R.P.F.III

FINAL REPORT

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1. Institute Code No: 1000766005 (Phy XXIX(231))

2. I.C.A.R.Code No.

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3. Name and address of Research Institute/Centre:

Central Plantation Crops Research Centre
Kudlu P.O., Kasaragod 671 124, Kerala.

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4. Project Title:

Genotypic variation in essential fatty acids and other nutrients at different stages of fruit development in coconut.

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5. Name & designation of Principal investigator:

Dr. Shamina Azeez
Scientist (Senior Scale) – Biochemistry (Plant Sciences)

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6. Name(s) and designation of Associates) and establishment(s) on which borne:

(a) Whole time: None

(b) Part time (indicate proportion of time to be devoted and other area(s): None

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7. Location of research project with complete address:(Division/Section/Sub Station)

Division of Physiology, Biochemistry and Post Harvest Technology
Central Plantation Crops Research Centre
Kudlu P.O., Kasaragod 671 124, Kerala.

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8. Date of start: October 1998 9. Date of termination: August 2006

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10. (a) Objectives: (not more than 150 words)

1. To compare the concentration of essential fatty acids in coconut oil from nuts of different maturity, in selected cultivars.

2. To study the lipid profile among the cultivars at different maturity.
3. To investigate the nutritional value of nuts of different maturity and cultivar.

Goal

1. Identify the coconut variety and nut maturity of optimal nutritional value, for consumption.
2. To help in breeding selection for optimal essential fatty acid and nutrient status.

(b) Practical utility (not more than 100 words)

The main constraint in the use of coconut oil as a dietary fat, apart from the controversy surrounding its hypercholesteremic property, is its low essential fatty acids content. Previous studies by Padua-Resurreccion and Banzon (Philippine Coconut studies 4(3): 1-15 1979) indicates greater essential fatty acids content in tender coconut (about 8 months from pollination). Similar changes exist in the other nutrients with respect to maturity levels, as revealed in studies abroad and summarized in the review by Shivasankar (Journal of Plantation Crops. 19(2): 102-119, 1991.)

This project intends to study the changes in the popular cvs/hybrids, released by CPCRI, in major nutrients – fats, proteins, carbohydrates, vitamins – and to identify the genotype with optimal nutrient content. This project is an offshoot of the QRT recommendation to look for scientific validation to promote tender nut consumption.

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11. Technical program (Indicate briefly plan of procedure, techniques, instruments and special materials, organism, special environments etc.)

I Year

1. Tagging of freshly fertilized coconuts of released cvs/hybrids (see below in 'Materials' for details) in the post-monsoon, pre-monsoon and monsoon seasons.
2. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in post-monsoon.

II Year

1. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in pre-monsoon.

2. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in monsoon.

III Year

Statistical analysis & report writing.

Materials

In the first phase of the experiment, i.e. October 1998 to October 2001, the cvs/hybrids studied were WCT, COD x WCT, LCT x GBGD, LCT x COD and WCT x GBGD. The project was extended to include the cvs/hybrids WCT x COD, LCT, COD and GBGD in the second phase of the experiment, i.e. March 2004 to August 2006.

Parameters

In nut water and kernel - fat content, fatty acid composition, vitamin C, total proteins, total amino acids, total soluble sugars and reducing sugars; nut water volume; kernel moisture, starch and dietary fiber were analyzed.

Seasons and Maturity

Freshly fertilized bunches were tagged in post monsoon (November), pre monsoon (May) and monsoon (June) seasons in both the first and second phase of the experiment and sampled on the 7th, 8th, 9th, 10th and 12th month after fertilization.

Techniques

Fat content: Soxhlet extraction and gravimetric analysis.

Fatty acid profile: Conversion of fatty acids to fatty acid methyl esters (FAME) and profiling in gas chromatograph (Banzon, J.A, and Resurreccion A.B.P. 1979. Fatty acid distribution in coconut oil obtained by four processing methods and secured from four Philippine types of coconuts. *Philippine Journal of Coconut Studies*. Vol IV No 2, pp. 1-8.)

Vitamin C: Spectrophotometry (Sadasivam, S. and A. Manickam.1996. *In*: Biochemical Methods. (II Edition). New Age International (P) Ltd. Publishers, New Delhi and TNAU, Coimbatore. Pp 185-186.)

Total protein: Spectrophotometry (Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193: 265.)

Total free amino acids: Spectrophotometry (Moore, S. and Stein, W.H. 1948. *In*: Methods in Enzymology. (Eds. Colowick, S.P. and Kaplan, N.D.) Academic Press, New York, 3: 468.)

Total soluble sugars: Spectrophotometry (Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 26: 350.)

Total reducing sugars: Spectrophotometry (Miller, G.L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*. 31: 426.)

Starch: Spectrophotometry (Hodge, J.E. and Hofreiter, B.T. 1962. Analysis and. preparation of sugars. *In*: Carbohydrate Chemistry (Eds. Whistler, R.L. and Be Miller, J.N.) Academic Press, New York.)

Dietary fiber: Gravimetric (Van Soest, P.J. and R.H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of Association of Official Analytical Chemists*. 50: 50-55.)

Instruments

Spectrophotometer (Shimadzu UV-160 A): Vitamin C, total proteins, total amino acids, total soluble sugars, reducing sugars and starch.

Gas chromatograph (Shimadzu GC 2010): Fatty acid profile.

Soxhlet extractor: Oil content.

Fibertec system M1017 (Tecator): Dietary fiber in kernel.

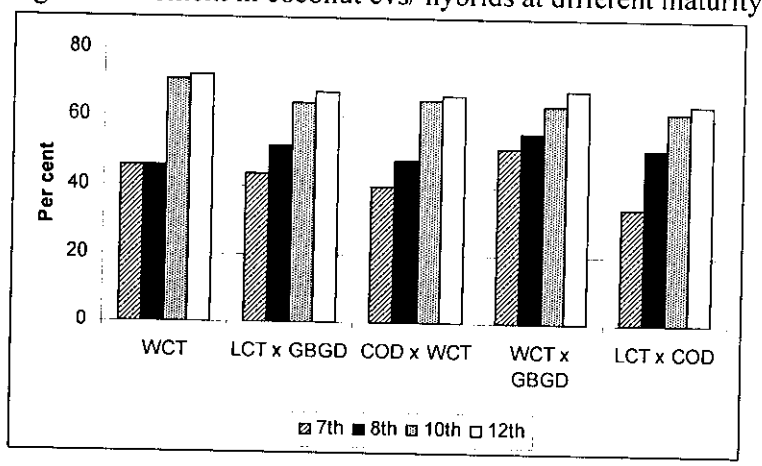
12. Final Report on the Project:
(A summary of results not exceeding 5 typed pages precisely and concisely stating the fundamental and/or practical significance thereof.)

As indicated in Sl. No. 11, the project was initiated (in October 1998) with the analysis of five released cvs/hybrids - WCT, COD x WCT, LCT x GBGD, LCT x COD and WCT x GBGD. With the conclusion of these analyses, the project was extended (in March 2004) to four more cvs/hybrids - WCT x COD, LCT, COD and GBGD. Freshly fertilized bunches were tagged in post-monsoon (November), pre-monsoon (May) and monsoon (June) seasons and the biochemical analyses of the nut water and kernel at 7, 8, 9, 10 and 12 months after fertilization, have been completed. The parameters studied in nut water and kernel were fat content, fatty acid composition, vitamin C, total protein, total amino acids, total sugars and reducing sugars, and nut water volume; and additionally in kernel, moisture, starch and dietary fiber.

The results of the first phase of the project – analysis of the nutrient content of the kernel – has been published and is attached (Shamina Azeez and John George. 2004. Changes in the composition of coconut kernel (*Cocos nucifera* L.) with maturity and season of fertilization in selected cultivars/hybrids. *Journal of Plantation Crops*. 32 (Suppl.): 433-436.) This phase did not include the analysis of nut water, kernel starch or the ninth month nuts and is therefore not reported. The results of the fatty acid profile (sent for publication) are given below.

Oil content: Fig. 1 gives the oil content of the maturing kernels of different varieties of coconut fertilized in monsoon. As already known the oil content increases with nut maturity and in the present study it increases from a low of 24% in the 7th month nut of the LCT x COD hybrid to a high of 72% in the fully mature nut of WCT. Oil content stabilizes around the 10th month (60-65%). The oil contents of nuts tagged in the pre-monsoon and monsoon seasons were on par, while that of nuts tagged in the post-monsoon season was significantly higher. Oil content was least in LCT x COD (24-68%) and maximum in LCT x GBGD and WCT (43-72%). The contents in WCT x GBGD and COD x WCT were on par (40-70%). The difference in oil contents of nuts differing in maturity was significant at both 5 and 1% levels.

Fig. 1. Oil content in coconut cvs/ hybrids at different maturity stages in monsoon.



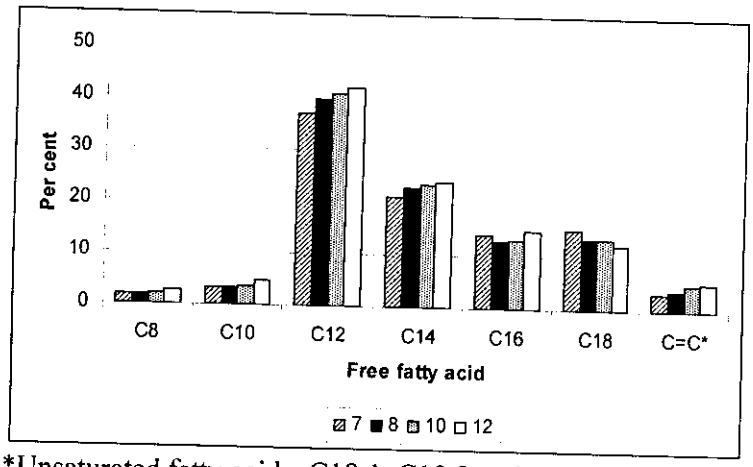
Fatty acid profile: The predominant fatty acids were the medium chain fatty acids lauric acid (C12:0) (31-47%) followed by myristic acid (C14:0) (18-25%), the concentrations of which increased with maturity and were on par in the nuts of 10th and 12th month maturity (Fig. 2). The concentrations of the small chain saturated fatty acids, viz., caprylic acid (C8:0), ranging between 1 to 7.5%, and capric acid (C10:0), ranging between 2 to 7.7%, were also found to significantly increase with the increasing

maturity of the nut. Though significant differences were observed in fatty acid concentrations of nuts of different treatments, the trend remained the same.

All the above fatty acids, except C14:0 were least in nuts tagged in post-monsoon season, while those tagged in pre-monsoon and monsoon seasons were on par, in all except C8:0. Of the five varieties, COD x WCT had lesser amounts of C8:0, C10:0 and C12:0 fatty acids; concentration of C8:0 and C10:0 were on par in COD x WCT, LCT x GBGD and LCT x COD, the varieties WCT and WCT x GBGD were on par.

The long chain fatty acids, palmitic (C16:0) and stearic (C18:0) acids ranged between 7 to 24% of the total fatty acids, while the unsaturated fatty acids oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids together made up only 2 to 6%. C16:0 and C18:0 were lesser in the more mature nuts, while the unsaturated fatty acids increased marginally with nut maturity, but the concentrations were on par among the 10 and 12-month-old nuts. The concentration of these fatty acids was higher in nuts tagged in the post-monsoon season, while those tagged in pre-monsoon and monsoon seasons were on par. No marked difference in concentrations of long chain fatty acids was found among the varieties.

Fig. 2. Fatty acid profile in WCT coconut oil at different maturity stages, fertilized in post-monsoon season.



*Unsaturated fatty acids: C18:1, C18:2 and C18:3

The results of the second phase of the project where the cvs/hybrids – WCT x COD, LCT, COD and GBGD – were studied are presented below. Here the nutrients in the nut water and the 9th month nuts were also analyzed. The result of the fatty acid profile of the post monsoon fertilized nuts alone is presented here, as the data of the pre monsoon and monsoon-fertilized nuts were lost when the gas chromatograph went out of order. As expected, nut water volume and kernel moisture decreased as the nuts matured, COD had considerably greater volumes than the other cvs/hybrids, followed by GBGD; WCT x COD had the least. Likewise the kernel moisture also decreased significantly with nut maturity; the dwarfs had greater moisture than talls, especially COD (Fig. 3.)

Fat content in kernel increased with nut maturity, with WCT x COD and GBGD having greater amounts. In the nut water minor amounts of fat were detected, increasing as the nuts matured; GBGD had the maximum amounts, followed by LCT, and the pre-monsoon nuts had significantly greater fat. An analysis of the fatty acid composition of the kernel and nut water revealed the following. In both kernel and nut water lauric acid (C12:0) formed the major fraction of the total fatty acid content. In the kernel, the short to medium chain fatty acids (C8:0, C10:0, C12:0) increased with nut maturity, while the long chain fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, C22:1 and C24:0) decreased with nut maturity. The essential fatty acids linoleic and linolenic acids (C18:2 and

C18:3 respectively) were in greater concentration in tender nuts. In the nut water generally very little fatty acids were detected, especially in the tender nut water (Fig. 4.)

Fig. 3. Nut water volume and kernel moisture content in COD, fertilized in post monsoon season.

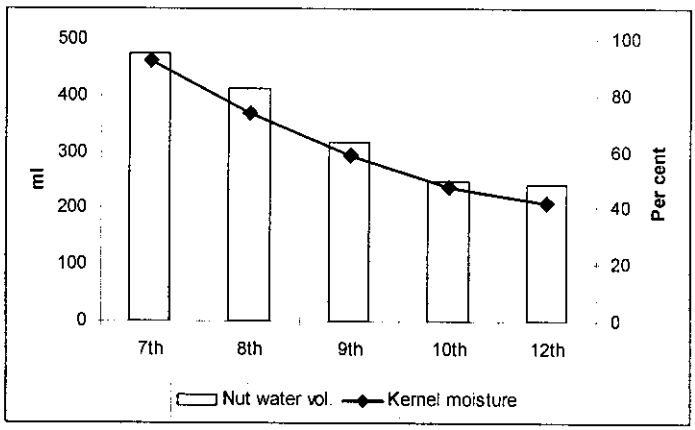
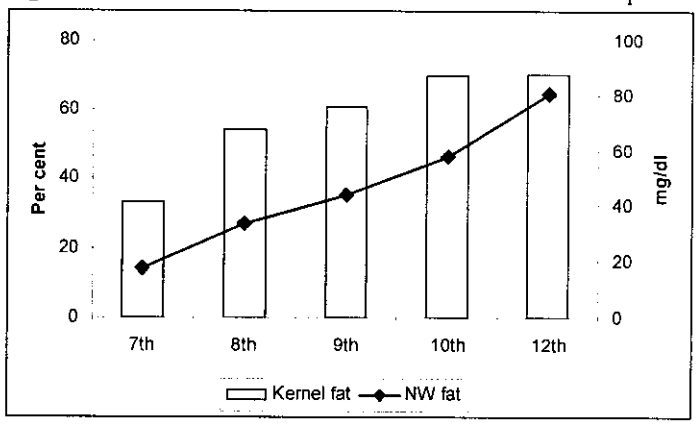
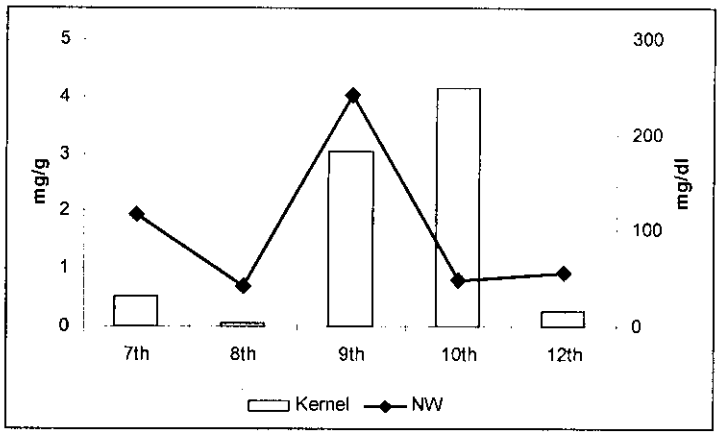


Fig. 4. Kernel and nut water fat in LCT fertilized in post monsoon season.



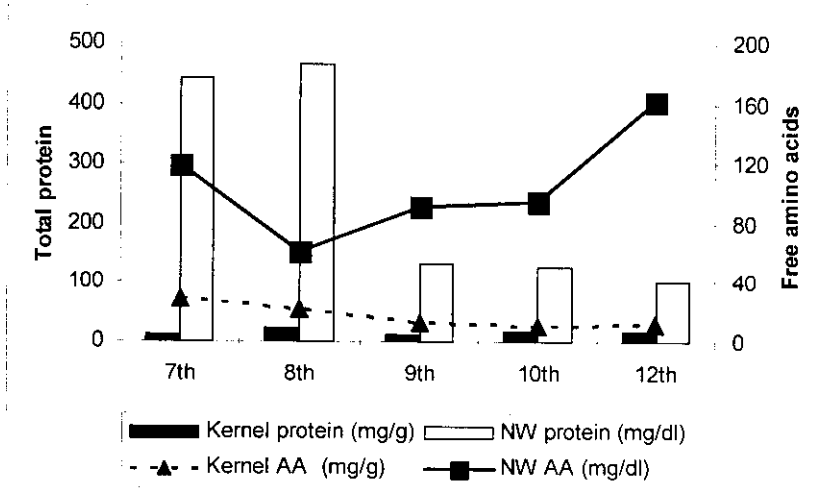
There were significantly greater amounts of vitamin C in the nut water compared to kernel, which decreased with maturity, drastically after the 9th month. GBGD contained maximum amounts, followed by WCT x COD and COD. Similarly in the kernel, though the trend was somewhat erratic, vitamin C decreased after the 9th month; the dwarfs, especially GBGD, containing greater amounts (Fig. 5.)

Fig. 5. Vitamin C content in kernel and nut water of GBGD nuts fertilized in post monsoon season.



In the kernel the total protein content increased to a maximum in the 9th month, after which it declined; LCT recorded the least, the rest being on par. In nut water total protein content decreased with nut maturity; there were significantly greater amounts in the talls, especially LCT. The free amino acid pool in the nut water and kernel showed reverse trends: while the kernel amino acid content decreased to a minimum by the 10th month (followed by an increase by 12th month), in nut water it increased up to the 10th month (the 10th and 12th month values being on par). In the kernel, the dwarf COD had greater amounts, while in nut water it was WCT x COD. In both the amino acids was less in post monsoon fertilized nuts.

Fig. 6. Kernel and nut water protein and amino acid content in LCT fertilized in monsoon.



The total sugars in kernel increased to a maximum in the 9th month and post monsoon fertilized nuts; there was no significant difference among the varieties. In the nut water it decreased with maturity and was maximum in GBGD, minimum in WCT x COD. Reducing sugars in both kernel and nut water decreased with nut maturity; while COD had maximum kernel reducing sugars, GBGD had the most in nut water (Fig. 7.) The starch content in the kernel decreased to a minimum by the 10th month, the 9th and 12th month contents being on par; it was greater in monsoon tagged nuts. The dietary fiber content of the kernel increased significantly with nut maturity, maximum being in COD and least in WCT x COD (Fig. 8.)

Fig. 7. Kernel and nut water soluble and reducing sugars in GBGD nuts, fertilized in post monsoon season.

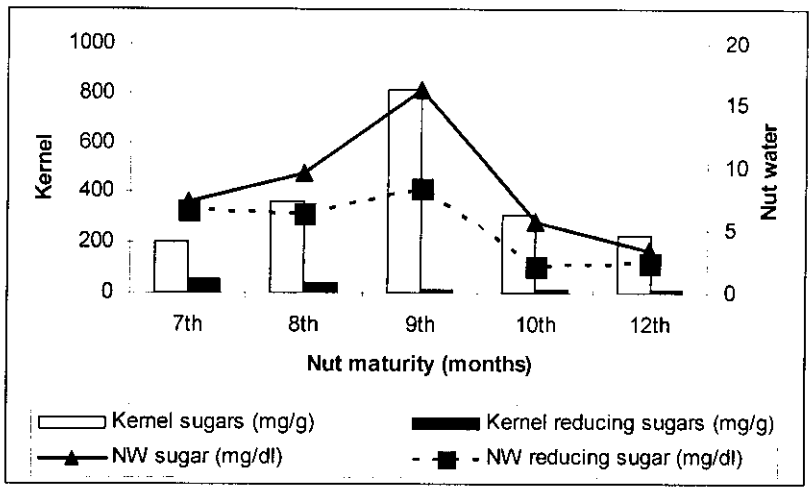
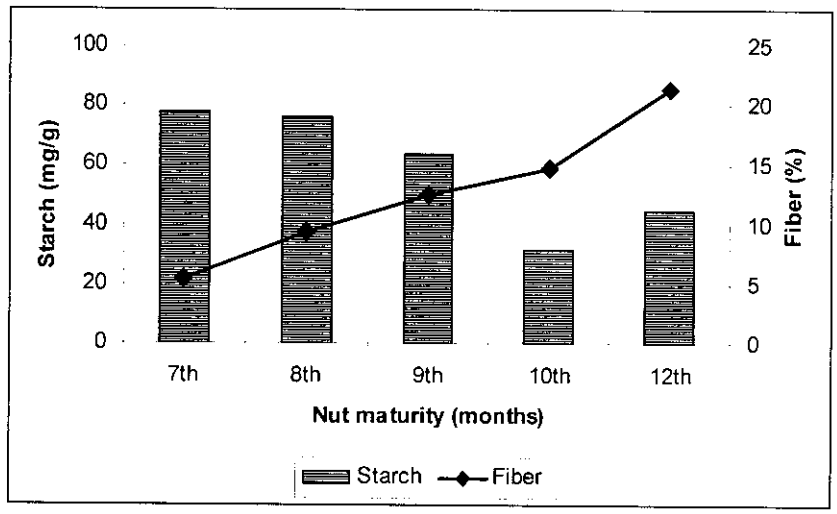
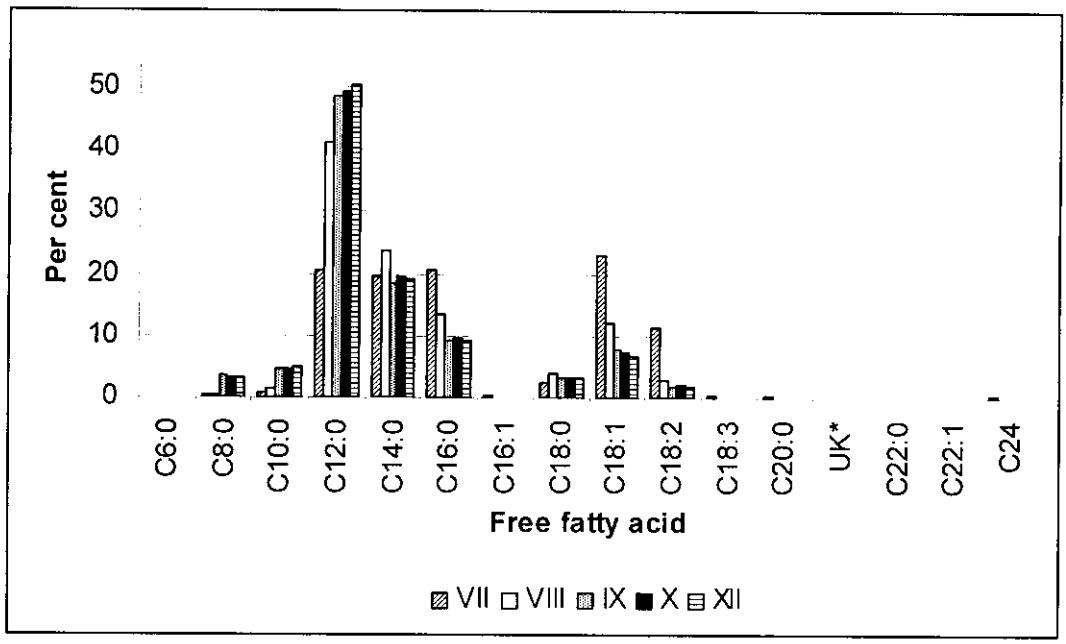


Fig. 8. Kernel starch and dietary fiber in COD, fertilized in monsoon season.



The fatty acid profile of the kernel and nut water of the second phase of the experiment could be done only for the post monsoon season (the reasons have been cited above), the result of which is presented below. C6:0 to C12:0 fatty acids increased, while C14:0 to C24:0 fatty acids decreased with nut maturity. The essential fatty acids (C18:2, C18:3) were greater in content in tender kernel. COD nuts have greater lauric acid content (Fig. 9.)

Fig. 9. Fatty acid profile in COD nuts fertilized in post monsoon season.



*UK- Unknown

The collaborative project to study the effect of coconut oil on the health of the heart, with the Cardiology division of KG Hospital, Coimbatore, is underway since April 2005. So far data of a total of 552 subjects – normal, cardiac patients both pre- and post- statin treatment – have been obtained and is being analyzed. The proforma included the following:

Personal details; Family profile; Habits like hobbies, exercises, intake of beverages etc.; Food habits including oil/fat used, quantity; Vegetarian/ Non-vegetarian; Calories intake/day and the

percent contributed by oil/fat; Clinical status/ Biochemical details; Medicines prescribed; Family history of ailments; Emotionally/physically compatibility with family members.

The details available so far are attached with this document as soft copy (File name: KG compiled data).

The conclusions obtained from this project are:

- In both kernel and nut water lauric acid (C12:0) was the predominant free fatty acid; the content was greater in the talls.
- The essential fatty acids linoleic and linolenic acids (C18:2 and C18:3 respectively) were in greater concentration in dwarf, tender nuts.
- Post-monsoon fertilized and GBGD nuts had maximum Vit. C in both nut water and kernel.
- In the nut water total protein content decreased with nut maturity, significantly greater amounts were present in talls. In kernel the protein content increased to a maximum in the 9^{th} month, least being in LCT.
- The free amino acid pool in nut water and kernel showed reverse trends: while kernel amino acid content decreased to a minimum by the 10^{th} month, in nut water it increased up to the 10^{th} month. COD kernel and WCT x COD nut water had greater amounts.
- The total sugars in kernel increased to a maximum in the 9^{th} month and post monsoon fertilized nuts; in nut water it decreased with maturity and was maximum in GBGD and least in WCT x COD.
- Reducing sugars in both kernel and nut water decreased with nut maturity; while COD had maximum kernel reducing sugars, GBGD had the most in nut water.
- Starch content in the kernel decreased to a minimum by the 10^{th} month; it was highest in monsoon tagged nuts; dietary fiber content was maximum COD and it increased significantly with nut maturity.

The above results obtained from this project will thus help in identifying the optimal maturity and season of fertilization in the released cvs/hybrids studied, for the nutrient content. This information should be useful for the farmer, the consumer, and the nutritionists/ scientists of allied fields.

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE

KASARAGOD-670124, KERALA

RPF III

Project No. 1000766005
Date of start: October 1998

Technical Programme:

I Year

1. Tagging of freshly fertilized coconuts of released cvs/hybrids in the post-monsoon, pre-monsoon and monsoon seasons.

2. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in post-monsoon.

II Year

1. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in pre-monsoon.

2. Analysis of nutrients in the kernel and water of 7, 8, 9, 10 and 12 month old nuts tagged in monsoon.

III Year

Statistical analysis & report writing.

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13. Progress of work in relation to the time targeted for completion of work and reasons for non-achievement of targets, if any.

All the above components of the technical program have been completed except the analysis of the fatty acid profile of coconut oil of 10^{th} and 12^{th} months of monsoon and 12^{th} month of pre monsoon tagged nuts in the second phase of the project i.e. in WCT x COD, LCT, COD and GBGD. As indicated earlier, the fatty acid profile of oil of the 7^{th}, 8^{th}, 9^{th} and 10^{th} month nuts of the pre monsoon tagged and the 7^{th}, 8^{th} and 9^{th} month nuts of the monsoon tagged nuts were completed. However the data could not be presented here, as it was lost when the gas chromatograph went out of order. But the fatty acid profiles of the post monsoon tagged nuts have been completed and the results presented above; the trend is the same as in the first phase of the project. As a consequence it may be presumed that the data lost also would have followed the same trend.

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14. Publications:

(a) Research papers: ~~Two~~ One

(b) Popular articles: One

(b) Reports

Two copies of **each** to be supplied with this proforma: Attached

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15. Details (Nos. etc.) of Field/laboratory Note Books and their final location.
Two.
In: Division of Physiology, Biochemistry and Post Harvest Technology,
Central Plantation Crops Research Institute, Kasaragod 671 124.

Shamina. A
18-8-'06

16. Signature of Principal Investigator

17. Signature of Head of Division/Station/section

18. Signature of Director