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Comparative Studies of the Thiamine and Riboflavin Contents in the Sap of *Raphia hookeri* Palm-by the Applications of Fluorimetric Method and Arrhenius Equation

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Abstract: Matured *Raphia hookeri* palms were selected by using the appearances of three inflorescences on the palms, which is an indication of their state of maturity. The palms were tapped and the saps were collected. The thiamine and riboflavin contents were determined fluorimetrically and compared. Arrhenius equation was used in comparing the activation energies of the extracted thiamine and riboflavin. The overall result indicated that the sap is a rich source of thiamine and riboflavin, which could enhance normal health.

Key words: *Raphia hookeri*, thiamine, riboflavin, fluorimetry, activation energy

INTRODUCTION

The *Raphia* is the largest palm in Africa and is one of the most useful economically (Otedor, 1976). Varieties of *Raphia* palm exist and have been described by Mann and Wendland (1864), Otedoh (1976) among others.

The exude which flows when an incision is made on the inflorescence of the *Raphia hookeri* palm is called the sap. The sap is sweet and colourless just like the sap of Elaeis guineensis (Eapen, 1982). In Africa, the sap is drunk as a beverage either in its unfermented or fermented states, depending on the choice of the individual. *Saccharomyces cerevisae*, is the yeast that ferment the sap of *Raphia hookeri*.

Thiamine and riboflavin are parts of the water-soluble vitamins. Due to their solubility in water, excesses of these vitamins are excreted in urine and so rarely accumulate in toxic concentrations. Their storage is limited for the same reason and as a consequence they must be provided regularly. They are not stable in alkaline or neutral solutions (Mayes, 1993; Rankin and Hildreth, 1976).

Fluorescence is initiated by the absorption of radiant energy and the re-emission of some of this energy in the form of visible light. The light emitted is almost always of higher wavelength than absorbed. The intensity of fluorescence is proportional to the number of fluorescent molecules and hence to the amount of light absorbed. Fluorescence is therefore proportional to concentration (Vogel, 1978; Plummer, 1971).

The activation energy is the minimum amount of energy the colliding reactant particles must posses before any reaction can take place. The energy barrier must be smaller than the activation energy for a particular reaction, before the process or reaction can take place. Every reaction is characterized by its activation energy. Usually, reactions with low activation energy will take place at room temperature, while reactions with relatively high activation energy will only occur during the application of energy in the form of heat, light or electrical energy (Ababio, 1985).

Apart from the water-soluble vitamins mentioned above, mineral nutrients have been detected in the palm wine (fermented sap) from *Raphia hookeri* palm by Ukhun *et al.* (2005). Ezeagu and Fafunso (2003) have also reported on the biochemical constituents of palm wine.

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The overall contents of the sap could therefore serve as a beverage source for various nutrients and possible use in food applications and medicine.

MATERIALS AND METHODS

Selection of Raphia hookeri Palms

The palms used for this study were located on the swampy area of the river at the Nigerian Institute for Oil Palm Research, near Benin City. They were selected by the appearance of three inflorescences on the palms, which is an indication of their state of maturity. At the above stage the palms were due for tapping.

The laboratory analyses were conducted at the Food Science Department, Obafemi Awolowo University, Ile-Ife, Nigeria, in the year 2004.

Tapping and Collection of the Sap of Raphia Hookeri Palms

The palms were tapped while they stood erect, without the application of fire at the tapping panel to stimulate the flow of the sap. The sap was collected from the palms throughout their productive life span, with the use of clean plastic jerry cans. In the tapping procedure, the stems of the plant, which varied in height between 10 and 13 m, were climbed, with a climbing rope, to the top where the branched leaves or fronds appeared. The tapping was done with a tapping knife. A plastic jerry can was hung at the base where the tapping was done, in such a way that the sap could flow into the container with the aid of a clean polythene sheet. A 20 L capacity container was used for the collection of the sap. After each tapping, the sap-collecting containers were washed with a long brush daily by using clean water. The tapping knife and panel on the stem were also washed daily with clean water. After tapping, the mouth of the hung container and the tapping panel were covered with clean polythene to prevent debris.

Extraction and Fluorimetric Assay of Thiamine and Riboflavin Contents in the Sap of Raphia Hookeri

The AOAC (2002) method of extraction of water-soluble vitamins from foods was adopted, for the extraction of thiamine and riboflavin from the sap of *Raphia hookeri* palm. The concentrations of the vitamins in the sap were detected fluorimetrically with Coleman Model 12-A fluorimeter.

Determination of the Activation Energies for Thiamine and Riboflavin in the Sap

The thiamine and riboflavin extracts containing 1g each in iso-butyl alcohol, were heated between 20 and 100°C at five degrees interval and the fluorimetric reading were read from Coleman Model 12-A fluorimeter. Four extract readings were obtained and their mean values were used in plotting the graphs presented in Fig. 1 and 2.

RESULTS AND DISCUSSION

The mean values of thiamine and riboflavin extracted from the saps of five palms were 0.407 and 0.212 mg 100 mL, respectively. The yeasts (*Saccharomyces cerevisiae*) cells in the sap of the *Raphia hookeri* obviously were lysed during the acid hydrolysis and autoclaving processes in these studies. Consequently, the vitamins assayed for by the fluorimetric method were released into the sap. These water-soluble vitamins may not have been destroyed during the acid hydrolysis because they are stable in acid medium but unstable in neutral and alkaline conditions (Rankin and Hildreth, 1976). The concentrations of thiamine and riboflavin extracted from the sap indicated that the sap of *Raphia hookeri* palm is richer in thiamine.

The fluorescence patterns of thiamine and riboflavin are presented in Fig. 1 and 2. The rates of reactions of the vitamins under reference were determined by measuring the amount of fluorescence, which changed proportionately with the mass of the reactants at corresponding temperatures (Ababio, 1985). Morris (1978) had reported that the activation energy of a particular reaction could be calculated, by mathematical manipulation of the Arrhenius equation, to yield a derived two-point

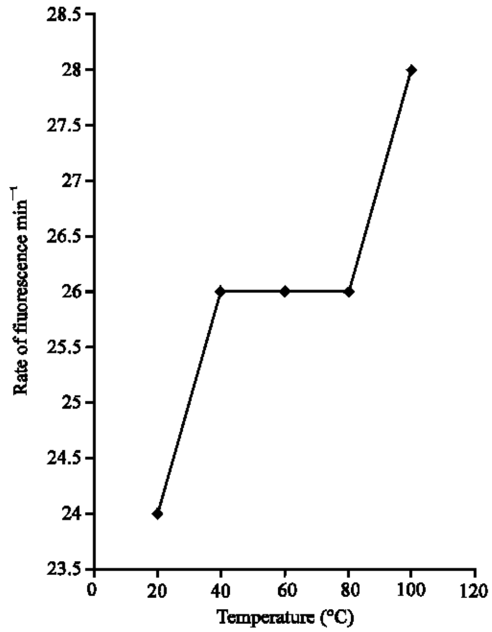

Fig. 1: Plot of fluorescence against changes in temperature (Thiamine)

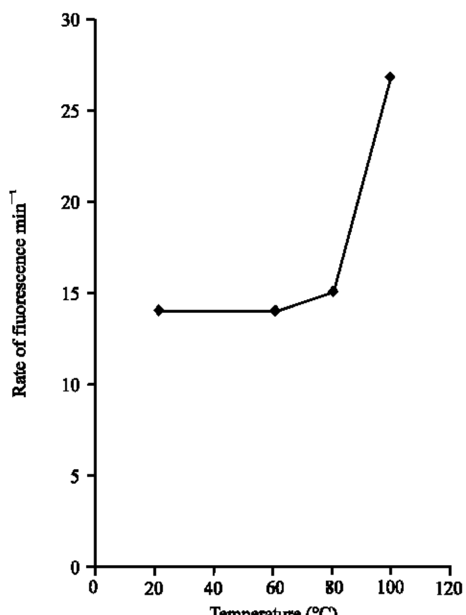

Fig 2: Plot of fluorescence against changes in temperature (Riboflavin)

Arrhenius equation. The results obtained for thiamine and riboflavin rate of reactions at corresponding temperatures were therefore substituted in the two-point Arrehnius equation. The values of 4.0 $kJmol^{-1}$ ±0.1 and 32.0 $kJmol^{-1}$ ±0.1 were obtained for thiamine and riboflavin, respectively.

Obviously, from the above results, riboflavin has higher activation energy than the thiamine extracted from the sap of *Raphia hookeri*.

The activation energies calculated for both vitamins would be useful in gaining insight into the stability of the vitamins in food systems, in terms of the energy barrier that will have to be overcome in the degradation reactions involving the vitamins. The vitamin content of food is one thing; the stability of the vitamin during storage is quite another thing. This is the importance of the activation energies for the vitamins as conducted and reported in these studies.

Physiologically, the active form of thiamine, thiamine pyrophosphate functions as the cocarboxylase in the oxidative decarboxylation of pyruvic acid. As a consequence of thiamine deficiency, pyruvic acid accumulates. Some of the accumulated pyruvic acid is converted to lactic acid, with little release of energy. The presence of thiamine in human nutrition is necessary to ensure that pyruvic acid catabolism can proceed via the citric acid cycle and the electron transfer chain (Rankine and Hildreth, 1976). Thiamine deficiency in human nutrition causes the disease condition known as beri-beri, a neurological disorder, resulting from carbohydrate-rich/low-thiamine diets (Mayes, 1993; Lehninger, 1990).

The physiological roles of riboflavin include its formation of the prosthetic group of flavoproteins which function as hydrogen carriers in the oxidation sequences of respiration, resulting in the release of hydrogen into the electron transport chain and thus combine with oxygen to form water. Deficiency symptoms of riboflavin include, angular stomatitis, cheilosis, seborrhoea and photophobia (Mayes, 1993).

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