DETERMINATION OF ACTIVE SWEETENING AGENT IN SOME NATURAL SWEETNERS USED AS SUBSTITUTE TO PROCESSED SUGAR.

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ABSTRACT

Natural sweeteners are preferable over artificial sweeteners because of its nutritional benefits, it Is of a great interest to research on replacement of artificial sweeteners with natural sweeteners. The self produced natural sweeteners was extracted from there various plant sources and they are date syrup, grape sugar alcohol,honey, coconut sugar and Jaggery. Further analysis were carried out on some of these natural sweeteners such as total sugar content determination using refractometer, reducing sugar determination using Benedict reagents and sensory evaluation test. The results of these analysis that was carried out on some of these natural sweeteners are as follows, for total sugar content determination, the results of date syrup, coconut sugar, honey, grape sugar alcohol and Jaggery are 83.17,84.83,81.85, 8.12 and 17.90 respectively. Benedict test that was carried out were determined by there colour change during heating process, were the colourchanges from colourless solution to either blue, orange, red PPT, and greenish yellow PPT, the results on the sweeteners shows blue, brick red PPT, green yellow PPT, orange and blue for coconut sugar, date sugar, Jaggery sugar, honey and grape sugar alcohol respectively while the sensory evaluation test that was carried out by volunteered persons results at 10%,1%,0.1% and 0.01% only grape sugar alcohols that maintain it's baseline test at 0.01%% while other samples are tasteless at 0.01%.natural sweeteners are highly nutritive and maintains it's vitamins even after processing.

Key words: Natural Sweeteners, Benedict Test, Refractometer, Glucose, Sensory Evaluation.

INTRODUCTION

Sweeteners are substances used to improve the palatability and shelf life of food products. (Michelle, 2002).

Sweetness balances bitterness, sourness, and saltiness, and most humans prefer sweet tastes. The osmolarity (the solute concentration) of sweeteners means that they usually inhibit bacterial and mold growth. (Abishak, 2009)

Sugars occur naturally in many plant foods; we get most common sweeteners by processing these plants (such as agave necter, maple trees, sugar cane, coconut, palms, sugar beets and corn) to extract and condense the sugars. In the case of honey, bees do the work of extracting and processing flower nectar.

One of the most important thing to understand about sweeteners is that their chemical structure affects the way the body processes it and stores them (Mayor, 2012).

For instance, although honey and maple syrup are both "natural", they have quite different sugar type profiles. Honey is about 50% fructose, while maple syrup is mostly sucrose (Whitney et al, 2011).

Sugar is sugar, and there is no difference between sucrose in sugar cane and sucrose in your sugar bowl. However, it is critical to understand that different sugars act differently in the body.

because sugars come from plants, it does not mean that they are good for you – especially in large amounts. For example, eating 5 bananas will give you 85g of sugar. That is ¾ of a cup of sugar (Robert, Z. (2011))

In fact, eating too many high fructose foods like sweet fruits also can give you diarrhea (Robert, Z. (2011)) the fructose combines with the fiber and intestinal water to create the "perfect storm".

In recent years, the need to increase the production and utilization of locally available sweeteners has been highlighted at different national and international flora; Natural sweeteners are underutilized source of sugar, locally available in Nigeria they aid in solving major nutritional problems through exploitation of its nutritional and economic potentials. The results of this study will provide a baseline data on types of natural sweeteners utilization and their active sweet components. It will go a long way to diversify its use and in turn lead to its increased production both at household and national levels, ultimately, to ensure food security. Furthermore, it is expected that through the knowledge of its composition, natural sweeteners may be exploited for use in the prevention and treatment of some non communicable diseases for example cancers, diabetes, hemorrhoids and cardiovascular disease.

The significant difference between sugar sources is not "natural" or "refined" but concentrated sugars such as honey, table sugar, concentrated fruit juices, corn sweeteners, and diluted sweeteners naturally occurring sugars in foods such as oranges, corn, milk, and potatoes.

Fructose, for example, is the major sugar in fruit and furnishes the same calories per grain as refined table sugar and honey. The difference is that sugar in fruit is diluted in a large quantity of water that also contains vitamins, minerals and fiber.(Robert, Z. (2011))

In humans, the carbohydrate's primary role is to supply energy; One grain of carbohydrate contains four calories (energy). In fact, the body prefers the source of energy over all other available sources of energy nutrients.

Carbohydrates are known as the "energy-sparing nutrient" because the energy available from them spares protein from being used for energy so that protein may build and repair body tissues. When a diet lacks carbohydrates, protein is used for energy.

Species in the fetus, Taste buds are developed by the 16th week of gestation, and the new born infant is able to produce favorable sweetened solutions. Sugar is a natural sweetener that provides 4 calories per gram. It is acknowledged that excess sugar ingestion, amounts to increased energy intake, which in turn, can lead to weight gain and chronic diseases associated with obesity and dental caries. Therefore, there is need for sugar substitutes, which can help reduce caloric intake, particularly in overweight individuals. The demand for new alternative "low calorie" sweeteners for dietetic and diabetic purposes has increased worldwide. As of mid-2002, over 100 plant-derived sweet compounds of 20 major structural types had been reported, and were isolated from more than 25 different families of green plants. Several of these highly sweet natural products are marketed as sweeteners or flavouring agents in some countries as pure compounds, compound mixtures, or refined extracts. Many synthetic sweeteners, which are widely used are proved to be carcinogenic and are non-nutritive. Hence demand greatly increased for natural sweetening agents, especially for non-sacchariferous sweetening agents, because they are highly potent, useful, safe and low-calorie sugar alternatives. Recently it was found that Himalayan forests are good sources of plants containing non-saccharide sweetening agents. (Bachman et al., 2006)

2. MATERIALS AND METHODS

2.1 PROCEDURE FOR PALM SUGAR PROCESSING

Cut diagonally the flower coconut palm, tie your container beneath the cut area, allow the drops for four hours, collect the nectar, weigh the sap/nectar collected, pour the liquid in a pan and boil, stir the solution

continuously with the stirrer, after about 40 minutes, pour thick brown solution in a sieve, allow the solution to cool, then pound in a mortar to a powdering form, sieve with a sifting tray, spread the powdering content to dry, sieve the dried palm sugar and dry again, then package palm sugar formed for consumption.

i. PROCEDURE FOR PROCESSING A COCONUT MILK SUGAR

Break four coconut, extract the milk, weigh the liquid extract and record, boil the milk for about 20 minutes, a thick solution is formed after evaporation, collect the milk sugar formed at the bottom.

ii. PROCEDURE FOR PROCESSING DATE SUGAR

Weigh 100g of date fruit, wash the fruit, allow it dry, grind in grinding machine, and spread the powder to dry, package the date sugar in containers.

iii. PROCEDURE FOR PROCESSING OF MOLASSES

Weigh 100g of sugar cane, mesh sugar cane, squeeze out the juice, boil the juicy solution until the solution evaporates, and collect the dark thick brown molasses sugar formed.

iv. PROCEDURE FOR PROCESSING OF SUGAR ALCOHOL

Wash and weigh one unripe paw-paw, peel the bark and remove the seed, cut the unripe paw-paw and soak for three days, collect the solution, sugar present in the solution.

v. PROCEDURE FOR PROCESSING GRAPE SUGAR

Collect four grapes and wash, cut the grape squeeze out, allow staying for three days in the fridge, sugar alcohol present in the solution.

2.2 TESTING THE PRESENT OF ACTIVE SWEET COMPONENT'S IN ABOVE PREPARED SWEETENERS

Testing active sweet component's in the above sample: This test is carried out to identify the active sweet components (fructose, glucose, sucrose, sorbitol, eryithol) present and their percentage in each fruit which helps to identify the ADI for human consumption. The compounds are placed in beaker s for further laboratory analysis.

Preparation of solutions for analysis of active sweet component

100g of each natural sweeteners was weighed out, the sweeteners were dissolved with 5ml of water in a beaker; these solutions were prepared for the determination of active sweet components in natural sweeteners.

2.3 DETERMINATION OF ERYTHRITOL, MALITOL, XYLITOL AND SORBITOL IN SOLUTION

Each beaker was labeled according to its content.

The samples were conducted by electrophoresis with capacitive detector (C`4`D).

Samples were simply obtained using ultra pure water and ultrasonic energy.

Linearity was assessed by calibration curves that showed R^z varying from 0,9920 to 0.9976.

The LOQs were 12.4, 15.9, 9.0, and 9.0micro Newton per gram for erythritol, maltitol, xylitol and sorbitol respectively.

PREPARATION OF BENEDICT'S SOLUTION:

One litre of benedict's solution was prepared from 100g of anhydrous sodium carbonate, 173g of sodium citrate and 173g of copper (ii) sulfate pentahydrate.

2.4 DETERMINATION OF GLUCOSE, FRUCTOSE AND SUCROSE IN THE ABOVE NATURAL SWEETENERS

- Pipette 5ml of benedict's reagent in three test tube each (20 *150mm)
- Add eight drops of each solution respectively in different test tubes of benedict's reagents.
- Heat carefully on flame of gas burner or boiling water for 5 to 10 minutes
- Cool under tap water or by placing in a beaker containing tap water.
- Observe the color change and precipitate formation.
- Analyze the test results

2.5 PREPARATION OF SOLUTIONS:

10g each of sugar powder and natural sweetener (sucrose) was weighed, 10%, 1%, 0.1% and 0.01% solutions of sugar and each of the sugar substitutes were prepared. These solutions were used to find out the threshold of taste (sweetness) for each substance.

MIXING THE SWEETENER SOLUTIONS

- Four plastic cups were labeled according to the solution that was obtained.
- The first cup- natural sweetener 10%, the second cup- naturalsweetener 1%, the third cup- sugar 0.1%, and the fourth cup -sugar 0.01%.
- 90ml of distilled water was measured into the 100ml graduated cylinder and poured into the cup labeled sugar 10%.
- 10g of sugar powder was then poured into the cup labeled sugar 10% and using a stirring stick the solution was stirred until it completely dissolved and the sugar granules in the bottom can no longer be seen. This gives a 10% by weight (w/w) sugar solution then filled up to the 100ml mark.
- 1%naturalsweetener solution was prepared: 90ml of distilled water wasmeasured into the 100ml cylinder and poured into the cup labeled sugar 1% using the 10ml graduated cylinder, 10ml of the 10% sugar solution was carefully measured
- Two liquids are completely mixed. This gives a 1% w/w solution.
- The l0ml graduated cylinder was thoroughly cleaned and dried to prevent cross- contaminations between the solutions.
- The 0.1% natural sweetener solution was made by measuring 90ml of distilled water in the 100ml cylinder and poured into the cup labeled sugar 0.1%. Using the 10ml graduated cylinder 10ml of 1% natural sugar solution was carefully measured and poured into the cup labeled natural sugar 0.1% and stirred until the two liquids are completely mixed. The result is a 0.1% w/w solution; the l0ml graduated is thoroughly cleaned and dried.
- Finally the 0.01% natural sugar solution was made by measuring 90ml of distilled water in the 100ml cylinder and poured into the

cup labelednaturalsugar 0.01%. 10ml of the 0.1%natural sugar solution in the 10ml graduated cylinder was measured and poured into the cup labeled natural sugar 0.01% and the solution stirred until the two liquids are completely mixed. The result is a 0.01% w/w solution.

- The same steps were repeated
- For date syrup, grape sugar alcohol andHoney, however, is a liquid, so the procedure to make the 10% solution was slightly different, because it was measured in milliliter and not in grams. The water wasalso warm in order to easily dissolve the honey.
- Four plastic cups were labeled, the first cup honey 10%, the second honey l%, the third honey 0.1%, the fourth 0.01%.
- 90ml of warmed distilled water in the 100ml graduated cylinder was measured and poured into the cup labeled honey 10%. 10ml of honeywas measured in the 10ml graduated cylinder and poured into the cup labeled honey 10%and stirred until the two liquids were completely mixed and the solution was a uniform light brown color. The result is a 10% by volume (v/v) honey solution.
- The 10ml graduated cylinder was thoroughly cleaned and dried.
- The steps that were used to prepare the 1%, 0.1% and 0.01% sugar

solutions were repeated to make up the rest of the honey solutions.

3. RESULTS AND DISCUSSION

The result obtained after the analyzing in methodology above are presented here in tables

Table 1: Determination of reducing / non reducing sugar present in some natural sweeteners, using benedict's reagent as the determinant.

SAMPLE	5ML OF SAMPLES	2 DROPS OF BENEDICT'S REAGENT
Coconut sugar	5ml of coconut extract	Turns blue
Date sugar	5ml of Date sugar extract	Turns blue colour to red brick
Honey Sugar	5ml of Honey Sugar extract	Turns blue colour to orange with red ppt

Jaggery sugar	5ml of Jaggery sugar extract	Turns blue colour to green /yellow ppt.
Grape solution	5ml of Grape solution extract	Turns blue.

Table 2: Result showing present/absence of reducing sugar in some of these natural sweetener.

	SAMPLE	**COLOUR CHANGE**	**LEVEL OF SUGAR**
	Honey	Orange / red ppt	Moderate
	Date	Brick / red ppt	Large
	Coconut Sugar	Blue	None
	Palm sugar	Green / yellow ppt	Traces of reducing sugar
	Grape	Blue	None

Table 3: Result of further analysis on determination of reducing sugar.

COLOUR OBSERVED	**SUGAR %**	**RESULT / INTERPRETATION**
Blue	Nil	Absent of reducing sugar
Green colour	0.5%	+
Green Ppt	0.5-1%	++

Yellow Ppt	1-1.5%	+++
Orange Ppt	1.5-2%	++++
Brick red Ppt	>2%	+++++

Table 4: Using Refractometer to Determine The Sugar Content In Some Natural Sweeteners And Their Various Percentages

SAMPLES(100 ML)	1ST TEST	2ND TEST	3RD TEST	TOTAL VALUE	MEAN
Honey	82.00	82.05	81.50	245.55	81.85
Jaggery	18.0	17.50	18.20	53.70	17.90
Date syrup	83.56	83.50	82.46	249.52	83.17
Grape	8.12	8.10	8.15	24.37	8.12
Coconut Syrup	85.01	84.50	85.0	254.81	84.83

TABLE 5: VOLUNTEERS' TASTE THRESHOLD FOR HONEY

NO OF VOLUNTEERS	SUBSTANCE	10% SOLUTION	1% SOLUTION	0.1% SOLUTION	0.001% SOLUTION
1.	Honey	Yes	No	No	No
2.	Honey	Yes	No	No	No
3.	Honey	Yes	No	No	No
4.	Honey	Yes	No	No	No
5.	Honey	Yes	No	No	No
6.	Honey	Yes	No	No	No
7.	Honey	Yes	No	No	No
8.	Honey	Yes	No	No	No
9.	Honey	Yes	No	No	No
10.	Honey	Yes	No	No	No

Table 6: Volunteer Taste Threshold For Jaggery

NO OF VOLUNTEERS	SUBSTANCE	10% SOLUTION	1% SOLUTION	0.1% SOLUTION	0.001% SOLUTION
1.	Jaggery	Yes	No	No	No
2.	Jaggery	Yes	No	No	No
3.	Jaggery	Yes	No	No	No
4.	Jaggery	Yes	No	No	No
5.	Jaggery	Yes	No	No	No
6.	Jaggery	Yes	No	No	No
7.	Jaggery	Yes	No	No	No
8.	Jaggery	Yes	No	No	No
9.	Jaggery	Yes	No	No	No
10.	Jaggery	Yes	No	No	No

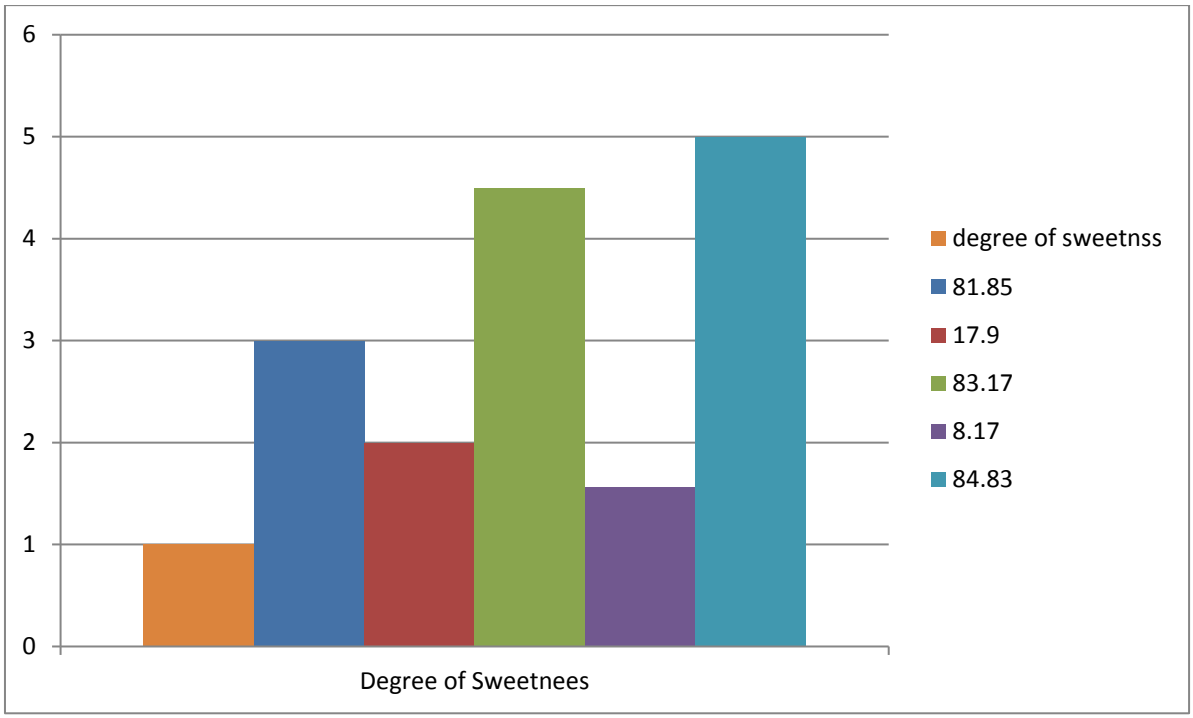

FIG. 1: Illustration of Some Natural Sweeteners Using A Bar Chart

4. Discussion

Identification of simple sugar is done by change of colour using Benedict's reagent. When the colorless solution turns blue and maintains its blue colour after heating, It shows absence of reducing sugar, Indicating that the solution contains disaccharide compound If the blue colour turns green with yellow ppt, it indicates traces of reducing sugar. If the green colour goes on to change into orange /red ppt, it shows moderate sugar present and when it goes further to change Brick/ red ppt after further heating it indicates large amount of reducing sugar.

Table 1: Benedict's Solution is designed to detect the presence of reducing sugar. In hot alkaline solutions, reducing sugars reduce the blue copper (II) ions to brick red copper (I) Oxide precipitate. As reaction proceeds, the colour of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the colouration developed and the amount of precipitate formed depends upon the amount of reducing sugar present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained.

This method measures all reducing substances present. Hence compound, other than reducing chemicals (e.g. ascorbic Acid), that can reduce the hot alkaline copper (II) ions may cause a positive error.

The extent of this reaction depends markedly upon the conditions of temperature, duration of heating and degree of alkalinity. Hence the specified conditions must be followed in all determinations and standards should always be heated together in the same boiling water bath.

Table 2- From the result obtained, the sugar levels were of the order coconut syrup> Date Syrup> Honey>Jaggery> Grape Solution. Similar work done in Abakaliki soft drinks other than natural sweeteners by Aloh *et al, (*2015), agree with the fact that sugar level in Juicy substances varies.

The results obtained from his work are comparable to those observed by Obuzor and Ajezi (2010) where the order of sugar level was in the order Amstel Malta< Hi Malt< Maltina< Grand Malt< Malta Guinness.

The result from the analysis does not mean that these sweeteners are unhealthy but it does suggest that different analytical equipment used in determining percentage of sugar in some natural sweetener may have different sensitivity and hence the result may vary

Table 3 – the baseline taste with distilled water which is used as the control.

In Table 4 -honey produces high degree of sweeteners as that of sugar in different dilution percentage. It contains some amount of vitamins and minerals and also can also function as antioxidants. It is recommendable for both young and adult.

Table 5: Though grape is included in Natural sugar, it belongs to this class of sugar known as sugar alcohol, which is used mostly in Breweries industries. its taste is persistence which was notice in the different percentages.

Table 6: There is a change in taste at 10% and 1% and the rest of percentage dilutions weren't noticed, this shows average degree of sweeteners which is very good for human consumption, most especially diabetic and obsessed patients.

5. Conclusion

Natural Sweeteners could be used in diets by young and old, pregnant and lactating mothers, for its high energy and vitamins content. Glucose content of natural sweeteners could be used to replace artificial sugar from other sources other than root, fruit, stem and animals. The effect of

processing of natural sweeteners may considerably apply in formulating diets for vulnerable groups (Diabetic and Obsessed Patients).

Palm sugar can be used as a substitute for aspartame and other table sugar honey can be used as excellent substitute for saccharin and for healthy diets.

Honey, Jaggery, Date Syrup, Grape Juice and Coconut Sugar are refreshing when used, because preservative agents are not used in there storage.

There is need for further development of product based on natural Sweeteners for household and commercial purposes to ensure food security.

These in turn will increase its production and utilization thereby making it more popular.

There is also need for further experimental investigation geared towards ascertaining nutritional value of some of these natural sweeteners and identify the various percentage of fructose, glucose and sucrose level in them.

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