بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

Sudan University of Science and Technology

Collage of Agricultural Study

Department of Food Science and Technology

Suitability of Using Carob *(Piliostigmareticulatum)* Fruits for Juice Production

امكانية استخدام ثمار الخروب في انتاج عصير

A Dissertation Submitted to Sudan University of Science and Technology in partial Fulfillment for the Requirements of B.Sc (Honours) Degree in Food Science and Technology

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الاستهلال

بسم الله الرحمن الرحيم

﴿ فَلْيَنظُرِ الْإِنسَانُ إِلَى طَعَامِهِ ﴿ 24 ﴾ أَنَّا صَبَبْنَا الْمَاء صَبّاً ﴿ 25 ﴾ ثُمَّ شَقَقْنَا الْأَرْضَ شَقّاً ﴿ 26 ﴾ فَأَنبَتْنَا فِيهَا حَبّاً ﴿ 27 ﴾ وَعِنَباً وَقَضْباً ﴿ 28 ﴾ وَزَيْتُوناً وَنَخْلاً ﴿ 29 ﴾ وَحَدَائِقَ غُلْباً ﴿ 30 ﴾ وَفَاكِهَةً وَأَبّاً ﴿ 31 ﴾ مَّتَاعاً لَّكُمْ وَلِأَنْعَامِكُمْ ﴿ 32 ﴾ .

صدق الله العظيم

سورة عبس الايات (24-32)

DEDICATION

To our Family

Teachers

Colleagues

and Friends …

With respect.

ACKNOWLEDGEMENT

Prayers and thanks to ALLAH who gave us good health and support to accomplish this study.

Grateful thanks to my supervisor **Dr. Salma Elghali Mustafa** who was too patient with us during this study, also for her unlimited assistance and guidance which made this study worth full.

Thanks are also given to any other one who had supported us during this study.

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Abstract

The main objective of this research was to assess the suitability of utilization of *Piliostigma reticulatum* (carob) fruits for production of fresh juice with high nutritional value in order to improve industrial uses and facilitate the domestic consumption of these fruits in Sudan. The chemical composition of carob fruit powder was determined. The total soluble solid, acidity and p H for carob were also identified. Moreover, the organoleptic characteristics of carob juice were investigated. The result indication that the carob fruit powder contains high percentage of moisture (11.43%), Crude fiber (11.83%), total sugar's (62.70%), percentage of protein (8.8%), fats (2.34%), and vitamin C (177.64%).Also the study indicated that the fruit juice could be easily extracted after soaking (2hr) in boiling distilling water. Two concentrates: A (1:10) and B (1:8) (carob: water, w: v)of carob juice were prepared. The nutritional values of carob juices showed that sample (B) has higher contents for total sugar, T.S.S, and vitamin C, (14.8,17.2,and25.6)respectively compared to sample A (13.9, 14.9,and17.6) respectively, while sample A hashigher moisture content (84.03%) than sample B (82.43%). Finally, the sensory evaluation results verified that sample A(1:10) got high acceptability from the panelist.

ملخص الدراسة

الهدف الاساسي لهذا البحث هو معرفة مدى امكانية استخدام ثمار الخروب لانتاج عصير جاهز ذوقيمة غائية عالية من اجل تحسين الاستخدامات الصناعية وتسهيلطريق الاستهلاك الغذائي لهذه الثمار في السودان .
تم تحديد المواد الصلبة الذائبة والحموضة ورقم الاس الهيدروجيني للخروب. علاوة على ذلك تم فحص الخصائص الحسية لعصير الخروب.
ولقد اوضحت نتائج الدراسة ان مسحوق ثمار الخروب تحتوي علي نسبة عالية من المادة الجافة (11.43%) والالياف الخام (11.83%) ,السكريات الكلية (62.7%) ونسبة بروتين تصل (8.8)% والدهون (2.34%) وفيتامين ج (177.64%).
كما اوضحت الدراسة أن عصير الفاكهة يمكن استخلاصه بسهولة بعد نقعه لمدة ساعتين في ماء مقطر مغلي' تم تحضير مركزين (وزن:حجم) العينة (A1:10) والعينة B (1:8) من عصير الخروب .
أظهرت القيم الغذائية لعصير الخروب ان العينة B تحتوي علي نسبة اعلى من السكريات الكلية والمواد الصلبة الذائبة وفيتامين ج(14.8,17.2,25.6) على التوالي مقارنة مع العينة A (13.9,14.9,17.6) على التوالي. بينما العينة A تحتوي على نسبة رطوبة اعلى (84.03%) من العينة B (82.43%).
اخيرا ،اكدت نتائج التقييم الحسي ان العينةA (1:10) حصلت علي قبول عالي من قبل المقيمين .

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CHAPTER ONE

Introduction

Piliostigmareticulatum (Carob) is a leguminous ever green shrub or small tree which grows wild in the tropics. One of common species *Piliostigma*(Hochst) is a branched tree up to 8 m high in the drier savanna areas of central Sudan in south Kordofan and south Darfur **(El-Amin,1990).** It is widely used in Africa as a traditional medicine for the treatment of a wide range of diseases including epilepsy, anxiety, and agitation. The leaf extract was found to have antimicrobial activity. In Sudan (Nuba mountains in particular), it is widely used to dress new wounds and as well puerperal sepsis. Moreover the fruit is eaten and used to prepare juice.However there is the little information or data published on the subject. Although carob iswidely speared in western of Sudan it is not very known in the other part. Moreover, carob has high nutritional value and anti-microbial and antioxidant activities.The purposes of this studywere to provide information on this subject and to assess suitability of using carob fruit powder to produce fresh juice with high nutritional value in order to improve industrial uses and facilitate the domestic consumption of these fruits in Sudan.

1.1 Specific Objectives:

The specific objectives of this research can be summarized under the following:

1. To study the nutritional value of carob fruits powder.
2. To examine the suitability of carob fruits for juice production.
3. To evaluate the chemical, physicochemical and organolepticproperties of the end product.

CHAPTER TWO

Literature Review

2.1 Classification of *Piliostigmareticulatum*(DC) Hochst

The tree is perennial in nature and its vernacularnames include Abefe, Monkey bread, Camel's foot, KalgoandOkpoatu and these vary according to locality**(Djuma, 2003).***P. reticulatum*has a close resemblance to *P. thonningii* to such an extent that theymay easily be confused with each other, although when examinedvery closely, distinguishing morphological features become readilyobservable.

Family: Caesalpiniaceae (Lequminosae – Caesalpinioideae)

Synonyms: Bauhinia reticulate DC, Bauhinia glabraA .chev, Bauhinia

Genus: *Piliostigma*

Species : . *reticulatum, thonningii*

Common name: Carob

2.2 Description of *Piliostigmareticulatum*tree

Every green shrub, occasionally a small tree with bush spherical crown,

few big knotty trees can be found. Bark dark grey to brown, fibrous corky,

slash dark red. Leaves grey- green, glabrous, 6 – 12 cm long, 4-8 cm

wide; Flowers dioeciou , clustered in short hairy racemes , axillary measuring 4-5 cm. Petals white with pink stripes. Fruit along,hard, straight, but mostly twisted pod, glabrous or weakly pubcent,ligneous, brownand indehiscent up to 25 cm long and 5 cm wide andpersisting, many seeded**(El-Amin, 1990).**

2.3 Origin and geographic distribution of *P.reticulatum tree*

*Piliostigmareticulatum*occur in the Sahel – Sudanian region from Senegal and Mauritania eastward to Sudan **(Baumer,1983).**There are two species in the Sudan *Piliostigmareticulatum*(DC) Hochst and *Piliostigmathonnigii*(Schum).Its occurs in the southern parts of center Sudan, running from east of south Kassala , south Blue Nile, south Kordafan , south Darfur (Jabel Mara) and extending southward Bahr Elghzal ,upper Nile and Equatorial . *Piliostigmareticulatum*(DC)Hochstmuch – branched trees up to 8m high, in the drier savanna areas of central Sudan in south Kordfan and south Darfur **(El-Amin,1990).**

2.4 Growth and development of *P. reticulatum*tree

The growth of *Piliostigmareticulatum*is recorded to be slow- flowering in the dry season. In drier areas *Piliostigmareticulatum*issemideciduous, losingmost of it is leaves at the end of the dry season but morehumid zones the leaves are often persistent. The plant resproutsafter thestem has been cut. Animals eating the fruits contribute to dispersal of the seeds **(Diack*et al*., 2000).**

2.5 Ecology of *P. reticulatum*tree

*Piliostigmareticulatum*occurs from sea level 2000m altitude in areas annual rain fall of (200-)400 – 1000 mm , mainly on heavy and poorly drained soils, but also on sandy soils. It is a pioneer species in woodland, wooded scrubland, wood grass land .Valleys and disturbed habitats such as cultivated fields fallows and roadsides**(Baumer, 1983).**

2.6 Propagation and planting of *P. reticulatum*tree

*Piliostigmareticulatum*propagated by seed, one kg contains 11000 to14500seeds. Germination is poor **(El-Amin, 1990).** Butcan be improved by soaking the seed successively in 90% H_2SO_4 for 30minutes and in water for 24 hours, or by soaking them in hot water overnight in98 % HCl for 90 minutes. Due to fast growth of the root system,seeding can be planted out already at 5-7 weeks after sowing.

2.7 Management of *P. reticulatum*tree

The tree is spared during land preparation and maintained inogroforestrysystem. The density of the species in parkland is very variable.

2.8 Harvesting of *P. reticulatum*tree

The bark is harvested by cutting the branches. It is hard to remove the bark when the stem is dry, for this reason it is recommended to harvest the bark as soon as the stem is cut. The bark is stripped off, dried and used as rope, mature fruits and

young leaves are directly harvested from the plant for various uses **(Baumer,1983).**

2.9 Handling after harvest of *P. reticulatum*tree

*Piliostigmareticulatum*is not explanted for industrial fiber production.

The park is directly collected from cut branches in the field; sometimes collectors take the whole stem to their houses before removing the bark.

The remaining wood is later use as fuel.

2.10 Genetic resources of *P. reticulatum*tree

*Piliostigmareticulatum*has a wide distribution and is common and locally abundant in its distribution area, it is not treated by genetic erosion.

2.11Prospects of *P.reticulatum*tree

*Piliostigmareticulatum*is a valuable multipurpose plant yielding a wide range of useful products. It is becoming Integration of*Piliostigmareticulatum*in the traditional agro forestry system in semi-arid countries is important for sustainable use of the species. Further research on the domestication potential of the species is worthwhile. The antimicrobialandanti- inflammatory properties warrant further research for pharmaceuticaluses, **(Baumer, 1983).**

2.12 Utilizatio of *P. reticulatum*tree

2.12.1 As food:

Theparts of *P. reticulatum*tree can be used as food by humans, animals and insects. However, humans eat the boiled pods and make drinks from it,animals eat the branches leaves and pound pods the termites and various insects eat the bark and wood **(El-Amin, 1990).**

2.12.2 As medicine:

The leaves cure cold-barks cure diarrhea- dysentery, ulcers, wounds and help on haemostatic to cure syphilitic cancer. the roots treat gonorrhea- and ascites and dropsy, roots decoction cure liver and gall complaints, antidote against plant poison , cure fever, cough, encephalitis poison, cure fever, cough, encephalitis **(Burkill,1995) .**

2.12.3 Other uses:

Piliostigmareticulatumis tree is also used in tying roof rafters producing articles (baskets, chair, mats, arrows, and masks), the wood for fire and tying up sheep and pigs to houses, the stems as toothbrushes **(VonMaydell, 1986).**

2.13 Fruit juice and drink:

Fruit juice and drinks are becoming very important in modern communities,because there are beyond its use as refreshing also contains several of minor ingredients, particularly vitamins and minerals, as well as carbohydrates, far

free and naturally occurring phytonutrients that contribute to the good health of the human being **(Frank *et al* .,2005and Ashurst,2005).**

2.13.1 Definitions

Fruit juice is the unfermented liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post-harvest surface treatment. Some juices may be processed with pips, seeds, and peel. A single juice is obtained from one kind of fruit. A mixed juice is obtained by blending two or more fruits juices and puree **(CODEX, 2005).** Also can be defined as the product from which water has been physically removed in an amount sufficient to increase the Brix level to a value at least 50% greater than the Brix value established for reconstituted juice from the same fruit. During the production of fruit juice that should be concentrated, suitable processes are used and may be combined with simultaneous diffusion of pulp cells or fruit pulp by water before the concentration process. Fruit juice concentrates may have restored aromatic substances and volatile flavor components, all of which must be obtained by suitable physical means, and all of which must be recovered from the same kind of fruit. Pulp and cells obtained by suitable physical means from the same kind of fruit may be added **(Babiker, 2013).**

A wide range of drinks can be made of using extracted fruit juice or fruit pulp as the base material. Many are drunk as the pure juice without addition of any other ingredients, but some are mixed with sugar syrup. The drinks are usually preserved by a combination of natural acidity, pasteurization and packaging in sealed containers. Some drinks (syrup and squashes) also contain a high concentration of sugar which helps to preserve them **(Azam, 2008).**

2.13.2Processing steps

2.13.2.1Juice extraction

The first stage is the extraction of juice or pulp from the fruit after selection of mature and undamaged fruits. The fruit juice may be obtained by diffusion in water. The solids content of the finished product shall meet the minimum Brix level.

2.13.2.2 Filtering

The extracted juice or pulp the filtered through a muslin cloth or a stainless steel filter that to make a clear juice. The juice of fruits is naturally cloudy; however some consumers prefer a clear product. It may be necessary to use pectin enzymes to break down the pectin so as to have a clear juice.

2.13.2.3 Preparation

Fruit would normally contain about 25% fruit material mixed with the sugar syrup to give a final product.The addition of sugar to the fruit pulp to achieve the

recommended levels for preservation must take in to a count the amount of sugar already present in the juice. The person square is useful tool to use with the batch formulation and to calculated the amount of sugar to be added for preservation.

2.13.2.4 Pasteurization

All the fruit juice and drinks need to be pasteurized at 80-95C°for 1-10 minutes prior to the hot filling.

2.13.2.5 Filling and bottling

The fruit juice and drinks products should be hot filled into clean, sterilized bottles. After filling hot, the bottles are capped and laid on their sides to cool prior to labeling.

2.13.3Quality aspect

The fruit juice should have the characteristic color, aroma and flavor of maintenance of the same kind of fruit from which it is made.

Authenticity is the maintenance of the product essential physical, chemical, organoleptical, and nutritional characteristic of the fruit from which it comes. Total soluble solids (T.S.S) contents are related directly to both the sugars and fruit acids as these are the main contributors. Pectin, glycosidic material and the salts of metals (sodium, potassium, calcium ect.) when present, will also register a small but insignificant influence on the solids figure **(Babiker, 2013).**

CHAPTER THREE

MATERIALS AND METHODS

3.1Materials

Carob fruits were obtained fromKadugli Central market in South KordofanState during the season 2019- 2020. The fruits were kept at room temperature until needed for the different investigations.

Gum Arabic, sugar and plastic containers were obtained from Omdurman local market. Sodium benzoate was obtained from college lab.

3.2 Methods

3.2.1. Chemical composition of carob fruit

The chemical compositions of carob fruit (moisture,protein, fat, fiber, ash) according to the methods mentioned below:

3.2.1.1 Moisture content

The moisture content was determined according to the standard method of the Association of Official Analytical Chemists (**AOAC, 2010**).

Five grams of sample 5g ±1mg was weighed into a pre - dried metal dish provided with a tight fit cover. The samples were placed into an oven (Model: Kat – NR. 2851, Electrohelios, Sweden) and left to dry at 105 ºC until a constant weight was obtained. After drying, the covered samples were transferred into a desiccator and cooled to room temperature before reweighing. The moisture content of the sample was calculated according to the following equation:

Moisture (%) = $\frac{(w2-w3)}{w2-m1}\times 100\%$

Where:

w_1 =weight of an empty dish + cover.

w_2 = weight of (dish + cover + sample), before drying

w_3 = weight of (dish + cover + sample), after drying

3.2.1.2 CrudeProteincontent

Sample crude protein was determined according to the standard method of the Association of Official Analytical Chemists **AOAC (2010)** using the macro-kjeldahl method.

A sample of 5.0g ± 1mg was transferred into a kjeldahl digestion flask together with 0.8 g of a catalyst mixture (96 % anhydrous Sodium sulphate, 3.5 % Copper sulphate and 0.5 % Selenium dioxide). Then, 20 ml of a concentrated H_2SO_4 was added to each sample. After that, the sample was placed into a kjeldahl digestion unit (Model: DS5. 50. Boh77 – Sweden) for 3 hours until a colourless digest was obtained. After cooling (at room temperature), the ammonia distillation was carried out by using a kjeldahl distillation unit into 30 ml boric acid (2 %) after addition 40 ml distilled water and 60 ml hydroxide solution (33 %) into each sample. Finally, the distillate was titrated against standard solution of 0.1 N HCl in the presence of 2 – 3 drops ofBromocreasolgreen and methyl red as an indicator, until a brown reddish colour was observed. Then, the crude protein of each sample was calculated from the following equation:

Crude protein (%) = $\frac{T\times N\times 1.4\times F}{1000\times \text{Sample weight}}$

Where:

T = Titre volume (ml) .

N = Normality of HCl.

1.4 = Each ml of 0.1 N HCl is equivalent to 1.4 mg nitrogen.

F =Protein conversion factor = 6.25 %.

3.2.1.3 Fatcontent

Total fat content was determined after extraction with Hexane for 16 hours in a Soxhelet device according to the standard method of the **AOAC (2010)**.

Five grams (5.0 ±1mg) from each sample were placed in a thimble covered by a piece of cotton and placed into a Soxhelet extraction tube. 50 ml of the hexane solvent (BP 60 -80 °C) were added into a pre-weighed Soxhelet flask and the flask was connected to the Soxhelet apparatus (Model: Electrothermal.Co.No.R/650l18, England). The extraction was carried out for 16 hours and after that the solvent was recovered from the oil. Finally, the samples were heated in an air oven at 105 $\pm$ 1 °C for 3.0 hours, cooled, reweighed and the oil content of each sample was calculated according to the following equation

$$\text{Fat content \%} = \frac{(W2-W1)\times 100\%}{\text{Sample weight(g)}}$$

Where:

W_1 = Weight of an empty flask.

W_2 = Weight of the flask with the extracted oil.

3.2.1.4 Crude fiber content

The crude fiber content was determined according to the standard method of the **AOAC (2010)**.

A sample of two grams (2.0 ±1mg) was accurately weighed and extracted with Hexane in Soxhelet extraction apparatus. Then, the air dried fat free sample was transferred into a fiber beaker connected with fiberic heat extractor system (Model: Laboconco – Stands - 83, England). The sample was digested with H_2SO_4 (1.25 %) for 30 minutes and washed twice with hot water, The digestion was also repeated for the same sample by using KOH (1.25 %) for 30 minutes and then washed twice with hot water. After that, the crucible with the digested sample was transferred into an oven (Model: LDO-03ON, Korea) at 105 $\pm$ 1 °C and left for overnight. Then, the sample was cooled, reweighed and ashed in a muffle furnace (No.20-3018870, Carbolite, England) at 550-600 °C until a constant weight was obtained. The crude fibre was calculated by using the following equation:

$$\text{Crude fibre \%} = \frac{\text{Loss in weight after sample ignition (g)} \times 100\%}{\text{Sample weight(g)}}$$

3.2.1.5 Total carbohydrates

Total carbohydrates were calculated by subtracting the sum of percentages of moisture, fat, protein, fiber and ash contents from 100% as described by **West *et al*. (1988).**

Total carbohydrates % = 100 - (Moisture + fat + protein + fiber+ ash)%

3.2.1.6 Available carbohydrates

The available carbohydrates were calculated by subtracting the fibre percentage from the percentage of total carbohydrates percentage as described by **West *et al*. (1988).**

Available carbohydrates% = Total carbohydrates (%) – Crude fibre (%)

3.2.1.7 Sugars determination

Total sugars, reducing and non- reducing sugars were determined following Shaffer-Somogyi method as described by the **AOAC (2010)**.

Principle: Reducing sugars in pure solution or in raw materials after suitable pre-treatment (to remove interference substances) may be estimated by using copper sulphate as oxidizing agent in standard Fehling's solutions.

3.2.1.7.1 Reducing sugars

A sample of five grams (5±1mg) was weighed and transferred to 250 ml beaker. Then, 50 ml water was added, boiled gently and left to cool to room temperature. After that, the sample solution was transferred to 250 ml volumetric flask and 2 ml of standard lead acetate was added with stirring and left to stand for 10 minutes at room temperature. Finally, the excess amount of lead acetate was precipitated by using an appropriate amount of potassium oxalate solution (22 %) and the solution was filtered and made up to volume.

3.2.1.7.2 Total sugars

From the previous sample solution, a volume of 50 ml was pipetted into a 250 ml conical flask and 5 g of citric acid and 50 ml water were added. Then, the mixture was boiled for 10 min to complete the inversion of sucrose and left to cool at room temperature. After that, the solution was transferred to 250 ml volumetric flask, neutralized with 1.0 N NaOH by using phenolphthalein as indicator and the sample was made up to volume.

Procedure: A sample of 5 ml was pipetted into a test tube. In the meantime, a volume of 5 ml Shaffer-Somogyi reagent [25 g anhydrous sodium carbonate + 25 g Rochelle salt + 75 ml copper sulphate solution + 20 g sodium bicarbonate + 5 g potassium iodide + 250 ml (0.1 N KIO_3)] was added and the sample was mixed well by swirling. In addition to that, a blank was prepared by using 5 ml water and 5 ml Shaffer-Somogyi reagent under the same conditions. The tubes were then capped with funnels and placed in boiling water for 15 minutes at 100 °C. After that, the tubes were removed carefully and cooled by running water for 4 min. Then, the funnels were removed and 2 ml of iodide-oxalate solution [2.5 g of each potassium iodide (KI) and potassium oxalate ($K_2\ C_2\ O_4$) were dissolved in water] was diluted to 100 ml and 3 ml $H_2\ SO_4$ (2.0 N) were added by the side of each tube without agitation. After that, the tubes were thoroughly mixed and left to stand in a cold water-bath for 5 minutes and mixed twice during that time. Finally, the solution was titrated with 0.005 N sodium thiosulphate ($Na_2\ S_2\ O_3$) standard solution using starch as an indicator.

The titre volume of the sample solution was subtracted from the blank volume and the amount of dextrose in 5 ml sample solution was determined from Shaffer-Somogyi dextrose-thiosulphate equivalent table.

Calculation:

$$\text{Reducing sugars (\%)} = \frac{\text{mg of dextrose} \times \text{Volume made up} \times 100\%}{\text{Titre} \times \text{Sample weight(g)} \times 100}$$

$$\text{Total sugars (\%)} = \frac{\text{mg of dextrose} \times \text{dilution} \times 100\%}{\text{Titre} \times \text{Sample weight(g)} \times 100}$$

Non-reducing sugars (%) = Total sugars (%) - Reducing sugars (%)

3.2.1.8 Determination of vitamin- C

Vitamin- C (Ascorbic acid) was determined according to**Pearson (1982)**.

Procedure: Ten grams (10g ±1mg) sample were made up to 100 ml in a graduated flask with 0.4% oxalic acid solution and blended for 2 min in a Warring blender. The blend mixture was made up to 500 ml in a volumetric flask with 0.4 % oxalic acid solution and filtered.

Twenty milliliters from the filtrate were titrated against a standard solution (0.05 %) of 2, 6- dichloroohenol indophenol until a faint pink colour was observed and considered as an end point.

Then, a sample of 0.05g pure ascorbic acid was dissolved in 60 ml of 20% metaphosphoric acid and diluted to 250 ml. Ten milliliters ascorbic acid solution were titrated against the standard 2,6 dichloroohenol- indophenol solution until a faint pink colour (persisted for 15 seconds) was observed. The result was expressed as mg / 100 g ascorbic acid according to the following equation:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre (ml) x dye strength x 100}}{\text{Factor}}$$

Where:

Factor = $\frac{\text{Sample weight (g) x sample taken for titration (ml)}}{\text{Total volume of the sample (ml)}}$

Dye strength = $\frac{1}{titre}$

3.2.2.9Titrable acidity

The total titrable acidity was conveniently determined according to **Ranganna (2001).**

Procedure: Ten grams (10g±1mg) of sample were added to150 ml of distilled water, stirred for 15 min and filtered using Whatman No. (4) filter paper. Ten milliliters from the prepared sample was titrated against 0.1 N NaOH in the presence of 1-2 drops of 1% phenolphthalein as an indicator, until a pink colour was obtained. The titrable acidity was calculated as percent citric acid according to the following equation.

Titrable acidity (%) = $\frac{\text{Titre (ml)x N (NaOH)x dilution factor x equivalent weight x 100\%}}{\text{Weight of sample (g)}\times\text{Sample Volume}\times 1000}$

Where:

N = Normality of NaOH

Equivalent weight of citric / malice acid

3.2.1.10 Ashcontent

The ash content was determined according to the **AOAC (2010)** method. A sample of five grams (5.0±1mg) was placed in an ash crucible and dried at 105±1 ºC for three (3) hrs. Then, the crucibles were placed into a muffle furnace (Mode: Carbolite – Bam ford S 30 2 AU, Sheffield, England) at 600 ºC and left until a

white ash with a constant weight was obtained. The weight of the residue after ashing was defined as ash content and expressed as a percentage based on the fresh weight of the sample.

Calculation:

$$\text{Ash \%} = \frac{\text{sample residue weight after ashing (g) x 100 \%}}{\text{Sample weight (g)}}$$

3.2.1.11 Food caloric value

Thecaloric value of the different samples was calculated based on Atwater factors for protein, fat and available carbohydrates as indicated by **Leung (1968).**

Protein = 3.87 K. cal/ g

Fat = 8.37 K. cal/ g

Carbohydrate = 4.12 K.cal/ g

3.2.2 Physical methods

Fruits diameter and length were determined by using stainless Virnier calipers (Model: E H B Stainless, Hardened, Germany), while the whole fruits, edible parts, peels and stones were weighed by using a top loading balance (Model: D0001-H R120, A&D Company, Limited E C).

3.2.2.1 Total soluble solids (TSS)

Total soluble solids (T SS) of fruits paste were measured with a Hand-type Refractometer (No.002603, BS Eclipse, UK) (0-50% °Brix) at 25º C and were expressed as percentage or degree Brix **(AOAC, 2010)**.

3.2.2.2 Hydrogen ions concentration

The hydrogen ions concentration**(** pH value) of the different samples were measured with a glass electrode pH- meter (Model: HANNA Instrument 8521 Portugal) at (20 º C). Five grams from the sample were diluted with 50 ml distilled water, and then it was filtered using a Whatman No. (1) Filter paper before determining the pH **(Egan *et al*., 1981).**

3.2.3 Carob juice extraction methods

1. weight the sample
2. Soaked in hot distilled water (100 ◦C) but for only two hours (2hrs) with fruits: water ratio of 1:8 and 1:10 (w/v).
3. The mixtures were blended for 5 min using a magnetic stirrer,strainer and thenfiltered.
4. Weighed and checked for its hydrogen ions concentration (pH), volume (ml), weight(g) , total soluble solids (T.S.S%) and yield (%).
5. Placed in stainless kettle and heated (100˚C) for 15min.

6. After that, sugar, gum Arabic 3% and sodium benzoate as preservative with immediately added. the mixture was boiled for 5min
7. Finally,fruits drink was filled in acleaned sterilized plastic container, tightly closed, cooled and stored at 4°C until needed for the different investigation.

Table (1) Carob fruit juice recipe

Ingredients	Sample A (g)	%	Sample B (g)	%
Carob fruit	500	21.27	500	29.41
Sugar	346	15	240	15%
Gum Arabic	70.5	3	51.1	3%
Sodium benzoate	0.58	0.25	0.43	0.25
Water	5000	60.48	4000	52.34

3.2.4 Organoleptic evaluation method

The organoleptic properties of carob natural juice were evaluated using hedonic scoring test method as described by **Watts *et al*. (1989).** In this method, 29 trained panelists from Sudan university of sciences and technology (college of agricultural studies) were asked to evaluate the products with regard to their color, flavor, appearance and overall acceptability using the following hedonic scale,1=unacceptable, 2=acceptable, 3=good 4=very good, 5=excellent.

3.2.5 Statistical analysis method:

The results were subjected to Statistical Analysis System (SAS) by using One-Factor Analysis of Variance (ANOVA). The Mean values were also tested and separated by using Duncan's Multiple Range Test (DMRT) as described by Steel *et al*. (1997).

CHAPTER ROUR

RESULTS AND DISCUSSION

4.1. Chemical composition of carob fruit powder

Table (2) shows the chemical composition of carob fruits powder on wet and dry basis. The moisture content, protein, fiber, ash, lipid, vitamin C and total sugar were found to be 11.43, 8.8, 11.83, 2.88, 2.34, 177.64, and 62.7, respectively on dry basis. There is no previous study carried out for the same generous or species.

4.2 Physical and physico-chemical characteristics of Carob fruits extract

The Physical and physic-chemical characteristics of Carob fruits crude extractwere shown in table (3).

4.3 Chemical composition of carob juices

Table(4) shows the chemical composition for carob fruit juices. sample A exhibited 84.03, 1.21, 0.28, 0.57, 2.14, 17.6, and 13.9% for moisture content, protein, ash, fiber, lipid, vitamin C, and total sugar; respectively. While sample B showed 82.43, 1.58, 0.42, 0.71, 2.15, 25.6 and 14.8% for moisture content, protein, ash, fiber, lipid, vitamin C, and total sugar; respectively. The results indicated that, there was no significant difference ($p<0.05$) between sample A and B for fiber, protein, ash, T.S.S, acidity and pH. On the other hand the two samples were revealed a significant difference ($p>0.05$) in moisture content, total sugar, reducing sugar, T.S.S, vitamin C, and sucrose. However, sample A has higher moisture content (84.03%) than sample B (82.43%). While sample B showed higher contents for total sugar, reducing sugar, T.S.S, vitamin C, and sucrose compared to sample A. These variations might be due addition of difference quantities of water to the difference sample which indicating different concentrations.

When comparing the results of chemical compositions for carob fruit powder and carob juice sample (A), it was found that there was declining on the chemical compositions concentrations of carob fruit powder compared to the juice. However, vitamin C concentration was dropped from 177.64 in carob fruit powder to 17.60 % in carob juice. Moreover, fiber content of the powder while decreased from 11.83 to 0.54 in the juice. These differences may be due to effect of processing method

4.4 Physico-chemical properties of carob juices

Table (5) represents the results for the physic-chemical properties of carob juices. The results showed that there is significant differences ($p<0.05$) between two carob juice concentrates.

Table (2) Chemical composition of carob fruits powder

Parameter	% on Wet basis
Moisture	11.34±0.20
Crude Protein	8.80±0.16
Crude Fat	11.83±0.73
Crude Fiber	2.88±1.3
Ash	2.34±0.14
Vitamin C	177.64±9.53
Total sugar	62.81±1.58

These values are means ± standard deviation

Table (3): Physical and physico-chemical characteristics of Carob fruits extract

Parameter	Values A	Values B
Carob fruit weight	500(g)	500(g)
Extract volume	2350(ml)	1700(ml)
Total soluble solids	2.2%	1.5%
pH	4.21	4.1

A=Carob Fruit Juice: water ratios (1:10)

B=Carob Fruit Juice: water ratios (1:8)

Table (4): Chemical composition and energy value of Carob fruit Juice

Parameter	**A**	**B**
Moisture	$84.03^{a} \pm 0.02$	$82.43^{b} \pm 0.20$
Protein	$1.21^{a} \pm 0.09$	$1.58^{a} \pm 0.4$
Fat	$2.14^{a} \pm 0.18$	$2.15^{a} \pm 0.06$
Ash	$0.28^{a} \pm 0.02$	$0.42^{a} \pm 0.07$
Total sugar	$13.9^{b} \pm 0.22$	$14.8^{a} \pm 0.22$
Reducing sugars	$2.3^{b} \pm 0.34$	$3.3^{a} \pm 0.33$
Sucrose	$11.4^{b} \pm 0.28$	$12.5^{a} \pm 0.56$
Total carbohydrates	$11.8^{b} \pm 0.46$	$12.76^{a} \pm 0.1$
Acidity	$0.65^{a} \pm 0.03$	$0.54^{a} \pm 0.04$
Vitamin C	$17.6^{b} \pm 0.87$	$25.6^{a} \pm 1.9$
Caloric value	455.8^{a}Cal/g	436.3^{b}Cal/g

These values are means ± standard deviation.Values in same row with different superscript letters are significantly different.

A=Carob Fruit Juice: water ratios (1:10)

B=Carob Fruit Juice: water ratios (1:8)

Table: (5) Physico-chemical properties of carob juice

Parameter	A	B
Hydrogen Ion concentration(pH)	$4.4^{b} \pm 0.07$	$4.6^{a} \pm 0.05$
Total Soluble solids%	$14.9^{b} \pm 0.76$	$17.2^{a} \pm 0.12$

These values are mean ±Standard deviation. Values in same row with different superscript letters are significantly different.

A=Carob Fruit Juice: water ratios (1:10)

B=Carob Fruit Juice: water ratios (1:8)

4.5. Organoleptic evaluation:

The resultsof organoleptic properties were mentioned in table (6).In general, both of carob juice concentrates were highly accepted by the panelists. On the other hand,there is significant differencein the flavor between the two products.

Table (6) organoleptic evaluation of carob fruit juice

Quality characteristics				
Sample	**Color**	**Flavor**	**Taste**	**Overall acceptability**
A	$4.32^{a} \pm 0.92$	$3.48^{b} \pm 0.78$	$4.06^{a} \pm 0.96$	$4.03^{a} \pm 1.01$
B	$4.58^{a} \pm 0.78$	$4.10^{a} \pm 0.97$	$4.20^{a} \pm 0.72$	$4.41^{a} \pm 0.68$
CV%	19.29	23.30	20.50	20.50
SE±	0.22	0.23	0.22	0.22
$LSD_{0.05}$	0.45^{NS}	0.46^{*}	0.44^{NS}	0.45^{NS}

Mean ±S.D value(s) bearing different superscript letter(s) within columns are significantly different ($p \leq 0.05$).
A=Carob Fruit Juice: water ratios (1:10)
B= Carob Fruit Juice: water ratios (1:8)

Scale:
1= unacceptable
2= acceptable
3= good
4=very good
5= excellent

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:

From the result obtained in this study it be can concluded that carob fruithas high level of vitaminC and energy, and issuitable for production juice with high acceptabilityby panelists.

5.2 Recommendations:

1. Further studies are needed to improve the industrial uses of Carob fruit so it can be used in different product.
2. More researches should be done to optimize methods for carob fruit juice extract.
3. More studies about the chemical and bioactive compounds are required.
4. Additional studies are definitely needed to ensure safety, storage conditions,and economic feasibility for the product.

REFRENCES

Ashurst, P.R. (2005). Chemistry and Technology of Soft Drink and Fruit Juices, SeconondEdn., Blackwell Publishing Ltd, London , UK.

AOAC (2010). Association of Official Analytical Chemists, Official Methods of analysis 18th ed. AOAC International Washington, DC, 2010.

Azam, A. (2008). Fruit juice processing. The Schumacher Center for Technology and Development, Bourton-on-Dunsmore Rugby, Warwyckshir, CV23 9QZ, UK..

Babiker, H. A. (2013). Nutritional Value of Doum Fruits (Hyphaene thebica) and their Suitable for Production of Ready-to-use Concentrated Drink, Department of Food Sci. and Tech., College of Agricultural Studies Sudan Univ. and Tech. of Sciences and Technology, Sudan.

Baumer, M.(1983). Notes on *Trees and Shrubs in arid and Semi- arid regions.*Ecological management, of arid and semi – arid rangeland in Africa and the Nearand Middle East (EMASAR) – Phase2. FAO, Bome , Italy. 270pp.

Burkill . H .M. (1995) . "*The useful plants of west tropical Africa" . 2nd Edition*.Volume 3 , Families J- L. Royal Botanic Gardens , kew , Richmond , UnitedKingdom .857pp.

Diack ,M, Sene ,M ., Badiane ,A.N ., Diatta, M. and Dick, R.P.,(2000). Decomposition of native shrub ,*Piliostigmareticulatum*,Litter in soils ofsemiarid Senegal . Arid Land Research and Management **14** (3) : 205- 218.

Djuma, 2003.Djuma Game Reserve Copyright(C) 1998–2003. Drummond, A.J., Waigh, R.D., 2000. In:S.G. Pandalai. Recent Research Developmentsin Phytochemistry, vol. 4. Research Signpost, India, pp. 143–152.

Egan , H ; Kirk , R .S . and Swager, R (1981). Pearson's Chemical Analysis of Food 18th edition Longman Science and Technology Group . W. K.

El-Amin, H. M. (1990): pTrees and shrubs of the Sudan. Ithaca press Execer.160-163.

Leung, W. T. (1968). Hand book on Food Composition for Use in Africa.

FAO, Rome and Wasing,D.C.,USA,P.23.

Neuwinger , H.D ., (2000) . African traditional medicine: a dictionary of plant useand application s .MedpharmScientific , Stuttgart, Germany .589 pp.

Pearson , D .(1982) . The Chemical Analysis of food. J. A. Church Livigston Hill, 104 Gloucester Place. London.

Ramganna, S .(2001) . Manual of Analysis of Fruits and Vegetables Products pp 7-15 . Tata McGrawm- Hill Publishing Company Limited. New Delhi.

Steel, R. D. G; Torrie, T. H. and Dickey, D.A. (1997). Principles and procedures of Statistics; In a Biometrical Approach. 3rd ed; published by McGraw-Hill, New York, USA.

Von Maydell, H. (1986). Trees and shrubs of the Sahel: their characteristics and uses .Schriftenreihe der GTZ No 196. Deutsche Gesellschaft fur TechnischeZusammenarbeit ,Eschborn , Germany .**525** pp.

West, C. E.(1988) . The Composition of foods Commonly Eaten in East Africa . Department of Human Nutrition. Wageningen Agricultural University.

Appendices

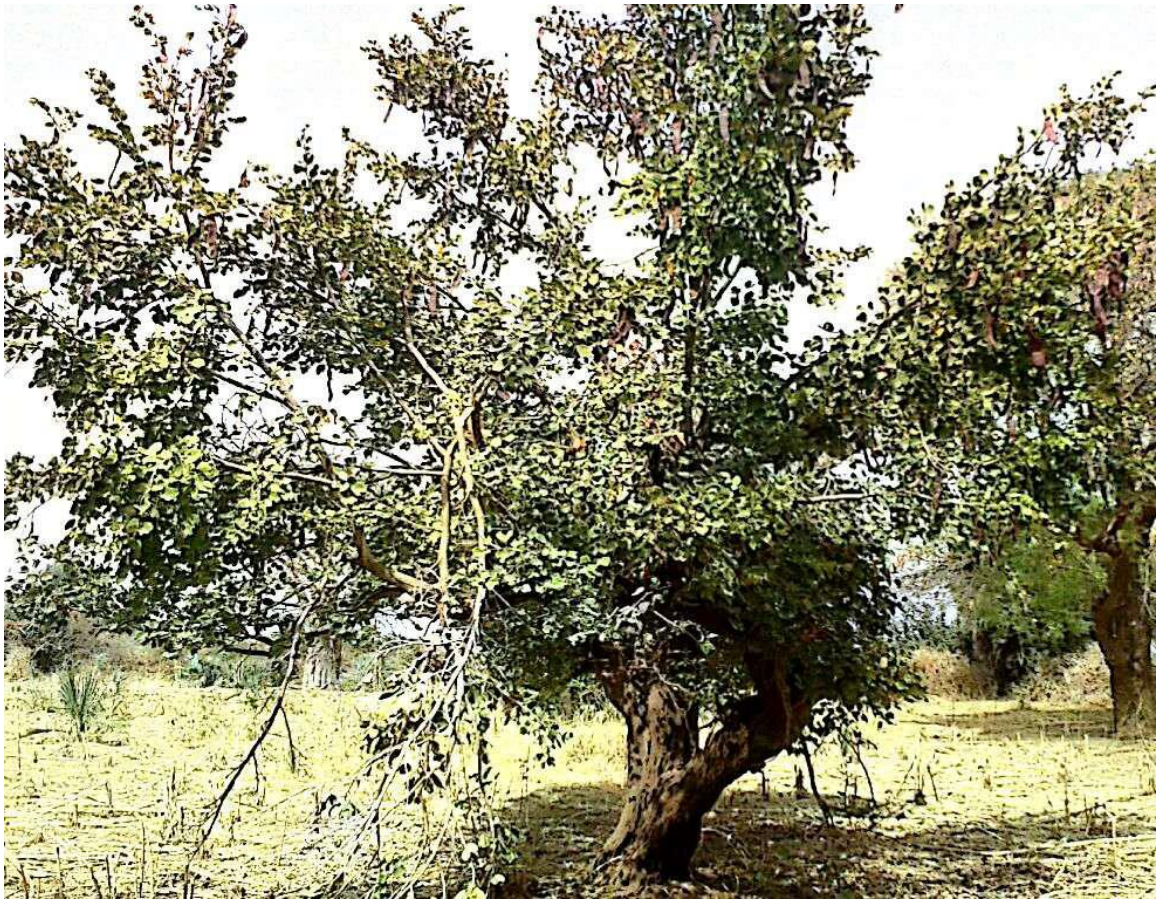

Figure (1):*Piliostigma reticulatum* tree

Figure (2) :*Piliostigma reticulatum* branch with fruit pods

Figure (3):*P.reticulatum*seeds

Figure (4): Sample A

Figure (5): Sample B