaki, 2014) that MCT is implicated as an important factor in this phenomenon. It is know that MCT plays a role in a number of dough properties. However, information regarding the interaction between MCT and bread dough requires to be studied.

The purpose of this study was to determine the functional food properties of MCT in baked bread in comparison with long-chain triacylglycerols (LCT) and butter by assessing the molecular structure and rheological properties of bread.

MATERIALS AND METHODS

Materials

Medium-chain triacylglycerols (MCT), and LCT were a kind gift from Nisshin OilliO Group Ltd. (Kanagawa, Japan). Spring wheat flour (Super King brand) was obtained from Nisshin Flour Milling Inc. (Chiyoda, Tokyo, Japan). The proximate analysis of the flour indicates the contents of protein, ash, lipid and water were 13.1% (Kjeldahl, N x 6.25), 0.4%, 1.8% and 15.0%, respectively. More than 95% of flour granules were sifted through a 132 mm mesh sieve. Dry yeast (*Saccharomyces cerevisiae*) was purchased from S. I. Lesaffre (Marcq-en-Baroeul, France). Other reagents used in this study were of analytical grade and were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Preparation of baked bread and freezing conditions

Bread dough was prepared using commercially available ingredients and by employing the straight dough method. The dough ingredients were comprised of lipids (MCT, LCT, and butter, at 4.0%), hard flour (80%), yeast (2.5%), water (70%), sugar (8%), salt (2.0%) and skimmed milk powder (4.0%). Dough temperature at the completion of mixing was 26°C, and the dough was fermented for 90 min at 28 to 30°C. It was then molded and subjected to a final fermentation for 60 to 70 min at 36°C and 75% humidity, followed by baking for 35 to 40 min at 230°C (top of oven) to 210°C (bottom of oven). After baking, samples were frozen and freezer-stored under conditions typically found in the baking industry. Also, pieces of baked bread (200 g) were frozen in a blast freezer at -40°C for 4 h. The temperature in the center of the sample reached -20°C after 2 h and -40°C after 2 h of freezing. Thereafter, the samples were packaged in plastic bags and stored in a walk-in freezer at -40°C. During the 60-day storage period, samples were taken at regular intervals and analyzed by cryoscanning electron microscopy (cryo-SEM).

Cryo-SEM

Samples were removed after 2 h of freezing in a blast freezer and immersed in liquid nitrogen or after 1 day and 40 days of frozen storage. Small rectangular pieces (120 mm^3) were cut from a frozen baked bread sample in the walk-in freezer to avoid thawing. All samples were taken from the center of the baked bread section, except in one case, where samples were also taken from the outer zone. For further cooling and transport, samples were stored in liquid nitrogen. The fresh reference sample (100 g) was frozen in liquid nitrogen for 20 min after proofing of the baked bread.

Samples were fixed on a precooled holder with O.C.T compound and fractured under liquid nitrogen. The holder was transferred under nitrogen atmosphere to the cryo preparation unit (SCU 020; Bal-Tec AG, Balzers, Liechtenstein). The sample was sputter-coated with 20 nm of gold at -40°C and then transferred to the cold stage of the SEM (JSM-7800F; JEOL Ltd., Japan). The samples were observed at temperatures below -60°C and at an acceleration voltage of 10 kV.

Water activity (a_w)

The water activity (a_w) of baked bread was determined at 25°C with a Hygro Palm 2 (Rotronic AG, NY, USA). Determinations were made in at least duplicate.

Determination of rheological properties of baked bread

The hardness and adhesion of the baked bread were measured using a rheometer (TPU-2S; YAMADEN Co., Ltd., Tokyo, Japan). The baked bread was placed in a rheometer cell, 42 mm across and 16 mm high. A cylinder-type plunger (diameter of 15 mm) compressed the dough in the cell at 5-mm intervals and at a compression rate of 1 mm per second. Quadruplicate replicates were carried out on each sample within 5 min of one another. Each value is expressed as the mean ± standard deviation.

Statistical analysis

All data were expressed as the mean ± standard deviation. Statistical analysis was performed using the unpaired student's *t*-test (KaleidaGraph, Ver. 4.0; Synergy software, PA, USA). Differences in mean values among groups were assessed using the Tukey-Kramer multiple comparison test (Instat Ver. 3.0; GraphPad software, Inc., CA, USA). The level of significance was set at $p<0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Rheological properties of baked bread

The hardness and adhesion of baked bread containing MCT, LCT or butter were rheometrically determined. The hardness of the LCT bread was increased compared to the MCT and butter breads (Figure 1); a significant difference ($p<0.05$) was observed between the MCT and LCT breads. In contrast, the adhesion of the breads did not significantly differ (Figure 2). These results confirm that type of lipids used for the preparation of bread is a major factor in its physical properties.

Water activity (a_W) of baked bread

Water absorption is the main limiting factor in the shelf life of baked confectionery products and bread. Baked breads absorb humidity in storage, consequently a_W decreased,

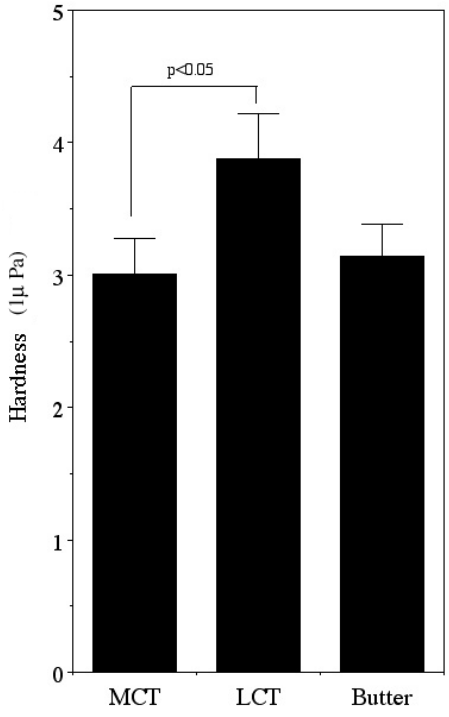


Figure 1. Effects of MCT, LCT or butter on the hardness of baked breads. Each value represents the mean ± standard deviation of triplicate measurements.

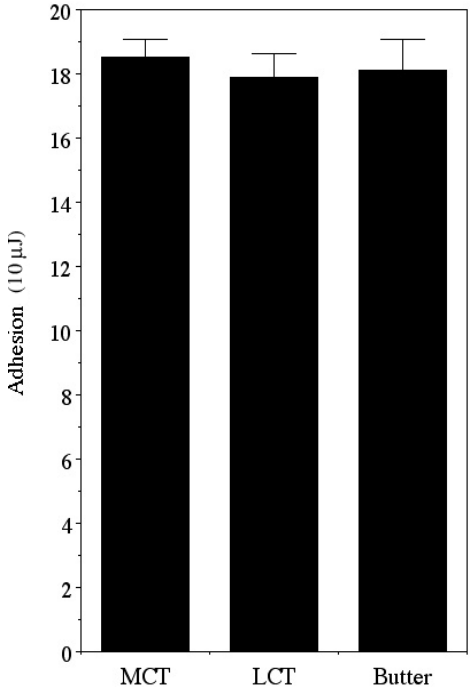


Figure 2. Effects of MCT, LCT or butter on the adhesion of baked breads. Each value represents the mean ± standard deviation of triplicate measurements.

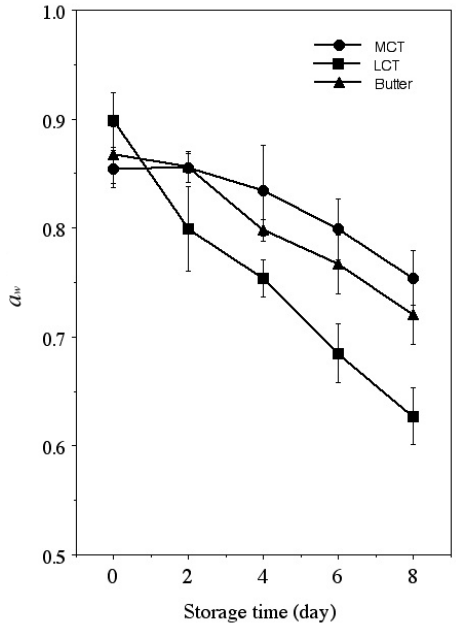


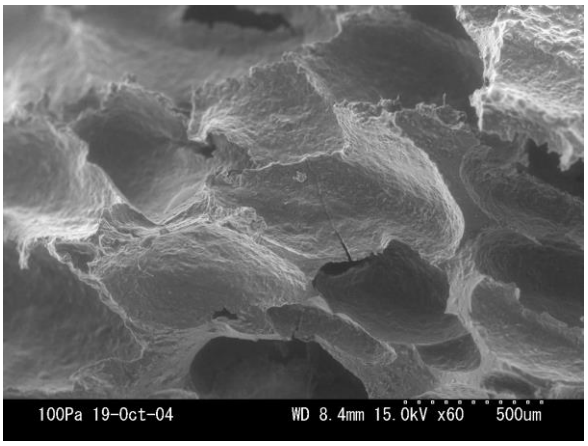
Figure 3. Effects of MCT, LCT or butter on the water activity (a_W) of baked breads stored for 8 days. Each value represents the mean ± standard deviation of triplicate measurements.

reaching an equilibrium after eight days; the a_W of LCT bread was 0.61 (Figure 3). In contrast, the MCT and butter breads showed a_W of 0.76 and 0.72, respectively. The decrease in a_W means a decrease of the mobility of the water molecule. The a_W is an important parameter in the microbiological stability of products (Fennema, 1993; Battaiotto et al., 2012). The a_W maximum of 0.6 as described in the present work, is sufficiently low to prevent microbial growth. However, in case of bread containing MCT or butter, the observed a_W maximums were insufficient to prevent microbial growth. Hence, the results of this study indicate that the differences in the properties of the lipid affect the level of water activity in the breads incorporated with them.

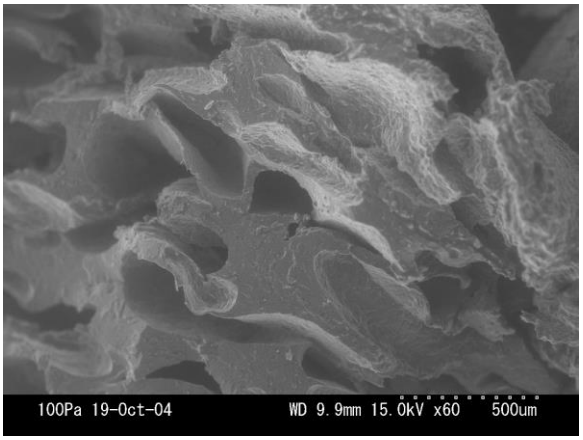
Microstructure of baked bread

As shown in Figures 4 to 6, cryo-SEM was employed to observe the fine structure of the baked breads and indicates the differences in the properties of lipids which affect the microstructure of breads. It is likely that differences in the fine structure are closely involved in the textural quality of the bread. In the cryo-SEM images of Figure 4, a number of pores were observed in the MCT bread. On the other hand, in bread containing butter, smaller numbers of pores were observed. Considering the fact that porosity had a major impact on the textural properties of the bread, the texture of the MCT bread was

MCT



LCT



Butter

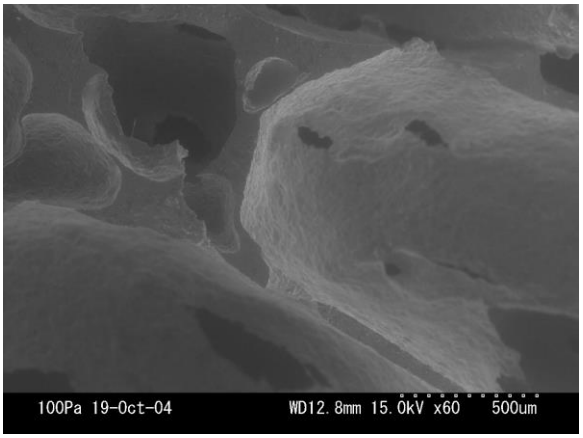
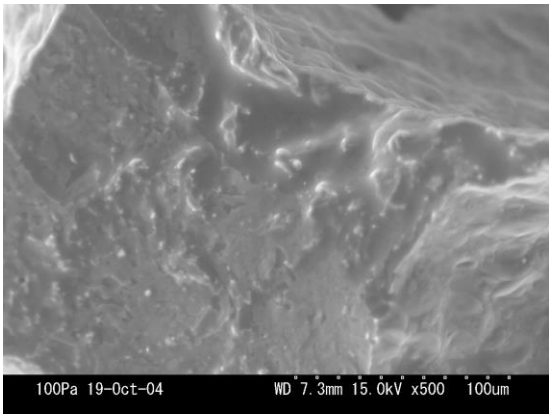
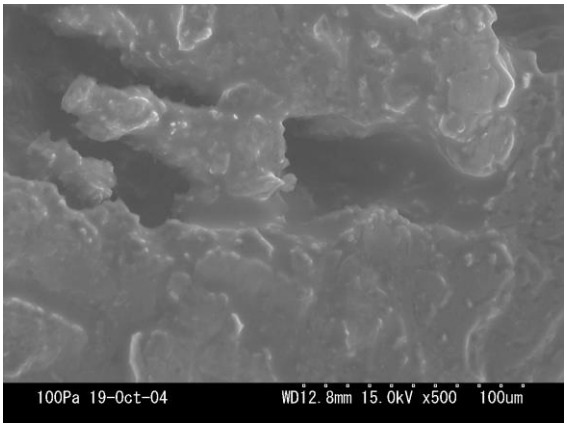


Figure 4. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent * 500 µm

MCT



LCT



Butter

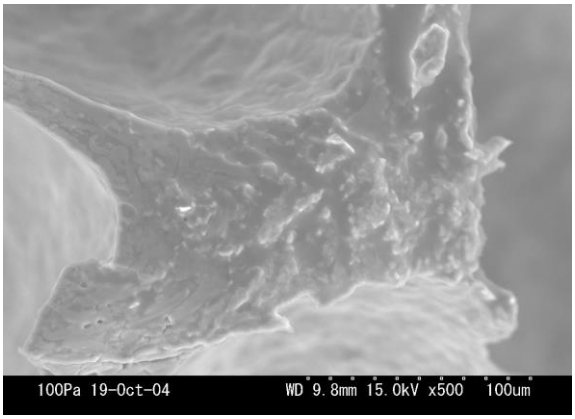


Figure 5. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent *100 µm.

presumed to be the best. Figures 5 and 6 show enhanced details of the fine structure of breads shown in Figure 4. Changes were observed in the bread microstructure depending on the lipid added. With respect to the fine structure surface, in the case of MCT bread, a smooth surface was formed compared to bread with butter or LCT. This may be that bread with MCT has good texture. Furthermore, the microstructure results are consistent with the results of bread properties (Figures 1 and 2).

Conclusions

The main aim of this research was to ascertain the

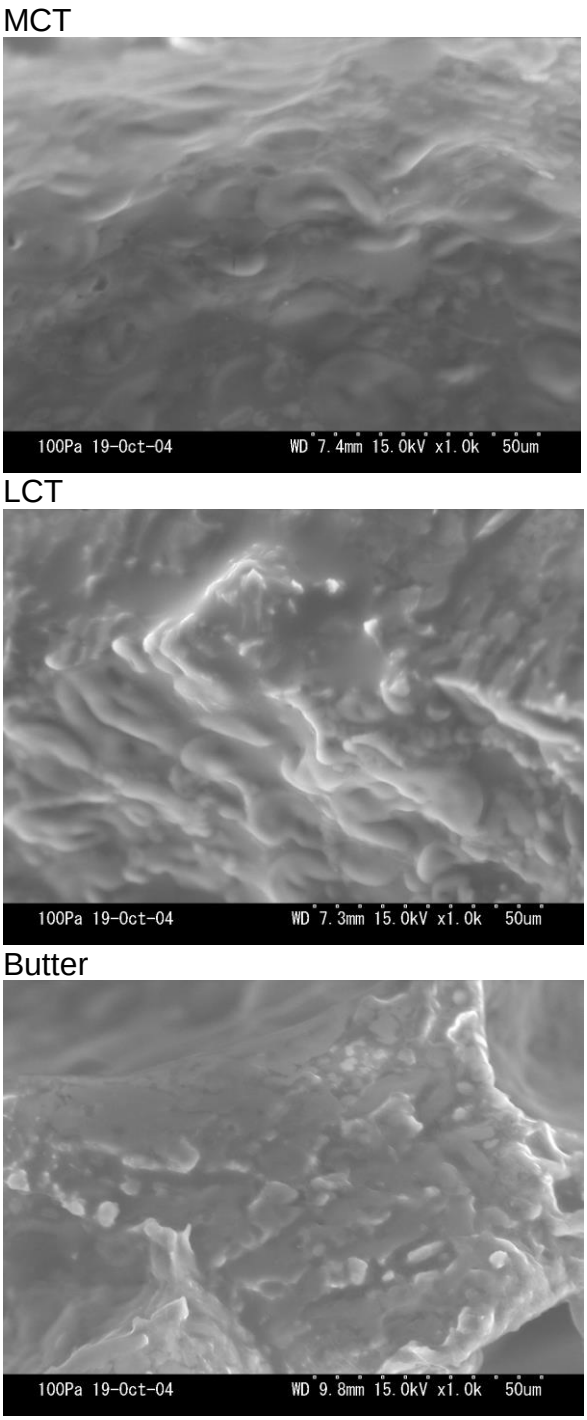


Figure 6. The microstructure of breads prepared with MCT (top), LCT (middle) or butter (bottom). Scale bars represent *50 µm.

functional characteristics of MCT in bread production. The microstructure formation and rheological properties of MCT bread were compared with those of bread containing LCT or butter. While the role of lipids in bread making has previously been poorly understood, the present study clarified that lipids affect the physical properties and microstructure of breads. This study suggests that the properties of MCT can be functionally applied to bread making.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Nutritional contents of *Balanites aegyptiaca* and its contribution to human diet

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Wild edible plants are used as food and energy sources. However, their uses are not as the potential inherent in the resources. *Balanites aegyptiaca* is a multipurpose species in semiarid areas including Ethiopia. Hence, quantitative nutrition study in specific habitat is essential for sustainable use of the species. Composite fruits sample of *B. aegyptiaca* was collected from six areas of east Shewa, Ethiopia for nutrient analysis following standard laboratory procedure. The results reveal that the fruits are rich in P, Ca, Fe, Zn, Cu, Na, K, Mg and Mn. The nutritional content varied (P<0.05) across land uses. Mean calculated energy value of lipids ranged from 0.09-027 kcal 4.2-7.68 for *B. aegyptiaca* and the total energy from carbohydrate was 342.2-354.24 kcal. Therefore, the fruit of *B. aegyptiaca* is promising in terms of nutrient content to human's diet diversification. It is a valuable species particularly during dry season for coping and adapting to climate variability/change. In spite of the promising potential, the nutritional contribution of this species to the people's diet remained underutilized. Therefore, the utilization of *B. aegyptiaca* is justified to be considered for integration in dryland agrobiodiversity systems and nutrition research to enhance the contribution to the diet of people and enhance its sustainable utilization.

Key words: Food security, nutrition, diet.

INTRODUCTION

Consumption of wild edible plants (WEPs) was practiced for ages. Nebel et al. (2006) explained that there has been renewed interest worldwide in consumption of wild food plants. Despite the increased reliance by agricultural societies' on conventional crop plants, the tradition of eating wild plants has not completely disappeared; their nutritional role and health benefits are being reported in many studies (Pardo-de-Santayana et al., 2007). Wild food plants are used as a source of food energy, sources of vitamins and minerals. They were important as dietary supplements, providing trace elements, vitamins and minerals (Gillman, 2008, Debela et al., 2011).

In spite of many studies on WEPs focused on the

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functions within ecology and ecosystems there are few research works that compared the food plants of various communities (Ladio et al., 2006). Some research works have compared medicinal floras and other useful plants (Pieroni and Quave, 2005). Such comparative studies therefore contribute to the understanding of why edible species are widely consumed or given little attention and provide insights into food and nutrition security potential of different communities.

Food and nutritional security are key issues for human wellbeing while WEPs are underutilized in many countries including in Ethiopia. Fentahun and Hager (2009) have reported that wild edible fruits contribute to nutrition and health security of rural people as they contain proteins, vitamins and minerals. In the first phase of this work which is already published in Debela et al. (2011) and others, the proximate (carbohydrate, lipids and total minerals) were addressed. However, the information on the fruits nutritional quality of the species is not exhaustively documented. The specific minerals and vitamins contents were not determined in this study. Therefore, these studies curve this research gap through further analysis of the nutrient composition macro and micronutrients of *Balanites aegyptiaca*. It also indicates future line of research on the contribution of *B. aegyptiaca* to nutrition and food security of the semiarid people. Therefore, this study was aimed at analysing the nutritional content of *B. aegyptiaca* and implications to food and nutrition of people in semiarid areas.

MATERIALS AND METHODS

The study area

The study was conducted in 'Fantalle' and 'Boosat' districts, in East Shewa Zone of Oromia National Regional State, Ethiopia. East Shewa is located between 7°12'-9°14'N latitudes and 38°57'-39°32'E longitudes in the northern part of the Great East African Rift Valley.

Collection and preparation of fruit sample for laboratory analysis

Frequency distribution and density of *B. aegyptiaca* in the local vegetation was systematically determined by total count of shrubs and trees from 20 x 20 m of 66 plots (33 from each district) laid at 200 m interval on the 5 km line transects.

Before undertaking laboratory nutrient analysis, fruit samples of *B. aegyptiaca* were identified through focus group discussion (FGD), interview and field observations as described in Martin (1995) and Cotton (1996) by repeated field visits between 2009 and 2010. Composite fruit samples of the species were collected in a sample bag from Fantalle (Galcha, Qobo and Dheebiti villages) and Boosat (TriiBiretti, DigaluTiyo and Xadacha villages) districts for nutrient analysis.

Minerals, vitamins A and C and tannin analysis

The mineral elements comprising sodium, calcium, potassium, magnesium, iron, zinc and phosphorus were determined according to the method of Shahidi et al. (1999), Sundriyal and Sundriyal (2004), AOAC (1990) and Nahapetian and Bassiri (1975). Two gram of each of the processed samples was weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace.

The resultant ash was dissolved in 5.0 ml of HNO_3/HCL/H_2O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman No.42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption. A 10 cm long cell was used and concentration of each element in the sample was calculated on percentage of dry matter, i.e. mg/100g sample. Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975).

For tannin determination samples were dried at a maximum of 60°C immediately after collection to minimize any chemical changes and extracted with 50%v/v acetone. Determination was by a modification of the vanillin method of Ranganna (1977) and Broadhurst and Jones (1978), which utilizes the formation of coloured complexes between vanillin and condensed tannins and Catechin was used for the standard, and results were expressed as catechin-equivalents.

Determination of vitamin A was carried out by spectrophotometer following Davies (1976) and (AOAC, 1990). Ascorbic acid (vitamin C) was determined by redox titration using iodate (I_3) following (Pearson, 1976) and Helmenstine (2007).

Determination of percentage titrable acidity and total soluble sugar (TSS)

Titrable acidity was determined by titration using 0.1 N NaOH. Titration was done by adding 0.1 N NaOH from a burette to the Erlenmeyer flask containing 10 ml of fruit solution prepared in measuring while swirling until the solution just starts to change colour to pink/purple (slightly pink). The amount of NaOH added to the flask was recorded immediately at this end point. Multiplied the volume of sodium hydroxide (volume of titre) added by acid conversion factor of the fruit acid to get the value of the acid (in grams per 100 ml); that is, TSS of fruit indicator used is 2-3 drops of phenolphthalein indicators from burette filled, along with phenolphthalein indicators (AOAC, 1990, OECD, 2005). Soluble solids (TSS) content was determined directly in the fruit juice, using a portable/handle held refractometer (Pocket PAL-1, UK) at room temperature in degree Brix (°Brix) following (Khemiss et al., 2005; Franco et al., 2009). Degrees Brix (°Bx) is a unit representative of the sugar content of an aqueous solution.

Determination of energy value

Energy of the WEPs fruits was calculated in kilocalories (kcal) multiplying by energy factor composition (4, 4 and 9) of percentage proteins, fats and carbohydrates respectively as used in FAO (1968, FAO, 2011), USDA (1999) and Asibey-Berko and Tayie (1999). The conversion factors are for physiological energy, which is the energy value remaining after losses due to digestion and metabolism and deducted from gross energy (USDA, 1999) where one kcal equals 4.184 kJ. Organic carbon (O.C) in the fruit was obtained by subtracting total ash from 100 (Adams et al., 1951).

Data analysis

Statistical analysis for nutritional content of *B. aegyptiaca* was done

through analysis of variance and means were separated by LSD at P< 0.05 using GenStat (VSN International, 2011). Organic carbon (O.C) in the fruit of threatened WEP was calculated using formula % O.C = (%VS/1.8) x100, where, %VS = (100-%Ash) following Adams et al. (1951). TSS obtained directly from Refractometer was expressed in °Brix. Titrable acidity was calculated by:

$$TA\,(\%) = \frac{mL\ of\ \ NaOH\ (Titre) \times 0.1N\ NaOH \times acid\ meq.factor}{mL\ of\ \ juice\ titrated} \times 100$$

and was expressed as g malic acid 100 g-1 fresh weight (F.W.) (AOAC, 1990; Franco et al., 2009).

RESULTS AND DISCUSSION

Abundance and distribution of *B. aegyptiaca*

The present study indicated that, the average % relative frequency is 24.24 and Av% relative density was 0.96 in settled farmers land use and av % frequency was 33.33 and Av % relative density was 2.27 in transhumance land use for *B. aegyptiaca*. Direct repeated field visit and observation in the study areas revealed that the natural forest is more degraded in settled farmers area due to charcoal and firewood production, expansion of agriculture and settlements in settled farmers areas. Commercial fuel wood (charcoal and firewood) production was observed. *B. aegyptiaca* has declined in natural forest due to overharvesting. Cutting dawn local vegetation for building of houses for the increased settlement in the area has affected *B. aegyptiaca*. As reported by informants during field observations, overgrazing/browsing by camels and goats is a serious threat to the vegetation of the area including *B. aegyptiaca*.

Local consumption of WEPs as food was not regarded as a threat to the survival of WEP species unless when demand becomes higher than sustainable harvest in the future. Gamado-Dalle et al. (2005) and Asfaw (2009) reported that local people maintain species which are useful to their livelihood at farm boarders, live fences and sacred areas.

Nutritional potential of *B. aegyptiaca*

The results indicate the existence of variations of nutrient and energy from *B. aegyptiaca* from different study sites. This can be attributed to environmental factors such as soil, temperature and rainfall. In spite of the variations within and between land uses, the result indicated that *B. aegyptiaca* has significant amount of nutrients and energy to supplement household nutrition and a good income source if properly valued.

Besides direct nutritional contributions through carbohydrates, lipids, proteins, minerals and energy; the diversity of wild fruits by itself is a source of variety and taste in the local meals of rural communities including Ethiopia. Earlier studies made by FAO (1995) on non-timber forest products focusing on nutrition, and in India on nutrient composition of specific plants (Parvathi and Kumar, 2002) have reached similar conclusion. In east Shewa, the nutritional contribution of *B. aegyptiaca* and value are hardly recognized in the formal production system as it is overshadowed by the values of conventional food crops and charismatic species such as *Eragrostis tef*, *Triticum aestivum, Sorghum bicolor, Zea mays* and others.

Comparison of the results of the present study with the nutrient content of some cultivated fruits noted by Srivastava and Kumar (1998) revealed that they are superior in protein content to banana (1.2 and 0.3%), guava (0.9 and 0.3%) respectively; mango (0.6 and 0.4%, respectively) and papaya (0.6 and 0.1%) respectively.

Test also contributes to the nutritional quality of a fruit. In this regard, the relatively high tannin content of *B. aegyptiaca* may be a point of argument to fully recommend the fruit for common use for human diet. Chapagain and Wiesman (2007) also reported nine compounds from *B. aegyptiaca* of which six of them were saponins with molecular masses of 1196, 1064, 1210, 1224, 1078 and 1046 Da.

The same report revealed that the compound of mass 1210 Da being the main saponin (ca. 36%). Saponins with masses of 1224 and 1046 Da have not been previously reported in *B. aegyptiaca.* Chapagain and Wiesman (2007) also reported that in all saponins, diosgenin was found to be the sole aglycone. Though people use *B. aegyptiaca* fruits, the controversial issues of saponins and consequent bitter test and the impact consumption pattern and nutritional quality is question not addressed by the present study.

Interaction effect of fruit of *B. aegypiatca* and land use on mineral and tannin contents

The mineral and tannin contents of *B. aegyptiaca* significantly varied across land uses (P<0.05) (Table 1). Phosphorus content is highest in *B. aegyptiaca* for sampled collected from transhumance land use. Sodium content was highest in *B. aegyptiaca* followed for samples collected from settled farming area (Table 1).

Interaction effect of land use on Vitamin A and C contents of *B. aegyptiaca* species

Vitamin C content of *B. aegypiatca* was 50.30 and 50.90 on mg/ 100 g dry matter basis for SF and TH land uses respectively and not significantly varied (P>0.05) across land uses. Vitamin A content (Beta carotine REs, Retinol Equivalents) for *B. aegypiatca* was 233.70 and 266.33 for SF and TH land uses respectively which significantly varied between land uses (P<0.05) with higher mean value from sample collected from transhumance land use systems.

Table 1. Interaction effects of fruit tree species with land use system on mineral and tannin contents of *B. aegyptiaca* in mg/100 g dry matter basis.

Nutrient contents across land uses	Nutrient and tannin contents									
	P	Ca	Fe	Zn	Cu	Na	K	Mg	Mn	CT
SF* *B. aegyptiaca*	102.625	74.550	39.700	0.5500	0.0950	22.100	1990.30	87.070	0.8200	1219.88
TH* *B. aegyptiaca*	103.55	76.650	40.360	0.3150	0.1002	20.100	1992.50	87.635	0.7850	1222.10
LSD (5%)	0.1806	0.7824	0.1594	0.8667	0.03726	0.1816	1.589	0.6627	0.05051	2.07
s.e.d	0.0783	0.3393	0.0691	0.03758	0.00808	0.0787	0.689	0.2874	0.02190	0.898
Sig*	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.005	<0.001	0.008

SF=settled farmers; TH=transhumant land uses; Means with the same letter are not significantly different ,P=Phpsphorus; Ca=Calcium; Fe=Iron,Zn=Zink,Cu=Copper; Na=Sodium; K=potassium; Mg=magnesium; Mn=manganese; CT=condensed tannin,*= significant at 0.05 level, s.e.d = standard error of the differences.

Percentage titrable acidity and total soluble solids in composite fruits pulp samples

Analysis of variance between locations revealed that in % TTA (1.37) and TSS (4.35) and TSS/ %TTA is not significant (P>0. 05). However, the mean values %TTA and TSS °Brix related that the fruits *B. aegyptiaca* have relatively high characteristics of sourness combined with a high degree of sweetness taste. By extrapolation, the total soluble solid was lower. The TSS/%TTA ratio (3.18) also indicated the fruit can be consumed by humans.

Comparison of energy content of *B. aegyptiaca* fruits

The result of the present study indicated that mean calculated energy value of lipids ranged from *B. aegyptiaca* from 0.09-027 kcal to 4.2-7.68 for *B. aegyptiaca.* Energy for total carbohydrate was 342.2-354.24 kcal for *B. Aegyptiaca* (FAO, 1968; 2011).

Conclusions

The fruit of *B. aegyptiaca* is promising in terms of nutrient content to humans and livestock. The results of the nutrient analyses showed that B. aegyptiaca fruits can be important indigenous sources of nutrients to supplement other major food sources. This is a valuable plant particularly during dry season for coping and adapting to climate variability/change. Though it has good potential, the nutritional contribution of this species to the human diets is not sufficiently utilized. On top of this, there is a need of more research on the identification of the bitter components and their effects on human nutritional utilization and quality. Hence, *B. aegyptiaca* need be considered for integration in dryland production system such as agrobiodiversity systems to improve the livelihoods of people and promote its sustainable utilization by improving nutritional quality through more research.

Conflict of interests

The authors did not declare any conflict of interest.

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Temperature and high pressure stability of lycopene and vitamin C of watermelon Juice

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The retention of nutrient components of fruit juices during processing is an important criterion to produce better quality fruit products. Stability of nutrient components during processing vary from product to product and processing methods. Information about kinetic study of particular nutrient component under different processing conditions would enable to optimize processing parameters for better quality retention. Therefore the objective of this research was to evaluate the effect of thermal and high pressure food processing methods on stability of vitamin C and lycopene of watermelon juice. Watermelon juice was subjected to different thermal (70 to 90°C) and high pressure (400 to 600 MPa) treatments for different interval of times and the residual vitamin C and lycopene concentrations were measured. The destruction of nutrients in both thermal and high pressure processing conditions obey first order reaction rate kinetic model. In both processing conditions lycopene remains more stable as compared to vitamin C. The degradation rates of both components were faster in thermal treatment as compared to high pressure. The *D* value of vitamin C ranged from 40 to 176 min ($z_{=}$ 30.8°C) and 4 to 24 h ($z_{p=}$ 257 MPa) in thermal and high pressure treatments respectively whereas the *D* value of lycopene ranged from 15 to 83 h ($z_{=}$ 24.5°C) and 61 to 258 h ($z_{P=}$ 318 MPa) for thermal and high pressure treatments respectively. Therefore high pressure ensures better retention of the nutrients as compared to thermal treatment and hence can be used to pasteurize better quality watermelon juice.

Key words: Vitamin C, lycopene, thermal, high pressure, destruction kinetics, watermelon.

INTRODUCTION

Food processing is one of the major means to produce safe foods for consumer's use. Most of the food products in stores are produced in either one or more than one type of food processing technologies. Thermal processing of foods is the major means of production of majority of foods. Particularly, in the canning industry thermal processing has enabled to produce safe and shelf stable products. However due to intensive heat treatment the process has also an effect on heat sensitive quality components of foods. For instance heating accelerates

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the oxidation process of vitamin C, thus thermal treatments result in loss of the vitamin content in fruits and vegetables and the milder the treatment, the better the vitamin C retention in juices (Dewanto et al., 2002).

In order to overcome the above mentioned limitations various novel thermal and non thermal processing technologies have been developed to preserve mainly liquid foods. One of the major non thermal processing technologies which have gain momentum and application from time to time is the use of high pressure processing technology. In this process the packaged or unpackaged beverages are subjected to pressures between 100 and 1000 MPa inside a cylindrical pressure vessel. Developed high pressure processing allows microbial inactivation at temperatures below those used during conventional thermal pasteurization, providing better retention of antioxidant compounds and essential nutrients, (Hancock and Stewart, 2010). The process is used on a variety of products including fruit juices, fruit purees and jams (Bertucco and Spilimbergo, 2006). It has been also generally admitted that high pressure affects minimally low molecular weight compounds such as vitamins, pigments, antioxidants and flavour components when compared to thermal processes, as it keeps covalent bonds intact. This effect is especially important in fruits and vegetables that are rich sources of these compounds. A study showed that the antioxidant capacity in carrot and tomato juice treated by high pressure was retained more compared to thermal treated samples (Saner, 2007). Bignon (1996) also observed that the decrease in vitamin C content of strawberries and guava puree treated with high pressure was much lower as compared to the fresh product.

Nowadays there is an increasing demand for high quality, nutrient and antioxidant rich fruit and vegetable products. Studies have shown that high consumption of fruits and vegetables can provide health benefits due to their antioxidant components including carotenoids, phenolic, flavonoids compounds and vitamins (Sánchez-Moreno et al., 2003). Watermelon (*Citrullus lanatus*) is a native plant of tropical Africa; it has relatively low vitamin C content compared with other citrus fruits. However, it is rich in carotenoids. Some of the carotenoids in watermelon include lycopene, phytofluene, phytoene, beta-carotene, lutein, and neurosporene. Lycopene makes up the majority of the carotenoids in the fruit and responsible for its red colour. The carotenoid content varies depending on the variety of the watermelon. Depending on the variety, carotenoid content in red fleshed watermelon varies from 37 – 121 mg/kg fresh weight, where as lycopene varies from (23.0–72.0µg/g of weight) (Xianquan et al., 2005). Carotenoids have antioxidant activity and free-radical scavenging property. Several researches have reported an association between dietary lycopene consumption and lower incidence in diseases such as prostate and oral cancers and may also help reduce risks of cardiovascular disease (Oms-Oliu et al., 2009). Maintenance of vitamin C and lycopene in thermally processed products has always been a major challenge in food processing. Vitamin C loss and undesirable degradation of lycopene in watermelon juice affects the health promoting ability, sensory characteristics and its natural appearance (Sharma, et al., 2008). Therefore, the objective of this study was to determine the degradation kinetics of vitamin C and lycopene of watermelon juice under different thermal and high pressure treatment conditions.

MATERIALS AND METHODS

Watermelon juice preparation

Watermelons (*Citrullus lanatus*) fruits were purchased at commercial maturity from a local supermarket (Montreal, Canada). Fruits cut into pieces and pulps were mixed in order to avoid fruit to fruit and maturity variation. Mixed pulp was stored at – 40°C until the required juice was made. Before experimentation the pulp was thawed and the juice extracted with a household juice processer. Double layer cheese cloth was used to filter the juice from the pulp. The natural pH of the juice founded around (5.48±0.02) and this relative high pH may support the growth of microorganisms and hence, the pH was adjusted to 4.4 with citric acid.

Thermal treatment

Juice (40 ml) was transferred into test tubes (15 cm length, 1.5 cm diameter and 0.1 cm thickness) and thermally treated at different temperatures (70, 80 and 90°C) for different pre set time intervals in a water bath (HAAKE P5, Type 003-5007, Karlsruhe, Germany). During heat treatment, the temperature was registered using data acquisition system and K- type thermocouples. For all temperatures investigated, a coming-up time of 2 min was taken into account and temperatures varied ± 1°C during the isothermal phase. Following heating, samples were taken out in regular time intervals and immediately cooled in an ice-water bath to avoid further heat degradation effect.

High pressure treatment

For the pressure treatments, 50 ml juice was filled into small flexible polyethylene plastic bags that were sealed, covered by another plastic bag and taking care to leave no air inside either of the two bags, to prevent them from breaking as a result of the pressure and prevent oxidation of nutrient components. The packaged samples were left at room temperature for 2 h to equilibrate to the ambient atmospheric condition (25°C). The initial water temperature (used as the pressure-transmitting fluid) was at 25ºC. Samples were pressurized using a laboratory pilot scale isostatic high pressure unit (ACP, TYPE ACIP 6500/5/12 VB, France, capacity with working pressure range of 100-600 MPa and temperature range of -20 to +80°C.), employing pressures from 400 to 600 MPa with pressure build up rate of 1 min, with different holding time (400 MPa (60, 80 and 100 min); 500 Mpa (30, 60 and 90 min); 600 MPa (15, 30 and 45 min). During pressure build up time a temperature increase to 30-34°C was observed and average of 32°C was taken as holding phase temperature. In order to exclude the effect of pressure build up phase samples at zero time were subjected for pressurization for 1 min and immediate depressurization without holding time. It was assumed that there were no vitamin C and lycopene degradation at

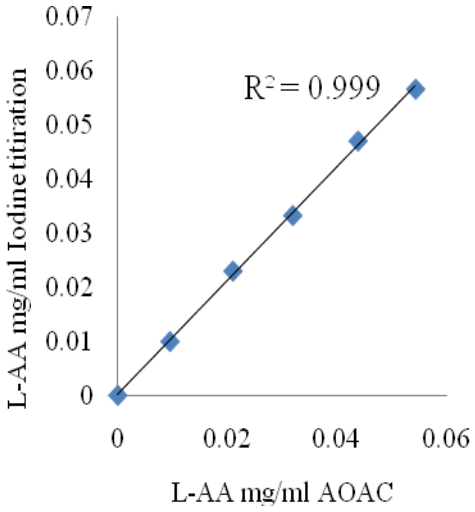

Figure 1. Vitamin C estimation capacity of iodine titration method as compared with official AOAC 967.21 method.

this temperature and all the effects were assumed exclusively from the pressure.

Vitamin C estimation

Due to the Red-Pink color of watermelon juice it is hard to identify the end point of titration (rose-pink color) using indicator solution (2,6-dichloroindophenol Na salt) if the official AOAC 967.21 method is applied. Because of this the vitamin C content of samples was determined using iodine titration method as indicated by Ajibola et al. (2009) and Spínola et al. (2012). Since this method is not an official method, its validity was cross checked with official AOAC 967.21 method using known concentrations (0.01 – 0.05 mg/ ml, pH 3.63) of standard vitamin C solutions. The result confirmed that the estimation capacity of iodine titration method was found almost equivalent to the official method with high degree of correlation (R^2=0.999) (Figure 1).

Extraction and quantification of lycopene

The low volume hexane extraction method with a little modification was performed as indicated in Fish et al. (2002). Approximately 0.6 g duplicate samples were weighed from each watermelon juice into two 50 ml centrifuge tubes covered by aluminum foil to exclude the degradation of lycopene by light. The mixture that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) dissolved in acetone, 5 ml of 95% USP grade ethanol, and 10 ml of hexane. After the samples were mixed with the extraction solution, samples covered by ice and thoroughly shacked using horizontal shaker (Belly Button, BBUAAUV1S, Greensboro, USA) for 15 min. After shaking, 3 ml of distilled water was added and the shaking continued for additional 5 min. Then the samples were left at room temperature for 5 min to make the phase separation. Eventually the absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm wave length using spectrophotometer (Novaspec II Visible, England). Before samples measurement, the spectrophotometer was calibrated with hexane as a blank. The final lycopene content of sample was analyzed as indicated in Fish et al. (2002) (Equation 1).

$$Lycopene(mg/kg(sample)) = \frac{A_{503} * 31.2}{g(juice)} \quad (1)$$

Where, 31.2 is the molar extinction coefficient of lycopene; *g* gram of fruit juice used for analysis, and A_{503} absorbance at 503 nm.

Data analysis

Vitamins and lycopene degradation values are generally considered to follow first order kinetics (Ahmed et al., 2002; Polydera et al., 2003; Sharma et al., 2008; Vikram et al., 2005). The rate of degradation of the components is mathematically expressed as:

$$-\frac{dC}{dt} = -k(C_o) \quad (2)$$

Where, *k* is the rate constant; *C* concentration at time *t*; *Co* initial concentration at time zero, *n* the order of reaction.

Under isothermal and isobaric conditions, the inactivation rate *k* is constant and for first order reaction *n* =1. The integration of Equation 2 is expressed in Equation 3 form.

$$\ln C = \ln C_o - kt \quad (3)$$

For the first order reaction, a plot of ln *C* versus *t* will be a straight line, and the rate constant is represented by the slope. The decimal reduction time (*D*) of the quality factors can be calculated from equation four or from the inverse slope of logarithmic order of residual concentration of the quality factors versus time. *D* value indicates the heating time results in 90% destruction of vitamin C or lycopene content as compared to time zero concentration at constant temperature.

$$D = \frac{2.303}{k} \quad (4)$$

Temperature dependency of the reactions can be explained either through thermal death time (TDT) or Arrhenius kinetic method. In the former case the temperature sensitivity of quality factors at different temperature levels as thermal resistance curve which is represented as log *D* versus temperature. From inverse slope of the curve line the temperature sensitive indicator (z value) can be calculated. The z value represents the temperature level increase required to achieve ten-fold decrease in *D* values. In the later case the Arrhenius kinetic model, is one of the most important model to predict the effect of temperature on specific reaction rate, *k* (min^{-1}). The effect of temperature (in absolute form) on the reaction rate constant is explained by Arrhenius, in Eq. (5 and 6).

$$k = k_o e^{-Ea/RT} \quad (5)$$

$$\ln k = \ln k_{Tref} + \left[\frac{E_a}{R}\left(\frac{1}{T_{Tref}} - \frac{1}{T}\right)\right] \quad (6)$$

Where, k_{Tref} is reaction rate constant at reference temperature, *Ea* is the activation energy (kJ/mole), and *R* the molar gas constant

(8.314 J/mole °K, *T* temperature (°K) at time *t*.

The graph *ln k* versus *1/T* will give us a linear line from which the inverse slope is used to calculate the E_a required for the reaction.

The effect of pressure on reaction rate constant at constant temperature can be expressed by theory of Eyring, where reaction rates are based on the formation of an unstable intermediate complex, which is in quasi-equilibrium with the reactants. At constant temperature, the theory is expressed in equations 7 and 8, (Heldman and Lund, 2007).

$$\left(\frac{d\ln k}{dp}\right)_T = -\frac{\Delta V}{RT} \quad (7)$$

The integration of Equation 7 yields Equation 8 and rate constant, *k* is expressed as:

$$\ln k = \ln k_{\Pr ef} + \left[\frac{V_a}{RT}\left(P_{\Pr ef} - P\right)\right] \quad (8)$$

Where, k_{Pref} is a rate constant at reference pressure P_{Pref}; V_a is the volume of activation, *R* is the molar gas constant (8.314 J/mole °K), *T* temperature (°K) at time *t*.

Based on experimental data, *k* and *D* values were calculated from linear regression of the natural (ln) and ten-based (log) logarithms of the concentration retention versus processing time. The E_a and *z* values were estimated from linear regressions of ln (*k*) versus (*1/T*) and of log (*D*) versus *T*, respectively. From a practical point of view, the activation volume can be determined from the slope (−*Va/RT*) of the plot of ln *k* versus *P* at constant temperature.

RESULTS AND DISCUSSION

Temperature stability of vitamin C and lycopene

The degradation of vitamin C during thermal processing operations has received much attention due to its instability to heat, light, metal catalysts, oxygen, and its relatively high water solubility.

Therefore studying thermal stability of vitamin C content of watermelon juice is an important input in processing of the fruit juice.

Temperature stability of vitamin C and lycopene of watermelon juice were studied after subjecting the samples to various temperatures and heating time conditions. The log-linear decrease of the vitamin C and lycopene as a function of heating time are illustrated in Figures 2 and 3. The figure shows that a decrease in the concentration of the vitamin and lycopene as a function temperature and heating time. The estimated kinetic parameters are summarized in Tables 1 and 2.

Previous works in orange juice confirmed that vitamin C degradation followed first order degradation kinetics (Polydera et al., 2003; Vikram et al., 2005) which was also observed in this work. The rate of the vitamin degradation in the study increases with an increase in temperature from 70 to 90°C (Table 1). The data obtained for the effect of temperature on vitamin C content were not comparable with reported values of Vieira et al. (2000) and Vikram et al. (2005). Difference in *D* and *k*

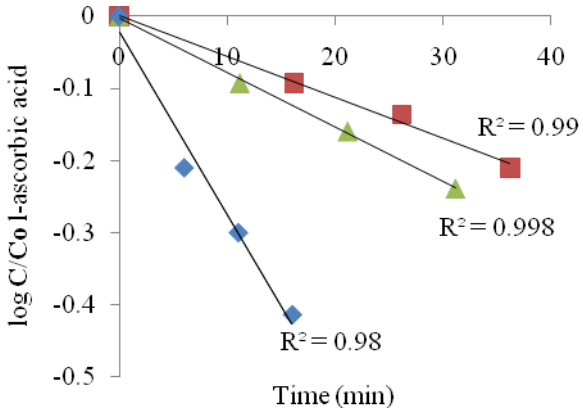

Figure 2. Semi log plot of thermal stability of vitamin C of watermelon juice under: (■) 70 °C, (▲) 80 °C, (♦) 90°C)) heated for different time durations.

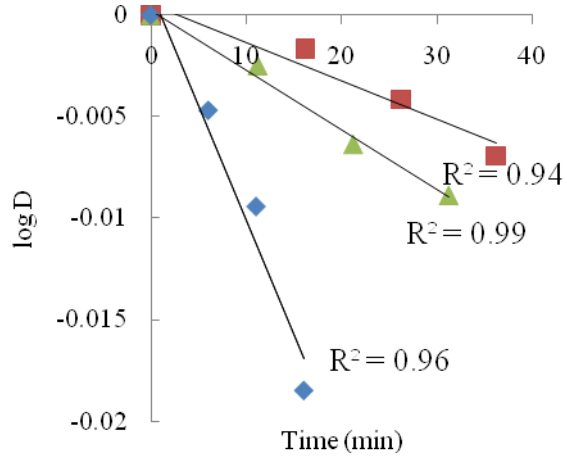

Figure 3. Thermal stability of lycopene of watermelon juice under different temperature: ((■) 70 °C, (▲) 80 °C, (♦) 90°C)) heated for different time durations.

Table 1. *D*, *k*, *z*, and *E*a values for the thermal degradation of vitamin C of watermelon juice.

T (°C)	*D* (min)	*k* (min-1)	R^2
70	175.97±2.5*	0.013093±0.00019	0.99
80	132.62±7.96	0.017491±0.0011	0.998
90	39.5±0.71	0.058393+0.0011	0.98
	z = 30.8±0.067°C	Ea = 18.37±0.057kcal/mole	0.89

*Standard error

value can be due to the fact that they measured vitamin C for different products. On the other hand, Van den Broeck et al. (1998) reported higher *D* values for the thermal degradation of vitamin C in squeezed tomatoes

Table 2. *D*, *k*, *z*, and *E*a values for the thermal degradation of lycopene of watermelon juice.

T(°C)	*D* (hour)	*k* (hour^{-1})	R^2
70	83.33±13.9*	0.02418±0.0017	0.94
80	57.5±1.03	0.03938±0.00035	0.99
90	14.5+0.06	0.1596±0.00035	0.96
	z=24.5°C±1.8	Ea=23.35±1.85 kcal/mole	0.92

*Standard error.

and oranges. For instance the vitamin C from orange found more heat sensitive as compared with tomato source which have been studied in temperature range of 120-150°C (Van den Broeck et al., 1998). This shows vitamin C stability varies from product to product which could be associated with total vitamin C content. Vitamin C from watermelon juice in this work found more temperature sensitive than a source from orange and tomato (Van den Broeck et al., 1998). This variation might be contributed due to agro ecological or agronomical difference where the fruits were grown. Furthermore a compositional variation in juices might play also a protective role in case of orange and tomato as compared to watermelon juice.

These days the nutritional and health benefits of lycopene of watermelon juice are well known. Xianquan et al. (2005) indicated that, under different food processing conditions, lycopene undergoes degradation via isomerization and oxidation, which impact its bioactivity and reduce the functionality for health benefits. Therefore, food processing technologies should ensure the availability of sufficient amount of lycopene after a given thermal process. For this reason it is pertinent to study thermal degradation behaviour of lycopene. Likewise vitamin C degradation, lycopene degradation also obeys first order reaction (Figure 3) and hence lycopene degradation is temperature dependent and increases with an increase in temperature. The rate constants (Table 2) show an increase with heating and this result is in agreement with earlier reports of Ahmed, et al. (2002) and Sharma et al. (2008).

Lycopene may be expected to undergo two changes during processing and storage: isomerization from all-trans to mono-cis or poly-cis forms, and oxidation (Cole and Kapur, 1957). Different reports indicated that isomerization is the main reaction during heating for short period of time at low temperature, but with an increase in treatment condition the degradation reaction dominated (Lee and Chen, 2002; D'Evoli et al., 2013). The all-trans isomer of lycopene is the most predominant geometrical isomer in fruits and vegetables and is the most thermodynamically stable form (Xianquan et al., 2005). Lycopene from canola oil treated at a temperature range of 100 to 180°C showed the degradation of trans isomer as compared to samples treated below 100°C (Shi et al., 2002). In a similar work lycopene from carrot source remained heat stable even treated at 70°C for 5 h, but the degradation was fast with enhanced degree of isomerization when temperature increased above 100°C (Mayer-Miebach, et al., 2005). Comparison results of Tables 1 and 2 indicate that the rate of thermal degradation of vitamin C is faster than lycopene. For instance at 90°C vitamin C required less than an hour but lycopene required more than 14 h to reach 90% degradation. Therefore vitamin C could be taken as indicator nutrient component as compared to lycopene to determine the effect of heat treatment on quality of watermelon juice.

The temperature dependence of the *D* value of the vitamin and lycopene are given by the *z* value. Estimated z value and activation energy of vitamin C in temperature range of 70 to 90°C were found to be 30.8°C and 18.37 kcal/mole respectively (Table 1). These values are in close agreement with vitamin C from orange and tomato sources (Van den Broeck et al., 1998), but they are lower than almost by half when compared with pure vitamin C tested in phosphate buffer (Oey et al., 2006). Meanwhile the z value and activation energy of lycopene for the same temperature range were estimated as 24.5°C and 23.35 kcal/mole respectively (Table 2). The activation energy in this work was found higher than lycopene from tomato olioresine (11.5 -15 kcal/mole) (Hackett et al., 2004). Furthermore the activation energy of all-trans lycopene degradation was reported as 14.5 kcal/mol for standard lycopene (Lee and Chen, 2002), 6.7 kcal/ mole in olive oil-tomato emulsion (Colle et al., 2010) and 19.8 kcal/mol in safflower oil (Henry et al., 1998). Generally, the degradation of lycopene is high with an increase in temperature and treatment time, particularly when the temperature is above 100°C. However under studied temperature range the rate of degradation was not high. There might be isomerization of the *trans* form in *cis*-forms with this temperature range which were not studied during the experiment.

High pressure stability of vitamin C and lycopene

High pressure processing is one of the non-thermal processing technologies which allows to process foods in low or reduced thermal effect for better quality retention. When the semi log residual concentration of vitamin C and lycopene versus time were plotted, both obey first order reaction model (Figures 4 and 5). The kinetic parameters, at different pressure levels are shown in Tables 3 and 4. In both cases the degradation rates are by far lower than thermal effect. There are several reasons indicated by different workers about the benefit of high pressure in retention of quality parameters as compared to heat treatment. However the main reason is, high pressure does not break down the covalent hydrogen, ionic or hydrophobic bonds. Covalent bonds are resistant to pressure, which means that low molecular

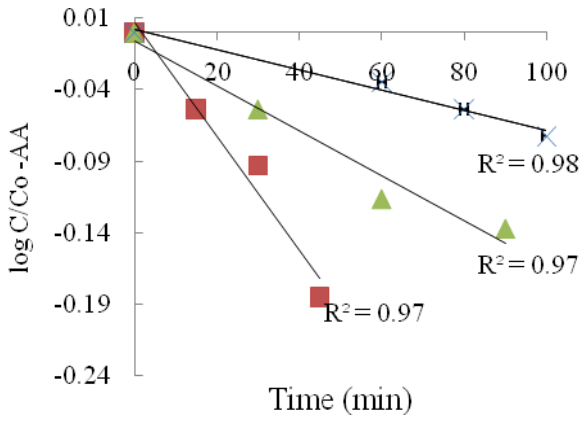

Figure 4. Effect of pressure and treatment time on stability of vitamin C of watermelon juice under different pressure: (×) 400 MPa, (▲) 500 MPa, (■) 600 MPa for different durations.

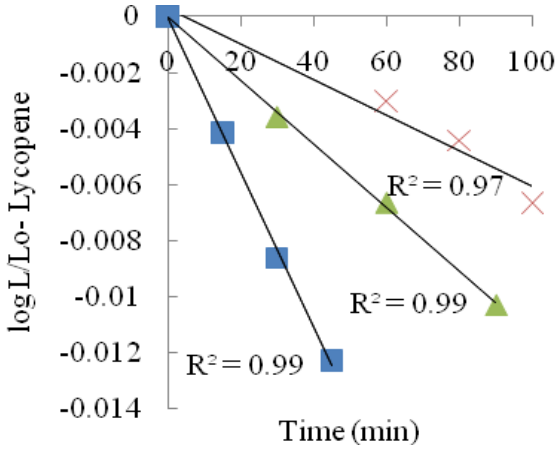

Figure 5. Stability of lycopene of watermelon juice under different pressure: (×) 400 MPa; (▲) 500 MPa; (■) 600 MPa for different durations.

Table 3. *D*, *k*, z_p, and V_a values for the high pressure destruction of vitamin C

P (Mpa)	*D* (h)	*k*(h^{-1})	R^2
400	23.71±2.34*	0.09811±0.0097	0.98
500	10.97±2.15	0.21832±0.043	0.97
600	4.27±0.56	0.54856±0.072	0.97
	z_p =257.3±37 MPa	V_a = -28.8±3.9 cm^3/mole	0.98

*Standard error.

Table 4. *D*, *k*, z_p, and V_a values for the high pressure destruction of lycopene.

P (Mpa)	*D* (h)	*k*(h^{-1})	R^2
400	257.95±19.85*	0.00898±0.0007	0.97
500	145.2±6.3	0.01589±0.038	0.99
600	60.6±1.1	0.038±0.0007	0.99
	z_p= 318.2±15.2	V_a= -24.24±1.01 cm^3/mole	0.98

*Standard error.

weight food components responsible for nutritional and sensory characteristics remain intact during pressure treatment, whereas high molecular weight components whose tertiary structure is important for functionality determination are sensitive to pressure (Tewari et al., 1999). Therefore, this behaviour plays a role for better retention of vitamin C and lycopene in high pressure processing as compared to thermal. This result is also in agreement with pressure stability of vitamin C of orange juice (Polydera et al., 2005).

The pressure dependency of *D* value is expressed by z_p value, which is defined as pressure increase required to reduce the *D* value by a factor of 10. Based up on this, z_p values of vitamin C and lycopene were estimated 257.95 and 318.2 MPa respectively. This result along with other kinetic parameters in table 3 and 4 confirm the variation in pressure stability of the two components of watermelon juice. Even though the stabilities of these components were better in pressure treatment as compared to heat, vitamin C was found relatively more sensitive to pressure effect as compared to lycopene. Hence likewise thermal treatment vitamin C can also be used as an indicator nutrient component to evaluate the effect of pressure treatments in processing of watermelon juice.

The kinetic degradation data could be used to calculate the activation volume *Va* of the pressure-induced destruction reaction using the *Eyring* equation (Equation 8). Activation volume was calculated for both components in studied pressure range (Tables 3 and 4) at constant temperature (32°C). According to the *Braun-Le Chatelier* principle under equilibrium conditions, processes associated with volume decrease are encouraged by pressure, whereas processes involving volume increase are inhibited by pressure (Butz and Tauscher, 1998). In this work a negative activation volume (*Va*<0) indicates that the nutrient degradation increases with an increase in pressure. The result confirms more negative shift in volume per mole of molecule for vitamin C (-28.8±3.9 cm^3/mole) than lycopene (-24.24±1.01 cm^3/mole). More shifts in decrease of volume for effect of pressure could result in change and modification of the inherent structure of the compounds and leads to more degradation.

Qiu et al. (2006) studied the effect of pressure and storage conditions of lycopene of standard solution and from tomato puree. He reported that lycopene from standard solution showed pressure stability up to 400 MPa (only 2% losses), but the loose jumped to 20.8 and

Table 5. Percent lycopene and vitamin C residue retained after different pressure and temperature treatments for time combinations.

Pressure (MPa)	Treatment time (min)	Lycopene %	Vitamin C %	Temperature (°C)	Treatment time (min)	Lycopene %	Vitamin C %
	0	100.0	100.0		0	100.0	100.0
	60	99.3	92.3		15	99.2	80.6
400	80	99.0	88.5	70	25	98.1	73.1
	100	98.5	84.6		35	97.0	61.5
	0	100	100		0	100	100
	30	99.2	88.4		10	98.5	78.9
500	60	98.5	76.9	80	20	97.6	69.2
	90	97.7	73.1		30	96.2	57.7
	0	100	100		0	100.0	100
	15	99.1	87.3		5	98.7	61.5
600	30	98.0	80.6	90	10	97.1	50.0
	45	97.2	65.3		15	95.8	38.5

56.3% at 500 and 600 MPa respectively. However the stability of lycopene from tomato puree remained persistent up to 600 MPa (only 5% loss) which is in close agreement with result of this work (Table 5). Therefore the presence of various macromolecules in the juices could play protective role to enhance pressure resistance of lycopene as compared to standard lycopene solution.

Conclusion

To summarize, although heat is the most common method for preservation, it is well known today that the consequences of conventional intensive heating are not necessarily good for the products in terms of consumers' acceptability. High pressure processing constitutes an alternative method to conventional thermal pasteurization for the preservation of watermelon juice. Vitamin C and lycopene degradation rates were found very low in high pressure processing. In addition to this, the stability of red lycopene rich component of watermelon juice for thermal treatment is very poor but remained stable in all studied temperature range. Therefore thermal treatment is not recommended to pasteurize watermelon juice. Therefore high pressure processing is recommended to produce stable nutrient rich and better quality juice. Various studies showed that, sensorial properties of pressure treated fruit juice samples are more or less comparable with control samples (Daoudi et al., 2002; Matser et al., 2004; Barba et al., 2012) due to minimal or limited effect of pressure on various chemical bonds. In terms of safety point of view, by choosing appropriate pressure treatment conditions (like higher pressure shorter time or vise versa), it is possible to kill pathogenic and spoilage vegetative cells to get shelf stable fruit juices stored at refrigerated temperature (Matser et al., 2004; Black et al., 2007). Overall, high pressure showed a potential in preserving and improving valuable attributes of watermelon juices as compared with conventional thermal treatment method which could be the same for other fruit juices.

Conflict of interests

The author(s) did not declare any conflict of interest.

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