A SEMINAR PAPER ON

Physiochemical, microbial and functional characteristics of Palmyra palm sap and pulp powder

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Physiochemical, microbial and functional characteristics of Palmyra palm sap and pulp powder[1]

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ABSTRACT

This study investigated the physicochemical functional microbial properties of Palmyra palm sap and pulp powder. Thus the fruits were procured from local market and several analyses such as ash, moisture, pH, protein, fat, total sugars, carbohydrate, starch, energy value, calcium, vitamin C using common scientific methods of food characteristics determination were performed. The results showed that the pH range between 5.5 to 6, TSS of the fresh pulp is 16.50 Brix, moisture content is 74-77%,ash content is 1.2 g, carbohydrate content is 22.5g, caloric value (energy) is 102.83 kcal/100g, reducing and non reducing sugars content is 9.5g and 13g, starch is 12.6g,Maltose is 0.5g, Ascorbic acid is 16mg and calcium is 8.76 mg. The water absorption capacity of the palmyra pulp powder is 18 % (2.5 ml/g), The fat absorption capacity was found to be 2.8 % , bulk density of the sample obtained from the study is 0.78 gcm-3, the powder has swelling power value of 4 and the percent foam capacity is about 2.5 %. Two dominant yeast species identified in the palm sap was *Saccharomyces cerevisiae*, and *Lachancea fermentati*, and dominant Lactic acid bacteria are *Leuconostoc mesenteroides* and *Fructobacillus fructosus* .

Keywords: Palmyra Palm; Fruit pulp; Physic chemical; microbial functional properties

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List of Abbreviations

a*	Redness and greenness
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
b*	yellowness and blueness
BSMRAU	Bangabandhu Sheikh Mujibur Rahman Agricultural University
BARI	Bangladesh Agricultural Research Institute
BARC	Bangladesh Agricultural Research Council
Cfu	colony-forming unit
DPPH	2,2-diphenyl-1-picrylhydrazyl
L*	lightness
HMF	Hydroxy Methyl Furfuraldehyde
mm	millimeter
M&Y	molds and yeasts
g	gram
w/w	weight by weight
w/v	weight by volume
%	percentage
µg/ml	microgram / ml
O.D	Optical density
I.e	latin phrase meaning "that is"
3,5-DNS	3,5-Dinitrosalicylic acid
PCA	Principal component analysis
TVC	Total Viable Count
YEPDA	Yeast Extract Potato Dextrose Agar

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CHAPTER I

INTODUCTION

The palmyra palm(*Borassus flabellifer L*) tree is a dioecious plant that grows indigenously in Bangladesh,India, Sri Lanka, Malaysia, Philippines etc. (Morton, 1988). The coconut like fruits are three sided when young, becoming rounded or more or less oval, 12-15 cm wide, and capped at the base with overlapping sepals (Morton, 1988).Palmyra has a great economic importance and every part of the palm is use full in one way or the other more than 88% of the Palmyra is used for the welfare of the people, it serves as food (fruit, sap, young shoots) as a building material (the stem, the leaves) It is also used in the pharmacopoeia (roots, male inflorescence) and the leaves are widely used to make variety of objects, brooms, baskets, fences and roofs palm wine extracted form Palmyra plays an importation role in the diet. Fruits mature during august and the ripe fruits fall from the palm during September and October. Each female palm may bear 10-20 bunches of about 200-300 fruits per year. When the fruit is very young, and the top of the fruit is cut off, you find usually three sockets inside and these contain he kernel which is soft as jelly, and translucent like ice, and is accompanied by a watery sweetish liquid. The mature fruit is usually tossed burning fire or embers to cook them mildly and skin is peeled off to expose the juicy fruit. This is squeezed and the pulp removed. The pulp in itself is sweet and creamy and is delicious to eat. The pulp is usually sucked directly from the fibres of the fruit. The fresh pulp is reportedly rich in vitamins A and C (Islam *et al., 2013).* Palmyra fruit pulp could be commercially utilized to produce food items and animal feed.

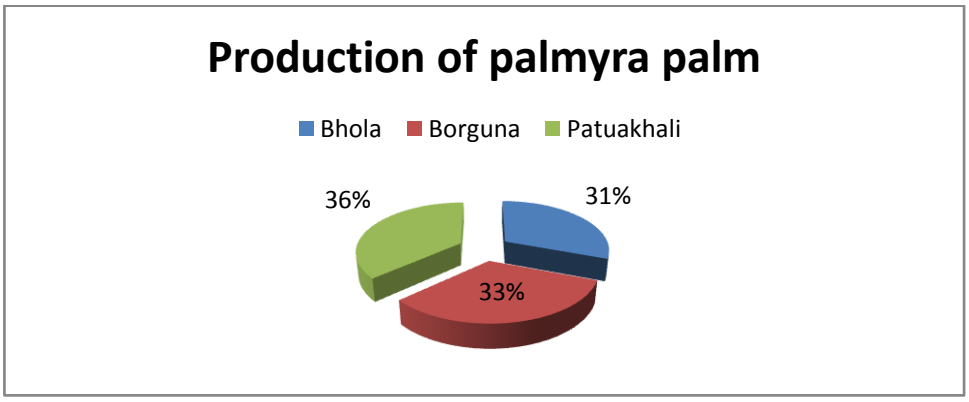

Figure 1: Production of Palmyra palm in Bangladesh (Source: Islam *et al., 2013)*

The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap, which was trapped from the flower part, has been used as a sweetener for diabetic patients (Masayuki et al., 2007). The different parts of the plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. It has anti-inflammatory effects (Nadkarni, 1954; Vaidyaratnam, 1994; Kapoor)

The whole fruit contains about 40% of undiluted pulp which is dark yellow in colour with its characteristic flavor and bitterness. The juice contain sugars, proteins, lipids, vitamin A, B-complex, vitamin C and others minerals (Barhet al., 2008). It has also been reported to possess immunosuppressant properties (Revesz et al., 1999).

The pulp is extracted manually with water. Palmyrah pulp is mixed with other fruits for making jam, cordial, cream etc. since its pulp is bitter in taste, it is better to prepare mixed fruit jam rather than Palmyra jam separately. To prepare cordials, citric acid is added to its diluted pulp and boiled. Well boiled cordial is bottled in white or amber colored bottles after adding approved food preservative (Masayuki et al., 2007).

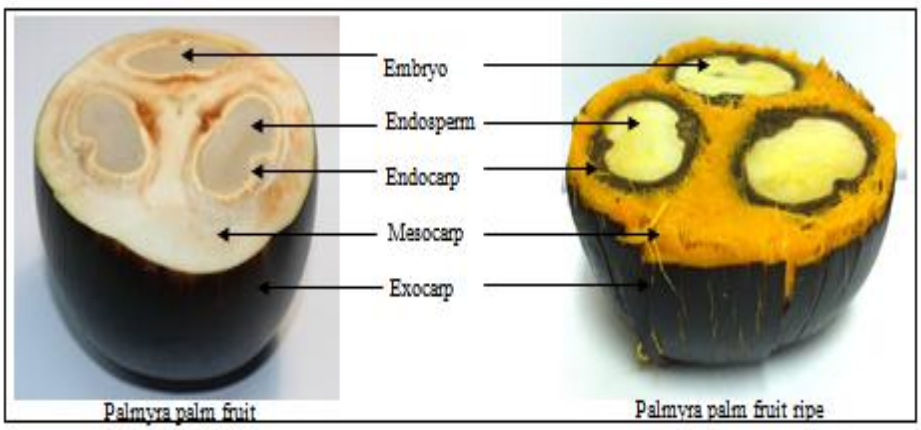

Figure 2. The palmyra palm fruit young (left) and palmyra palm fruit ripe (right)

(Source: Islam *et al., 2013)*

Nutritionally rich palm sap is very good source of medium for the microorganisms to grow. Freshly tapped sweet palm sap contains 11.36% (w/v) of Total sugar, 0.96% (w/v) of reducing sugar, 0.35% (w/v) of protein, 0.056% (w/v) of nitrogen, 0.14% (w/v) of phosphorus, 0.54% (w/v) of mineral ash, 0.4% (w/v) of iron, 13.25% (w/v) of Vit-amin C, 3.9 IU of Vitamin B1 and pH of 7.25(Masayuki et al., 2007).. Even though reducing sugar is found in traces in fresh palm sap, rapid fer-mentation by microbes hydrolyses half of sucrose to glucose and fructose within 24 hours resulting increase in the reducing sugar and production of lactic acid of 0.05-4.78% (w/v) and acetic acid of 0.01-0.24% (w/v) along with the ethanol of 0.21- 5.28% (w/v) reduces the pH of the palm sap to about 5, might makes

palm sap unacceptable to consumers (Barhet al., 2008).Microorganisms like *Schizosacharomyces pombe*, *Saccharomyces cerevisiae*, *Saccaromyces chevalieri*, *Debaryomyces hansenii*, *Geotrichum lactis*, *Zygosaccharomyces rouxiiin, Kloeckra apiculata, Bacillus cereus*, *Bacillus Sphaericus, Leuconostoc palmae*, *Fructobacillus fructosus* and *Bacillus firmu*s were isolated in Palm (Barhet al., 2008). Although utilization palmyrah fruit pulp is extensive, the literature on physicochemical functional and microbial properties is very limited.

Hence this study was carried out to know the essential information of produce and fruit pulp for emergent of value added products.

SPECIFIC OBJECTIBES

- To Review the physico-chemical and functional properties of Palm fruit pulp and sap
- To highlight the process for enzymatic extraction of juice from Palm fruit.
- To know microbial properties of Palmyra Palm sap and powder.

CHAPTER- II

MATERIALS AND METHODS

This seminar paper is exclusively a review paper so all of the data and information has been collected from the secondary sources. During preparation of this paper I went through various relevant books, journals, proceedings, reports, publications etc. Findings related to my topic have been reviewed with the help of the library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) and Bangladesh Agricultural Research Institute (BARI). Information also collected from Bangladesh Agricultural Research Council (BARC). I have also searched related internet web sites to collect information. I got valuable suggestion and information from my major professor and course instructors. After collecting all the available information, I myself compiled and prepared this seminar paper.

Chapter III

Review of Findings and Discussion

3.1 Juice extraction Process:

Enzyme Assisted Extraction

Polysaccharides: ***Cellulose, Hemicelluloses, Pectin*** etc. present in cell walls of fruits and vegetables, These are responsible for ***Haziness*** of the juice. ***Clarification is needed.*** Enzymes: ***Hemicellulase, Pectinase, Cellulase*** helps in degrading the cell wall materials.

Many advantages of using enzymes are

- Increase in yield
- Clear juice
- Minimum waste
- Better taste and flavor
- Better storability
- Improves quality

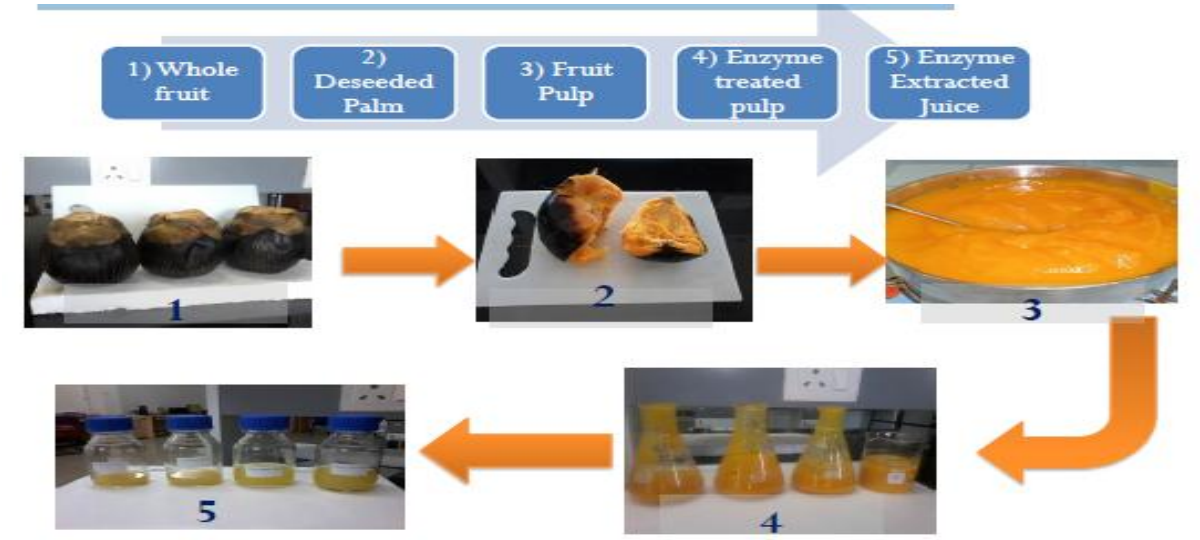

Fig 3: Enzymatic Extraction of palmyra palm

(Source: Ogbuagu A. S. *et al., 2014)*

Table 1: Nutritive value of palm fruit per 100 g of raw ingredient

Nutrition	Amount
Water	77 g
Protein	1 g
Fat	0.8 g
Carbohydrates	21 g
Fiber	2 g
Calcium	8.76 mg
Phosphorus	33 mg
Thiamine	0.04 mg
Riboflavin	0.02 mg
Niacin	0.3 mg
Vitamin C	5 mg
Energy	102.83kcal

(Source: Ogbuagu A. S. *et al., 2014)*

3.2 Palm sap and its quality

The most important product of palmyra palm is the sap or juice. The tapping process involves the staining of the core of the developing inflorescences by means of a made of wood mallet or tong, thereby stimulating sap flow. Sap is collected by cutting the inflorescences grown with a very sharp sickle or knife at the apex of the palm tree. Sap collector cut the outer end of the inflorescence for collecting sap.

The chemical composition of palm sap is mentioned in the Table 2.

Table 2. Chemical Composition of palm sap

Qualities	**Values[1]**	**Values [2]**	**Values [3]**	**Values [4]**	**Values [5]**	**Values [6]**
pH	5.09	7.55	4.69	5.76	5.63	5.60
Total soluble solid (^{o}Brix)	13.80	13.50	13.93	11.20	12.67	16.00
Total sugar (%,w/w)	12.34	13.48	11.54	10.91	11.72	15.82
Reducing sugar (%,w/w)	-	-	0.78	0.67	0.44	1.79
Total acidity (%,w/v)	0.036	0.068	0.098	0.032	0.051	0.022
Total solid (%,w/w)	-	-	-	-	1.27	-
Protein (%,w/w)	0.37	-	-	-	-	-
Ash (%,w/w)	1.04	-	-	-	-	-
Moisture (%,w/w)	84.47	-	-	-	-	-
Vitamin C (mg/ml)	0.084	-	-	-	-	-

{Source: [1] Jamfa (2002) [2] Jitbunjerdkul (1989)[3] Jitbunjerdkul (1989) [4] Tiapaiboon (2004) [5] Loetkitsomboon (2004) [6] Chantachum (2004) }

3.3 Physico-chemical Charecteristics of pulp from palmyrah

The ripped fruits data showed that 74% of them were large 3 seeded fruits with weight ranging from 450 g to 2200 g and a mean weight of 950 g of the residual 18% were two seeded fruits and 8% were sole seeded fruits were around 300 g in mass. In this study the average weight of palmyrah a seed was found to be 214 g and the average pulp weight per fruit was about 350 g ripe fruits and their seeds are used on a fairly large and profitable scale.

Moisture: The study was done by AOAC methods, about 10 g of the material is weighed (M1) into porcelain crucible and placed in an oven at 100-105°C and collected in a desiccators. The process of heating and cooling is repeated till a constant weight is achieved (M2) the moisture percentage is given in Equation 1.

$$\text{Moisture (\% wet basis)} = \frac{(M2-M1)\times 100}{M1} \qquad (1)$$

Moisture provides a measure of the water content of the pulp and for that matter its total solid content. It is also an index of storage stability of the pulp. The moisture content of the fresh pulp was 74.77% (Table 1). The lower the moisture content, the better its shelf stability and hence pulp should be dried for storage.

Protein by lowry method: This method was determined by Lowry et al. the lowry folin-ciocalteu (FC) reagents enables the determination of phenolic groups of tyrosine the fresh pulp sample was extracted with buffer and pipette out 0.1 to 0.2 ml of sample extract and make up to 1 ml with water. Addition of 5 ml of Alkalin copper and incubate for 10 min then addition of 0.5 ml of FC regent and incubate for 30 min read the O.D at 660 nm. The working standard proteins were prepared with 200 µg/ml.

The per cent crude protein of the pulp was 1.236% (Table 1). The value obtained was however lower than that obtained by Sankaralingam et al. The difference observed may be contributed by varietal differences, maturation of the seeds and environmental conditions.

Lipid extraction: Lipids were extracted according to the method using soxhlet apparatus . A mass M of each dry sample was weighed and introduced in to a previously weighed wattman cartridge. A cotton swab is then placed on top of the cartridge to prevent the rise during heating soxhlet apparatus and extracted with anhydrous ether for about 16 hr. The ether extract is filtered in to a weighed conical flask (M1) after an extraction time the solvent was evaporated on a rotary evaporator. The flask is dried in a desiccators for 2 hours and then weighed with the fat (M2). The fat content is given by the equation 2

$$T = \frac{(M2-M1)\times 100}{M1} \qquad (2)$$

Crude fat

The fat content of the Palmyrah fresh pulp was 0.8% (Table 3). This value is comparatively high when compared to other pulps and similar to that observed by Sankaralingam et al.

Ash: The ash content was determined by AOAC method. About 5 g of pulp powder was calcified in a muffle furnace at 450°C during for 3-6 hr. The residue was weigh and converted to a percentage of ash.

Crude ash

The per cent ash content of the pulp was 1.20% (Table 1). The ash content is the organic residue remaining after the organic matter has been burnt away. It is not necessarily of exactly the same composition as the mineral matter present in the original pulp as there may be losses due to volatilization or some interactions between constituents.

Carbohydrate: The carbohydrate content was calculated by difference method.

I.e.=100 - (m.c%+fat%+ protein%+ash%)

The major component of the pulp was carbohydrate. The value obtained from the study was 22.5% on fresh pulp and it shows higher carbohydrate content (Table 3).

Identification of carbohydrates: The qualitative tests were performed to identify the carbohydrates i.e Molish test, Fehcing's test, Bendicts test, Seliwanofii's test, Barfoed's test, Bia's test, Inversion test and osazone test, to identifying the Amino Acids i.e Ninhydrin test, Xanthoprotic test, Millons test, Hopkins cole test, Paulys test, Selivons test, Ehrlichs test, Nitrotrusside tests were performed.

Total sugars: Fresh pulp of 100 mg was hydrolyzed by keeping it in a boiling water bath for 3 hr with 5 ml of 2.5 n HCL of cool to room temperature. Then neutralized it with solid sodium carbonate and make up the vol to 100 ml of centrifuge. Then collection of the supernatant and taking of 0.5 ml of 1 ml of aliquots for analysis. Standards was prepared by taking '0' as blank and 0.2 to 1 ml of working standard glucose (0.1 mg/ml) make up the volume to 1 ml by adding water. Then addion 4 ml of anthrone reagent heat for 8 min in a boiling water bath. By Cooling rapidly and reding the green to dark green color at 630 mm.

Reducing sugars using 3,5 DNS method: 100 mg of fresh pulp sample was extracted with 80% alchohol pipette out 0.5 to 3 ml of sample of make upto 3 ml with H_2O. Then addition of 3 ml of DNS and heat for 5 min. The addition 1 ml of 40% Rochelle salt solution and cooling the tubes and take OD at 540 mm using spectro photometer. The standard curve obtained with 1 mg/ml of glucose is used to determine the concentration of reducing sugars sample.

9.5g Reducing sugar was prevent in the sample (Table 3)

Table 3: Physico-chemical composition of pulp from Palmyra.

Parameter	Values for 100 g
Moisture	74-77%
Ash	1.2g
Fat	0.8g
Total Carbohydrates	22.5 g
Reducing sugar	9.5 g
Non Reducing Sugar	13g
Starch	12.6g
Maltose	0.5 g
Protein	1.24g
Ascorbic acid	16 mg
Calcium	8.76 mg
Energy	102.83 k.cal

(Source: Sankaralingam et al.., *2010)*

Starch by anthrone method : The fresh pulp taster was extracted with 80% hot ethanol by addition of 5.0 ml of H_2O and 6.5 ml 52% perchloric acid kept at 0°C for 20 min and centrifuge save the supernatant repeat the extraction using fresh perchloric acid. Pipette out 0.1 or 0.2 ml of supernatant and make upto 1 ml with water. The standard glucose sample are prepared with the concentration of 0.1 mg/ml. Then add 4 ml of anthrone and heat for 8 min. cool the tubes and take O.D at 630 mm . Starch was 12.6g in the sample (Table 3)

Non reducing sugars: By subtracting the reducing sugars from the total sugars given the value of Non reducing sugars. 13g Non reducing sugars was in the palmyra (Table 3)

Maltose by 3, 5 DNS method: The fresh pulp sample was extracted with 80% warm ethanol and centrifugation and collection the supernatant and make up to 2 ml with water. Then addition of 2 ml of DNS reagent and cover with marble keep in boiling water bath for 10 min.

cool and dilate to 10 ml with water and measure the OD at 520 nm. Calculation was done using standard graph maltose concentration is 1 mg/ml range from 0.1 to 2. maltose concentration was 0.5g in palmyra palm (Table 3)

Vitamin-C: The pulp sample is extracted with 4% oxalic acid. Then centrifuge pipette out 5 ml of supernatant and 10 ml of 4% oxalic acid and titrate adjacent with the dye i.e. 2, 6 dichlorophenol indophenols, observe the pink color. The working standard prepared with ascorbic acid and titrates against with the dye. Ascorbic acid content was determined 16 mg in palmyra palm (Table 3)

Calcium: Pipette 20 to 100 ml of ash solution into 250 ml beaker with addition 25 to 50 ml of H_2O if necessary add 10 ml of saturated ammonium oxalate and 2 drops of methyl red indicator. Adding of dil ammonia and a few drops of acetic acid until the colour is fainst pink. By Heating the solution to the boiling point. Allow to stand for overnight or 4 hr at room temperature. Filter through what man No-42 paper wash with water, till the filtrate is oxalate free. Break the point of the filter paper with platinum wire or pointed glass rod. Washed the precipitate first using hot diluted H_2SO_4 from wash bottle into the beaker in which the calcium was precipitated. Then wash with hot water and titrate while still hot (Temp 70-80°C) with $KMNO_4$ to the first stable pink color. Calcium was 8.76mg in the Palmyra palm (Table 3)

Energy: This determination was made according to the method of Atwater which gives the following heat flow coefficients.

1 g of Carbohydrate Provides - 4 kcal

1 g of fat provides - 9 kcal

1 g of protein provides - 4 kcal

The caloric value (energy) of the Palmyrah pulp was 102.8 kcal/100 g (Table 3) on fresh weight basis. Also fresh pulp of 100 g having significant amount of sugars and minerals i.e Reducing sugar is 9.5 g, non-reducing sugar is 13 g, starch is 12.6 g, Maltose is 0.5 g, Ascorbic acid is 16 mg and calcium is 8.76 mg.

3.5 Functional properties of Palmyra pulp

Color: The Color of the fresh pulp is observed visually and % transmission**.**

The colour of the pulp is light orange colour and gives orange colour wavelength in spectrophotometer. The solubility of the pulp is partially soluble in water, alcohol and acid solutions completely soluble in ether and chloroform. The pH of palmyrah fruit pulp powder

is slightly acidic i.e pH range Between 5 to 6 as shown in Table 4. T.S.S. of the fresh pulp was 16-16.5 (Brix), with these enviable characteristics; the pulp can be used for food preservative to enrich dietary values .

Solubility: The solubility tests were conducted with water, alcohol and acids. Completely soluble in ether and chloroform

pH: The pH of fresh pulp was measured with digital pH meter using standard procedure .It was 5.5 -6 (Table 4)

Table 4 : Functional properties of pulp from Palmyra

Parameter	**Values**
Color	Light Orange
Solubility	Completely soluble in ether and chloroform
T.S.S	16.5 Brix
Water absorption capacity (%)	3
Fat absorption capacity (%)	2.8
PH	5.5 to 6
Bulk density (g/cm3)	0.78
Swelling Power(g/g)	4
Foam capacity (%)	2.5

(Source: Vengaiah PC et al., 2015)

TSS (total soluble solids): The T.S.S. of the fresh pulp was measured with hand refractrometer . Total soluble solids was 16.5 Brix (Table 4)

Water absorption capacity: This was determined using methods described by Beuchat . 1g sample was weigh into 25 ml graduate conical centrifuge tubes and about 10 ml of water added. The suspensions were allowed to stand at room temperature (30+2°C) for 1 hr. The suspension was centrifuge at 200xg (2000 rpm) for 30 min. The volume of water on the residue was calculated and the water absorbed is articulated as percent water absorption based on the original sample weight.

The water absorption capacity for the Palmyrah pulp powder was 18% (2.5 ml/g) (Table 4). Water absorption capacity describes pulp water association ability under limited water supply. The result obtained shows that the pulp has a good ability to bind water. This result suggests that Palmyrah pulp powder could be used in bakery industry.

Bulk density: This was determined by the method of Narayana and Narasinga Rao. A graduate cylinder tubes were weighed and powder sample filled to 5 ml by tapping until there was no additional change in volume. The contents were weighed and the difference in weight determined. The bulk density was computed as grams per milliliter of the sample Bulk density is depended upon the particle size of the samples. The value obtained from the study was 0.78 g/cm3 (Table 4). Bulk density is a measure of heaviness of a pulp sample. It is important for determining packaging requirements, material handling and application in wet processing in the food industry. Since pulps with high bulk densities are used as thickeners in food products, the Palmyra pulp could be used as a thickener.

Swelling power: This was unwavering with the method described by Leach et al. with alteration for small samples one gram of the sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. The mixture was repeatedly dazed the heating period. After heating, the suspension was centrifuged at 1000 x g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as swelling power=weight of the paste/weight of dry sample.

The result for swelling power is presented in Table 4. The Palmyrah pulp powder has swelling power value of 4. Swelling power is a measure of hydration capacity, because the determination is a weight measure of swollen starch granules and their occluded water.

Foam capacity and foam stability: The method was used for the determination of foam capacity (FC) and foam stability (FS) Two grams of pulp sample was added to 50 ml distilled water at 30+ 2°C in a 100 ml measuring cylinder. The deferment was mixed and properly traumatized to foam and the volume of the foam after 30s was recorded. The foam volume was recorded in 1 hr after whipping to settle on the FS as a percentage of the initial foam volume. The foam capacity of the Palmyrah pulp is shown in Table 4. The percent foam capacity is about 2.5% which is lower . Foamability is reported to be related to the quantity of solubilized protein.

3.7 Physico chemical characteristics of palm sap

Freshly tapped palm sap collected from inflorescence of *Borrasus flabellifer* was transparent without any colour and less viscous. The pH of the fresh samples was ranging between 7-7.4 and near to neutral **(**Table 5). Fresh palm sap samples were estimat-ed for total sugar, reducing sugars, non-reducing sugars, glu-cose and non-glucose reducing sugar content in cell free me-dium. Non-reducing sugar in the palm sap is sucrose. Content of reducing sugars is subtracted from the content of total sug-ar to calculate the content of non-reducing sugar.

Total sugar content in the fresh palm sap was ranging between 09.88±0.08% (w/v) and 17.32±0.04% (w/v)(Table 5). Here, One way ANOVA with *post hoc* Tukey's test was able to establish a significant(p<0.05) difference in the total sugar content amongst the samples collected from seven different palm trees. Non-reducing sugar content in the palm sap estimated immediately after the tapping was ranging between 8.49±0.06% (w/v) and 2.64±0.01% (w/v), wich is mainly sucrose.

Table 5 : Physico chemical characteristics of palm sap.

Parameter	**Values/ranges**
pH	7-7.4
Total sugar(w/v)	09.88±0.08%
Non-reducing sugar(w/v)	8.49±0.06% - 2.64±0.01%
reducing sugar(w/v)	1.38±0.03% - 2.64±0.01%
Protein (mg/mL)	0.99±0.76 - 2.90±0.45
lipid	0.027±0.002% - 0.09±0.002%
Vitamin C (mg/mL)	0.04 ±0.005 - 0.11 ±0.005

(Source: Rahman M et al., 2010)

The overall variation of non-reducing sugar levels amongst the samples collected from seven different palm trees remained at 5% level of significance, as indicated by One way ANOVA with *post hoc* Tukey's test. Here, of the total sugar content of palm sap 86.41±0.99% of content of is non-reducing sugar. Content of reducing sugar in the palm sap was varing between 1.38±0.03% (w/v) and 2.64±0.01% (w/v), of which percentage of glucose varied between 0.69±0.01% (w/v) and 01.32±0.06% (w/v), and non-glucose reducing sugar varied from 0.53±0.02% (w/v) to 1.32±0.06% (w/v). We have recorded a significant (p<0.05) variation in the level of reducing sugars collected from seven palm trees. Here, percentage of reducing sugar is 13.38±0.98% of the total sugar content of the palm sap. Of the total reducing sugar content of the palm sap samples, 49.84±0.14% of content was glucose and 40.46±3.08% of content is non-glucose. Moreover, it very interesting here to record that even

though there was a significant (p<0.05) variation in the content of given type of the sugar between the samples collected from seven palm trees, One way ANOVA with *post hoc* Tukey's test was not able to establish any significant (p>0.05) variation the the ratio of the reducing sugar or non-reducing sugars to total sugar content of the palm sap. Protein content of palm sap collected from seven different trees varied significantly(p<0.05) between 0.99±0.76 mg/mL and 2.90±0.45 mg/mL, and lipid content varied significantly (p<0.05) between 0.027±0.002% and 0.09±0.002%. Similarly, Vitamin C in palm sap collected from seven different trees varied significantly(p<0.05) between 0.04 ±0.005 mg/mL and 0.11 ±0.005 mg/mL(Table 5).

3.8 Microbial Characteristics of palm sap

Total bacterial count reported between palm sap collected from seven different palm trees varied from 2X 10^3 cfu/mL to 3X10^6 cfu/mL, and total mould count reported between palm sap collected from seven different palm trees varied from 8X10^2 cfu/mL to 9X10^5 cfu/mL. Bacterial or mould population of the freshly collected palm sap varied significantly amongst the samples of seven different palm trees. Freshly tapped palm sap did not record significant (p<0.05) level of alcohol, as indicated by One way ANOVA with *post hoc* Tukey's test. Two types of yeast were isolated from YEPDA at 30^0 C. Isolate with smooth colonies, round colony margins, with budding sperical cells were identified as *Saccharomyces.* The other yeast colony was creamish, smooth, glucose, spindle shaped cell with ascospores, galactose and sucrose assimilating isolate was identified as *Lachancea sp.* The two types of bacterial colonies were isolated on MRS medium incubated at 30^0C. Small, whitish, convex, circular, and smooth colonies were Gram positive cocci with the chain of up to 40 cells, non-motile, non-spore forming, facultaive anaerobic, catalase negative, resistant upto to 3% of Nacl, that produced lactic acids and ethanol were identified as *Leuconostoc sp.*, marked as MRS1. Where as Small, whitish, convex, circular, and smooth colonies were Gram positive bacilli that produced acetic acids were identified as *Fructobacillus sp* .

3.9 Microbial status of processed Powder during storage

The number of yeast and bacterial colonies in palm increased from 0.0 to 2.17 and 0.33 to 7.50 cfu×10^3/g, respectively at the end of ambient storage (Fig. 4) while from 0.0 to 4.67 and 0.33 to 10 cfu×10^3/g, yeast and bacterial colonies, respectively at the end of refrigerated storage (Fig. 4). The bacterial population was higher than the yeast as the higher pH level is

more favourable for bacterial growth. Bhagirathi et al. (1993) reported the safe limit of a reconstituted vegetable gravy mix with 8.9×103, 2.5×103, 6.3×103 and 2.4×103 in order of coliforms, faecal coliforms, yeast and mold, respectively. Similar microbiological evaluation of processed products of minor fruits stored under both ambient and refrigerated conditionswas earlier investigated by Monteiro et al. (2005). However, the present study showed acceptable limit (ICMSF 1997) with respect to the yeast and bacterial count up to their respective storage period. The presence of microbes up to the above limit is negligible and safe for consumption within the mentioned period at both temperature regimes.

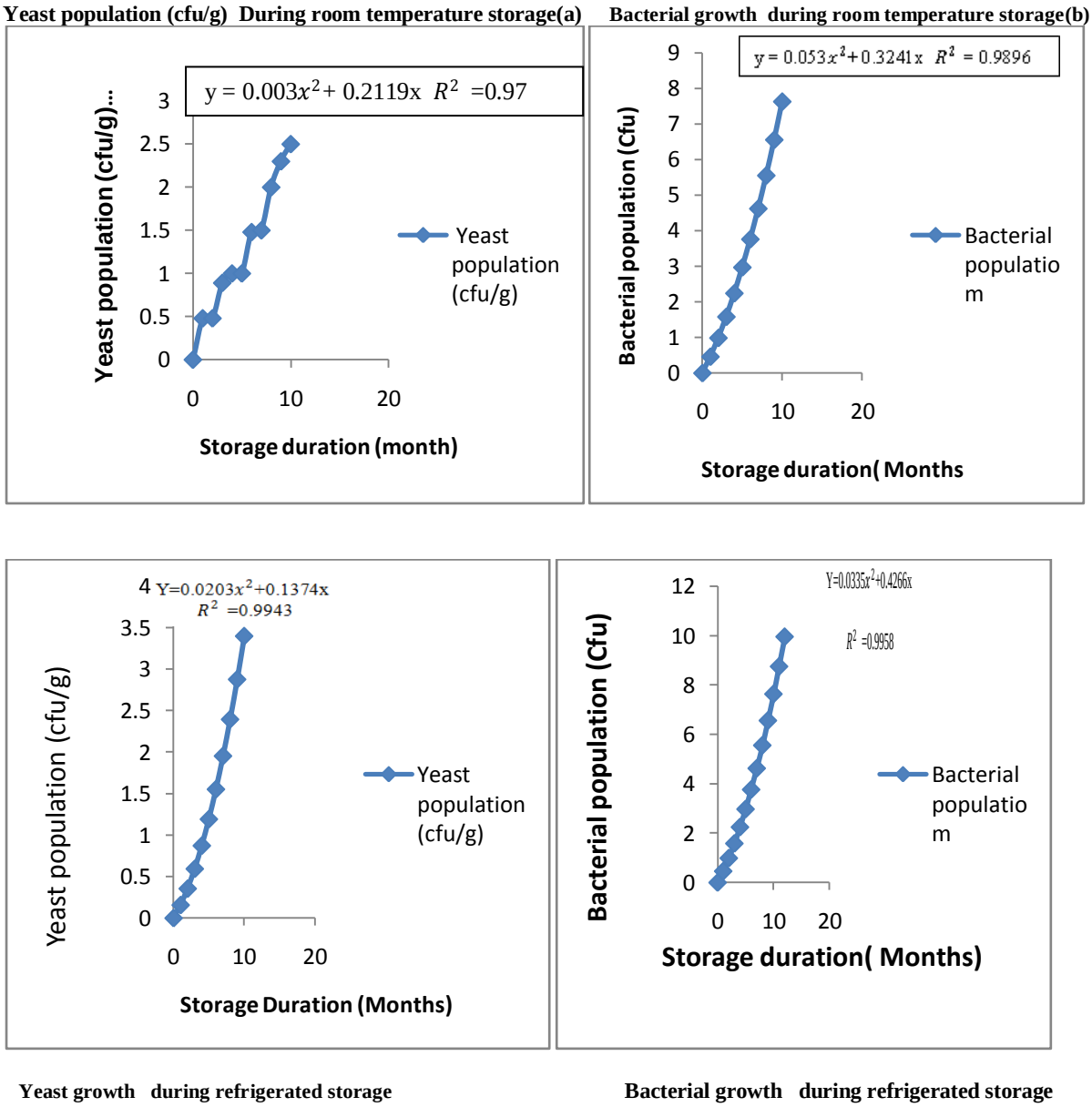

Fig. 4 Growth of yeast and bacteria in palm during room (25–37 °C) and refrigerated (8–10 °C) temperatures (Source: Monteiro et al. (2005)

Table 6 shows the physical properties of pasteurized palm sap samples including L*, a*, b*, transmittance value and browning index. The results indicate a large variation in the quality of pasteurized palm sap among the provinces ($p<0.05$). Normally, fresh palm sap is oyster white in color and translucent, with nearly neutral pH. However, pasteurized palm sap samples tend to show a red color shade as indicated by the positive a* values. The red shade of pasteurized palm sap was mainly affected by the harvesting procedures and pasteurization process . In addition, an enzymatic browning reaction can take place during the collecting of palm sap [13-14]. Polyphenol oxidase is responsible for this reaction. This enzyme catalyzes the hydroxylation of monophenols (from the metabolite of the plant and Kiam wood) to *o*-diphenols and the oxidation reaction of *o*-diphenols to *o*-quinones. Quinones are very reactive compounds which strongly interact with other molecules, leading to a high level of pigment with a high molecular weight with marked red to brown coloring. Moreover, a Maillard reaction could take place during pasteurization, resulting in the brown color formation.

According to our survey data, the producers usually added sucrose to the palm sap before pasteurization to adjust the sweetness. The reducing sugar content in palm sap can be reduced by the addition of sucrose. Since sucrose is a non-reducing sugar, therefore it cannot participate in a Maillard reaction, leading to the reduction in brown color formation.

Table 6 Physical quality profiles of pasteurized palm sap

Physical quality	Ranges
L* (lightness)	44.12-73.51
a*(redness and greenness)	0.48-1.14
b*(yellowness blueness).	11.08-13.56
Transmittance value (%)	32.43-75.29
Browning index	0.31-1.02

(Source : Borse et al., 2006)

The clarity of the palm sap was measured in term of the transmittance value (%). More clarified juice was found with a high transmittance value. In general, the presence of cell fragments has been found to be responsible for the clarity of fresh juice. Additionally, haze formation causes a reduction in the clarity of juice. The clarity of palm sap depends greatly on its protein concentration and the polyphenol compounds. An interaction between protein and polyphenol can be induced and, therefore, a large colloid size or haze can be developed. The clarity of pasteurized palm sap was also mainly influenced by the harvesting procedures of palm sap and the processing conditions. From the results, the lowest clarity was found in samples collected from Ayutthaya province. This result could be because of the use of red lime paste and Takian wood as antimicrobial agents during the collection of palm sap, palm sap was pasteurized and then placed at ambient temperatures to allow the undissolved particles to settle down. After that, the supernatant was packed in bottles.

Volatile flavour compounds in ten palm sapsamples were investigated using Headspace soild phase microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GCMS). Similar profiles of volatile flavour compounds from 10 palm sap samples were obtained. Volatile flavour compounds were commonly found in all samples, consisting of alcohols, aldehydes, esters, ketones, acids, and terpenes as shown in Table 7 . Ethanol was mainly presented in all samples. Ethanol in these categories may produced by fermentation from carbohydrates with microorganisms.

Table 7 Volatile flavour compounds of 10 palm sap samples

Volatile flavor compounds	Attribute	Area (%)
Alcohols	Ethanol alcoholic	57.68
Isoamyl alcohol	fermented	13.24
Aldehydes Acetaldehyde	fruity	0.26
Acids Acetic acid	sour	1.23
2-Heptanone	cheesy	0.44
Ethyl acetate	sweet, fruity	13.91
2-butanone 6-Methyl-5-hepten-2-one	citrus, herbal	1.48

(Lasekan *et al.,* 2006)

Table 8 Chemical composition profiles of pasteurized palm sap

Chemical quality	Ranges
pH	5.03-8.11
Total acidity (%)	0.11-0.23
Total soluble solid (^{o}Brix)	14.60-20.30
Total sugar (%)*	14.23-20.15
Reducing sugar (%)*	0.25-6.37
Sucrose content (%)*	14.38-19.05
Glucose content (%)*	0.14-3.45
Fructose content (%)*	0.17-3.21
Polyphenol content (mg/g) *	0.65-1.03
HMF (mg/kg) * HydroxyMethylFurfuraldehyde	4.24-10.22

*These values were calculated in term of dry basis.

(Source : Borse et al., 2006)

The pH of all pasteurized palm sap samples was significantly different among the samples ($P<0.05$) while total acidity showed a narrow range. Pasteurized palm sap a high pH, while lower pH values were found in all samples . Normally, natural palm sap showed a neutral pH of approximately 7 as reported by Jitbunjerdkul and Lasekan et al. However the growth of microorganisms during collection caused a decrease in the pH due to the conversion of sugars to acids . The alkaline pH found in all samples might be due to the addition of red lime paste to the bamboo tube added before harvesting in order to retard microorganisms.

The total soluble solid (TSS) of all pasteurized palm sap samples varied from 14.60^{o}Brix to 24.10^{o}Brix. Mainly, the initial TSS of palm sap and the processing conditions affected the TSS of pasteurized palm sap. Normally, palm sap contains TSS at approximately 10-18^{o}Brix. Microorganism contamination during collection is responsible for the low TSS of palm sap due to the effects of the sugar fermenting process. In addition, processing conditions, such as the processing temperature and processing time, also influenced the TSS of pasteurized palm sap. During pasteurization palm sap was boiled in an open container, resulting in the removal of water, especially at high processing temperatures and long processing times. This result could be explained by the long processing time (approximately 1 hour) used during palm sap pasteurization. All samples were boiled for approximately 30 minutes according to our survey data. Therefore, delays occurred in the times of collection.

Total sugar, the reducing sugar as well as fructose and glucose contents of all pasteurized palm sap samples, were analyzed in this study and showed significant differences across the samples ($P<0.05$). A positive correlation between the total sugar and TSS was observed, suggesting that the highest proportion of the soluble solid in pasteurized palm sap were sugars.

The most abundant of the sugars found in palm sap was sucrose. The presence of fructose and glucose can come from its natural state and the inversion reaction caused by invertase activity and acidic conditions. The occurrence of invertase in palm sap was due to its natural presence and it was also synthesized by microorganisms. Sucrose can be converted to glucose and fructose by invertase via microorganisms and thus yield organic acids and alcohols in palm sap. The fermenting organisms, particularly *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, are considered as primary sources of inverts.

In addition, the inversion of sucrose could take place significantly during thermal pasteurization. The highest reducing sugar content, as well as the glucose and fructose content, was found in all samples . This result suggests that the low pH of palm sap and a long heating time could promote the inversion reaction of sucrose. A low reducing sugar content was observed in all samples , indicating that alkaline conditions and a short heating time could minimize the formation of glucose and fructose. A high content of reducing sugars present in the pasteurized palm sap influenced the brown color development of a sample during storage via a Maillard reaction.

Table 10 shows the microbiological quality of pasteurized palm sap samples including TVC, M&Y and LAB. The Product Standards require that TVC and M&Y in pasteurized palm sap samples shall not be more than 500 cfu/g and 100 cfu/g, respectively. The microbial load was generally reduced after pasteurization. However, some spoilage microorganisms may have survived and developed, leading to a limited shelf life. In addition, inadequate *sanitation* and hygiene *practices could affect the safety and quality of the product.* According to the survey data, poor sanitation and facilities are the main factors affecting the microbial load of pasteurized palm sap. As mentioned previously, all the samples collected from received from various areas. This involved with long transportation times and improper transportation conditions, such as insufficient ice in iceboxes or lack of temperature control during transportation and storage.

Table 10 Microbiological quality profiles of pasteurized palm sap

Microbiological quality	Ranges
TVC (cfu/g)	1.40×10^{6}-8.60×10^{6}
M&Y (cfu/g)	1.20×10^{8}-7.90×10^{8}
LAB (cfu/g)	4.30×10^{4}-2.10×10^{5}

(Source Faparusi: S.I., 2006)

To study the effect of tapping season in total acidity and total sugars and microbial counts, an average value was calculated, results are shown in figure 5. The standard Plate Count Agar counts obtained were not affected by tapping seasons. The average counts of yeasts and moulds were around 6 log10 cfu/ml for the different samples. In spite of the average value for total aerobic mesophilic bacteria and yeasts and moulds are slightly higher in springtime, no significant difference has been noted between seasons ($P < 0.05$). However, microbial counts remain constants, significant changes in total acidity and total sugars are noted ($P < 0.05$). Palm sap harvested in springtime contains more sugars with lower acidity than the one harvested in winter.

This fact can be explained by difference in nutritive needs of palm tree that vary between seasons and enhance the degradation or the storage of sugars as reserves.

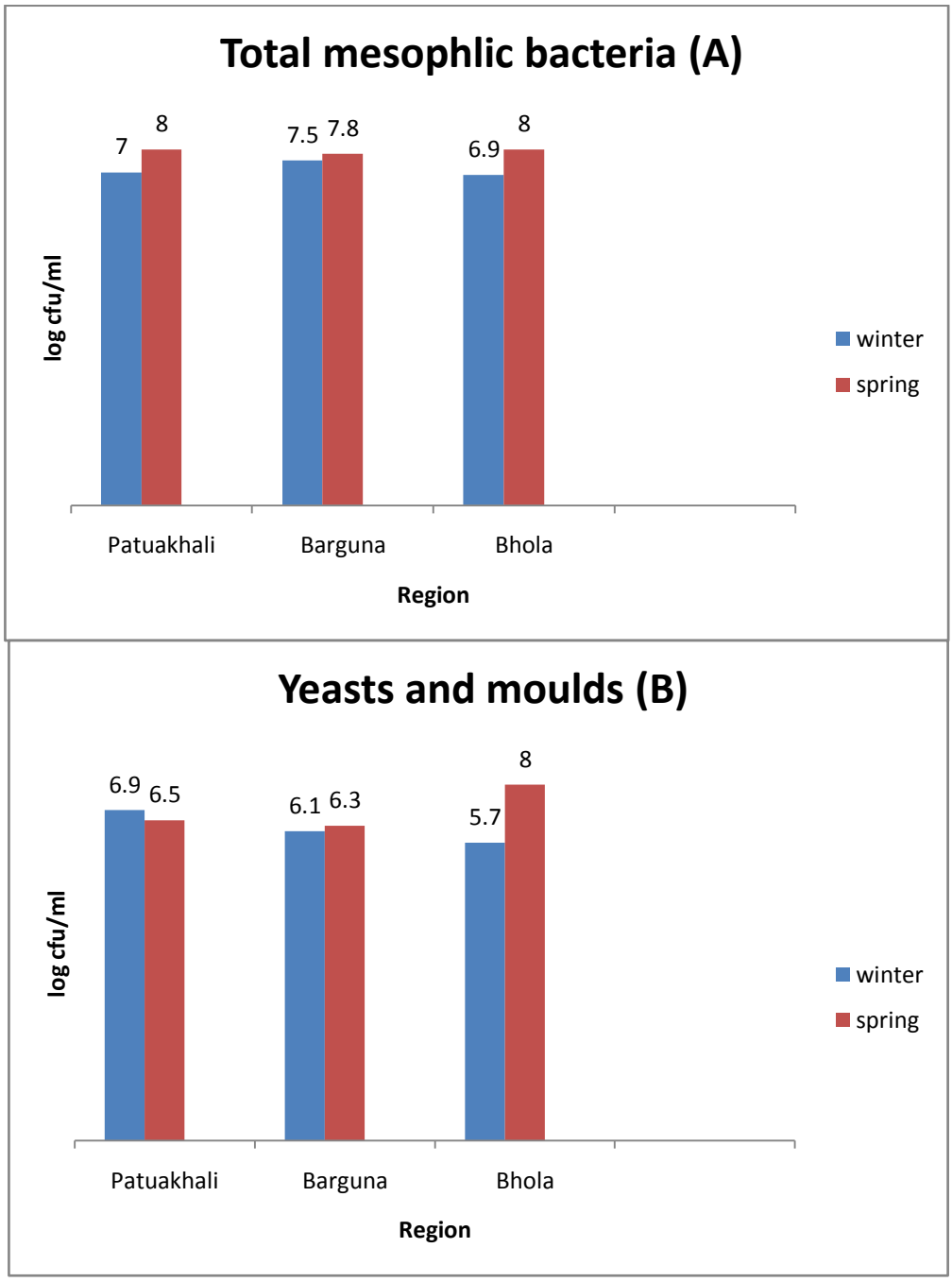

Fig 5 : Comparison in microbial counts (total mesophlic bacteria (A), Yeasts and moulds (B) palm sap harvested in winter and springtime come from different region .

(Source: Aziz et al., 2016)

3.10 Changes in characteristics of palm sap during exposure to heat

Changes in the total protein content in the palm sap kept at 30^0C, and those samples exposed to 60, 70, 80, 90, 100, 110 or 120^0C temperatures of moist heat for 5, 10, 15, 20 or 25 min is illustrated in the figure 6 Protein content in samples kept at 30^0C was 1.60±0.05, 1.56±0.04, 1.44±0.06, 0.9±0.07 and 0.6±0.04 mg/mL, during 5, 10, 15, 20 or 25 min, respectively. On 25 minutes of exposure of palm sap to moist heat at 60, 70, 80, 90, 100, 110 or 120^0C, the protein content decreased to 0.20±0.5, 0.60±0.4, 0.40±0.3, 0.67±0.4, 0.20±0.1, 0.02±0.5, and 0.02±0.3 mg/mL, respectively. Total protein content of the sample at 30^0C did not vary significantly (p>0.05) during 30 min of incubation.

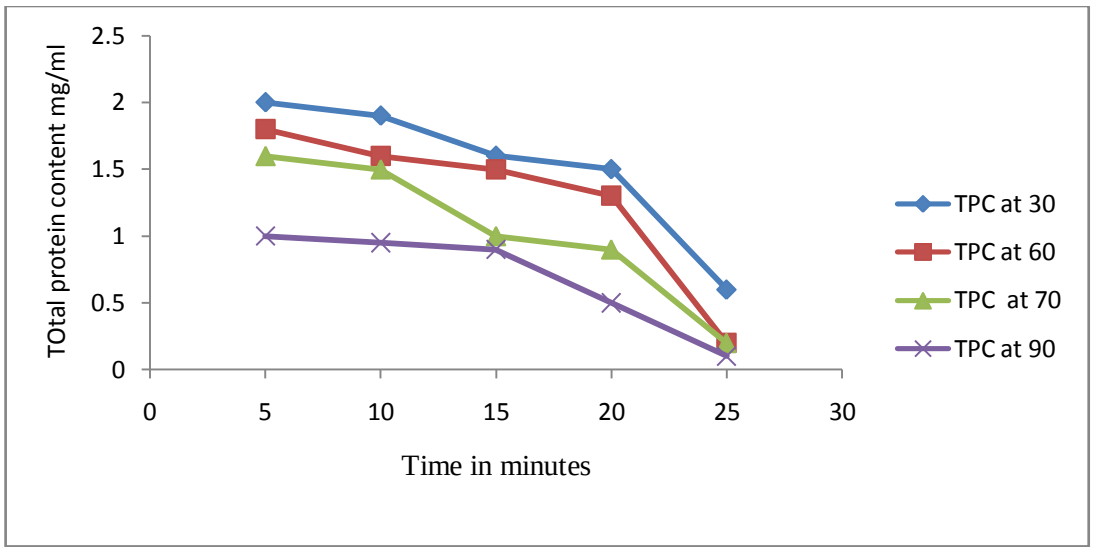

Figure 6: Changes in total protein content in palm sap during exposure to moist heat
(Source: Cammerer *et al.*, 2010)

Changes in the Vitamin C storage at and in samples sterilized at 60, 70, 80, 90, 100, 110 or 120^0C C for 5, 10, 15, 20 or 25 min is illustrated in the figure 7.

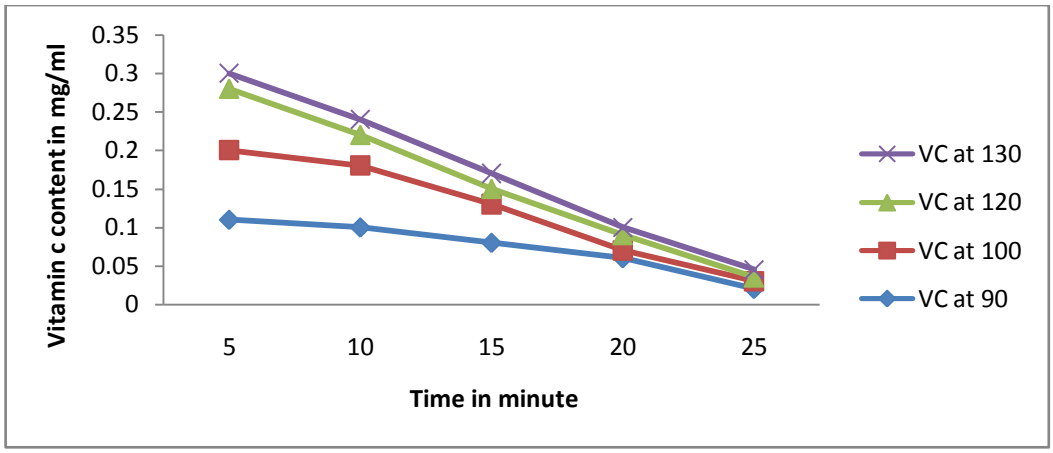

Figure 7: Changes in total vitamin c content in palm sap during exposure to moist heat
(Source: Cammerer *et al.*, 2010)

Vitamin C in palm sap during storage at 30^0C remained constant at level of 0.61±0.001 mg/mL during 25 min of stor-age. Thermal reduced the Vitamin C levels to 0.016±0.5, 0.043±0.4, 0.033±0.03, 0.027±0.03, 0.016±0.01, 0.011±0.05, and 0.003±0.02 mg/mL, respectively. Here, overall significant ef-fect of moist heat on the thermal degradation of protein re-mained at 5% level of significance, as indicated by One way ANOVA with *post hoc* Tukey's test.

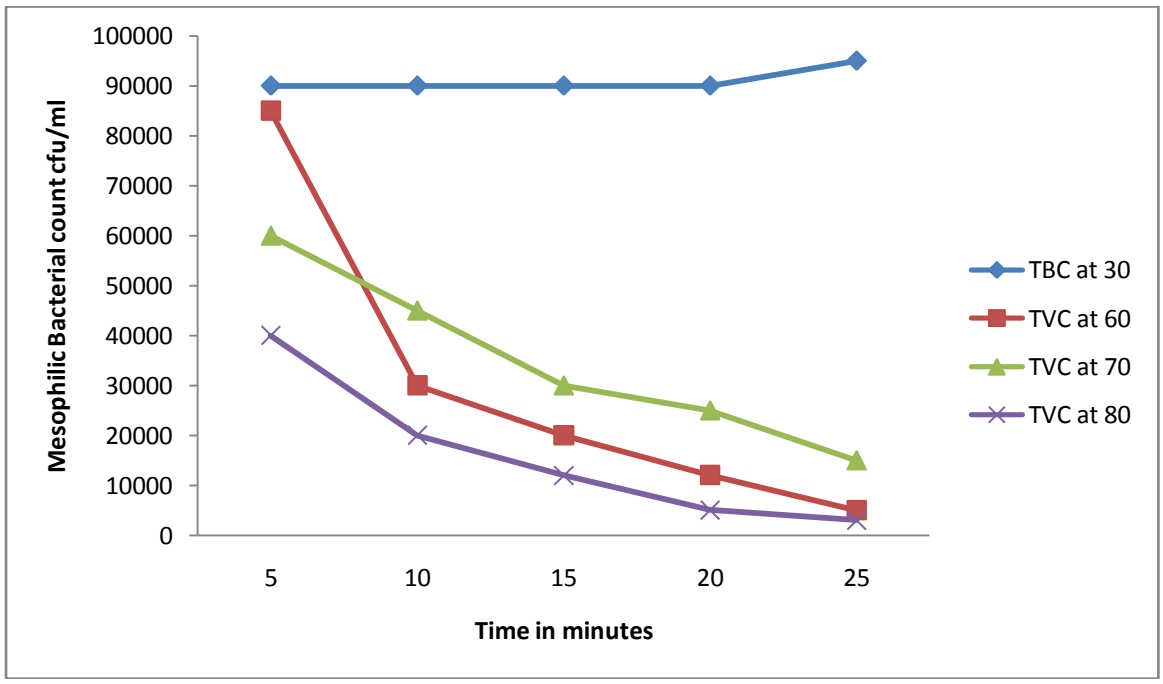

Figure 8: Changes in total Total microbial count in palm sap during exposure to moist heat (Source: Phaichamnan *et al.*, 2010)

The growth of microorganisms in fresh palm sap samples at 30^0C with and without moist heat treatment was investigat-ed. Samples of palm sap was recorded the initial microbial load of 105 cfu/mL on MRS agar and 106 cfu/mL on YEPDA agar. Different batch of samples were thermally treated at 30, 60, 70, 90, 80^0 C of moist heat for 5, 10, 15, 20 or 25 min. Lactic acid bacterial load reduced by 20 folds at 60^0 C for 5 min. For the same thermal treatments, yeast populations were reduced by more than 100 folds at 60^0C in 5 min. The results of this study on palm sap demonstrate that Yeast population is more sensitive than the lactic acid bacterium in for thermal treatment in figure 8.

CHAPTER- IV

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

1. Form this study it was observed that the fresh pulp powder contains, the moisture content was 74.5%. The ash and fat contents (wet matter basis) were 1.2% and 0.8%, respectively. The protein content and carbohydrate content were 1.25% and 22.5% respectively. The caloric value obtained was 102.83 kcal/100 g. The pH value was 5.5.
2. Enzymatic extraction could give a better quality juice from Palm.
3. *Saccharomyces cerevisiae , Lachancea fermentati i*solate, *Leuconostoc mesenteroids a*nd *Fructobacillus fructosus* . Freshly tapped palm sap is sweet and clear, but microbial activity changes it to milky white and sour. Degradative activity of yeast and lactic acid bacteria can be intervened by thermal treatment by moist heat below, at and above This study showed that pH of the medium reduced during thermal treatment at and above 80^0C with the samples more translucent and milkish whit to brownish white. Vitamin C, protein and glucose thermally degraded above 60^0C at pH 7.

5.2 Recommendation

1. There are few research was done with palm sap as well as palmyra pulp, So proper and regular research is needed for making value added product with palmyra palm as it is very nutritional fruit compared to other fruit grown in Bangladesh.
2. More microbial studies is needed for the storage of palmyra palm and for the import of palm processed products.

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