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SOME FACTORS AFFECTING THE DEVELOPMENT OF PINK COLOUR IN COCONUT WATER

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Abstract: Young coconut water (and king coconut) water maintained free of microbial growth tends to produce a pink colouration. The substance giving rise to the pink colour was quantified by precipitation at pH 9.5, re-dissolving in 0.1M HCl and measuring absorbance at 500nm. Using this simple method of quantification some factors affecting the colour reaction were defined viz., it had a pH optimum 5.0, a temperature optimum at 50° C, and appeared to develop best at the 'Kurumba stage' (6-7 months). Time for development varied from 1-4 days. Analysis of amino acids, with and without colour development, showed that free tyrosine was not the precursor of the red colouring matter.

Introduction

Young water when maintained under sterile conditions develops a pink colouration.[1,2] This was first reported by JeyaRaj and Jansz[1,2] who tentatively suggested that the pink colouration was due to a 5,6 indole quinone produced by oxidation of tyrosine. A method of suppressing the reaction in bottled nut waters had been patented.[3] More recently, the colour development had proved detrimental in the export of frozen nut waters and refrigerated whole young coconut (personal communications) and bottled mature coconut water.[4] No attempt had been made to previously quantify the colour formation, apparently for want of a procedure to concentrate the pigment.

In this study the main factors affecting the development of the pink colour are quantified and evidence is provided to rule out free tyrosine as a precursor of the red pigment.

Materials and Methods

Samples

Young coconut (kurumba) and young coconut (thambili) were obtained from home gardens and were freshly picked on the day of use.

Development of colour

Sodium benzoate (1g/1000ml) was dissolved in young coconut water and the latter filtered. The pH was adjusted to 4.5 with 2M HCl and aliquots (50ml) were

introduced into sterilized bottles (125ml in capacity). The cotton wool stoppered bottles were left to stand for 2 to 3 days at ambient temperature (30°C). The extent of pink colour development was very variable and too low for accurate direct spectrophotometric determination for comparison purposes.

Measurement of colour

The pH of the solution (50 ml) was adjusted to pH 9.5 with 3M NaOH and the mixture allowed to stand for 30 min. at 10°C. The resulting precipitate was centrifuged and the supernatant discarded. The precipitate was re-dissolved in 0.1M HCl and made upto 5 ml. Absorbance was measured at 500 nm with Miton Roy spectronic 21 uv/visible spectrophotometer. Absorbtion was corrected for any particulate matter by measuring light scattering at 700 nm. The absorbtion spectrum of the red pigment was also measured using a Perkin-Elmer uv/visible spectrophotometer Model 552.

Amino acid analysis

Free amino acids were isolated by passing filtered coconut water (200 ml. pH adjusted to 4) through a column (approx. 200g) of amberlite CG-50 (100-200 mesh) cation exchange resin (H^+ form). Having absorbed the cations, the column was washed with distilled water (200 ml) and eluted with ethanol: ammonia (1:1, 100ml). The eluate was concentrated under reduced pressure to dryness using a rotary evaporator (Rotovapor-R. Buchi).

The mixture of free amino acids was dissolved in acetate triethylamine buffer pH 6.4 (5 ml) and 10μl injected into a waters "pico-tag" LC gradient amino acid analyser using standard techniques, i.e. derivatization with phenyl isothiocyanate, separating the mixture on a column (15 cm) using a gradient of sodium acetate and acetonitrile:water (60:40) as calibrated for 17 amino acids (i.e. all the normal protein amino acids except Asn, Gln, Try, Cys).

Results and Discussion

The estimation of colour

On dissolving the precipitate formed at pH 9.5 in 0.1M HCl the uv spectrum showed a shoulder between 484 and 500nm. On cooling this extract in a refrigerator a red powder separated, which on dissolution in methanolic HCl gave a broad symmetric absorbtion peak in the visible region with a λ max at 500 nm. Thus 500 nm was selected as the wavelength for determination. This method of concentrating the red compound is superior to the one described previously.[2]

Analysis of replicates gave a coefficient of variation ($\frac{sd}{\bar{x}}$ x 100) of between 13% and 16%. However, this was not a reflection of the concentration technique as deviations in colour development in unconcentrated replicates were visible to the eye.

Further after precipitation of the pigment, the supernatant showed no absorbance (< 0.01) at 500 nm on readjustment of pH. At pH values above 5.5 spoilage of the coconut water occurred. As this resulted in turbidity readings above this pH are not reliable. In hindsight it may have been preferable to use methanolic 0.1M HCl instead of aqueous 0.1M HCl for dissolving the precipitate because the former dissolved less impurities. However, this observation was not quantitatively confirmed.

It was presumed that the precipitate at pH 9.5 is a coprecipitate of basic proteins (histones?) and the pigment. Alternatively the pigment may be a part of the peptides or proteins that precipitate.

It was noticed that independent of: (i) whether the sample was young coconut (kurumba) or king coconut (thambili) water and also (ii) colour intensity, the precipitate at pH 9.5 can take two forms (a) an off-white profuse precipitate that forms immediately or (b) a blue precipitate that only forms on cooling at 10^0C.

It was felt that this was due to maturity and also genetic factors affecting the amount of protein in the young coconut water sample.

Effect of pH

Preliminary experiments showed that the optimum was between 4.5 to 5.0. Results (Figure 1) confirmed this.

Effect of temperature

Results (Table 1) showed that maximum colour developed at 50^0C.

Table 1 : Effect of temperature on colour development

Incubation Temperature (^{0}C)	Corrected Absorbance at 500 nm
12	0.034
29	0.068
37	0.098
50	0.116
60	0.095

pH 4.5; incubation time, 2 days

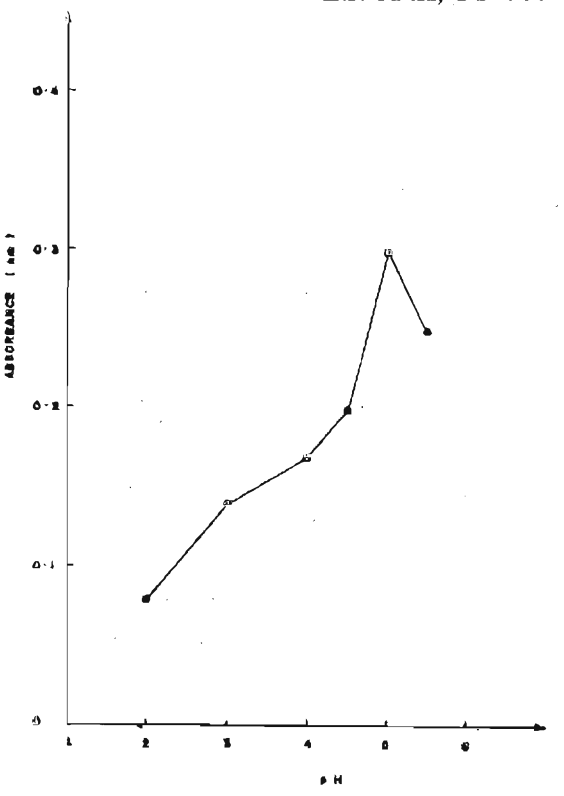

Figure 1: Effect of pH on colour development
Temperature, 29-30^{0}C; Incubation time, 3 days

Effect of time and colour development

Results (Table 2) show that by 29 h. the colour had reached nearly the full intensity and remained approximately constant upto 4 days.

It should be noted that if fruits have not been harvested on the same day, colour development can be quicker.

Effect of maturity

Nuts picked from 4 successive bunches from 4 months onward gave results as in Table 3, showing that colour development by coincidence was at a maximum at the best stage of maturity for drinking purposes. It was further found that the colour reaction also takes place to a slight extent in sterile mature coconut water after filteration- a reaction very similar to nuts > 8 months of age.

Table 2 : Effect of incubation time

Time (h)	Corrected Absorbance at 500 nm
0	0.071
23	0.270
29	0.296
54	0.345
71	0.318
95	0.317
100	0.274

pH 4.5; Temperature, 29-30^{0}C

Table 3 : Effect of Maturity

Approximate age (months)	Common Name	Corrected absorbance Absorbance at 400 nm
4	Late button	0.063
5	Wawara	0.135
6	Early Kurumba	0.237
7	Late Kurimba	0.210

pH 4.5; Incubation time 3 days; temperature, 29-30^{0}C

Other factors

The presence of turbidity due to not filtering or poor filteration reduces both the rate and extent of colour formation. Previous studies had shown that re-introduction of filtered material inhibits pink colour development and that antioxidants completely inhibit the reaction.[2]

It is possible that this effect of filterable material is due to either reduction of oxygen tension or the promotion of completing reactions for the precursor. The previous report[2] that microbial contamination reduces or eliminates the reaction fits well with the latter. The hypothesis that tyrosine was the precursor was next tested.

Amino acids

The free amino acid profile of samples before and after pink colour development is shown in Table 4. Results show that free tyrosine concentration in the young coconut water is low and that this is no significant decline of free tyrosine when the pink colour has formed, thus ruling out free tyrosine as the precursor of the red compound. This

is further supported by evidence that tyrosine levels in the mature nut water (much less prone to the colour change) is higher than the young nut water.[4]

Table 4 : Free amino acid analysis

Amino acid	n moles/100ml Young king coconut	Young coconut	Young coconut (pink)
Aspartate	-	2.05	-
Glutamate	-	12.54	2.05
Serine	17.54	13.97	1.28
Glycine	2.27	-	-
Histidine	10.85	12.24	11.56
Arginine	-	-	-
Threonine	16.18	85.90	-
Alanine	3.96	13.95	7.77
Proline	43.22	38.99	48.17
Tyrosine	4.41	2.92	2.42
Valine	3.68	3.88	8.56
Methionine	-	0.70	-
Cystine	-	-	-
Isoleucine	-	2.90	2.92
Leucine	-	-	-
Phenylalanine	1.66	4.19	3.79
Lysine	20.66	34.06	24.23

-, not detected

Tryptophan (tentative) was approximately 25, 11.5 and 12 nmoles/100ml respectively.

The findings are consistent with the multistep reaction with at least one enzymic step. The variations of colour development observed in research and industry can be explained by the effect of pH, temperature, maturity, freshness of the nut and oxygen availablilty, and also other undefined factors such as reactions competing for the precursor or an intermediate.

References

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