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Physico-chemical characteristics of Ethiopian date palm fruit as compared to imported Date palm fruits from Dubai and Saudi.

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Declaration

- I. The undersigned, declare that this thesis is my original work, and has not been presented for degrees in any other university and all sources of material used for the thesis have been duly acknowledge.

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This thesis has been submitted for examination with my approval as a university advisor.

Name of the thesis supervisors

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Dedication

This work is fully dedicated to my family:

For everlasting support and true love they had for me

My today's success is their last work

Acknowledgments

First and foremost, I would like to thank almighty God for giving me the strength and ability to perform this research. Without his blessings, it would not have been possible.

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List of acronyms and abbreviations

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BC	Before Christ
CE	Catechin Equivalent
CHO	Carbohydrate
DPF	Date Palm Fruit
DPPH	1, 1-diphenyl-2-picrylhydrazyl
GAE	Gallic Acid Equivalent
GI	Glycemic Index
HPLC	High Performance Liquid Chromatography
IC50	50% Inhibition Concentration
Kcal	Kilocalorie
L	Lightness
UV	Ultraviolet
RSD	Relative Standard Deviation

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Abstract

The consumption and production of Date palm fruit is very low, despite its long history of domestication in Ethiopia. This is mainly due to lack of awareness on the nutritional and health benefits, scant studies on physicochemical characteristics, and shortage of DPF plantation. The present study aimed to evaluate the physical characteristics, nutritional composition, antioxidant properties and glycemic index/load of Ethiopian harvest in comparison with the imported DPFs from Dubai and Saudi Arabia. Most of the physical characteristics were measured by gravimetric methods, AOAC 2000 methods were used for majority of the analysis, HPLC method for sugar profile, the average fruit weight of the DPF harvested in Saudi Arabia, Dubai, Ethiopia was 6.36 g, 5.61 g, and 4.56 g, respectively. The seed weight of the respective three DPFs was 0.84, 0.60 and 0.92g with flesh weight of (5.46, 6.41, and 3.63) g. This resulted the highest and lowest seed to flesh ratio in the Ethiopian (0.29) and Dubai (0.07) harvests, respectively. The average fruit length was 2.86mm, 2.72mm, and 3.06 mm for the Saudi, Dubai and Ethiopian harvests. The average kernel weight of the DPFs was (350.85, 311.20, 227.75)g for the Dubai, Saudi and Ethiopian harvests, respectively. The Ethiopian harvest had the smallest date size of 5.99mm. The Dubai harvest had the biggest diameter of 14.66mm. The Ethiopian and Saudi harvest DPF had bigger length than the Dubai harvest. In contrast, the Dubai harvest DPF had larger diameter (14.66 mm) than the Ethiopian (5.90mm) ($p < 0.05$) and Saudi (13.76mm) harvests, the result of the texture implies that our date palm fruit is harder, brittle and it has high shearing and cutting effect but the date from Saudi is the stickiest and adhesive when we compare with the two date palm fruits. The Lightness redness and yellowness values of the Ethiopian, Dubai and Saudi harvest DPFs were (44.19, 4.44, 20.48), (45.15, 4.84, 8.00), and (55.50, 6.31, 7.50) ($p > 0.05$), respectively. Having the lowest L value the Ethiopian harvest was darker in appearance than the other dates. The Dubai and Saudi DPFs had the highest moisture content of 16%; whereas the Ethiopian harvest had the lowest value of 11%. Accordingly, the DPF harvests in this study were semi-soft dates. Interestingly, the Ethiopian harvest DPF had the highest crude fiber content of 6.1%. The total and utilizable carbohydrate contents were the highest in Ethiopian harvest DPF (85.5, 79.4) %, respectively. The gross energy results increased from 311 kcal for the Dubai and Saudi harvests to the highest energy content in Ethiopian harvest DPF with 330 kcal. The fructose content in the Ethiopian, Dubai, and Saudi harvest DPFs was 12.20, 2.58, and 24.90%, respectively. Meanwhile the glucose

content was 12.42, 27.05, and 26.06% for the respective DPFs. But the sucrose content was small as compared to the two reducing sugar (glucose and fructose). The dominance of reducing sugars indicated that the studied dates were soft date. The Dubai harvest DPF had the highest total sugar content (54.57%), while the Ethiopian harvest had the lowest sugar amount (29.57%). The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was in order of Dubai < Ethiopia < Saudi Arabia ($p < 0.05$). The Ethiopian harvest DPF had the highest total flavonoid and total phenol content (9.86%, 14.70%) respectively ($p < 0.05$). The mean glycemic index of the dates were 54.2, 52.1, and 55.0 (i.e. low GI food) for Ethiopian, Dubai, and Saudi Arabia harvest DPFs ($p > 0.05$), respectively. The present study demonstrated the nutritional and physical quality and potential of Ethiopian DPF, which has low production and consumption in the country.

Key words: date palm fruits, nutritional compositions, physical characteristics, antioxidant activity

1. Introduction

1.1. Background and justification

Date palm fruit (DPF) is the basic dietary food stuff in desert and semi desert parts of the world. The species is scientifically known as *Phoenix dactylifera*L. This belongs to the genus Phoenix and the family Arecaceae. It is widely cultivated in Middle East and Asia. Its plantation has started since 3000 BC along Nile River and production is increasing worldwide (Al-Shahi, 2003). Here The majority of date palm-growing areas are located in developing or underdeveloped countries (Pruessner *et al.*, 2001)Based on a comparison of 119 countries in 2013, Egypt ranked the highest in date consumption followed by Saudi Arabia and China(FAO, 2018). In Ethiopia, date palm fruit was introduced, 200 years back by the Yemen and Sudan traders. Currently, a substantial amount of the plantation is found in Afar region with an altitude between 300 to 350m above sea level and 220mm average rain fall. The local farmers used plant suckers as seedling which provided less fruit yield and longer time to produce fruit than the seed plantation (Johnson *et al.*,2013)Also, the DPF seed to produce the fruit takes about 2-3 years earlier compared to the suckers. The seeds should be planted three feet to the ground which enhances water storage at the ground. Water is provided to the plant once per 7 days in the arid season (Hussen, 2010).

Date palm fruit is a high-energy fruit which contains natural sugars including glucose, fructose and sucrose (Abbes *et al.*, 2012). It is also a source of vitamins A, B1, B2 and Nicotinic acid. The flesh of DPF on average has moisture, ash, fiber, protein, carbohydrates, and lipid contents of 13.80%, 2.13%, 5.20%, 3.00%, 73.00%, and 0.50%, respectively. The concentration of minerals like magnesium, phosphorus, selenium, potassium, calcium and iron is significant in DPF. Also DPF contains high fiber, which helps to regulate digestion and bowel movement. Overall, DPF is an important source of vital nutrients and has biological beneficial functions including antibacterial, antifungal, and anti-inflammatory (Al-Farsi, 2007).

Approximately, there are 3,000 different cultivars of date palm, in which about 60 are extensively cultivated (Moussouni *et al.*, 017). Apparently, according to the variety and growth conditions, date fruits vary in shape, size, and weight. Quality profile evaluation of DPF relies on the color, size, shape, texture, taste, and the seed to flesh ratio. Also the nutritional content is important to grade DPF's quality. As to our knowledge, in Ethiopia there is no study conducted

on evaluating the physicochemical quality of Ethiopian harvest DPF. In fact, in Ethiopia the production and consumption of DPF is also limited among small number of the society. Most of the DPF in the country is imported. This is due to low production and lack of awareness on its health benefits. Thus, evaluating the physical characteristics and nutritional composition of Ethiopian DPF is important to sustain demand and improve production. Again as to our knowledge no reports are available concerning health benefits of Ethiopian harvest DPF. Recently, worldwide studies on the phytochemicals and potential health benefits of DPF are increasing. In an attempt to further contribute towards this effort, the current study aimed to evaluate the physical characteristics, nutritional composition, antioxidant properties and glycemic index of Ethiopian harvest DPF in comparison to the imported ones.

1.2. Statement of the problem

Despite the fact that DPF has been domesticated 200 years back in Ethiopia, still the consumption and production is very low. The main production areas in the country are Afar and Benishangul Gumuz regions (Biglari, 2008). The demand of DPF in the country is fulfilled by import from the popularly producing countries during Ramadan month of Muslim fasting season. The consumption of DPF among our society is limited due to many reasons including lack of awareness on the nutritional and health benefits, scant studies on physicochemical characteristics, and shortage of DPF plantation (Hussen, 2010). Thus, the necessity of research on evaluating the physicochemical composition and health benefits of Ethiopian harvest DPF is unquestionable. However, except few surveys on production and post-harvest management of DPF (Lemlem *et al.*, 2018), as to our knowledge in Ethiopia there is no study conducted on evaluating the physicochemical quality and health benefits of DPF. There was an old compositional study conducted by Ethiopian Nutrition Institute (ENI) back in 1998, as part of the national Food Composition Table (FCT). In addition to being a bit old this report was limited with few nutrients and no information on the physical qualities and health benefits of DPF (Ethiopian Food Composition Table, 1998). Recently, many activities have been undertaken to provide knowledge on the phytochemicals and potential health benefits of DPF. In an attempt to further contribute towards this effort, the present study aimed to evaluate the physical characteristics, nutritional composition, antioxidant properties and glycemic index/load of Ethiopian harvest DPF compared to the imported ones.

1.3. Objectives

1.3.1. General objective

- To evaluate the physico-chemical characteristics, antioxidant properties and glycemic index/load of Ethiopian harvest DPF in comparison with the imported ones (2021).

1.3.2. Specific objectives

- To evaluate the physical characteristics of Ethiopian harvest and imported DPF.
- To investigate the nutritional composition of Ethiopian harvest and imported DPF.
- To evaluate the antioxidant property of Ethiopian harvest and imported DPF.
- To investigate the glycemic load and index of Ethiopian harvest and imported DPF.
- To compare the Physico-chemical characteristics, antioxidant properties, and glycemic index and load of Ethiopian harvest DPF with the imported one.

1.4. Significance of the study

- The study Increase awareness on the health benefits of Ethiopian date palm fruits, Contribute scientific evidence to establish a basis for consumers and producers in selecting high-quality DPF and also it Contributes for DPF standard development and revisit of Ethiopian Food Composition Table lastly it paves the way to identify major focus areas for further investigation on DPF.

1.5. Scope of the study

- This study will determine the physicochemical characteristics of the Ethiopian date palm fruit in comparison with imported date palm fruits from Dubai and Saudi Arabia ,scares in time and financial resources; the study is limited in its sample size and the date varieties in the world. Furthermore since Ethiopia has areas which are suitable environmental conditions for date plantation and productions the government should work on this area, the result of the study may have limitations to make generalizations as the study is among three date palm fruits which were harvested from other two countries is not enough However, it may be useful as a base line for other studies will be conducted in our country.

2. Literature review

2.1. Farming of palm date in Ethiopia

Palm date is cultivated dominantly by nomadic people in Afar, Somali, Gambella, Dire Dawa, and BenishangulGumuz regions (Demeli, 2013).The production of palm date mainly is undertaken along awash river in Afar region as it is named as wild plant in Afambo,Aysaita, Gewane, and Amibara(Salah, 2015) in Danakil Depression which are date palm production areas (Hussen, 2013). Furthermore, flow of Awash River in the region provides great opportunity for establishment and expansion of small-scale and large plantation of date palm in the region (Salah, 2015)

Date production in Ethiopia is low due to many reasons such as due to less attention is given to the plant by the government and also by the region, the development of date palm production in the country is extremely low where the exact annual production is not registered in any statistical database. No researches and extension activities have been conducted by governmental as well as non-governmental organizations. In Afar region DPF are cultivated by agro-pastoralists using primitive farming practices which are acquired from their parents and grandparents through time (Zaid *et al.*, 2002).

Generally, improper cultural and poor handling of the fruit after harvesting and use of local varieties and thus low yields characterize date palm production in the region (Salah, 2015). As the region has suitable environmental condition, long experience of agro pastoralists, substantial amount of benefits of date fruits in different aspects, date palm production in the region helps to improve income of the agro pastoralists this helps them to reduce poverty in this areas and also reduce malnutrition this leads to improvement of food security for poor agro pastoralists., date palm production has a great role to keep our environment as it has benefits in the control of desertification and as means of land reclamation in the country at large and in Afar Region in particular(Butler *et al.*, 2007).

2.2. Production and post harvest handling of date palm fruits in the world

The production of date palms around the world is on the rise. For example, it boosted production from 1.8 million metric tons in 1961 to 2.8 million metric tons in 1985, and then to 5.04 million metric tons in 2001. On around 1.149 million hectares of land, the world's date palm production

reached about 8.06 million metric tons (FAO, 2013). Date palm cultivation is the most important agricultural activity in the world's hot dry regions, and it enhances countries' foreign exchange earnings in such a tough environment (A. Botes *et al.*, 2002). According to a survey by Pariona (A. Zaid *et al.*, 2002), Egypt tops the globe in date palm output, producing over 1.1 million metric tons per year and earning \$41.8 million in fresh date fruit exports. Saudi Arabia and Iraq are in a similar situation.

Dates are farmed in more than 30 nations throughout the world, and the date fruit has become increasingly important in global trade in recent years. The global output of dates has more than doubled in the last two decades. The bulk of date palm-growing areas are in poor or undeveloped countries, where date fruit is a primary food crop, and consequently plays a significant role in these people' nutritional status. Harvesting, cleaning, grading, packaging, processing, and transportation/shipment to local or export markets are just a few of the tasks involved in date marketing. Issues relating to postharvest handling methods, usage of proper processing and packaging technologies, food safety elements, and quality assurance are some of the key obstacles confronting date fruit cultivation and commerce.

2.3. Nutritional composition of Date palm fruit

Table 1. Nutritional composition of date palm fruit

Nutrient	Content (per100g)
Macronutrients	
Energy	282 Kcal
Carbohydrates	75.03g
Sugars	63.35g
Dietary fiber	8.00g
Fat	0.39g
Protein	2.45g
Vitamins	
Beta-carotene	6.00µg
Vitamin A	10.00IU
Thiamine (vitaminB1)	0.052mg
Riboflavin (vitamin B2)	0.066mg

Niacin (vitaminB3)	1.274mg
Pantothenic acid (vitamin B6)	0.589mg
Vitamin B6	0.165mg
Folate (vitamin B9)	19.00µg
Vitamin C	0.40mg
Vitamin E	0.05mg
Vitamin K	2.7µg
Minerals	
Calcium	39.00mg
Iron	1.02mg
Magnesium	43.00mg
Phosphorus	62.00mg
Potassium	656mg
Sodium	2.00mg
Zinc	0.29mg
Water	20.53g

Source: USDA Nutrient Database, 2019

2.3.1 Carbohydrate

The total sugar of dates which are ripe is about 80% the remaining 20% includes protein, fat, fiber, and minerals such as copper, sulfur, iron, magnesium, and folic acid (El-Nakhal *et al.*, 1989). DPF is high sweet as its flesh have high amount of sugar about 70-85% with large amount of reducing sugars (glucose and fructose) and very small quantity of non-reducing sugars (sucrose) (Al-Farsi and Lee, 2008; Rastegar *et al.*, 2012), and minute quantities of polysaccharides such as cellulose and starch (Najeh *et al.*, 2003) Sugar content of date is the most significant commercial characteristics that are significant for both fresh consumption and fruit consumption (Fadel, 2008), the type and quantity of sugars vary according to the cultivar and their specific fruit developmental stages (Amoros *et al.*, 2011). A single date contains about 5 grams of carbohydrates, which is sourced from the total sugar of the date. Furthermore, a date consists of high amount of fructose when we compare with the two monosaccharide's this result in the sweetness of date palm fruits. During ripening of date palm fruits, the amount of the sugar increases as the fiber content decreases.

2.3.2. Fiber

Date palm fruit contains substantial amount of fiber mainly in the leaves (Agoudjil, 2011).in ancient time Date palm fiber is considered as an output waste in agricultural sectors in Saudi Arabia, and some Asian countries (Mahdavi *et al.*, 2010).Date palm fruits are the most important tropical fruits exist in the market next to pineapple and citrus fruits., The main components of the fiber in DPF include holocellulose (60-75%), lignin (20%) (AL-Oqla *et al.*, 2014)

2.3.3. Protein

Dates contain low amount of protein (1-7%).But it contains amino acids (Bacha *et al.*, 2010) including aspartic acid, proline, glycine, histidine, valine, leucine, and arginine. It also contains small amounts of threonine, serine, methionine, isoleucine, tyrosine, phenylalanine and alanine. Upon maturation, the total amount of protein decreases due to pigments and phospholipid by free radical scavenging system (Prochazokova *et al.*, 2012).

2.3.4. Fat

Dates are not a significant source of fat, with a maximum content 1%(Hun *et al.*, 2006).Date palm fruits contain different fatty acids like oleic acid, palmitic acids, linoleic acids (Ogungbenle, 2011). Both the seed and flesh contain fatty acids. The seed contains 14 different fatty acids, but only eight of which are found in the flesh in very little amount. Some of these fatty acids are palmitoleic, oleic, linoleic, and linolenic acids. Specifically, the oleic acid concentration in the seeds ranges from 41.1 to 58.8%, suggesting that date seeds could be exploited as an oleic acid source (Walid Al-Shahib, 2003).

2.3.5. Antioxidants

Palm date fruits contain antioxidant compounds which have great health benefits. These antioxidants neutralize the free radicals and can prevent oxidation of biological molecules (Mansouri *et al.*, 2005). Dates possess important radical scavenging activity due to the presence of non-enzymatic antioxidant compounds (phenolic, flavonoid, and ascorbic acid) and enzymatic antioxidants (catalase, peroxidase, and superoxide dismutase), (Biglari *et al.*, 2008; Awad *et al.*, 2011a) known to have health benefits (Vayalil, 2011).

However, limited studies have been done on antioxidant activity, total phenolic contents, and sugar profiling of date fruits. Total phenolic content of date fruit varies with ripening, cultivation regions, environmental factors, storage time and conditions (Biglari *et al.*, 2009). Phenolic

compounds including cinnamic acids, flavonoid glycosides, flavonols are significantly available in DPF (Vyawahare *et al.*, 2009). Siddeeg *et al.* (2019) reported an average total phenolic and flavonoid content of 62.50mgGAE/100g and 3.20mg/100g in DPFs, respectively.

2.3.6. Minerals

Date palm fruits are rich in mineral salts and vitamins (Bij, 2010) Dates contain significant amount of minerals including phosphorus, potassium, calcium and magnesium Calcium is required as structural part of teeth and bones (Jackson *et al.*, 2006), and also to reduce blood pressure and risk of colon cancer (Lappe *et al.*, 2007). On dry weight basis, different varieties of DPF have average calcium content of 81.40mg/100 g (Dora variety), 195.18mg/100 g (Dhakki variety), (178.95mg/100 g) (Karblain variety) and (181.73mg/100 g) (Hillavi variety), (Table 1). These amounts can contribute almost 25% of daily requirement of calcium in adults. DPFs are also good sources of the important micronutrient, iron. The iron concentration in different DPF varieties is on average 0.46mg/100g (Desi black variety) and 2.52mg/100g (Dora variety). Recommended daily allowance of iron is 10 mg/day for a man while a woman needs 15 mg/day.

2.4. Health benefits of Date Palm fruits

The date palm fruits have very essential functional and nutritional values (El-Sohaimy and Hafez, 2010). Palm dates have important role for effective digestion by preventing constipation. It promotes regular bowel movements by contributing to the fecal density. Furthermore, and the fiber in dates may be beneficial for blood sugar control, by reducing digestions rate. Thus, dates are categorized as low glycemic index (GI) food (Brianna, 2018).

Dates contain several types of antioxidants that help to prevent the development of certain chronic illnesses, such as heart disease, cancer, Alzheimer's and diabetes. The three most potent antioxidants in DPFs are flavonoids, carotenoids, phenolic acids. Flavonoids are powerful antioxidants that prevent inflammation and have a great role to reduce risk of diabetes, Alzheimer's disease and certain types of cancer. Carotenoids have significant role to keep healthy heart function and may also reduce the risk of eye-related disorders, such as macular degeneration. Phenolic acids are known for anti-inflammatory properties, hence lowering the risk of cancer and heart disease (Vayalil, 2002).

Dates promote and ease natural labor for pregnant women when consumed during the last few weeks of pregnancy. Eating these fruits throughout the last few weeks of pregnancy may promote cervical dilation and lower the need for induced labor. They may also be helpful for reducing labor time. This role of dates in pregnancy is likely due to compounds that bind to oxytocin receptors and appear to mimic the effects of oxytocin in the body. Oxytocin is a hormone that causes labor contractions during childbirth. Additionally; dates contain tannins, which are compounds that have been shown to help facilitate physiological effects (Ibrahim, 2020).

The polar extracts of DPFs contain bioactive compounds such as caffeic acid, gallic acid, coumaric acid, chlorogenic acid, catchin and quercetin (Abd El Azim, 2014). Thus, one can claim that DPFs have high nutritional and therapeutic values with significant antioxidant, antifungal and anti-proliferative properties (Reem *et al.*, 2017). Also due to the presence of phenolic compounds, date palm fruits are antioxidant-rich with potent bioactivities against several bacterial pathogens (Selim *et al.*, 2012). Consumption of date palm fruits may promote health condition of the colon by increasing the growth of beneficial bacterial and inhibiting the proliferation of colon cancer cells (Eid *et al.*, 2014). In general, in recent years, a huge interest in health-promoting properties of date fruit had led to many pharmacological studies (*in vivo* and *in vitro*) as well as the identification and quantification of different classes of phytochemicals (Cho and Krueger, 2007).

2.5. Physical characteristics of date palm fruit

Physical features are used to determine the quality of dates (NurAshikin *et al.*, 2020). Physical characteristics of date palm fruits include dimensions (length and diameter), color, texture, and thickness (Elmardi *et al.*, 2006). There are a lot of studies which include the determination of physical properties of different date palm fruits with varietal differences. Few among them includes GashGaafar, Gash Habash, Shahal, Bushibal and Lulu (Al-Hooti *et al.*, 1997), mangocv. Dashehari (Jha *et al.*, 2006), three sweet cherry varieties (*Prunus avium* L) (Vursavus *et al.*, 2006), guna fruits (Aviara *et al.*, 2007), Dairi (Jahromi *et al.*, 2008), Irvingia gabonensis and wombolu (Ehiem and Simonyan, 2012).

2.6. Sugar profile of date palm fruit

On dry basis DPFs had an average sugar content of 78.32%, with glucose (51.80%), fructose (35.78%) and other sugars (47.50%). In fact, there is an apparent variation in sugar content with varieties of DPF (i.e. glucose content of Zaidy, Dora Basraywal, Desi red tiny varieties was 44.44%, 35.29%, and 26.75%, respectively). Similarly, variation in fructose content was observed in Dora and Dhakki varieties with contents of 32.78 % and 32.78%, respectively. While the Dora desi was found to have the lowest fructose content of 19.9% and also contains a significant amount of sucrose 29.63% (Mohammad *et al.*, 2019). Thus, DPF is an excellent material for producing refined sugar, concentrated juice, confectionery pastes, and fermentation products (Samarawira, 2011).

The monosaccharide's glucose and fructose reach up to 80% upon maturity of date fruit (tamer stage) (Al-Shahib *et al.*, 2003). This is due to the activity of invertase enzymes, which speeds up the conversion of sucrose to glucose and fructose. The glucose content of some date varieties ranged from 16.41 to 54.23 g/100 g, and the fructose content ranged from 12.62 to 16.41 g/100 g. In fresh fruits, it ranges from 43.31 g/100 g to 43.31 g/100 g (Sahari *et al.*, 2007).

2.7. Glycemic index

The glycemic index of a food is based on the digestion and absorption of carbohydrates. A single food's GI is determined by quantifying the increase in blood glucose level after ingesting a food containing 50 grams of carbohydrates (Astonmetal *et al.*, 2008). Using glucose as a reference, a GI of 55 is considered low, and 56-69 is considered medium. Many commonly used varieties of DPFs have low to medium GI (Alkaabi *et al.*, 2011). The glycemic index of date varieties from various African regions ranges between 47.6 and 57.7. (Ali *et al.*, 2009).

3. Materials and methods

3.1. Site of laboratory analyses

Majority of the laboratory analyses were conducted at Center for Food Science and Nutrition of Addis Ababa University. Main analyses done at the Center included proximate composition, antioxidant property, physical characterization, and glycemic index. Iron and calcium quantifications were handled at Department of Chemistry, Addis Ababa University. Color and thickness measurements were done at Melkasa Agricultural Research Center. Crude fiber and (texture and sugar profile) were conducted at Debrezeit Agricultural Research Center and Holeta Agricultural Research Center, respectively.

3.2. Sample collection

The local harvest DPF (10 kg) was collected from Afar plantation. Meanwhile, the imported DPF (10kg, Dubai and Saudi origin) were purchased from Merkato market, Addis Ababa, Ethiopia. Purposive sampling technique was applied. The samples were kept in polyethylene bag until analysis at room temperature

3.3. Sample preparation

The flesh of the DPF was hand peeled with knives (to remove the seed), the sliced flesh of the date was juiced (CAINAXIAMEN, Y-962-1, CHINA) with water(50g in one litter water), and kept at -20⁰C refrigerator for 48 hrs. This was followed by freeze drying (MINI LYODEL, DIGITAL HI-PR-PIRAN, CHINA). The dried sample was milled (SYNCHK, G119743, INDIA) and stored at 4°C in a polyethylene bag until analysis. The DPF flour processing flowchart is summarized in Figure 2 and Figure 3 depicts the flesh and seeds of the three DPF harvests after peeling off.

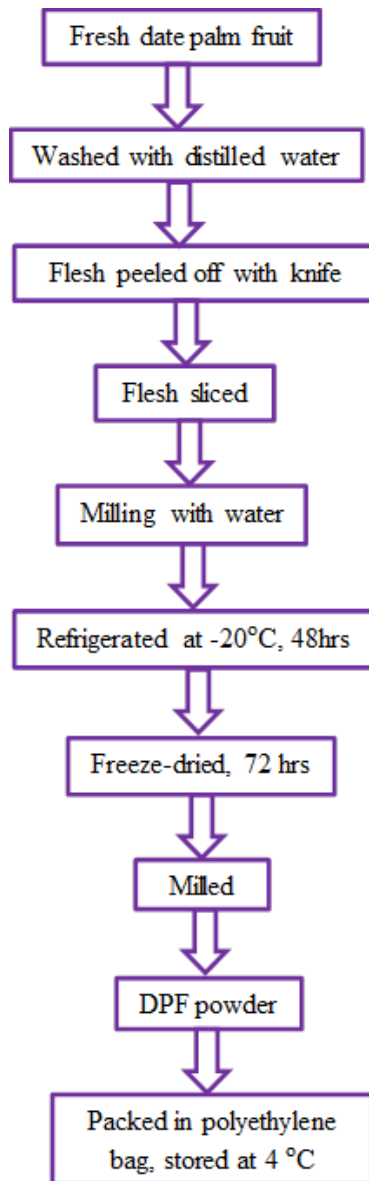


Figure 1. Flow diagram of DPF flour preparation

Source: laboratory



Figure 2. Date palm Fruit flesh from (a) Saudi Arabia (b) Dubai and (c) Ethiopia

3.4. Physical characteristics of DPF

The physical characteristics of the three DPF harvests determined included texture, color, fruit weight, fruit length and diameter, kernel weight, seed to flesh ratio and thickness (Cunningham and Sobolewski, 2011).

3.4.1. Color analysis

Color was measured using a Hunter Lab Ultra-scan Pro-colorimeter (ARS00073, hunter lab, USA) attached with easily match quality control software. The result was expressed following CIELAB system using the rates of L, a and b parameters. “L” values measured the lightness with a range from 0 for black until 100 for white. The ”a” values range from -60 for greenness to +60 for redness, whereas the “b” values range from -60 for blueness to +60 for yellowness(instruments manual).

3.4.2. Texture determination

The texture of the whole DPFs was measured using a texture analyzer called taxi plus 100(texture profile analyzer 1000, surrey GU71YL, UK). The equipment evaluated the force required for probe penetration into the product. The texture analyzer was set to 0.05 N trigger e and 30 mm target values respectively (Hematian, 2010).The Extended Craft Knife Blade is fitted to the load cell. As the Texture analyzer was used in target distance mode, a probe height calibration was conducted as shown in the calibration curve. The DPF was placed on the base of the instrument and positioned centrally below the probe. Ten DPFs and 5 fruit bar pieces were tested. The test began with the probe moving towards the sample surface at the test speed of 5 mm/sec. As a button trigger is being used, data was recorded as soon as the probe started to move from the start of the test. When it comes into contact with the specimen surface, the force

exerted on the probe by the sample started to increase. The probe moved to the target distance of 19 mm (1 mm above the base of the PTFE plate) and then returned to the start position at the post-test speed of 5 mm/sec.

3.4.3. Fruit, flesh, seed and kernel weight measurement

Analytical balance (PGL2002, Adam, China) was used to determine the seed to flesh ratio, as well as the weight of the fruit, kernel, and seed. The weight measurements were done with 10 randomly selected DPFs (Desai *et al.*, 2019). Then, the flesh and seed of the DPF samples were separated and the ratio was calculated. Similarly, using 50 DPFs from each harvest the kernel weight was measured. The fruit weight and seed weight were measured by weighting 10 DPFs from each harvest.

3.4.4. Thickness determination

The length and breadth of the DPF were measured with a Vernier Caliper with a precision of 0.01 mm by randomly selecting 10 DPF from each harvest (Desai *et al.*, 2019). The principal dimensions of the date fruit was determined by measuring three linear dimensions specified as length, width and thickness.

3.5. Determination of proximate composition

3.5.1. Moisture content

Moisture was determined according to AOAC (2000) method 925.09. A clean dried and covered flat aluminum dishes (W_1) were weighed and about 5gm of the sample were transferred to the dish (W_2). The dish then placed in the oven (DHG-9123A Michel, England) at 105°C for 3hrs and then cooled in desiccators and re-weighed (W_3). Then, the moisture content was estimated by the formula 1:-

$$\text{Moisture}(\%) = \frac{w_2 - w_3}{W_2 - w_1} * 100(1)$$

Where:

W_1 = weight of the empty container and cover (g)

W_2 = weight of the container, its cover and the sample before drying (g)

W_3 = weight of the container, its cover and sample after drying (g)

3.5.2. Crude protein

Protein content was determined according to AOAC(2000) method 979.09.A digestion flask containing about 0.5 g of sample, to which 6 ml of acid mixture (conc. sulphuric acid and about 3g of catalyst mixture (K₂SO₄ and Cupper) were added and exposed to about 370^oC in order to allow digestion. Then, distillation took place in Kjeltex(Gerhard ,7050/1 120146 , Germany).By adding 25 ml of 40% NaOH and using 25 ml of boric acid with 10 drops of indicator solution. Finally, the distillate was titrated with standardized 0.1N hydrochloric acid to a reddish color. The crude protein content was estimated using the formula 2, 3

$$\text{Nitrogene}(\%) = \frac{(V_{HCl} - V_{black}) * M_{HCl} * 14 * 100}{W_O} \quad (2)$$

Where:

V = volume of HCl consumed to the endpoint of the titration (ml)

M= morality of HCl

W_O= sample weight on dry matter basis (g)

14 = molecular weight of nitrogen

$$\text{Crudeprotein}(\%) = 6.25 * \% \quad (3)$$

The crude protein content was determined by multiplying percentage nitrogen content by a constant factor of 6.25.

3.5.3. Crude fat

A clean and dried thimble containing about 2g of dried sample (W₁) and covered with fat free cotton at the bottom and top was placed in the extraction chamber in petroleum ether. Then the petroleum was evaporated from the extraction flask .Then, extraction took place for at least 4 hrsaccording to official method (AOAC, 2000) method 920.39.The amount of fat was quantified gravimetrically and calculated from the difference in weight of extraction flak before and after extraction as percentage. The crude fat content was determined by the formula 4:-

$$\text{Crude fat}(\%) = \frac{(W_2 - W_1)}{W} * 10 \quad (4)$$

Where:

W1 = weight of the extraction flask (g)

W2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of the sample (g)

3.5.4. Total ash.

The porcelain dish used for the analysis was washed by dilute hydrochloric acid on boiling. And it was washed with distilled and de-mineralized water respectively by AOAC (2000) method 923.03. Then dried at 105°C in an oven (DHG-9123A Michel, England) and ignited at 550°C in Muffle furnace, (Carbolite CSF 1200 Germany) for 30 minutes. The dish was then removed from furnace and cooled in desiccators. The mass of the dish was measured using analytical balance (M1). About 2.5 gm of sample powder was weighed into the porcelain dish (M2). The sample was charred at 20°C on hot plate until the whole content becomes carbonized. Then the sample was placed in a furnace at 550°C until whitish color appears for 5 hours. Then the sample was removed from the furnace and placed in desiccators. Finally the mass was weighed as (M3).

$$\text{Total ash} (\%) = \frac{M_3 - M_1}{M_2 - M_1} * 100 \quad (5)$$

Where:

M1 = weight of crucible (g)

M2 = weight of fresh sample and crucible (g)

M3 = weight of crucible and ash (g)

3.5.5. Crude fiber

Crude fiber analysis was conducted using the method of AOAC (2000) method 962.09. About 1.6g weighed sample (W₁) was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml 28% KOH was added and boiled gently again for another 30 minutes. Subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. After, filtering it was then dried in an electric oven for 2 hrs. Furthermore, it was cooled at room temperature for

30 minutes in desiccators and weighed (W_2), then transferred the crucibles to muffle furnace for 30 minute ashing at 550°C . Finally, it was cooled again in desiccators and reweighed (W_3). The crude fiber content was determined by using the formula 6

$$\text{Crude fiber}(\%) = \left(\frac{W_2 - W_3}{W_1} \right) * 100 \quad (6)$$

Where:

W_1 = weight of a sample (g)

W_2 = weight of crucible and sample after drying (g)

W_3 = weight of crucible and sample after ashing (g)

3.5.6. Total and utilizable carbohydrate

Total carbohydrate content was determined by subtracting the sum of the percentages of moisture, ash, crude protein, and crude fat from 100% (formula 7).

$$TC(\%) = 100 - (\%moisture + \%ash + \%crudeprotein + \%crudefat)(7)$$

Where:

TC = total carbohydrate

Similarly, the utilizable carbohydrate content was determined by subtracting the sum of the percentages of moisture, ash, crude protein, crude fat, and crude fiber from 100% (equation 8).

$$UC(\%) = 100 - (\%Moisture + \%Ash + \%CrudeProtein + \%CrudeFat + \%CrudeFiber)(8)$$

Where:

UC = utilizable carbohydrate

3.5.7. Total energy

The gross energy of each sample was determined based on the values of crude protein, crude fat, and total carbohydrate, which were multiplied with factors of 4, 9, and 4 respectively as shown in equation 9.

$$Energy \left(\frac{kcal}{100g} \right) = (4 * gprotein) + (4 * gcarbohydrate) + (9 * gFat)(9)$$

3.6. Iron and calcium determination

Iron and calcium content was determined according to the method of AOAC (2016). About 2.5gm of the sample was charred on hot plate (Bicotestuart UCLS2, UK) at 200°C to remove organic matter. Then, ashed into muffle furnace (Carbolite, 5302RR, England) at 550°C for 5hrs. Three drops of 1M HNO₃ acid and few drops of deionized water added to the sample in each of the crucibles. The ash was digested by using 3M and 6M hydrochloric acid, respectively. The digested sample was filtered using the Whatmann filter paper (42mm) followed by adjusting the total volume to 50ml with deionized water. For calcium determination 2.5ml of 10% LaCl₃ was added. The iron concentration was determined using Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, Lambda 950 US). Using AAS a calibration curve was prepared by plotting the absorption values against the metal concentration in mg/100g for all the minerals.

Thus, reading was taken from the graph which depicted the metal concentrations that correspond to the absorption values of the samples and the blank. The metal contents were calculated by using equation 10.

$$MineralContent \left(\frac{mg}{100g} \right) = \frac{(a-b)*V*df}{10*Swt} (10)$$

Where:

a = concentration of sample solution (ppm)

b = concentration of blank solution (ppm)

V = total volume

Df = dilution factor

S = sample weight (g)

3.7. Determination of antioxidant activities

3.7.1. Sample extraction

Samples were extracted based on the procedures outlined by Woldegiorgis *et al.* (2014). Homogenized dried DPF sample, 5g was extracted by 50ml of methanol at 25°C at 150rpm (CART STRIKE 300, US) for 24hrs using an incubator shaker (ZRICHEG, ZHWY-103B, SHANGHAI) and then filtered through whatman No.1 filter paper. The residue was then extracted with

additional 50 ml of methanol as described above. The combined methanolic extracts were evaporated at 60°C dryness using rotary evaporator (CART STRIKE 300, US) and re-dissolved in methanol at concentration of 50mg/ml and stored at 4°C until analysis.

3.7.2. Determination of free radical scavenging activities by DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) scavenging activity of the methanolic extract of the DPF was measured according to the method of AOAC (2000) as cited in Woldegiorgis *et al.*, (2014). With this assay the concentration of the extract necessary to decrease the initial concentration of DPPH by 50% was calculated. The hydrogen donation ability of the corresponding extracts and ascorbic acid was measured from their bleaching capacity of the purple colored methanol solution of DPPH. Methanolic DPPH solution of 4ml, (0.004%) was mixed with 1ml of various concentrations (2-12mg/ml) of extracts in methanol with vortex mixer. Then, the whole content was incubated (ZRICHEG, ZHWY-103B, SHANGHAI) for 30min in dark at room temperature. Ascorbic acid standard was prepared by dissolving 0.3mg into 1ml of methanol. Scavenging capacity was read by using UV-Vis spectrophotometer (PERKIN ELMER, LAMBDA 950,US), monitoring the decrease in absorbance at 517nm. Inhibition of DPPH free radical (I %) was calculated using equation 11

$$I(\%) = \frac{(Ac-As)*100}{Ac} \quad (11)$$

Where:

Ac = absorbance of control reaction without test extract

As = absorbance of the test extract

3.7.3. Total polyphenol content

The total phenol content was determined by the method of Siddhuraju (2003). Aliquots (100 µl) of each extract were taken in test tubes and made to volume of 1 ml with distilled water. Then, 0.5ml of Folin-Ciocalteu phenol reagent (1:1 with water (v/v)) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Rapidly after vortexing (STUART EW-04729-03,UK) the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 765nm against reagent blank using UV-Vis spectrophotometer (PERKIN ELMER, LAMBDA 950 US). Gallic acid was used to construct the standard curve, and the result was expressed in milligram of gallic acid (GAE) equivalent.

The gallic acid standard was prepared by dissolving 0.05g of Gallic acid in 1ml methanol and then diluted to 10ml with deionized water (5g/L) final stock. Then, 0.1, 0.2, 0.5 and 1.0ml were dissolved in water to final concentrations of 50, 100, 250, and 500 (ppm) respectively (Biglari *et al.*, 2008). All determinations were done in triplicate. The total phenolic content was determined using equation 12.

$$TPC(mg\ GAC/100g) = \frac{GAC*V}{W}(12)$$

Where:

TPC = total phenolic content

GAC = gallic acid equivalent

V = total volume of extract (ml)

W = sample weight (g)

3.7.4. Total flavonoid

Flavonoid content was determined according to the method by El Guichee *et al* (2015). Briefly, an aliquot (150 µl) of each extract or standard solution was mixed with 1.25 ml of deionized water and 75 µl of 5% NaNO₂ solution. After 6 min, 150µl of 10% AlCl₃.H₂O solution was added. After 5 min, 0.5 ml of 1 M NaOH was added and then the total volume was made up to 2.5 ml with double distilled water. Quercetin standard (10-1000µL) was prepared in methanol from 1mg/ml stock solution. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm using UV-VIS spectrometer (Perkin Elmer lampda 950, US). The total flavonoid content was calculated using equation 13

$$Flavonoid\ content(mg\ QE/100g) = \frac{QE*V}{W}(13)$$

Where:

QE = quercetin equivalent (mg/ml)

V = total volume (ml)

W = sample weight (mg)

3.8. Sugar profile analysis

Sugar profile was determined using HPLC method. Briefly, 5g of homogenized sample in 100ml beaker was dissolved with 40ml of solvent mixture(methanol: water, 25:75%).Then, the

solution was transferred into 100ml tube and volume was adjusted to the mark with the solvent mixture. The whole content was sonicated (Ramsit GST-36ADL, India) until the solute was dissolved completely. After adjusting the volume to the mark with the solvent mixture, the sample solution was collected through syringe filter (0.45µm). The sugar content was determined using HPLC (Infinite series, Agilent 1260, Germany) with RI detector, sugar peaks were identified on the basis of their retention time.

HPLC conditions:-

Mobile phase: Acetonitrile: water (70:30,v/v)

Column type: carbohydrate 4.6×150mm, 5µ particle size

Flow rate: 0.5 mL/min

Column and detector temperature: 30⁰c

Injection volume: 10µL

Detector: Refractive index detector

Run time: 15min

HPLC method validation

HPLC method was used to detect and quantify monosaccharide (glucose and fructose) and disaccharide (sucrose). The HPLC method was validated for linearity, limit of detection, limit of quantification, accuracy, precisions, and recovery.

Identification

Identification of the mono- and disaccharide was done based on their retention time at different time of injecting at the same condition and its precision determined by the percent relative standard deviation (%RSD).

Precision

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The precision of the method was evaluated through the repeatability of the method by assaying 8 replicate injections of sugar standard at the same concentrations (5, 10, 20, 40, 50, 80, and 100 ppm) within the same day under the same experimental conditions to obtain an acceptable % Relative Standard Deviation (RSD).

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity determined by injecting a series of (5, 10, 20, 50, and 80) ppm of sugar standards. The concentration range (5-80) ppm and a regression equation were found by plotting the peak area (Y) versus the beta-carotene concentration (X) expressed in ppm.

LOD and LOQ

Limit of detection is the lowest concentration of analyte that the analytic process can reliably differentiate from background levels. The (background) level measured in the substrate blank plus 3 standard deviations of this baseline level. LOD was determined using serial dilution of 0.08 µg/ml, 0.05 µg/mL, 0.025 µg/mL, 0.0125 µg/mL and 0.0025 µg/mL (ppm) of sugar standard from 5ppm to obtain the lowest amount of analyte greater than three times of noise level $S/N > 3$ and run each of them seven times. Therefore, the signal (S) to noise (N) ratio should be higher than 3 ($S/N > 3$). In the same way, LOQ was determined by injecting 0.025 ppm of sugar standard, to obtain the lowest amount of analyte which can be reproducibly quantified above the baseline noise (i.e. $S/N > 10$).

Accuracy and recovery

The recovery was used to determine the method accuracy. Accuracy is the degree of average measurement with an accepted reference. In this study, the accuracy of the technique was evaluated in terms of % recovery values between 70% and 120% were considered as an acceptable range. The recovery was performed by injecting a known amount of sugar standard and % of recovery was calculated using equation 14.

$$\% \text{Recovery} = (\text{Obtained Concentration} / \text{Spiked Concentration}) \times 100\% \quad (14)$$

3.9. Glycemic index analysis

Glycemic index was determined using internationally recognized GI methodology and method used by Arvidsson-Lenner *et al.*, (2004). The experimental procedures used were according with international standards for conducting ethical research with humans and was approved by Ethics

Review Committee of College of Natural and Computational Sciences, Addis Ababa University (Appendix VII).

Study participants

A group of 12 healthy, non-smoking individuals aged between 20-30 years were recruited. The volunteered participants who excluded if they were overweight, dieting, had existing medical conditions such as diabetes, suffering from any illness, food allergy, or were regularly taking any medication. During data collection the participants were well informed about the purpose of the study, procedure of data collection and were assured on confidentiality issues. All participants were given a written consent before taking part in the study (Appendix VI).

Test foods

White bread (50 g carbohydrate equivalent) was used as standard reference material and provided to the participants with pure water. The participants consumed the white bread on empty stomach and their blood glucose level was measured between 30 minutes interval. Similarly in another day, the 50 g carbohydrate equivalent test food (DPF) was consumed by each study participant and the level of blood glucose was measured in 30 minutes interval. The study participants were informed to consume the entire portion of the food.

Calculation of glycemic index

The GI was calculated by the method of Jenkins *et al.* (1981). The values of blood glucose level were plotted against time. For each individual, the area under the curve of 2hrs blood glucose response after consuming white bread (white bread AUC) and DPF (DPF AUC) was measured. The glycemic index was calculated for each person by dividing the AUC of the DPF to white bread. The final glycemic index value for the test food (DPF) was the mean glycemic index value of the 12 individuals. The GI was calculated by following equation 15

$$Glycemicindex(\%) = \frac{AUCofDPF*100}{AUCofwhitebread} \dots \dots \dots (15)$$

Where:

AUC of DPF = area under the curve of date palm fruit

AUC of white bread = area under the curve of white bread

Calculation of glycemic load (GL)

The GL was calculated as the GI (%) multiplied by the grams of carbohydrate in the serving of food eaten (equation 16).

$$\text{Glycemicload} = \frac{\text{Carbohydratecontentofthefoodserved*GI}}{100} \dots \dots \dots (16)$$

Where:

GI = Glycemic index

3.10. Statistical analysis

The nutritional composition, physical properties, mineral concentration, antioxidant properties and glycemic index/load data collected from the DPF samples were subjected to analysis of variance (ANOVA) by using SPSS software version 22.0. One way ANOVA was carried out to determine the level of significance difference between means of triplicate measurements between the local and imported DPFs. Values are presented as mean ±standard deviation (SD). P-values < 0.05 were considered statistically significant.

4. Results and discussion

The consumption and production of DPF is very low, despite its long history of domestication in the country. This is mainly due to many reasons including lack of awareness on the nutritional and health benefits, scant studies on physicochemical characteristics, and shortage of DPF plantation (Hussen, 2010). Recently, many activities have been undertaken to provide knowledge on the phytochemicals and potential health benefits of DPF. In an attempt to further contribute towards this effort, the present study aimed to evaluate the physical characteristics, nutritional composition, antioxidant properties and glycemic index/load of Ethiopian harvest and imported DPF. The results on the mentioned physical, nutritional and functional attributes are described and discussed in this section As form of tables and figures.

4.1. Physical characteristics of date palm fruit

The physical features of the three DPFs (local harvest, imported from Dubai and Saudi Arabia) is presented in Table 4. The parameters evaluated included fruit weight, seed weight, flesh weight, fruit length, kernel weight, seed to flesh ratio, dimension (length and diameter), color (L, a, and b values), and texture.

Table 2. Physical characteristics of date palm fruits harvested from Ethiopia, Dubai and Saudi Arabia

Source of date palm fruit	Physical characteristics					Dimension		Color		
	FW (g)	SW (g)	FLW (g)	KW (g)	STF (g)	Length (mm)	Diameter (mm)	L* (Lightness)	a* (redness)	b* (yellowness)
Ethiopia	4.56 ± 0.6 ^b	0.92 ± 0.2 ^a	3.63 ± 0.5 ^b	227.75 ± 0.0 ^c	0.20 ± 0.0 ^a	38.72 ± 2.3 ^a	5.90 ± 0.66 ^a	44.19 ± 0.7 ^a	4.44 ± 3.5 ^a	20.48 ± 1.5 ^a
Dubai	7.07 ± 1.9 ^a	0.61 ± 0.2 ^b	6.40 ± 1.9 ^a	350.85 ± 0.0 ^a	0.09 ± 0.0 ^c	30.53 ± 1.2 ^a	14.66 ± 0.7 ^{ab}	45.15 ± 1.5 ^a	4.84 ± 2.8 ^a	8.00 ± 1.1 ^a
Saudi Arabia	6.36 ± 1.7 ^a	0.84 ± 0.2 ^a	5.46 ± 1.6 ^a	311.22 ± 0.0 ^b	0.13 ± 0.1 ^b	38.54 ± 6.7 ^a	13.76 ± 0.8 ^a	55.50 ± 1.8 ^b	6.31 ± 5.3 ^a	7.50 ± 0.9 ^b

Data are expressed as mean ± SD ($n=3$). Mean values within the same column with different superscripts are significantly different at $p<0.05$.

FW-fruit weight; SW-seed weight; FLW-flesh weight; FL-fruit length; KW- kernel weight; STF-seed to flesh ratio

As presented in Table 2, average fruit weight of the DPF of Sufri variety harvested in Saudi Arabia, Bukary variety harvested in Dubai, and Bolo variety harvested in Afar Ethiopia was 6.36g, 5.61g, and 4.56g, respectively. Average fruit weight of DPF reported by Al-Shahib and Marshal (2003) was in a range between 6.5 to 10.6g, which is a higher value than the values in the present study. The seed weight of the respective three DPFs was 0.84, 0.60 and 0.92g with flesh weight of (5.46, 6.41, 3.63) g. Apparently this resulted the highest and lowest seed to flesh ratio in the Ethiopian (0.29), and Dubai (0.07) harvests, respectively (Table 2). Similarly, Ramadan (1995) reported an average flesh weight of DPF varieties in a range of 3.43 to 7.30g. The average fruit length was 2.86mm, 2.72mm, and 3.06 mm for the Saudi, Dubai and Ethiopian harvest DPFs. These values are within the range reported by range Nadeem *et al.* (2011) Ismail *et al.*, (2006) and, Al-Shahib and Marshal (2003), which was between 2.65 to 3.88mm. The average kernel weight of the DPFs was (350.85, 311.20, 227.75)g for the Dubai F, Saudi and Ethiopian harvests, respectively (Table2)

With respect to the dimension parameters, the Ethiopian harvest had the smallest date size of 5.99mm. The Dubai harvest had the biggest diameter of 14.66mm. The Ethiopian and Saudi harvest DPF had bigger length than the Dubai harvest. In contrast, the Dubai harvest DPF had larger diameter (14.66 mm) than the Ethiopian (5.90mm) and Saudi (13.76mm) harvests. Apparently the Ethiopian harvest had the smallest size (Table 2) when we compare the results with codex standards which indicate our DPF samples are within the range of the standard for instance the minimum size of the fruit is 4.0g, significant difference in weight and length based on the nature of the fruits. There was no significant difference in the color values (L, a, and b) between the three DPF harvests ($p>0.05$). The L, a, and b values of the Ethiopian, Dubai and Saudi harvest DPFs were (44.19, 4.44, 20.48), (45.15, 4.84, 8.00), and (55.50, 6.31, 7.50), respectively (Table 2). Having the lowest L value indicated that Ethiopian harvest was darker in appearance than the other studied dates. Conversely, the L value for the Saudi harvest was the highest. This indicated that this variety was pale and brighter in color than the others. Similarly, the lowest a value was observed for the Ethiopian harvest, whereas the Saudi harvest showed the highest value. With respect to b parameter, the Saudi harvest displayed the lowest value and the highest b value was observed for the Ethiopian harvest. The positive values for a and b indicated the absence of green and blue tints in the DPF. The results obtained were consistent with the data previously reported by Al-Jasass *et al.*, (2015), which examined the colors of Moroccan DPF.

According to Biglari *et al.*, (2008), the difference in colors of the dates is mainly due to genetic variation and the cultivar type.

International standards do not suggest any limit for dates color ,however American standards for dates` recommended a good homogeneity in date color ,requiring that light amber colored dates` must present no more than 5%of dark colored dates present no more than 5%of light colored dates .

Overall, the local harvest (Afar) DPF has significantly lower fruit weight, flesh weight, kernel weight than the imported ones from Dubai and Saudi Arabia ($p<0.05$). In contrast, the seed weight of the local harvest DPF was significantly higher than the Dubai harvest ($p<0.05$), though no significant difference was observed with the Saudi harvest ($p>0.05$). Accordingly, the highest seed to flesh ratio was obtained with the Ethiopian harvest (Table 2).

Table3. Texture of date palm fruits harvested from Ethiopia, Dubai and Saudi Arabia

Source of date	AP	DP	AN (-)	F5	GA
palm fruit					
Ethiopia	4785.33± 0.0 ^a	8.00 ± 0.0 ^a	50.22± 71.2 ^a	650.71 ± 520.1 ^a	1194.61± 0.0 ^a
Dubai	863.16± 245.3 ^c	7.99± 0.0 ^a	65.17± 5.19 ^a	1184.07± 66.5 ^a	215.99± 60.9 ^c
Saudi Arabia	2412.25± 735.2 ^b	7.98 ± 0.0 ^a	174.9± 105.1 ^a	453.85± 69.9 ^a	669.79± 215.1 ^b

Data are expressed as mean ± SD ($n=3$). Mean values within the same column with different superscripts are significantly different at $p<0.05$.

AP-absolute positive; AN-absolute negative, GA-gradient to absolute positive, DP-distance at positive force F5-force at 5mm

The measurement of the force indicated that the hardness or firmness of the date palm fruits is related to tissue`elasticity from the data from the table the highest value of force indicates the hardness of the fruits our date is the hardest of others, the stickiness is measured by absolute negative, the date from Saudi have highest absolute negative so this sample is the stickiest, the adhesiveness of the sample is expressed by the absolute positive so the date from Saudi have is most adhesive ,cutting and shearing is expresses the gradient the date from Ethiopia have highest cutting and shearing value, in the aspect of brittleness Ethiopian date palm fruit have high value of DP so this date is the most brittle.

4.2. Nutritional composition of date palm fruit

The proximate analysis was done by assessing the amounts of moisture, ash, crude protein, crude fat, crude fiber and total and utilizable carbohydrate in the three DPF harvests. The moisture level in food products is considered to be a critical aspect in preserving food and thus affects the consumers' preferences (Agboola and Adejumo, 2015). The Dubai and Saudi DPFs had the highest moisture content, 16% where as the Ethiopian harvest displayed the lowest level of moisture, 11%. Lower moisture content implies a longer shelf life but resulting in the development of dry dates, which are less preferred by the consumers (Assirey, 2015). In fact, DPF are categorized into three groups based on moisture content; soft ($\geq 30\%$), semi-soft (10% to 30%) and dry dates ($\leq 20\%$) (Habiband Ibrahim, 2011) according to codex standard the moisture content should be less than 26% in this aspect our DPF is categorized as dry DPF. Hence, it can be proposed that the DPF harvests in this study were semi-soft dates. The ash content of the Ethiopian, Dubai, and Saudi harvest DPFs were 1.2, 1.7, and 2.4%, respectively. There was no significant difference in crude fat content between the three DPF harvests ($p > 0.05$). The DPFs in this study can be classified as having a low fat content, which was consistent of the fat content in Sudanese dates by Suleiman *et al.* (2011). NurAshikin *et al.* (2020) reported a $< 1\%$ crude fat content in Saudi DPF varieties.

Dates are not considered to be good sources of protein (Agboola and Adejumo, 2015). Likewise, the protein content of Ethiopian, Dubai, and Saudi harvest DPFs was 2.1, 2.1, 1.9%, respectively (Table 4). Mia *et al.* (2020) also reported similar content of crude protein in DPF between range of (1.8-3.0)%. The highest value for crude fiber, 6.1% was observed in the Ethiopian harvest DPF. Both the Dubai and Saudi harvests had crude fiber content of 4.1%. The total and utilizable carbohydrate contents were the highest in Ethiopian harvest DPF (85.5, 79.4) %, respectively. These results are similar with the report by NurAahikinet *al.* (2020) and Jamilet *al.* (2010). Carbohydrates in dates provide essential calories in the human diet (Agboola and Adejumo, 2015). The gross energy results increased from 311 kcal for the Dubai and Saudi harvests to the highest energy content in Ethiopian harvest DPF with 330 kcal. The energy values mainly depended on the amount carbohydrates. Any differences in nutrient content might result from effect of the varieties, cultivation practices and environmental conditions (Gromskiet *al.*, 2015).

Table 4. Proximate composition of Ethiopian, Dubai and Saudi Arabia harvest date palm fruits

Source of date palm fruit	Moisture (g/100g)	Crude fat (g/100g)	Total ash (g/100g)	Crude protein (g/100g)	Crude fiber (g/100g)	Total carbohydrate (g/100g)	Utilizable carbohydrate (g/100g)	Gross energy (kcal/100g)
Ethiopia	10.7 ± 1.4 ^b	0.5 ± 0.0 ^a	1.2 ± 0.0 ^c	2.1 ± 0.0 ^a	6.1 ± 0.1 ^a	85.5 ± 0.4 ^a	79.4 ± 0.0 ^a	330.5 ± 0.1 ^a
Dubai	16.9 ± 0.7 ^a	0.5 ± 0.0 ^a	1.7 ± 0.1 ^b	2.1 ± 0.1 ^a	4.2 ± 0.1 ^b	78.8 ± 0.2 ^b	74.7 ± 0.9 ^b	311.2 ± 3.4 ^b
Saudi Arabia	15.6 ± 0.3 ^a	0.5 ± 0.0 ^a	2.4 ± 0.0 ^a	1.9 ± 0.1 ^b	4.2 ± 0.1 ^b	80.1 ± 0.3 ^b	76.0 ± 0.2 ^b	311.3 ± 0.9 ^b

Data are expressed as mean ± SD ($n=3$). Mean values within the same column with different superscripts are significantly different at $p<0.05$.

4.3. Iron and calcium content

The nutritional quality of date palms is, in part, associated with their mineral composition. Dates contain essential minerals including phosphorus, potassium, sodium, zinc, manganese, magnesium, copper, and iron. Mineral content varies depending on the cultivar, ripening stage, agronomical practices and environmental conditions (Dghaim *et al.*, 2021). The mineral composition (calcium and iron) of the three DPF harvests is presented in Table 7. The Ethiopian, Dubai and Saudi harvest DPFs had calcium concentration of 2.04, 1.58, and 45.74 mg/L, respectively. The Saudi harvest had the highest calcium concentration. Dghaim *et al.* (2021) reported calcium content of DPF varieties in a range of (571.95-766.00 mg/kg), which is much higher concentration than in the values in the present study the is due to the soil which the plant grows. The Iron concentration in the respective three harvest were 4.51, 2.03, and 1.77 mg/L. Iron content of different DPFs varieties within range of 4.71-10.17 mg/kg was reported by Dghaim *et al.* (2021). Results of the one-way pooled ANOVA showed a significant difference in the concentration means of calcium and iron ($p<0.05$) between the three different date harvests according to codex the mineral impurity is no more than 1g/kg, this implies date palm fruits have a composition of many minerals like iron and calcium this study supports the standard and also our date is advisable for its mineral benefit.

Table 5: Mineral concentration of Ethiopian, Dubai and Saudi Arabia harvest date palm fruits

Source of date palm fruit	Calcium (mg/L)	Iron (mg/L)
Ethiopia	2.04 ± 0.04 ^b	4.51 ± 0.01 ^a
Dubai	1.58 ± 0.10 ^c	2.03 ± 0.08 ^b
Saudi Arabia	4.54 ± 0.14 ^a	1.77 ± 0.05 ^c

Data are expressed as mean ± SD ($n=3$). Mean values within the same column with different superscripts are significantly different at $p<0.05$.

4.4. Sugar content

Chromatographic method validation was done by checking different parameters such as identification, accuracy, recovery, linearity, LOD and LOQ. This is important to measure the analytical performance of the instrument and to validate the method used to analyze sugar content from DPF samples.

Identification

Identification of glucose, fructose and sucrose from the test sample was done according to their retention time on the HPLC chromatogram. This was obtained after running different concentration of the sugar standards (5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm, and 100ppm). According to the result, the retention time of glucose, fructose, and sucrose was 1.4, 2.1, and 0.9 respectively. The precision of the retention time measured by using percent relative standard deviation was 2.1, 1.4, and 0.9 for fructose, glucose, and sucrose standards, respectively (Table 6). Percent relative standard deviation below 2% is acceptable (FDA, 2002).

Precision

The repeatability of the analytical method was tested by injecting 7 replicates of 5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm and 100ppm of the sugar standard under the same analytical condition within the same day. As FDA standard the acceptable level of percent relative standard deviation for precision is ≤ 2 . The result obtained after the assay is described in (Table 6), for the peak area percent relative standard deviation is less than 2 which is acceptable.

Limit of Detection and Limit of Quantification

Among the concentration of 0.08, 0.05, 0.025 and 0.0125 ppm of sugar standard the ability of the instrument to detect the smallest change on the analyte was 0.0125 ppm with a signal to noise ratio of 8.375 which is greater than 3. The limit of quantification of the instrument was 0.025 with a signal to noise ratio 17.6 which is greater than 10 and acceptable validation according to (FDA, 2002).

Linearity

The coefficient of determination (r^2) is the main criteria that FDA uses to check the acceptability of linearity data, which obtained from y-intercept of the linear regression line for the peak area versus concentration plot. As the data described below in the Table 6 the coefficient of determination lie between 0.9945-0.9999, which indicated the presence of a strong relationship between the concentration of the analyte and peak area. The coefficient of determination > 0.998 is generally acceptable as FDA standard (FDA, 2002).

Accuracy and recovery

According to FDA (2002) mean percent recovery was lay within the range of 70-120 which was acceptable as the Table 6 Indicated that the method was accurate within the desired recovery range that set by FDA and the percent relative standard deviation was less than 1 (FDA, 2002).

Table 6. HPLC method validation for sugar analysis

LOD and LOQ		
Sugar analyzed	LOD (g/100g)	LOQ (g/100g)
Fructose	0.03	0.09
Glucose	0.05	0.15
Sucrose	0.08	0.25
Repeatability and reproducibility		
Sugar analyzed	Repeatability	Reproducibility
Fructose (32g/100g)	0.8	2.1
Glucose (31g/100g)	1.1	1.4
Sucrose (2.8g/100g)	0.4	0.9
Recovery	(85-105)%	

LOD-limit of detection; LOQ-limit of quantification

Sugar profile was determined using HPLC method and the result is shown in Table 9. The fructose content in the Ethiopian, Dubai, and Saudi harvest DPFs was 12.20, 2.58, and 24.90%, respectively. Meanwhile the glucose content was 12.42, 27.05, and 26.06% for the respective DPFs. But the sucrose content was small as compared to the two reducing sugars (Table 6). Likewise, Ziadi *et al.*, (2014) and El-Sohaimy (2010) highlighted that glucose and fructose have lower and higher quantities at khalal and tamr stages of maturity respectively, whereas sucrose was present only at the khalal and rutab stages. The most likely explanation of the sudden drop in the non-reducing (NRS) (sucrose) and the increase in reducing sugar (RS) (glucose and fructose) at the tamr stage is the rising activity of the invertase enzyme that hydrolyzes the sucrose into glucose and fructose (Sawaya *et al.*, 1982). Interestingly, the proportion of RS/NRS is commonly used to gain an idea about the softness or dryness of a fruit. The higher the proportion, the softer the fruit (Mrabet *et al.*, 2017). Consequently, the dominance of RS in all the studied samples pointed out that our cultivars belong to the soft date type. With the summation of the tested sugar types, the Dubai harvest DPF had the highest total sugar content (54.57%), while the Ethiopian harvest had the lowest sugar amount (29.57%) according to codex standard date palm fruits are majorly consisting of glucose and fructose so the study is majorly consisting the two monosaccharides (Table 7).

Table 7. Sugar profile of Ethiopian, Dubai and Saudi Arabia harvest date palm fruits

Source of date palm fruit	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	Glucose to fructose ratio
Ethiopia	12.20	12.42	4.90	1.02
Dubai	26.58	27.05	0.94	1.02
Saudi Arabia	24.90	26.06	–	1.05

“–” below detection limit

4.5. Antioxidant activity

The antioxidant property of the three DPFs was evaluated using DPPH free radical scavenging activity assay. Saudi harvested date palm fruit had higher anti-oxidant activities than the other two samples As shown in Table 8the Saudi Arabia harvest DPF showed the highest ability to inhibit DPPH radical at 0.2696 mg/ml.. The DPPH scavenging data suggested that the extract was capable of scavenging free radicals, hence preventing the initiation and propagation of free-radical-mediated chain reactions due to their content of bioactive compounds such as phenolic acids, carotenoids, flavonoids, and polyphenols. These bioactive substances can absorb and neutralize free radical intermediates resulting from oxidation reactions in the human body. The higher antioxidant activity (lower IC₅₀ value) in the three DPF harvests was in order of Dubai<Ethiopia<Saudi Arabia the study result is related with different studies Effective scavenging concentration (IC₅₀) on DPPH radical ranged from 10.83 to 21.27 mg/l, the IC₅₀ values (Ghiaba *et al.*, 2012),

Table 8. Antioxidant activity of Ethiopian, Dubai and Saudi Arabia harvest date palm fruits measured by DPPH method

Source of date palm fruit	IC ₅₀ (mg/mL)
Ascorbic acid	0.111849
Ethiopia	0.291171
Dubai	0.375814
Saudi Arabia	0.269635

IC₅₀-concentration for a 50% inhibition of DPPH free radical

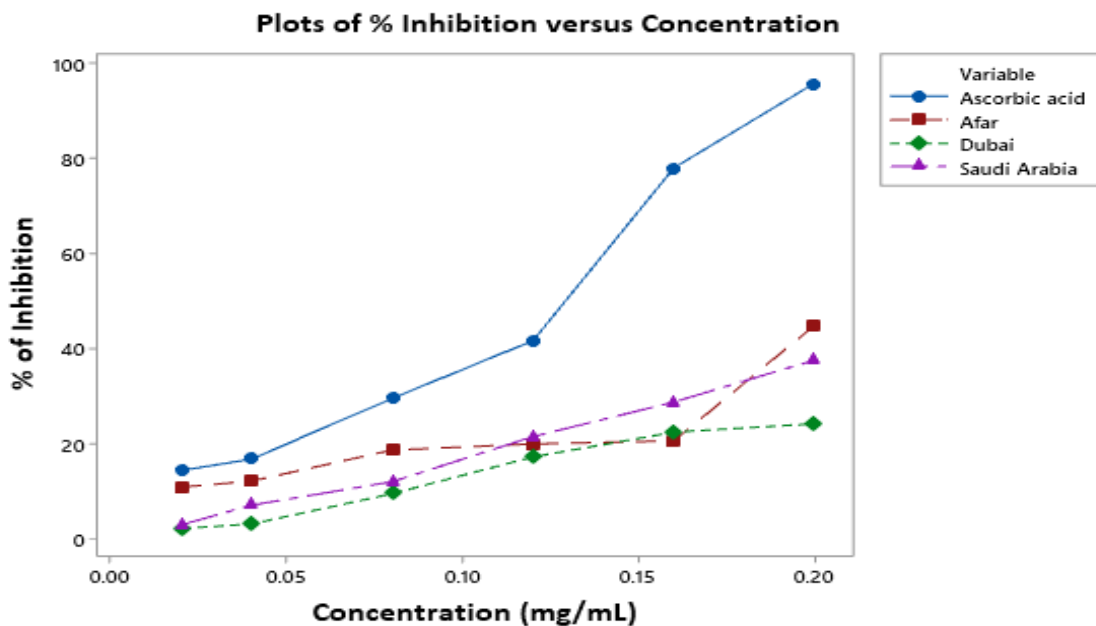


Figure 3. Plots of percent of Inhibition versus concentration of ascorbic acid and date palm sample

4.5.1. Total phenolic and flavonoid contents

It is clearly shown that the total flavonoid content of the Ethiopian harvest DPF was higher than that of Dubai and Saudi harvests ($p < 0.05$). The total phenol content of the DPFs harvests were in order of Ethiopia > Dubai > Saudi Arabia ($p > 0.05$) (Table 9). Factors such as variety, growing conditions and maturity among others, might be responsible for the observed differences in TPC and TFC values (Al-Farsi *et al.*, 2007). Indeed, natural drying occurred during dates ripening is regarded as unfavorable for antioxidants, due to the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds (Al-Farsi and Lee, 2008). The Ethiopian harvest DPF had the highest TPC and TFC.

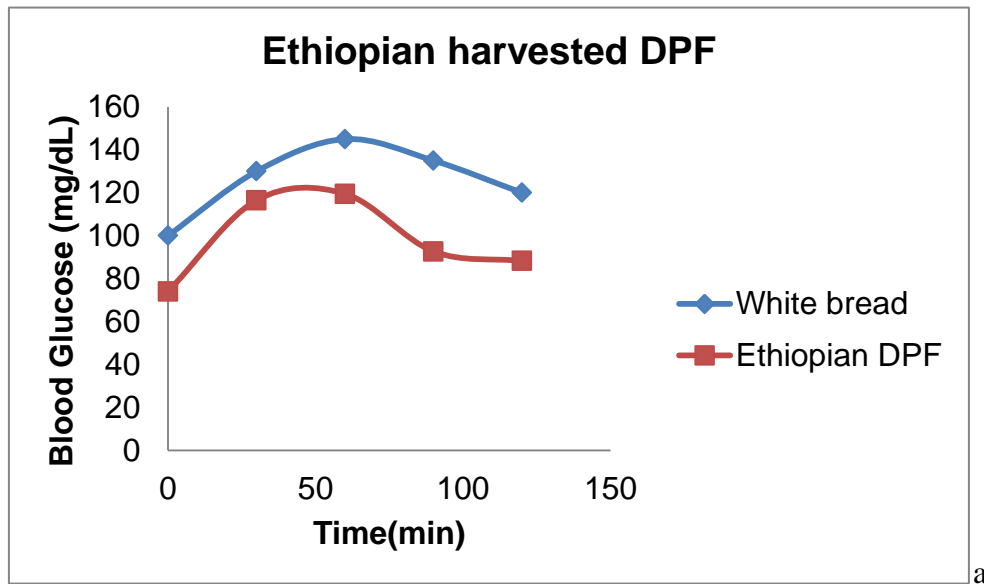
Flavonoids possess diverse health benefits, which include antioxidant and radical scavenging activities. However, the composition of flavonoids and other antioxidant phenolics in fruit changes dramatically during ripening (Brady, 1987).

Table9. Total phenol and flavonoid contents of Ethiopian, Dubai and Saudi Arabia harvest date palm fruits

Source of date palm fruit	Flavonoid (mg/100g)	Total phenol (mg/100g)
Ethiopia	9.86 ± 1.12 ^a	14.70 ± 2.59 ^a
Dubai	8.69 ± 0.86 ^b	12.40 ± 3.68 ^a
Saudi Arabia	6.68 ± 1.41 ^b	11.80 ± 2.49 ^a

Data are expressed as mean ± SD (*n*=3), on dry basis. Mean values within the same column with different superscripts are significantly different at *p*<0.05.

4.6. Glycemic index



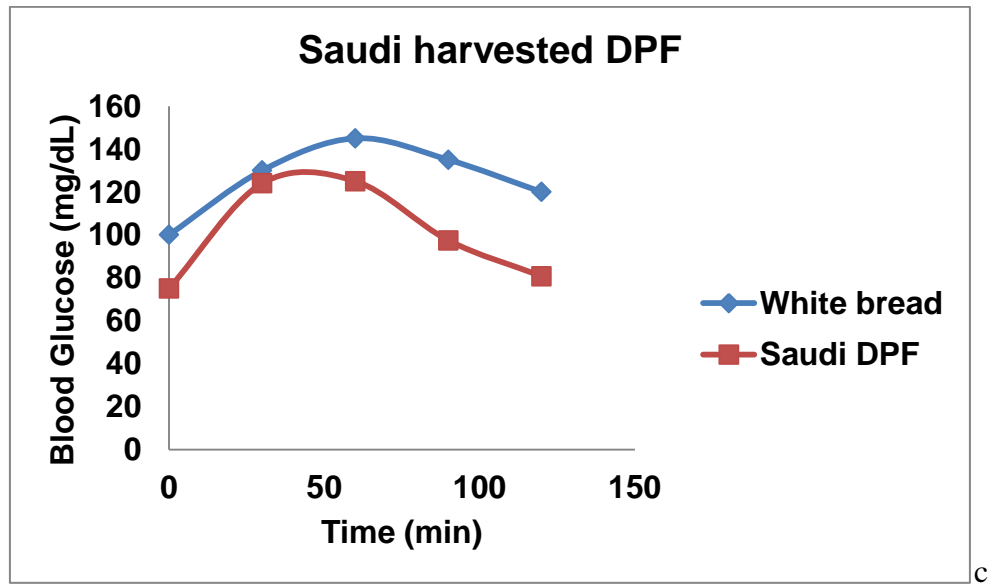
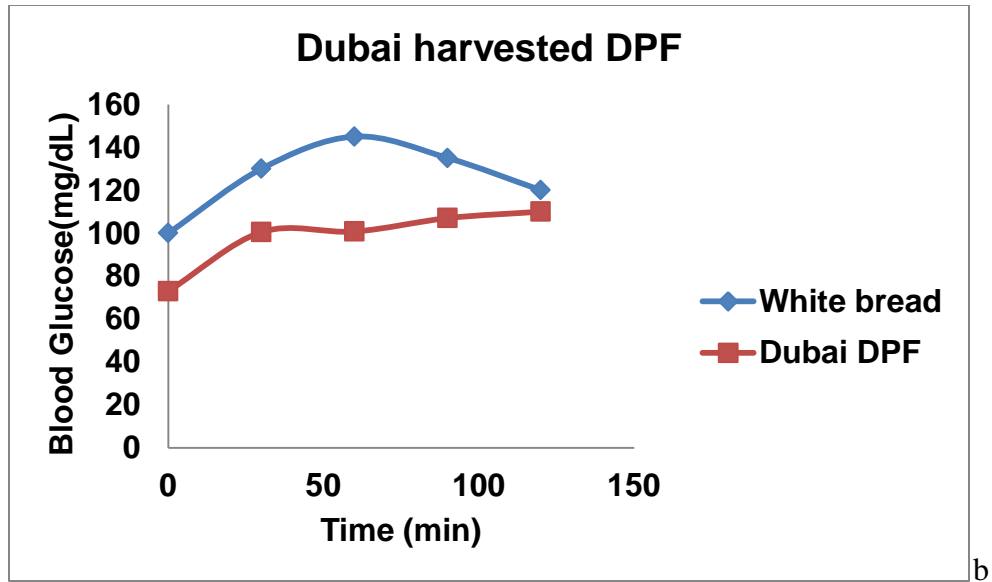


Figure 4. Glycemic index graphs of (a) Ethiopian (b) Dubai and (c) Saudi harvest DPFs using glucose as a standard

Data are expressed as the changes in glucose concentration from the fasting baseline concentration. Each data point represents the mean GI value of each DPF. The GI value in all the three DPFs in this study was under the category for low GI foods (0-55%). The low GI of dates can be attributed to their high fructose (Walton *et al.*, 2021)

The study participants for the GI determination were 12 healthy subjects (6 females and 6 males) with a mean \pm SD age of 20 ± 10 . Figure 5 shows the GI results for the three types of dates in healthy subjects. The mean \pm SD GIs of the dates among healthy individuals were 54.2 ± 6.5 , 52.1 ± 6.5 , 55.00 ± 6.2 for Ethiopian, Dubai, and Saudi Arabia harvest DPFs, respectively. There were no statistical differences between the GI values of the three dates ($p > 0.05$). The respective glycemic load was 27.10, 26.01, and 27.50. Conversely, the glycemic load of the three DPF harvests were above 20, which is a category for high glycemic load foods considering individual food portion (Bernard *et al.*, 2006)

Similarly, the GIs of three varieties of dates collected from various regions of Oman ranged between 47.6 and 57.7 (Ali *et al.*, 2009), which is a low GI food category. The low GI of dates can be attributed to their high fructose and dietary fiber content. In our study, the glucose:fructose ratio was approximately 1:1 consistent with previous reports (Al-Farsi and Lee, 2008; Hätönen *et al.*, 2006), Ahmed *et al.* (1995). A diet low in GI may decrease the risk of coronary heart disease, gallbladder disease and breast cancer. Furthermore, a low GI diet demonstrably improves HbA1c levels, body weight and lipid profile (Alkaabi *et al.*, 2011). 50% carbohydrate was used for GI determination, which is the possible maximally eaten at a single sitting by Ethiopian consumers. Though the three DPFs had low GI, their caloric content should be accounted for the glycemic load they might exert. Dates are rich in energy i.e. 100 g of flesh can provide approximately 314 kcal i.e. 11-15% of the total energy requirement per day for adults (Al-Farsi and Lee, 2008). Therefore, though low in GI considering their high carbohydrate contents, dietary intake should be optimized. It would be interesting to further examine the GI of DPFs at different stages of maturation.

5. Conclusions and recommendations

5.1. Conclusions

In this study the physical characteristics, nutritional composition, antioxidant properties and glycemic index/load of Ethiopian harvest and imported DPF were comparatively evaluated. The local harvest (Afar) DPF has significantly lower fruit weight, flesh weight, kernel weight than the imported ones from Dubai and Saudi Arabia. In contrast, the seed weight of the local harvest DPF was significantly higher than the Dubai harvest, though no significant difference was observed with the Saudi harvest. Accordingly, the highest seed to flesh ratio was obtained with the Ethiopian harvest. Similarly, the Ethiopian harvest had the biggest fruit length, but with the smallest date diameter. Having the lowest L value the Ethiopian harvest was darker in appearance than the other DPFs. The Dubai and Saudi DPFs had the highest moisture content, whereas the Ethiopian harvest had the lowest value. Based on moisture content, all the three date harvests in this study were semi-soft dates. The DPFs in this study can be classified as having a low fat and protein contents. Interestingly, the Ethiopian harvest had the highest crude fiber content. Similarly, the total and utilizable carbohydrate, and gross energy contents were the highest in Ethiopian harvest DPF. The sucrose content was small as compared to glucose and fructose in all the three DPF harvests. This indicates the highest maturity level of the harvests in the study which categorize them into soft date type. Overall, the Dubai harvest DPF had the highest total sugar content, while the Ethiopian harvest had the lowest content. The antioxidant activity of the three DPF harvests was in order of Dubai < Ethiopia < Saudi Arabia. The Ethiopian harvest DPF had the highest TPC and TFC compared with the two. All the three DPF harvests had low glycemic index. Therefore, the present study demonstrated the nutritional and physical quality and potential of Ethiopian DPF, which has low production and consumption in the country.

5.2. Recommendations

- Sensory evaluation of the DPFs was not conducted in this study, which is one of the major limitations. Hence, in the future the sensory acceptability of the local harvest date should be investigated.
- The nutritional and physical quality of DPFs depends highly on maturity level. Thus, in the future, the biochemical composition and physical properties following the maturity level should be investigated.
- Also the shelf-life of the local harvest DPF needs to be investigated considering different storage conditions and packaging materials.
- DPFs can be ingredients in different healthy food formulations. Accordingly, future studies on the formulation of food products with DPFs as ingredient should be carried out.
- The nutritional contribution of the local harvest DPFs along with cost analysis need to be evaluated as well.
- The anti-nutritional factors of the Ethiopian date should be investigated.

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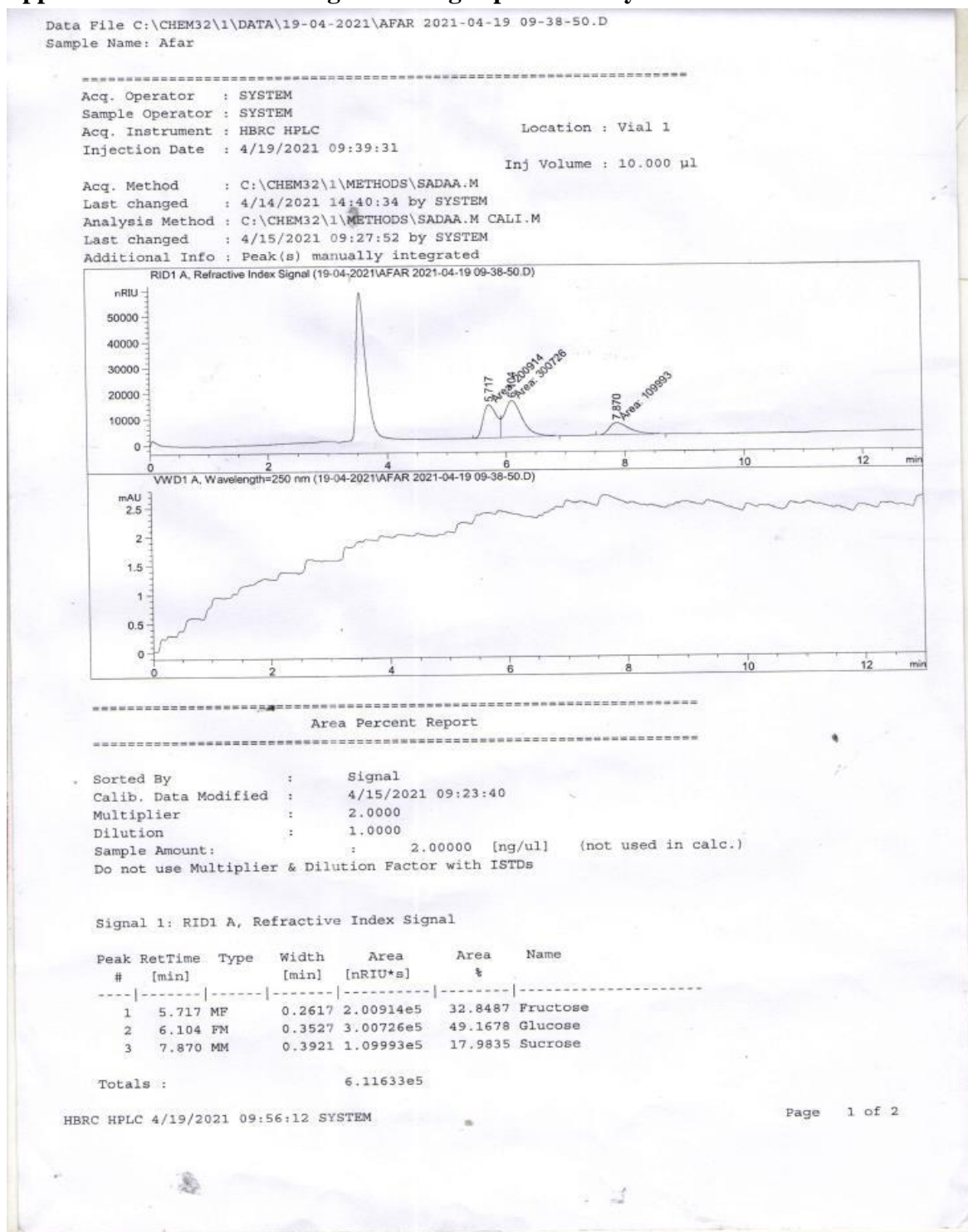
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Appendices

Appendix I HPLC chromatogram of sugar profile analysis

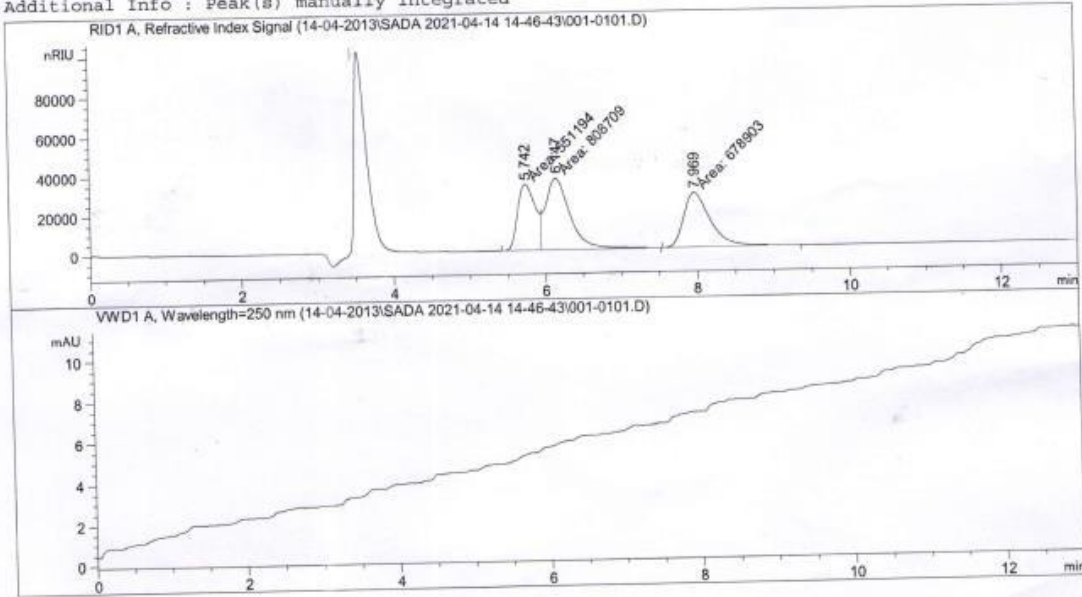


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Area Percent Report

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Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.742	MF	0.2725	5.51194e5	27.0352	Fructose
2	6.147	FM	0.3669	8.08709e5	39.6658	Glucose
3	7.969	MM	0.4030	6.78903e5	33.2990	Sucrose

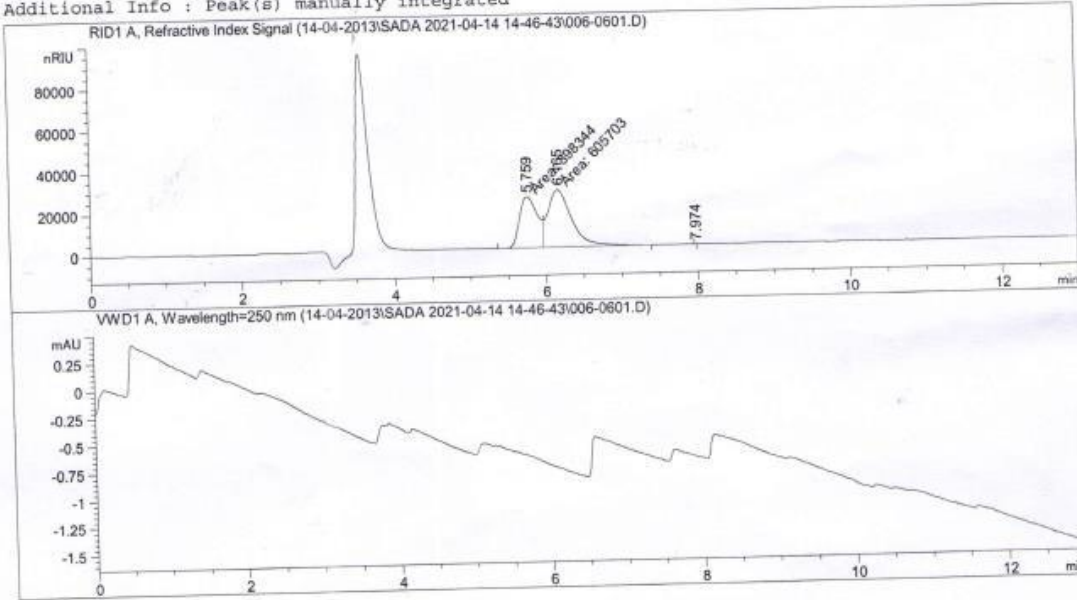
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 Sample Name: saudi

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Additional Info : Peak(s) manually integrated
  
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Area Percent Report

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Dilution       : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.759	MF	0.2700	3.98344e5	39.6737	Fructose
2	6.165	FM	0.3641	6.05703e5	60.3260	Glucose
3	7.974	BV	0.0152	3.24635	3.233e-4	Sucrose

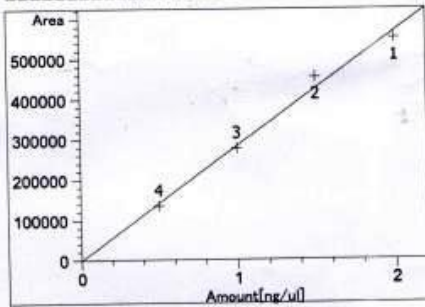
Totals : 1.00405e6

RT	Sig	Lvl	Amount [ng/ul]	Area	Rsp.Factor	Ref	ISTD #	Compound
			2	1.50000	6.87313e5			
			1	2.00000	8.08709e5			
7.969	1	4	5.00000e-1	1.71697e5	2.91210e-6	No	No	Sucrose
			3	1.00000	3.35933e5			
			2	1.50000	4.78883e5			
			1	2.00000	6.78903e5			

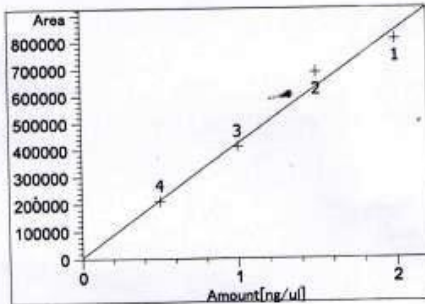
Peak Sum Table

No Entries in table

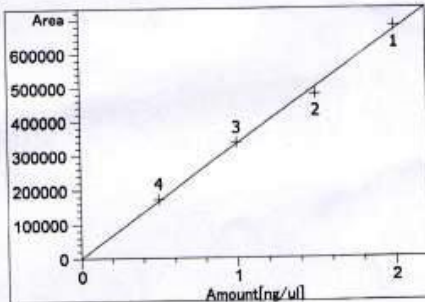
Calibration Curves



Fructose at exp. RT: 5.742
 RID1 A, Refractive Index Signal
 Correlation: 0.99702
 Residual Std. Dev.: 20092.05803
 Formula: $y = mx + b$
 m: 284502.78125
 b: -613.00625
 x: Amount
 y: Area



Glucose at exp. RT: 6.147
 RID1 A, Refractive Index Signal
 Correlation: 0.99520
 Residual Std. Dev.: 37614.24952
 Formula: $y = mx + b$
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 b: 5025.27500
 x: Amount
 y: Area



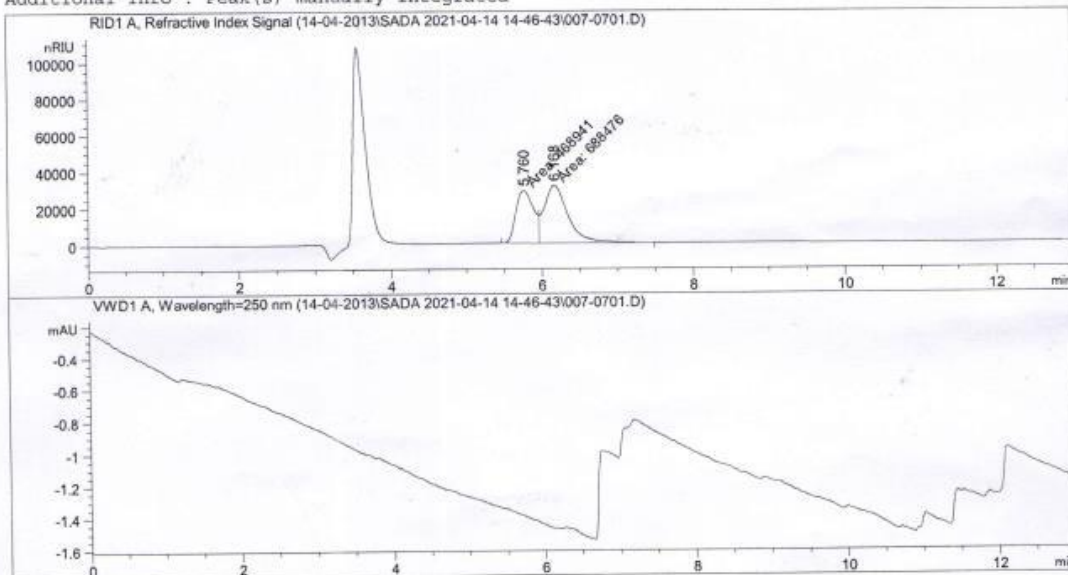
Sucrose at exp. RT: 7.969
 RID1 A, Refractive Index Signal
 Correlation: 0.99887
 Residual Std. Dev.: 14458.28890
 Formula: $y = mx + b$
 m: 332998.13438
 b: 85.07500
 x: Amount
 y: Area

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 Sample Name: dubai

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                                           Inj Volume: 10.000 µl

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Area Percent Report

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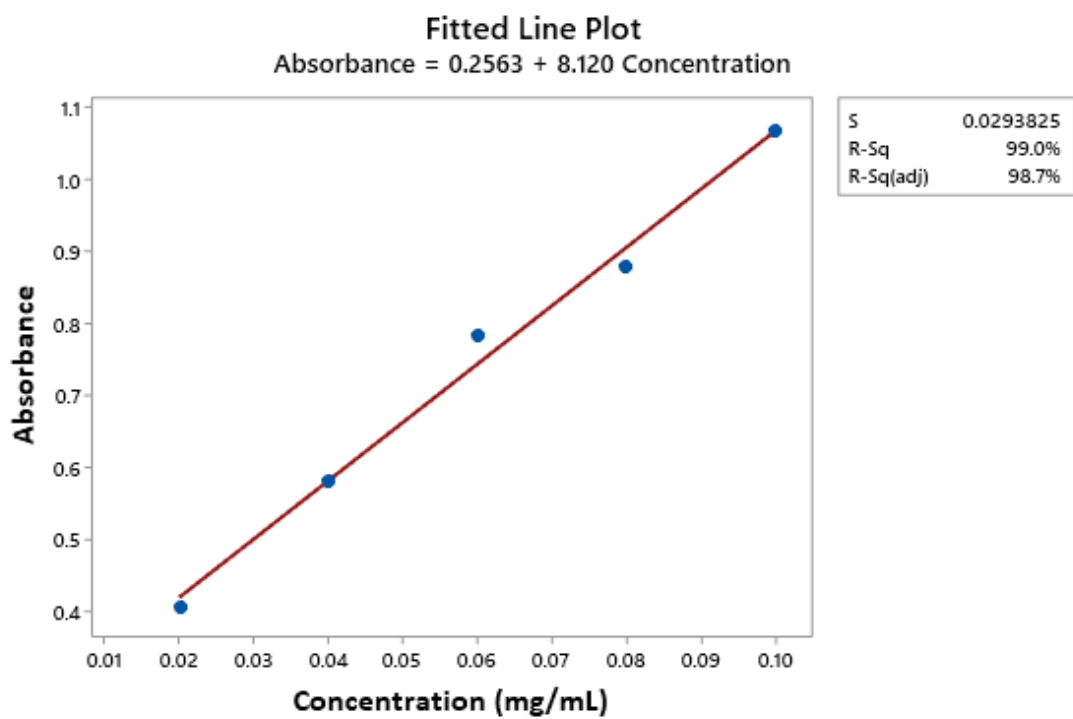
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Do not use Multiplier & Dilution Factor with ISTDs
  
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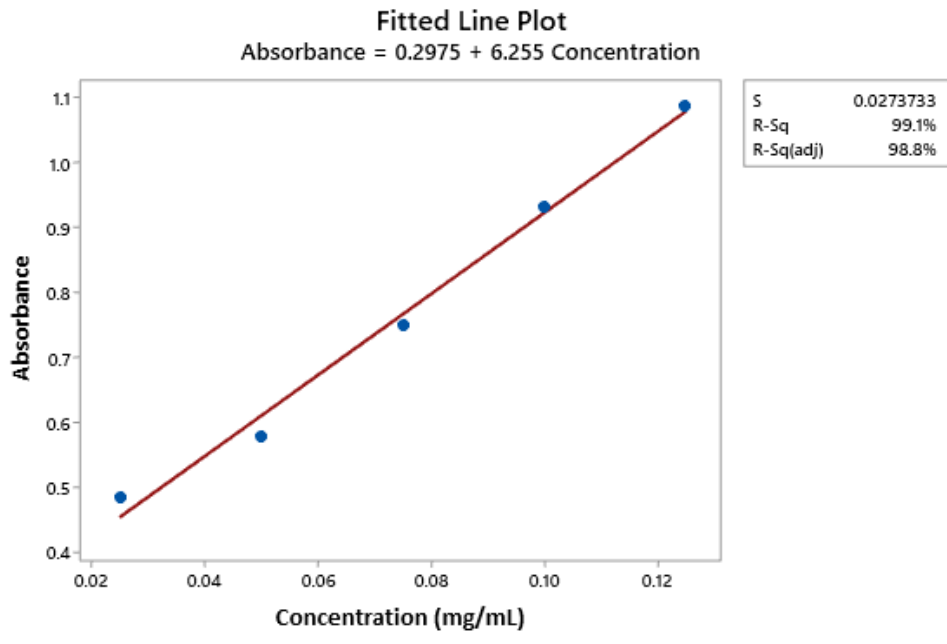
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.760	MF	0.2725	4.68941e5	40.5162	Fructose
2	6.168	FM	0.3640	6.88476e5	59.4838	Glucose
3	7.969		0.0000	0.00000	0.0000	Sucrose

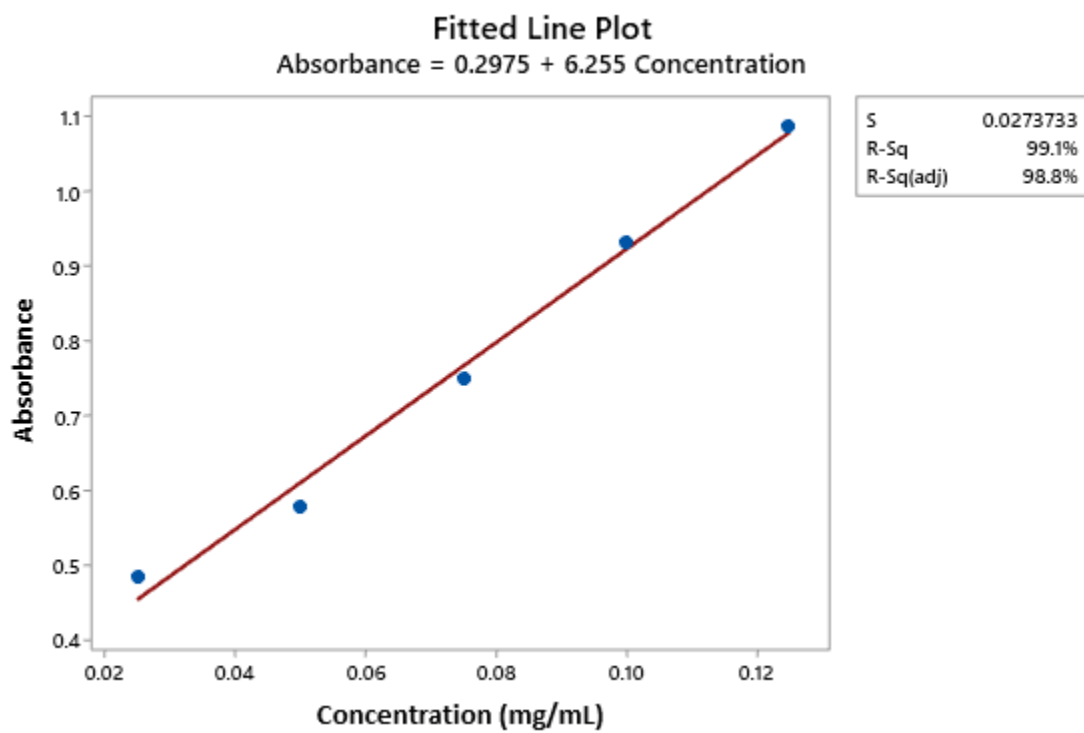
Totals : 1.15742e6

Appendix II Calibration curve used for total phenolic content determination





Appendix IV Calibration curve of standards



Appendix V Informed consent form for glycemic index test participants

Dear sir/madam,

You are invited to participate in a study involving the evaluation of the glycemic index and load of Date Palm Fruit (DPF). The overall objective of the study is to evaluate the level of Glycemic Index of local-harvest and imported DPF in our country. You will be asked to be on fasting 8 hours prior to consuming the samples. Once consuming the bread, capillary blood from fingertip will be collected every fifteen minutes and analyzed by glucometer for rest of two hours. If you have prior experience of any allergic reactions to DPF, you should not participate in this study. If you experience allergic reactions any time during the study, you should discontinue the study. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason. We also reserve the rights to terminate your participation of the study at any time and for any reason.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance. Names or other identifiable information will not be disclosed or published.

You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. Questions can be addressed to Ms. SadaHussen (+251964161705, sadahussen56@gmail.com).

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of this consent form.

Signature

Date

Appendix VI Laboratory procedure and selection criteria for glycaemic index and factors affecting level of GI

Parameter	Comments
Subjects	> 10 test subjects Healthy subjects appropriate (lower within-subject variation than e.g. diabetic subjects using drugs)
Time of day	Mornings (more pronounced glycaemic response)
Background diet	Fasting subjects Standardised evening meal may reduce within-subject variation
Physical activity	Standardisation so far not successful in decreasing variation
Determination of glycaemic carbohydrates	"By difference" (total CHO minus dietary fibre): Acceptable estimation of digestible CHO in most normal foods, but may overestimate digestible CHO in products with undigestible CHO not determined as dietary fibre (e.g. oligosaccharides, resistant starch, and sugar alcohols). Specific assay of the CHO profile recommended for scientific purpose
Carbohydrate load	Typically 50 g glycaemic CHO (linear response for 25–50 g)
Reference product	White bread or glucose Other reference than glucose: GI characteristics of the reference maintained over time and recalculated/disseminated using glucose reference
Blood sampling/analyses	Capillary blood (preferable): higher postprandial glucose concentration, less variation Venous blood: lower glycaemic response, larger variation, higher GI than capillary blood Blood glucose analyses based on approved analytical methods, not enzymatic recognition in dry systems
Calculation	Typically 2 h incremental area (3 h area may be useful for products with extreme lente characteristics)

CHO = carbohydrates.

Appendix VII Ethical clearance

