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9. ABSTRACT

Reports on investigations to develop a commercially viable process for the quantitative recovery of high quality coconut oil as well as food grade protein products. A large segment of the population of coconut producing countries consume diets inadequate in protein. Many of these countries import large quantities of protein foods. If an alternative to imported protein could be developed from indigenous crops it would improve the import-export balance while producing new industries and new income for the people. The common method of processing coconuts causes considerable loss or damage of coconut oil. The investigations reported here focus on four areas: fundamental properties of coconut proteins, the principle of separating the oil and protein phases by centrifugal force in aqueous medium, the recovery of food grade protein products by more traditional technology based on the use of desicated coconut of food grade copra, and the ultrastructure and biochemical development of coconut meats to provide information useful in the production, harvesting and processing of coconut.

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FINAL REPORT

to the

US Agency for International Development

For a Project Entitled

DEVELOPMENT OF A PROCESS FOR THE PREPARATION OF COCONUT PROTEIN PRODUCTS FOR USE IN FOODS

(Contract csd/2804)

from

Texas A&M Research Foundation

College Station, Texas 77843

March 1976

This report is intended to satisfy the contract requirements for "a final report which summarizes the accomplishments of the assignment, methods of work used and recommendations regarding unfinished work and/or program continuation." (General Provisions for USAID contracts, clause No. 16)

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REPORT SUMMARY

1. Development of a Process for Preparation of Coconut Protein Products for Use in Foods. Contract csd/2804.

2. Project Coordinator: Karl F. Mattil; Principal Investigators: Carl M. Cater, J.W. Dieckert, Robert D. Hagenmaier, Food Protein R&D Center, Texas Engineering Experiment Station, Texas A&M University, College Station, TX 77843.

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NARRATIVE SUMMARY OF ACCOMPLISHMENTS AND UTILIZATION

All of the originally stated objectives of this project have been attained. The physical characteristics and the chemical and nutritive properties of coconuts and/or processed coconut products have been determined. The technical feasibility of key unit operations of each of the following processes: the expeller process, the dry and solvent extraction process and the aqueous process, have been investigated along with the processing economics and the potential marketability of the products of each process. The food use potential of the derived protein products have been investigated and the information has been widely disseminated. Selected scientists and engineers from coconut producing countries have received training in the general techniques of oilseed processing and in the processing of coconuts for the recovery of food protein products. Of the processes evaluated, the one which has shown the greatest potential for early commercialization is the aqueous process for the simultaneous recovery of coconut oil and food grade protein products from fresh coconuts. Several engineering consultants appointed by USAID intensively reviewed the aqueous process and declared it to be technologically feasible. As a consequence, the government of the Philippines has funded a pilot plant which is currently in successful operation under the direction of a member of our staff. Representatives

of the Philippine government and of the food industry of the Philippines have shown a substantial amount of interest in the product from this pilot plant. Simultaneously conducted experiments with the utilization of food grade copra have shown that this concept is at least technologically feasible. This process is now ready for field demonstrations in a coconut producing country. The results of our studies on the developing coconut show that endosperm production per seed and probably the protein content are functions of environmental factors coming to bear at specified phases of endosperm growth.

FINAL RESEARCH REPORT

A. General Background

Coconuts are an important agricultural crop in many tropical countries, particularly the Philippines, Indonesia, Thailand and Sri Lanka. Like other oilseeds, the coconut is frequently processed for oil alone with the oilseed meal not finding use in human food. The consequence is a waste of a food resource. In the coconut producing countries, large segments of the population consume diets which are inadequate in protein. It is generally believed that if their diets could be improved by supplying low cost nutritious protein foods their total quality of life could be moderately to substantially improved with accumulative benefit to the economic and social stability of their countries. Further, many of the countries now import large quantities of protein foods such as non-fat dry milk or condensed milk. If an alternative to the imported protein foods could be developed from indigenous crops it would effect an improvement on their import-export balance while producing new industries and new income for their people.

Coconuts are commonly processed by drying in the sun or with smokey, hot air to make copra. During this drying coconut oil is lost or damaged by hydrolysis, physical losses and microbial action. The copra is pressed and/or extracted to recover crude coconut oil and coconut meal. Because of insect and microbial infestation, the copra meal is unfit for human consumption and can only find use as animal feed or fertilizer.

The loss of food is considerable. Coconut oil enjoys a good world market position and represents a major proportion of the export trade of some of the coconut producing countries. However, its quality has been damaged and its value reduced in the copra drying process. Annual world production of copra meal is about one million metric tons and contains about 20% of good quality protein which is not now available for use in foods. What is needed and has been the objective of this project is a commercially viable process or processes for the quantitative recovery of higher quality coconut oil as well as food grade protein products.

B. Statement of Project Objectives as Stated in the Contract

1. Conduct a thorough laboratory investigation, building upon the laboratory work conducted under AID Contract EA-84, of the physical characteristics and the chemical and nutritive properties of coconuts and/or processed coconut products;

2. Investigate the technical feasibility of key unit operations

of each of the following processes: the expeller process, the dry and solvent extract process and the aqueous process;

3. Evaluate the processing economics of the three above-proposed processes;

4. Investigate the food use potential of the protein products of each of the above three processes and disseminate information and limited quantities of material to other research groups in university, government and industry laboratories for product evaluation and development work;

5. Train selected scientists and engineers from coconut producing countries in the general techniques of oilseed processing and in the processing of coconuts for the recovery of food protein products;

6. Prepare selected scientists from coconut producing countries for the initiation of research activities on coconut processing at a research center in a major coconut producing nation;

7. Define the parameters for a pilot plant to demonstrate the commercial viability for the most favorable processing alternative, if warranted by investigation of the unit operations.

C. Continued Relevance of Objectives

In our judgment, the relevance of the objectives have been established beyond reasonable doubt. The orderly stepwise approach through the phases of fundamental scientific investigations, resolution of problems related to individual unit operations, to the final consolidation into a continuous process has once again proven to be the most efficient approach to the development of a new processing technology. As a consequence of this approach, we have been able to design at minimum expense a pilot plant for operation in the Philippines which at the date of this report is now processing about 2,200 coconuts per day. This is about 20 times the daily capacity of our facilities at Texas A&M University. The objective of the production of food grade copra for conversion into food grade copra flour by prepress solvent extraction remains relevant but is at a point where it must now be demonstrated to be workable under conditions existing in coconut producing countries.

D. Accomplishments to Date

In 1969 the US Agency for International Development asked the Food Protein Research and Development Center of Texas A&M University to undertake the development of a process or processes for the recovery of food grade protein products from coconuts. Inasmuch as

this has been the objective of research projects in a number of coconut producing countries for many years, a substantial amount of technology had already been developed. We have sought continuously to build on the knowledge and experience that have been reported and continue to be reported from these other laboratories. Our investigations have followed four main channels: a) investigation of the fundamental properties of coconut proteins; b) investigation of the principle of separating the oil and protein phases by centrifugal force in an aqueous medium; c) the recovery of food grade protein products by more traditional technology based either on the use of desiccated coconut or food grade copra; and d) an investigation of the ultrastructure and biochemical development of coconut meats to provide information useful in the production, harvesting and processing of coconuts. Our research has included the development of fundamental information, process technology, economic analyses of the processes, investigation of the food uses of the products and efforts to move this process technology into coconut producing countries.

The amount of work that has been done on this project has been far too extensive to be any more than superficially covered in this report. The detailed data have been reported in quarterly reports, in publications in scientific journals and in theses and dissertations. Copies from publications in scientific journals are appended to this report. In the body of the report these publications will be referred to by letter (a, b, c, etc.). The dissertations and theses are voluminous and in the main contain data that are reported in the publications. Therefore they and the publications along with selected other references will be referred to in the body of the report by number (1, 2, 3, etc.).

Properties of Coconut Proteins

Studies were made on nitrogen (protein) solubility of coconut meal in aqueous media over a range of pH's. The point of minimum solubility was found at pH 3.9 and nitrogen solubility increased toward both the acidic and basic ranges. Coconut proteins displayed a markedly different pH-nitrogen solubility profile in salt solutions, showing minimal solubility at pH's at or below three with a sharp rise to a maximum solubility at neutrality. Coconut meal prepared from fresh coconuts with and without testa removed, from parings and from a sample of sun dried copra showed comparable solubility characteristics. However, meal prepared from commercial desiccated coconut showed lower solubility even under acid and alkaline conditions (pH 2 and 10). The most efficient solvent-to-meal ratio was found to be 20:1 (v:w) for a single extraction, and a two-step 15:1 process for multiple extractions. The addition of various salts caused marked alterations in the protein solubility

profiles of coconut meal. Of the salts tested, each had a different effect. Osborne classification studies of coconut meal indicated that 90% of the proteins could be classified as albumins and globulins (1,A).

Further work showed that there are at least two classes of globular proteins. The major fraction consists of 84% of the proteins and has an average molecular weight of 150,000. This major fraction is only about 50% soluble at neutral pH and low salt concentration (0.1M NaCl). Solubility is increased at acidic or alkaline pH values, or with added salt. Solubility is considerably decreased by heating above 80°C at pH 5.0 to 7.5. A second fraction, containing 14% of the proteins has molecular weight of 24,000. This fraction remained soluble under all conditions of pH, salt concentration and temperature. The two classes of proteins have grossly different amino acid patterns, with the small molecular weight fraction containing more lysine and cysteine and less threonine and methionine. These differences in solubility and amino acid composition have important implications for aqueous processing and for the production of protein concentrates and isolates. It must be assumed that different protein fractions obtained from coconuts would be expected to have different nutritive values (2,B).

In attempts to determine the heat tolerance of coconut protein, coconut meats and coconut meal were subjected to several temperatures for varying lengths of time. Properties evaluated were browning (by visual observation), odor, lysine destruction, protein solubility at pH 2, 8 and 10.5 and nitrogen solubility index. From the experiments it was concluded that fresh coconut meats can tolerate up to 100°C air temperature (coconut layer temperature, 78 to 83°C) without significant loss in protein solubility. At 110°C air temperature, there is a loss of solubility at pH 2 and 8, and at 120°C protein solubility significantly decreased under all pH conditions examined. There was a marked loss of lysine availability when the coconut meats were heated in hot air at 120°C. Coconut meal can tolerate 105°C air for at least 60 minutes without significant loss of protein solubility. At 120°C for 15 minutes or longer, protein solubility decreased (3,C). These data combined with those above were critical to the design of processing conditions that could produce undenatured coconut protein products.

Early consideration was given to the possibility of the production of coconut protein isolates either directly by aqueous processing or indirectly by extraction from defatted, desiccated coconut or food grade copra meal (4,5,D). In the case of the latter two, several extraction and precipitation procedures were evaluated based upon previously determined protein solubility characteristics. A yield of about 63% of protein isolate based on the total protein

available was obtained either by extracting the meal at pH 10.5 directly or by sequential extractions at pH 8.0 and 10.5. These isolates had solubility characteristics and amino acid compositions similar to that of the original coconut meal. Attempts to produce an isolate by extraction at pH 2.0 showed that at this pH, coconut protein is partially denatured causing it to have a considerably reduced solubility in the alkaline range. Further work with isolates was set aside for several reasons. The principle reason was that a cost analysis showed that isolates prepared either by the aqueous process or from defatted, desiccated coconut would probably be too expensive. Further, in the production of isolates nutritionally valuable protein and other nutrients would remain dissolved in the aqueous extract and at that time there was no technology available to recover these valuable nutrients from the whey. On the other hand, an economic analysis suggested that an isolate produced from food grade copra might be commercially competitive and find a market. Again, at that time we had no technology for the production of food grade copra. It is our present recommendation that the possible production of coconut protein isolate not be considered a closed book. As will be pointed out later, we now believe that technology has been developed that markedly increases the feasibility of food grade copra. Further, in other research in the Food Protein R&D Center, technology has been developed for the recovery of proteins and other nutrients from the by-product wheys resulting from the production of vegetable protein isolates under conditions that look economically attractive. Consequently, all of the necessary technological pieces are available to put together a process for the production of protein isolates from food grade copra at an economically competitive cost. Whether or not this ever becomes a reality will depend upon the potential commercialization of food grade copra which will be discussed in more detail later in this report.

Aqueous Processing

The science and technology of aqueous processing of fresh coconut meats has received major emphasis during the entire project. The emphasis shifted from the recovery of an isolate to the recovery of the total non-oil solubles of the coconut. Utilizing the fundamental information that has been generated, a practical, economic process has been evolved for the production of oil and a protein food product. The process consists of two principle phases. In the first phase, the objective is to separate the oil and the protein from the fibrous components of the coconuts by repeatedly mixing the ground coconut meats with water and pressing much as one would rinse out a sponge. In this step, water, oil, soluble carbohydrates and minerals are separated from the fibrous residue in the form of an emulsion commonly called coconut milk. The second critical phase of the process is to break the emulsion so that the

coconut oil can be separated from the aqueous phase which contains the dissolved protein, carbohydrates and minerals.

Resolution of the technology for the first phase proved to be somewhat easier than we had anticipated based on reports we had heard from others who had worked on this process. However, separation of the emulsion proved to be more challenging. The emulsion is quite stable and others had found demulsification to be a major problem. Several methods of demulsification were tried with varying degrees of success. Mild heating of coconut milk (up to 85°C) or adjustment of pH were ineffective demulsification steps. The effectiveness of the various treatments tried was evaluated by centrifuging the samples for 10 minutes at 9,500 x g in a laboratory centrifuge.

Cooling the emulsion to crystallize the oil was found to be effective provided the coconut milk was first concentrated by centrifugation to reduce the moisture content to about 25%. The procedure used was to extract the milk, concentrate it by centrifugation, cool to about 10°C, warm to about 30°C and then centrifuge. This method was not further pursued because of the amount of heat exchange and special equipment required for the cooling and warming steps. However, the procedure remains a possible alternative to the method finally adopted. The cooling method for demulsification was later reported in the literature by another research group (6).

Another method used was fermentation which was inspired by work reported by Timmins (7) and Puertollano, et. al. (8). Coconut milk was incubated without inoculation before centrifuging. Samples incubated over night at about 45°C showed maximum separation of oil. Samples incubated below 20°C or at 90°C did not become demulsified. It was observed that the pH dropped to about 4.1 during successful incubation. After incubation and centrifugation the oil phase contained 94% of the oil from the coconut milk. However, demulsification by incubation was not adopted into our final process because of the probability of flavor problems resulting from bacterial action. Also, to be considered is the fact that Puertollano, et. al. have shown incubation to be somewhat unreliable in breaking the coconut emulsion even when a known culture of bacteria is added.

The demulsification technique which was finally incorporated into the aqueous process involves the use of shear to invert the stable oil-in-water emulsion into an unstable water-in-oil emulsion, which can be separated by gravity or by centrifugation. The emulsion as recovered from the cream separator contains about 35% moisture and agitation will not break this emulsion. It was found necessary to reduce the moisture content and it was determined that the simplist means to accomplish this was by the addition of coconut oil to the system before agitating. Laboratory scale experiments

indicated that the moisture must be reduced to about 25% to obtain good demulsification (9).

The individual unit operations that were successfully developed as described above have been combined into a processsing technology a simplified flow diagram of which is shown in Figure 1 (10,Graph). The coconuts were shelled manually and the coconut water saved for use in processing. Washed meats were ground in a hammer mill with 0.25 inch diameter holes in the screen, followed by grinding in a disc attrition mill with the discs set at minimum clearance. The ground coconut meats were briefly mixed with heated coconut water (80°C), or with a corresponding amount of heated tap water when the coconut water was not used. The mixture was pressed at 50 psi in a pulp press against a screen with holes 0.01 inch diameter. Extraction with hot water followed by the pressing was repeated twice more. The two materials resulting from the pressing operation are the emulsion (called coconut milk) and the wet solids (called residue). The residue came from the press at 70 ± 10% moisture and was dried in a rotary drier in a flow of hot air (inlet air is about 100°C). The dried residue is a final product.

The coconut milk emulsion was filtered through a vibrating screen (120 mesh) to remove the last traces of residue. The milk was centrifuged in a three-phase, desludging type separator. The model used develops a centrifugal force of 4,700 x g at the radius of the hole in the disc stack, has a bowl capacity of 3.2 liters and was operated with a feed of 8 liters per minute. Three phases emerged from the separator: 1) the lightest phase usually called coconut cream which is about 65% oil on a wet basis; 2) the aqueous phase usually called coconut skim milk which is a solution of the water soluble components of the coconut with some suspended particles; and 3) a solids phase consisting mostly of insoluble protein which is dried to give a final product.

The coconut cream is an oil-in-water emulsion with an average oil globule size of about 10 nm in diameter. Coconut oil was added to this emulsion to reduce the moisture content and the mixture then agitated with a high velocity pump to invert the emulsion. Best results were achieved when the temperature was raised to about 80°C before the agitation. After inversion of the emulsion, oil and water were separated by first allowing a gravity separation (which took several minutes) into two phases: an aqueous phase and an oil phase. The oil phase was purified by centrifugation, heated to drive off residual moisture and decanted from the settled solids. Inasmuch as the quantity of insoluble protein recovered from centrifugation of the coconut milk was so small it was dried by freeze drying. The coconut skim milk was dried by spray drying with an air outlet temperature of about 85°C and air inlet temperature of about 170°C.

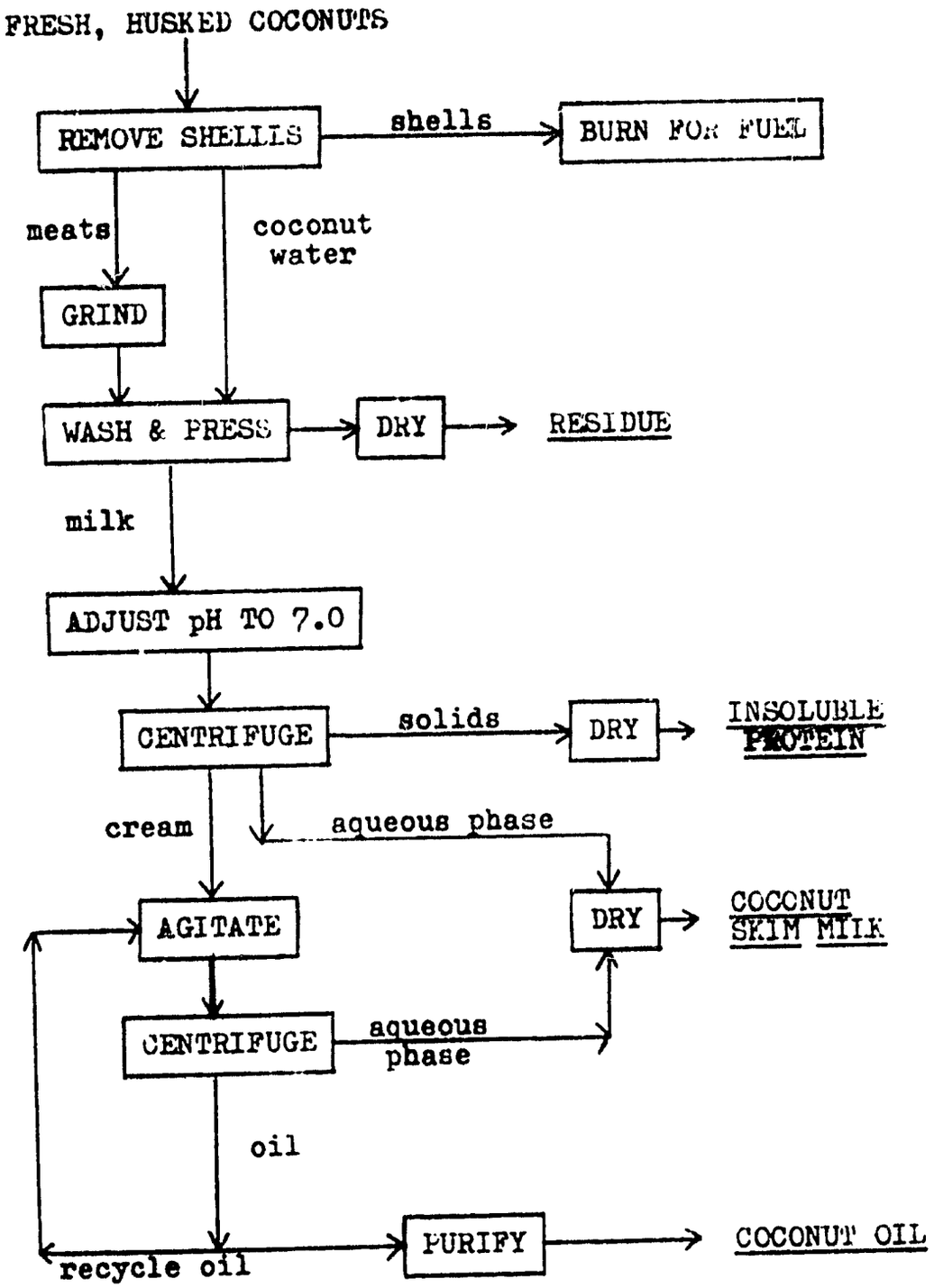

Figure 1 - Simplified flow diagram for the aqueous processing of coconuts. The shells were discarded in our operations, but would be burned for fuel in commercial processing.

The most critical step in aqueous processing is the efficient extraction of oil from the fresh meats. Repeated extraction of the coconut meats gradually reduced the oil content. The residue from the first extraction contains 46% oil on a dry weight basis. The second residue contains 32% oil. The fifth residue has its oil content reduced to 21%. Major expenses would be involved in so many extractions in a commercial operation. Therefore, a compromise would have to be made between the cost of the loss of oil against the cost of the recovery. The gradual reduction in oil can be rationalized by assuming that subsequent extractions remove exposed oil globules and protein, leaving only the oil and protein remaining in unruptured cells; which would be 5% of the oil and 7% of the protein. However, as pointed out by Adair and Timmins (11) it is still unknown whether size reduction must be sufficient to break all cells, or whether globules can be leached out of larger clusters of cells.

The dehydration and packaging of coconut skim milk powder is made difficult by its tendency to take up water. However, this difficulty can be avoided if the product is prepared as an intermediate moisture concentrate with inclusion of coconut water solids. This concentrate has decreased shelf life, principally because of its tendency to become dark in color (12,G). The product is stable to microbial growth for at least one month. For many applications the economy gained from elimination of the spray drying step would seem to more than balance out the reduced shelf life.

Considerable work has gone into the characterization and utilization of coconut skim milk. Spray dried coconut skim milk powder (at 3% moisture) is a hygroscopic powder that contains up to 30% protein, 7% fat, 0.03% crude fiber and 9% ash (13,H). This powder when mixed with water forms an acceptable beverage with a sweet coconut taste. It can also be used in preparation of an acceptable pudding without the use of either eggs or milk (I). The corrected PER is 2.0 at a low spray drying temperature. However, a value of 1.5 was found for product dried at 110°C outlet air temperature. Since coconut skim milk is being advocated as a practical replacement in many uses for non-fat dry milk, it is of interest to compare their properties. This is done in Table 1. On a direct weight to weight basis non-fat dry milk does provide more and better protein and also more calcium. However, in a coconut producing country, the coconut skim milk provides far more nutrition per unit of cost to the consumer.

A second by-product obtained from aqueous processing of coconuts is the residue or fiber fraction. Yields of residue and of dry coconut skim milk are each about 30% of the yield of coconut oil. The composition of the residue (at 5% moisture) is 5% protein, 24% fat,

Table 1 - Comparison of Dehydrated Coconut Skim Milk with Non-Fat Dry Milk

	Coconut Skim Milk	NFDM
Protein		
Crude Protein (%)	25 - 30%	36%
Corrected PER	2.0	2.5
Limiting Amino Acid	isoleucine[a]	sulfur containing[b]
Carbohydrate		
Carbohydrate by difference	52 - 59%	52%
Principal sugar	sucrose	lactose
Fat	5 - 7%	0.8%
Minerals		
Calcium	0.06 - 0.2%	1.3%
Phosphorus	0.5%	0.9%
Potassium	3.3 - 3.6%	1.8%
Sodium	0.9 - 1.4%	0.5%
Chloride	1.6%	1.1%

[a] Isoleucine is first whether based on FAO Pattern or egg values.

[b] From FAO, Amino Acid Content of Foods.

25% crude fiber and 0.6% ash. The principle component of the fiber is a carbohydrate of which mannose is the principle (74%) component. As a food component, this fiber would probably contribute little or no net caloric value. Evidently the small amount of nutritive value in the residue is just about cancelled out by its characteristic of absorbing nutrients and thereby causing them to leave the intestinal tract undigested (14,J).

The residue after hexane extraction and grinding has been used as an additive to white bread (15). Residue prepared from pared coconuts is white and does not discolor the bread. Preliminary results indicate that the bread has good loaf volume and is otherwise acceptable. Major food companies have shown an interest in the coconut residue as a source of fiber in the diet.

Coconut skim milk products are colored but possibly acceptable in some uses when prepared from coconut meats from which the testa has not been removed. The residue is white if the coconuts are pared but has a dark color if the coconut testa is included. To make acceptable residue, the testa probably will have to be removed. This effect of testa on color is a point which will be brought up later in this report.

An economic analysis has been made of aqueous processing using as the model a plant operating in the Philippines which is capable of processing 250 tons per day of husked coconuts (16,K). This plant was estimated to retire a total capitalization of $2.7 million (mid 1974 prices). Coconut oil would provide 63% of the income and coconut skim milk solids (selling at $.50 per kg) the other 37%. On this basis, the pre-income tax return on investment is calculated to be 20%. In this analysis, the residue was assumed to have no economic value. Based on medical reports subsequent to that economic analysis and on the degree of interest which has been shown by the food industry, it would now appear that the residue would have economic value which would add to the return on investment. Therefore, even though the analysis which was made was based deliberately on pessimistic assumptions, it was concluded that aqueous processing of fresh coconut is economically feasible.

Because the economic analysis did not assume a continuation of the high 1974 prices of oil, it is not affected by the subsequent decline in coconut oil prices and therefore very little updating is needed. In June 1975, the domestic coconut oil price in the Philippines was ₱ 0.30 per kg. The value used in appendix K was ₱ 0.28 per kg. Use of the sensitivity analysis in appendix K indicates that use of the true oil price would raise pre-tax return on investment by 0.5%.

Energy costs in June 1975 are higher than those shown in

appendeix K. Petroleum products are up about 60% and electricity is up 100%. Therefore operating expenses increase about $100,000 annually reducing the rate of return from 20% to 16%. No other major changes have occurred that should affect the economic analysis as shown. The point should be made again that by calculations it has been shown that the coconut shells contain enough energy value to operate the entire plant. Calculations were made on the basis of purchased energy because we are not yet prepared to recommend the use of burning coconut shells as a fuel because of our lack of experience and experimentation with this possibility. However, this alternative should be taken into consideration in designing a new plant.

Samples of the coconut skim milk products produced in the pilot plant were regularly analyzed for microbial content in order to assure constant sanitation in the pilot plant and the safety of the products produced for use in foods. In addition, a study was made of the entire process to point out those areas where microbial control were most needed (17). It was found that the main source of contamination was the coconut shells. Coconut meats and/or water contained few or no microorganisms if removed under aseptic conditions. Spray dried and intermediate moisture concentrates were produced with aerobic plate counts of less than 10,000/g. Little if any microbial growth took place during extended storage of these products at 21 or 35°C. Food grade products could be made even without pasteurization because the vacuum evaporator provided enough heat to serve as a kill step. Neither Salmonella nor coagulase-positive staphylococci were found in any samples. Proper equipment cleaning and sanitation were essential factors in maintaining these acceptable microbiological indexes.

Because of limitations in the facilities of the pilot plant, it was not possible to handle the residue samples under adequate sanitary control. As freshly produced, the wet residue had satisfactory bacterial analyses. Therefore, in a plant properly designed for aqueous processing, the residue could be maintained in food grade quality with little difficulty.

As a consequence of these activities outlined above, there has been some fairly serious interest shown in the implementation of the aqueous processing technology. The strongest interest to date has been shown by the Philippine government which has inaugurated a pilot plant at the University of San Carlos, Cebu City, Philippines. Funds for the pilot plant equipment and operation have been provided by the Philippine government. USAID is now participating in the program by providing technical management and backup support by Texas A&M University. It is strongly recommended that USAID continue its support of the Philippine project until the issue of success or failure of the aqueous process has been resolved. Further, it is

recommended that if the Philippine project is successful that AID promote use of the process in other countries (however not by proliferation of more pilot plants). This promotion could take the form of supporting travel of technicians from other countries to the Philippines and continued technical support from Texas A&M University if necessary.

Conventional Processing (During and Prepress Solvent Extraction)

A significant amount of research effort has been directed toward the science and technology of the production of coconut flour by more conventional processing consisting of drying the coconuts to remove water followed by prepressing and hexane extraction to remove the oil. The drying step may be performed in either of two ways. The first is by hot air drying as used in the desiccated coconut industry. Use of samples of especially prepared material (by a coconut desiccator) has indicated that this type of drying can be used without reducing the solubility of the protein in the coconut meats. The other type of drying would be the more traditional sun drying which for the purposes at hand would have to be under sanitary conditions to produce a food grade copra.

Coconut flour has been produced successfully on a small scale by at least two manufacturers in the Philippines using desiccated coconut as the raw material. This coconut flour has been used in the Nutribun in various nutrition programs in the Philippines. However, as yet, no significant market demand has materialized. It appears that the coconut flour made from desiccated coconut cannot be offered at a sufficiently low price to make it attractive. An economic analysis of coconut flour produced by the prepressed solvent extraction of desiccated coconut has been made based on approximately the same set of assumptions as in the economic analysis for aqueous processing (18,L). In order to attain a 20% pre-income tax return on investment, the flour would have to sell for $315 per metric ton. This makes it a rather expensive substitute for wheat flour.

At our present state of technology, an equally definitive cost analysis cannot be made for coconut flour derived from food grade copra. However, preliminary calculations indicate that the costs would be far below those for product derived from desiccated coconut. This potentially lower cost of coconut flour from food grade copra has provided the motivation for research on this type of product. It has the additional advantage which can also be a disadvantage that more of the preliminary processing would be done at or near the point of production of the coconuts perhaps by the coconut producers themselves. This would provide the coconut producers with a greater share of the benefits and consequently a greater incentive than they now have to produce improved quality copra.

The idea of producing a food grade copra is not new. Subrahmanyan, et. al., reported in 1966 that by dipping coconut meats in a solution containing either a mixture of 5% sulphuric acid and 7% acetic or a solution of 30% sodium carbonate would effectively retard microbial growth during the drying stages in the production of copra (19). However, a commercial trial with copra prepared under these conditions was unsuccessful. We have shown that the dip solutions used by Subrahmanyan, et. al., had two disadvantages. One, they reduced the solubility of the protein and two, they toughened the structure of the copra to the point where prepressing was made more difficult which would cause a higher level of oil to be left in the prepressed meal and probably slow down the throughput of the expellers. In a search for alternative antimicrobial dipping agents, we have found that a solution of propionic acid appears to offer good protection of the meats without causing subsequent problems in processing nor a reduction in the protein solubility (20). On balance, the optimum level of propionic acid in solution appears to be about 6%. The propionic acid dipping effectively reduces bacteria, yeasts and molds and prevents their growth during the drying of the copra. Food grade copra prepared by the propionic acid dip procedure is substantially lighter in color than is normal copra. Further, it is neither rancid, moldy nor dirty. However, the propionic acid dip is not effective if the copra is exposed to rain during drying, inasmuch as the propionic acid is primarily on the surface of the coconut meats and is washed off by the rain. Therefore, we feel that it is now necessary to test the effectiveness of this approach under field conditions in the Philippines.

There are a number of advantages in the concept of producing food grade copra by sun drying. It can be done at or near the point of production of the coconuts and thus lower transportation costs. It is less capital intensive and more labor intensive than the desiccated coconut approach. It requires a lower expenditure of energy and results in lower costs. On the other hand, there are some disadvantages which many believe may be insurmountable. The chief of these is that it would be necessary to change the generations-old production habits of the coconut farmers. Further if it would become possible to produce food grade copra at the site of production, it would also require that there be improvements in the sanitation of transportation facilities. It is possible that food grade copra could only become a reality through the efforts of farsighted progressive producer cooperatives.

Coconut flour made from pared coconuts is very white. If the parings are not removed, the flour contains large quantities of dark specks of testa and would probably be unacceptable for use in many foods. However, experiments have demonstrated that as much as 75% of a first grade coconut flour free of any visible specks of testa can be recovered from food grade copra flour by a multi-stage air classification process (21).

It is our recommendation that careful consideration be given by interested parties to the feasibility of the production of coconut flour from food grade copra and the potential markets for such a flour. We believe that all of the technological components are now available. The coconut meats can be protected from microbiological deterioration by dipping in 6% propionic acid solution provided they are handled in a sanitary manner. This would preclude traditional copra drying with its attendant exposure to insects, rodents and birds. It would suggest the use of a simple small-scale hot air dryer similar to the Tapahan type that is now being used in many instances. This would compensate for the inability of the acid-dip method to cope with the rainy season. This type of small-scale operation would be consistent with the needs for labor intensive processes in developing countries. The dried coconut meats (food grade copra) would need to be transported under sanitary conditions to the manufacturing point. It has been demonstrated that such food grade copra can be prepressed under sufficiently mild conditions neither to darken the coconut meal nor to reduce the solubility of the protein. The prepressed meal can be solvent extracted to produce a flour containing 1% or less of oil. This coconut flour could be sold and used as is if the specks of testa are not objectionable or could be put through an air classifier to remove the specks and produce a white coconut flour. This coconut flour could also be used as a source of coconut protein isolate if a market for the latter were developed. We believe that a careful economic analysis would show that this is by far the least expensive method to produce coconut protein isolate. Any progress in the direction of producing a food grade copra flour would of necessity have to be based on adaptive technology conducted with the full cooperation of interested and progressive coconut producers. Without the full cooperation and an active follow up by the latter, this phase of the project could be a technological success and a practical failure.

Biochemical and Structural Changes in Maturing Coconut Endosperm

The growth and development of the coconut seed and its content of solid endosperm is a complex well-ordered process. Once the spathe opens and the female flowers are pollinated, the fruit grows rapidly. The ovular cavity, limited by the seed coat, enlarges for about 240 days after the spathe opens. The endocarp hardens from the distal end toward the proximal end. This process is complete shortly after the ovule ceases to enlarge. The growth of the solid endosperm lags behind the growth of the ovule. Under favorable conditions only about 20% or less of the maximum growth of the endosperm is completed after 90% or more of the growth of the ovule is achieved. The increase in weight of the endosperm ends about 365 days after spathe opening.

Ultrastructural analysis shows that, except for a few cell layers adjacent to the seed coat, the cells of the mature endosperm are necrotic. Practically all of the intracellular substance that stains like protein is located in watery vacuoles with a single unit membrane similar to the tonoplast. The protein in the aleurone vacuole consists of a crystalline core surrounded by an amorphous layer. The lipids are non-osmiophilic and are located in fat droplets, the spherosomes, in the cell and are separate from the aleurone vacuoles. What few plastids, mitochondria, nuclei and other cell organelles that remain are necrotic and obviously non-functional. The cell walls are thick and woody.

In the early stages of growth the endosperm is gelatinous. The cells have active mitochondria, plastids, dictyosomes and nuclei. The cell walls are very thin, which explains the gelatinous quality of the tissue. The rough endoplasmic reticulum is not obvious, and there are no amorphous or crystalline protein deposits in the vacuoles. The gelatinous endosperm grows over the surface of the inner seed coat from the distal to the proximal end of the ovule. It takes roughly 31 days for the endosperm to cover the inner surface of the seed coat, once the rapid growth of the endosperm starts.

After the endosperm is established on a given region of seed coat, it differentiates. The cell walls thicken, the rough endoplasmic reticulum proliferates, the dictyosomal vesicles develop material that stains like protein and biphasic protein deposits similar to those in the aleurone vacuoles of mature endosperm appear. The latter signals the beginning of the process of synthesis and sequestration of the principal reserve aleurins of the mature seed. The endosperm increases in thickness and characteristic changes occur in the consistency of the tissue. Approximately 105 days are required for the conversion of the gelatinous endosperm to hard endosperm in a given region. Ultrastructural analysis suggests that the synthesis of reserve aleurins continues from the cooked egg white stage that follows the gelatinous phase of endosperm differentiation to the early hard phase of differentiation. The process continues for about 112 days in a given region. The synthesis of the reserve aleurins begins about 200 to 210 days post spathe opening in the distal region of the ovule and ends in the proximal region at about 330 to 340 days post spathe opening. Relating ovular growth to reserve aleurin synthesis, the ovule is formed first; then, the vacuolar proteins are synthesized and packaged. There is some time overlap in the two processes.

The ultrastructural pattern of the endosperm producing reserve aleurins is similar to that of embryos of dictyledonous seeds producing aleurins. Classical aleurone grains do not form in mature coconut endosperm, however, since the latter tissue does not desiccate with maturation. A comparison of SDS gell electrophoretograms of crude protein extracts of coconut endosperm in the cooked

egg white and hard phases show the presence of the principal coconut seed proteins in each. It appears that the principal reserve proteins are synthesized and sequestered by coconut endosperm in essentially the same way as in developing embryos of the dicotyledons. It is proposed that the aleurins of the coconut endosperm are synthesized by polyribosomes attached to the outer surface of the endoplasmic reticulum, transported to the dictyosomes, concentrated and/or modified by the dictyosomes and deposited in the aleurone vacuoles by a process of membrane fusion. Such a model is an aid in understanding the coconut seed proteins and the effects of environmental stress on the deposition of these commercially valuable proteins.

The bulk aleurins of seeds are thought to function as a source of organic nitrogen for the germinating embryo. In most seeds that have been studied carefully, the number of individual reserve aleurins is small. As pointed out earlier, ultrastructural analysis confirms that the principal proteins of mature coconut solid endosperm are aleurins. There is little doubt that they serve as a nutritional reserve for the germinating coconut embryo. Analysis of the crude proteins of mature coconut endosperm by SDS gel electrophoresis shows a relatively simple pattern. Most of the protein is in three molecular weight classes: 51,500, 30,000 and 20,500 daltons. Careful salt fractionation resolves the native proteins into one fraction bearing the 51,500 subunit plus impurities and another containing the 30,000 and 20,500 molecular weight classes. The latter isolate was fractionated into the two molecular weight classes by a new system of preparative SDS gel electrophoresis of our invention. The amino acid composition of each subunit and the starting material was determined. The amino acid composition of the starting material could be accounted for satisfactorily by the amino acid compositions of the two subunits and the assumption that the starting material contained equimolar quantities of each. Presumably, the native protein is of the form $(P_{20} \cdot P_{31})_n$. The Metzger index was used to compare the amino acid composition of $P_{20} \cdot P_{31}$ with legumin type proteins from peanuts, beans and cottonseed. Low values of the Metzger index (D.I.<15) are presumptive evidence that two proteins are homologous (i.e., arise from genes with a common ancestral gene).

By the Metzger criterion the following aleurins are homologous proteins: $P_{20} \cdot P_{31}$ (C. nucifera); AB (legumin, Vicia sativa); AC (legumin, V. sativa); αβ (legumin, Vicia faba); glycinin (Glycine max); P_{67} (arachin, Arachis hypogaea); and P_{40} (acalin B, Gossypium hirsutum). The difference indices ranges from 9.04 to 12.45. The amino acid composition of P_{20} and P_{31} were also compared with the individual subunits of the legumin type proteins. Two potential homologous series are apparent. One consists of P_{20}, A (V. sativa), β (V. faba) and P_{24} (A. hypogaea); and the other series consists of P_{31}, B (V. sativa), C (V. sativa), α (V. faba) and P_{43} (A. hypogaea).

In the first series the difference indices ranged from 13.37 to 13.67. In the second series they ranged from 9.84 to 12.40. No data were available for the individual reduced subunits of P_{40} from acalin B. These values of the difference index are respectably low when the phylogenetic distance between the coconut palm and the other species is considered. The apparent homology between P_{20} · P_{31} and the legumin type aleurins lends additional support to the concept that all are produced and sequestered by a common process.

A natural experiment on the effects of water stress on the production of solid endosperm is repeated yearly on the coconut palms on St. John Island. A considerable body of circumstantial evidence is adduced supporting the hypothesis that water stress at key times during flowering and fruit development severely limits the production of solid endosperm. The most sensitive phases are related to periods of rapid growth. Water stress during periods of spathe enlargement increases the time elapsed between the opening of successive spathes, thereby reducing the number of clusters of coconuts produced per year per tree. The interval between spathe openings can be lengthened from 26 days to 47 days, depending upon the severity of the water stress. Such an effect can cause serious economic losses. When water stress occurs during the period of rapid ovular growth, the size of the ovule at maturity is greatly reduced. Consequently, the quantity of endosperm produced per mature seed is lowered correspondingly. As much as a 60% reduction in the fresh weight of the endosperm per seed at maturity was noted for palms suffering water stress during the period of maximum ovular growth. Most of the growth of solid endosperm and the synthesis and segregation of the coconut seed protein occurs after the ovule reaches maximum size. Water stress during this interval reduces the growth rate of the solid endosperm and probably reduces the rate of synthesis and deposition of the vacuolar proteins. The effect of water stress on the growth rate of the solid endosperm is less dramatic than on the growth of the ovule (MN).

E. Dissemination and Utilization of Research Results

This project has achieved wide recognition in the coconut producing countries. One of the important vehicles for dissemination of research results was through the distribution of our quarterly reports. We had a response from one university professor that he used these quarterly reports almost as a textbook in a class that he taught. Perhaps the most important vehicle was through the publication of research results in scientific journals and distribution of reprints of the articles. The most significant of the articles have been referred to in this report and are appended, thereto.

We have responded to numerous requests for reprints and other information on coconut processing and products. The most direct

use of the research results was the decision by the government of the Philippines to establish a demonstration pilot plant. At last report, that pilot plant was operating three days per week with a capacity of about 2,200 per processing day. Considering the time frame involved, the need to train personnel, the turnover in some key personnel, the difficulty of providing maintenance parts and the fact that the pilot plant in the first place was built at minimum expense we must concede that the progress to date has in fact been beyond our expectations. This has been due not only to the excellence of the leadership of Dr. Robert Hagenmaier but also to the excellent cooperation of the officials of the Philippine government and the capability and enthusiasm of the staff operating the pilot plant.

The success of the pilot plant has not gone unnoticed. They have a steady stream of visitors and many requests for samples.

F. Statement of Expenditures and Obligations

Summary of Costs by Category 7-1-74 to 6-30-75:

Salaries and Wages	$ 59,390.50
Fringe Benefits	3,904.19
Consultant Fees	-0-
Overhead - 44.24% of S&W	24,151.83
Travel and Per Diem	4,271.67
Equipment, Supplies & Materials	15,095.55
Participants	-0-
Other Direct Costs	3,982.75
TOTAL	$110,796.49

G. Forecast

This has been a most satisfying research contract. It is always satisfying to a research group to attain its stated objectives. It is especially satisfying when these are attained in spite of many predictions of failure. We are most appreciative of the continued support of the US Agency for International Development in the face of these negative predictions.

While we can feel this sense of satisfaction from technological

accomplishment, the entire project will have been an exercise in futility if we do not succeed in convincing business interests to convert this technology into products for sale and distribution. It is only when the coconut protein products and fibrous products are feeding hungry people that the true objective of this project will have been attained. This phase of follow through is the heart of the cooperative agreement between the government of the Philippines and the US Agency for International Development in which Texas A&M University is now participating under USAID contract No. EA-C-1073. Therefore, in recognition of the rate at which businesses can move even when highly motivated, it will be at least another three to five years before we will know whether this project has provided true service to mankind.

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A. S. SAMSON S. J.,[1] R. N. KHAUND, C. M. CATER and K. F. MATTIL
Food Protein R&D Center, Texas Engineering Experiment Station
Texas A&M University, College Station, TX 77843

EXTRACTABILITY OF COCONUT PROTEINS

SUMMARY--Studies were made on nitrogen (protein) solubility of coconut meal in aqueous media over a range of pH's. The point of minimum solubility was found at pH 3.9 and nitrogen solubility increased toward the acidic and basic sides. Coconut proteins displayed a markedly different pH-nitrogen solubility profile in salt solutions, showing minimal solubility at acidic pH's (pH < 3), a sharp rise of solubility and a maximum at neutrality. Coconut meal prepared from fresh coconuts with and without testa removed, from parings and from a sample of sun-dried copra showed comparable solubility characteristics. However, meal prepared from commercial desiccated coconut showed low solubility, even under acid and alkaline conditions (pH 2 and 10). The most efficient solvent-to-meal ratio was found at 20:1 (v:w) for a single extraction, and a two-step 15:1 process for multiple extractions. Extractions using salt solutions show that the efficiency of extraction is greatly dependent upon pH; concentrations > 0.25M effect maximum extraction. Osborne classification studies of coconut meal indicate that 90% of the proteins would be classified as albumins and globulins.

INTRODUCTION

COCONUTS are an indigenous product and a major article of commerce in many countries of the world which are suffering from protein deficiency. A conservative estimate, probably low, of world coconut production in the early 1960's gives 5.6 million tons a year. This is equivalent to more than 200,000 tons of crude protein, only a minor proportion of which is presently being utilized for human food consumption (Orr and Adair, 1967; PAG, 1965; Teply, 1967).

The major proportion of coconuts is converted to coconut oil and coconut meal via copra. Commercial processes for extracting coconut oil commonly utilize expellers at temperatures which denature proteins. Furthermore, prevailing world practice in handling coconuts for the production of copra are not conducive to further utilization of the coconut meal for human consumption.

These studies were undertaken as a necessary preliminary step to the study of basic properties of coconut proteins in connection with the larger objective of finding methods for the better utilization of coconuts as protein-food sources. To insure that the present work was being conducted on good quality coconut protein, care was taken to prepare coconut meal by processes not expected to denature proteins.

Previous studies on coconut meal have commonly used meal prepared from commercial coconut oil processing (Chelliah and Baptist, 1969) or from sun-dried copra (Butterworth and Fox, 1963), or have made use of meats dehydrated by the application of heat (Sison et al., 1968). Studies by Chandrasekaran and King (1967) suggest that enzymatic processes may be required for the efficient extraction of coconut protein (from a control value of 50-60% to 85%), however, they do not indicate how their starting coconut flour has been prepared, except that it was supplied by the Central Food Technological Research Institute, Mysore, India.

EXPERIMENTAL

Preparation of coconut meal

The coconuts used were food-grade fresh coconuts obtained from the wholesale market in Houston, Texas. Indications are the coconuts came from Central America. As is common with commercial coconuts, these were received dehusked. The meats (kernel) are enclosed in a hard shell (endocarp). Between the shell and the kernel is a thin, brown seed coat (testa) which adheres firmly to the kernel. In the manufacture of desiccated coconut, the testa is removed by paring.

Coconut meal was prepared from the fresh coconuts by procedures that would not be expected to denature the proteins. The nuts were cracked and the coconut water discarded. The meats were removed from the shell, washed with water and then sliced into smaller fragments using an Urschel mill (cutting head 2-J-030510B or 2-K-020060B). The shredded meats (which included the testa) were dried under vacuum at 40°C for 24 hr to a moisture content of less than 2%. The dried meats were further comminuted by means of the Urschel mill, this time using a finer cutting head (2-K-020035C or 2-K-010030B), prior to defatting by several extractions with hexane. Residual solvent was removed with dry air.

Typical analyses of coconut meal prepared in this fashion are given in Table 1. Moisture, ash, oil and crude fiber analyses were made by standard AOCS (1969) procedures. Table 1 also gives typical analyses of fresh coconut meat and desiccated coconut meats involved in the preparation of coconut meal. It is to be noted that the moisture content of the dried meat equilibrated to a level of 7-10% on standing at ambient temperature and humidity.

Extraction of protein

Extraction of coconut proteins from coconut meal was carried out at room temperature for at least 30 min, using a solvent meal ratio of 20:1 (v:w), unless otherwise specified. The predetermined pH of extraction was obtained by addition of 0.5N NaOH or 0.5N HCl solution; the pH was rechecked and readjusted after 30 min of stirring. Sample size was 2.0g and the volume of solution was brought to 40 ml after the final pH adjustment. After centrifugation at 4300 × G (Sorvall RC2-B Refrigerated Centrifuge) for 20 min, the supernatant extracts were filtered through Whatman Filter Paper No. 1 to remove flocculent material. A 20 ml aliquot of each extract was taken for nitrogen analyses. Nitrogen was determined either by macro-Kjel

Table 1 - Coconut meal Proximate analyses

Sample	Moisture and volatile material %	Oil %	N %	Protein (%N × 6.25) %	Ash %	Crude fiber %
Fresh meats	47.0					
Desiccated meats[a]	1.8	73.0				
	1.0	71.1				
	1.5	69.3				
Coconut meal A[b]	9.9	1.1	3.6	22.2	5.2	7.5
Coconut meal B[b]	7.2	4.8	3.8	23.9	3.9	5.9
Coconut meal C[b]	5.1	0.4	3.8	23.8	4.4	7.6

[a] These are analyses of different lots dehydrated in the freeze drier.
[b] Coconut meals A, B and C are different samples, prepared from three lots of fresh coconuts obtained at different times.

[1] Present address: Dept. of Chemistry, Ateneo de Manila University, Loyola Heights, Quezon City, Philippines.

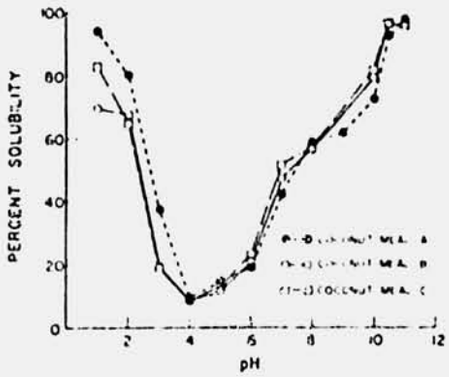

Fig. 1–Protein solubility profiles of coconut meals prepared from three lots of fresh coconuts obtained at different times (see Table 1)

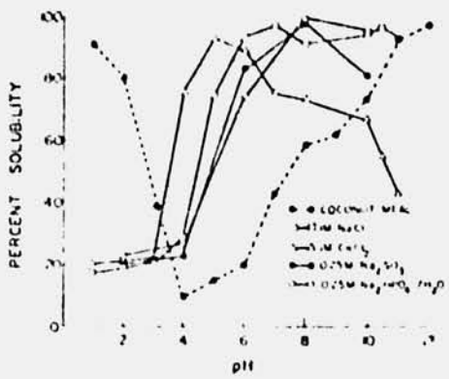

Fig. 2–Protein solubility profiles of coconut meal in several different salt solutions at varied pH's adjusted by the addition of 0.5N NaOH or 0.5N HCl.

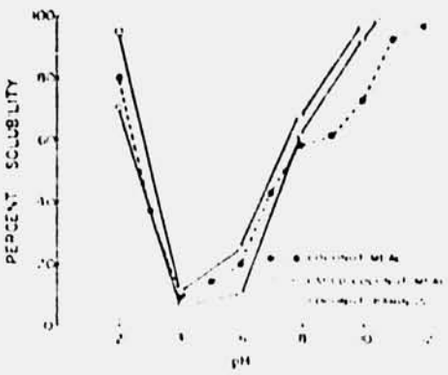

Fig. 3–Protein solubility profiles of a desiccated coconut and a specially prepared copra

dahl (AOAC, 1965; AOCS, 1969) or micro-Kjeldahl methods (Ammco, 1959; Miller and Houghton, 1948). In most cases, the data shown represent duplicate analyses, using two samples of meal.

RESULTS & DISCUSSION

pH-Nitrogen solubility profiles

Studies were made on nitrogen (calculated as protein using the customary 6.25 factor) solubility or extractability of three coconut meals in aqueous media over a range of pH's. The data shown in Figure 1 are for extracted meals prepared from three different lots of coconuts. The solubility profile for coconut meal C represents the average of four replications, the 95% confidence limits for a single mean is ± 2.95%.

The point of least solubility was further refined by extractions at pH's 3.0, 3.3, 3.6, 3.9, 4.2, 4.5, 4.8 and 5.0. Minimum solubility was obtained at pH 3.9. [Peters (1960) found maximum precipitation at pH 3.9. Sison et al. (1968) reported the best pH for protein precipitation in a citrate-phosphate buffer at pH 3.4–3.8. Chellah and Baptist (1969) precipitate coconut protein at pH 3.8.]

Nitrogen solubility increased with increasing acidity and alkalinity. The inflection in the curves near pH 8 suggests that there may be two main protein fractions, one soluble at pH 8–8.5 and the other at pH 10.5–11.

The solubility profile of coconut meal is strikingly different in salt solutions (Fig. 2). In 1M NaCl solution, there is rather low solubility on the acid side, up to pH 4. This is followed by a steep rise in solubility to over 90% at pH 6 and more alkaline conditions. Solubility in 1M $CaCl_2$ displays a similar low solubility at acidic pH's, followed by high solubility near pH 5–6. An unexplained decrease in solubility at more alkaline pH's is also observed.

Similarly, coconut proteins show low solubility at lower pH's for 0.25M Na_2SO_4 and 0.25M dibasic sodium phosphate. The pH-solubility profile rises steeply beginning at pH 4 and reaches a maximum of almost total solubility at pH 8. A decrease of solubility at more alkaline conditions follows.

The unique solubility characteristics of coconut proteins in salt solution have been used to prepare an interesting isolate by extraction in 1M NaCl at pH 7 followed by precipitation at pH 2. The preparation and characteristics of this and other isolates prepared by extractions at pH 2, 8, and 10.5 are the subject of another manuscript now in preparation.

The classical Osborne classification of proteins (Lund and Sandstrom, 1913), as applied to coconut meal, contrasts the solubility data obtained in aqueous and salt media. Table 2 indicates that over 90% of the proteins in coconut meal would be classified as albumins and globulins. The sodium tungstate fed indicate

Table 2–Classification of the proteins of coconut meal according to solubility[a]

Fractions	Extraction conditions	% of Total N
Albumin	CO_2-free water, pH 6.7	30.6
Globulin	1M NaCl solution, pH 7.0	61.9
Prolamine	70% aqueous ethanol	1.1
Glutelin	0.2% NaOH solution	4.7
Insoluble residue	---	1.8
Nonprotein nitrogen	12% sodium tungstate to precipitate proteins	0.1

[a]Osborne classification.

Table 3–Pared coconut meal and coconut parings: Proximate analyses

Sample	Moisture and volatile material %	Oil %	N %	Protein (%N × 6.25) %	Ash %	Crude fiber %
Fresh pared meats	47					
Fresh testa	35					
Desiccated pared meats	1.1	73.5				
Desiccated testa	2.2	54.7				
Pared meal	10.6	5.2	3.6	22.5	4.2	5.6
Defatted testa (meal)	8.9	3.6	3.2	20.0	2.9	12.1

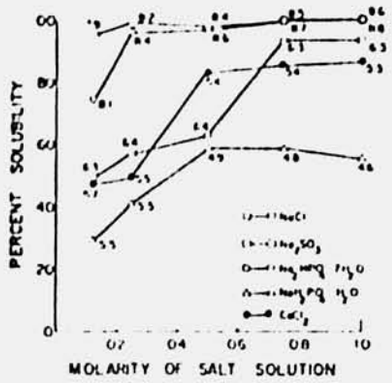

Fig. 4 - Solubility of the proteins of coconut meal in different concentrations of several salts. (Data next to solubility points indicate resulting pH's.)

that there is very little nonprotein nitrogen in coconut meal.

The solubility curves (Fig. 1 and 2) were made on coconut meal which included the testa. In addition, some coconut meal was prepared with the testa removed by manual paring. The parings were also dried and defatted and the solubility profile of the proteins determined. Table 3 summarizes some properties of coconut meal without the testa, and also of the coconut parings. The pared coconut meal gave a slightly higher protein analysis than the parings (22.5% protein compared to 20.0%, 22.8% protein compared to 17.7% in another determination). The solubility curves of pared coconut meal and of the parings are comparable to those of coconut meal with the parings.

Solubility profiles of the proteins in meals prepared from commercial coconut products have been obtained. Figure 3 gives the solubility profile for coconut meal obtained by defatting commercial desiccated coconut and sun-dried copra prepared under a "heat tent." The meal from sun-dried copra gave a pH-solubility profile comparable to the laboratory-prepared coconut meal. That from desiccated coconut, however, displayed rather low solubility at both acidic and basic pH's. The very low solubility (26%, compared to about 70% in the laboratory-prepared meal) at pH 8 is also striking.

The behavior of commercial desiccated coconut is very puzzling, inasmuch as the product is perfectly white. Preliminary work done at Texas A&M University (to be reported elsewhere) has indicated that browning might be a useful indication of incipient denaturation, as shown by loss of solubility. This, however, does not seem to be the case with commercial desiccated coconut, which, though pure white, has rather poor solubility.

Efficiency of protein recovery

Protein solubility or extractability is not the only factor governing efficiency of protein extraction. Other variables such as length of time of extraction, meal:solvent ratio, temperature of extraction and the effect of added salts are also very important.

Table 4 gives data obtained by varying the meal:solvent ratios, while extracting at pH 8 in aqueous solution and pH 7 in 1M NaCl (conditions chosen as representative for aqueous and salt extraction). The sample size for these extractions was 10g. After centrifugation and gravity filtration, the supernatant volume was measured. Under these conditions, three to five times the weight of coconut meal (i.e., 30–50g) was absorbed by the meal, and was not recovered as part of the separable solution. The amount not separable under practical conditions would vary depending upon the efficiency of the separation techniques used. The data in Table 4 indicate that a solvent:meal ratio of 20:1 (v.w) would be the most efficient, for single extraction.

Table 5 gives efficiency of protein

Table 4 Effect of solvent:meal ratio on extraction

Solvent:meal ratio (ml:g)	At pH 8		At pH 7, 1M NaCl	
	% N recovered[a]	% N extracted[a]	% N recovered[a]	% N extracted[a]
5:1	11.9	60.9	17.1	95.1
7.5:1	25.8	62.3	38.3	101.0
10:1	32.2	60.1	53.6	97.4
15:1	40.8	61.2	63.3	94.0
20:1	47.1	63.9	75.0	97.4
25:1	46.6	59.2	77.9	91.7
30:1	49.4	62.4	83.6	100.0

[a]The percent nitrogen extracted (or soluble) is based on the total nitrogen present in the coconut meal sample and the total volume of solvent used for extraction. The percent nitrogen recovered refers to that nitrogen in the separable supernatant extract, excluding the amount of extract (solvent) absorbed by the meal. The difference between the percent nitrogen extracted (dissolved) and recovered represents the nitrogen in solution in the absorbed solvent. Percent nitrogen recovered is the more useful data for process development purposes.

Table 5 Effect of successive extractions with different solvent:meal ratios

Solvent:meal ratio (ml:g)	Extraction	At pH 2 % N recovered[a]	At pH 8 % N recovered[a]
10:1	First	45.8	33.7
	Second	17.7	12.5
	Third	7.1	8.2
	TOTAL	70.6	54.4
15:1	First	65.5	40.7
	Second	17.9	13.3
	TOTAL	83.4	54.0
	(Third)	5.9	9.5
20:1	First	61.3	43.6
	Second	14.1	14.8
	TOTAL	75.4	58.4

[a]Based on actual recovery of extract.

Table 6 Effect of time on extraction

Time (min)	At pH 8 % N extracted[a]	At pH 7, 1M NaCl % N extracted[a]
5	58.6	96.9
15	59.6	99.5
30	58.4	99.0
45	59.3	98.6
60	60.5	103.0
90	65.4	104.0

[a]Based on total solvent used.

Table 7 Effect of temperature on extraction[a]

Temperature (°C)	% N extracted[b]
4	49.0
10	57.7
15	61.5
29	71.6
52	96.1
75	92.3

[a]Solvent: 0.2M phosphate buffer, pH 7.0
[b]Based on total solvent used

extraction and recovery with multiple extractions, using 10:1, 15:1 and 20:1 ratios. From these data it appears that a two-step 15:1 extraction may be most efficient. Though using one step less than a triple 10:1 extraction, the overall yield of the two-step 15:1 is almost as high. Compared to the two-step 20:1 extraction, it uses almost a third less solvent, with only a small loss of efficiency of protein recovery. The most practical extraction conditions would be selected on the basis of total process economics.

Other factors affecting protein solubility

In determining the effect of length of time of extraction on nitrogen solubility, it usually took at least 5 min to attain the desired pH, and then 15 min for centrifugation. Thus, the "time" variable employed was artificially set as actual stirring time after the desired pH had been attained. Table 6 indicates that maximum nitrogen solubility is attained in 15 min or less.

Table 7 summarizes the effect of temperature on nitrogen solubility. A temperature increase to about 50°C increases nitrogen solubility. The decrease observed at 75° may be due to partial coagulation of protein at the higher temperature. A similar decrease in solubility at higher temperatures has also been reported in studies on sunflower seed proteins in the same laboratory (Gheyasuddin et al., 1970).

The effect of various added salts on nitrogen solubility has also been studied. From Figure 2, giving the solubility curves of coconut proteins under varying pH's for different salt solutions, it is evident that these salt solutions, except toward the acid end of the pH scale, are rather effective solvents for coconut meal.

Figure 4 summarizes the effect on nitrogen solubility of several salts at various concentrations. The tests were run using the salts without adjusting the pH. In Figure 4, data next to the solubility points indicate the resulting pH at each salt concentration.

Concentrations at 0.25M or higher of dibasic sodium phosphate or sodium sulfite extract most of the available nitrogen in coconut meal. NaCl is most efficient at concentrations of 0.75M or higher. $CaCl_2$ solutions, at 0.5M or higher concentrations, effect over 80% extraction. Monobasic sodium phosphate, however, reaches a maximum extraction of a little less than 60% of the available nitrogen, at concentrations of 0.5M or higher. This difference in solubility could probably be explained in part by noting the resulting pH's: both Na_2SO_3 and Na_2HPO_4 attain pH's on the basic side of neutral, in which coconut protein is very soluble (as seen in Figure 2) while NaH_2PO_4 results in pH's between 4.6-5.5. Perhaps by coincidence, $CaCl_2$ and NaCl effect pH's at or near the highest points in their pH-solubility profiles (see Fig. 2).

It should be noted that the very high solubility of coconut proteins in dibasic sodium phosphate and sodium sulfite is in marked contrast to the rather poor solubility of sunflower seed proteins under similar conditions, in work done in the same laboratory (Gheyasuddin et al., 1970). This confirms the need to study each plant seed protein system individually and makes generalizations impossible prior to actual experimental work.

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ROBERT HAGENMAIER, C. M. CATER and K. F. MATTIL
Food Protein Research & Development Center, Texas A & M University, College Station, TX 77843

A CHARACTERIZATION OF TWO CHROMATOGRAPHICALLY SEPARATED FRACTIONS OF COCONUT PROTEIN

INTRODUCTION

THE TWO CHIEF coconut producing countries are the Philippines and India, both of which suffer from a shortage of edible protein (Abbott, 1966). In the Philippines, almost all (Nyberg, 1968) coconuts are dried to make copra, which is subsequently pressed to recover the oil. The resulting protein is generally unfit for human consumption.

Although the protein content of the coconut is low (about 4%), the quantity of coconuts grown makes recovery of the protein desirable. In 1965 the Philippines produced 7.0 billion grams of coconut protein, which is estimated to be enough for 10g per Filipino per day. According to the data of Abbott (1966) concerning the protein food supply of the Philippines, those 10g would increase the protein supply of the Philippines by 22%.

However, utilization of coconut protein for food is dependent upon the development of a process whereby both oil and food-grade protein are recovered from the coconut. One of the alternate processes often considered is a process whereby the coconut oil and protein are removed from the solids as an emulsion: the emulsion is then centrifuged to yield oil and proteins (Sreenivasan and Rajasekharan, 1967). Some additional knowledge of the solution properties of the coconut proteins are important toward the development of an aqueous process.

Previous investigations have emphasized classification of the coconut protein by solubility behavior (Samson et al., 1971a; Strength, 1970; Butterworth and Fox, 1963), by amino acid analysis (Strength, 1970; Samson et al., 1971b; Rajasekharan, 1964; Peters, 1960) and heat denaturation by heating of the dry meal (Samson, 1971; Butterworth and Fox, 1963).

The purpose of this research is to better characterize the coconut proteins, principally with respect to some of the parameters of interest in the aqueous processing and use of coconut proteins for food, i.e., solubility, heat denaturation, viscosity and amino acid analysis of the individual protein components.

MATERIALS & METHODS

THE ENDOSPERM (with testa) of fresh coconuts was freeze dried; the oil was then extracted with hexane at ambient temperature. The protein was dissolved by extracting the meal with 1M NaCl at pH 7.0 for about ½ hr. The residue was removed by filtration, the pH raised to 8.0 and the solutions clarified by centrifugation. The resulting solution was concentrated by ultrafiltration against a membrane designed to pass molecules with molecular weight below 10,000 (UM-10 membrane, Amicon Corp.). The concentrated solution was then filtered through a 0.22 μm pore size filter (Millipore Corp.) and dialyzed against 1M NaCl. After this procedure the solution contained 90 ± 2% of the total nitrogen in the coconut. Approximately 5% was not separated from the solids and 3% was lost in ultrafiltration.

Analyses of protein concentrations were made by ultraviolet absorption or by the Lowry method (Lowry et al., 1951). Both methods were calibrated with solutions of coconut protein of known nitrogen content, as determined by Micro-Kjeldahl analysis. The average error in determining nitrogen content was 4% by the Lowry method, and about 2% by ultraviolet absorption.

In heat coagulation experiments the protein solutions (aliquots of a stock solution) were heated for 10 min, then cooled to room temperature and centrifuged. The amount of protein remaining in solutions was taken as a measure of undenatured protein.

Protein solubility was determined by preparing approximately 1% suspensions of the protein in solvent, adjusting the pH of aliquots to the desired value and then agitating for about ½ hr at room temperature. The final pH was read and the suspension centrifuged; then the dissolved protein content of the aqueous phase was measured.

Viscosity was measured in an Ostwald Viscometer, with the time recorded to the nearest tenth of a second on a stopwatch. The average of at least three measurements was used. The standard deviation in measurement of viscosity was 0.1%. The temperature was 30°C.

Amino acid analyses were performed with the Beckman Model 120C analyzer. Samples were hydrolyzed in constant-boiling HCl for 24 hr, under a nitrogen flush. Cysteine was analyzed after oxidation of cysteine to cysteic acid with performic acid. Tryptophan was analyzed by the method of Kohler and Palter (1967). Methionine values were corrected for the ob-

Table 1—Some properties of the protein components

	Unfractionated	Large mol wt components	Small mol wt components
Approx mol wt (at pH 6.8)	–	1.5×10^5	2.4×10^4
Intrinsic viscosity[a]	4.3 cc/gm	4.7 cm/gm	–
Relative abundance	100	84 ± 3	14 ± 3
Percent nitrogen of dried, salt-free protein	19.2 ± 0.4	18.8 ± 0.4	19.4 ± 0.4
Extinction coefficient[b]	3.76 (at 280nm)	3.58 (at 280nm)	2.43 (at 276nm)
Amount sol in 0.1M NaCl, at pH 7.0	–	53 ± 7%	100%

[a]The value of $(\eta-\eta_o)/\eta_o$, extrapolated to zero protein concentration by least-square analysis of data, where η is viscosity and η_o is viscosity of the solvent (1M NaCl); pH was 7.0; temperature, 30°C

[b]The slope of a graph of absorbance vs. concentration, obtained by least-square analysis of data. Concentration in mg N/cc

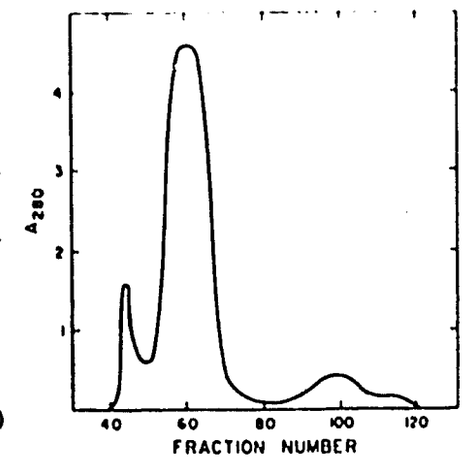

Fig. 1—Sephadex G-200 separation of coconut proteins on a 77 × 5 cm column in 0.95M NaCl buffered at pH 8.2 with 0.01M Na_2HPO_4. Peaks contain 1.6%, 84%, 13.5% and 0.7% of total protein eluted from the column.

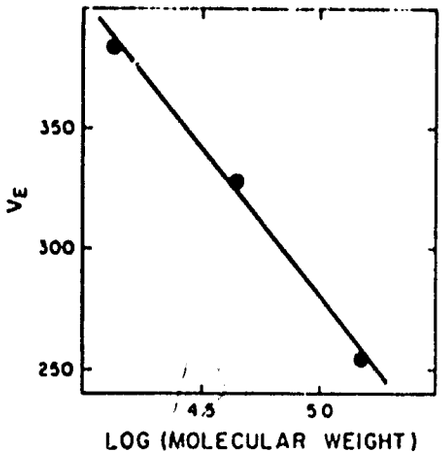

Fig. 2—Calibration curve for determination of molecular weight, calibrated with aldolase (mol wt 158,000), ovalbumin (mol wt 45,000) and ribonuclease (mol wt 13,700) on a 2.5 × 90 cm column of Sephadex G-200, with phosphate buffer (0.4 ionic strength) at pH 6.8. V_0 was 188 cc.

Table 2—Amino acid analyses of protein fractions[a]

Amino acid	Large mol wt component	Small mol wt component	95% Confidence interval
Lysine	3.44	5.01	0.14
Histidine	2.20	1.05	0.13
Arginine	14.70	22.03	0.74
Tryptophan	0.89	1.02	0.10
Aspartic acid	9.47	2.60	0.21
Threonine	3.29	1.13	0.11
Serine	4.89	1.28	0.09
Glutamic acid	17.94	35.51	0.47
Proline	3.60	2.17	0.33
Glycine	4.41	3.13	0.13
Alanine	4.30	0.59	0.15
Cystine	0.98	3.45	0.21
Cysteine	0.16	0.00	0.03
Valine	6.06	0.80	0.24
Methionine	2.84	0.37	0.16
Isoleucine	4.12	0.28	0.33
Leucine	8.15	1.94	0.72
Tyrosine	2.87	2.61	0.13
Phenylalanine	5.10	0.66	0.18
Total	99.41	85.63	2.32

[a]In grams amino acid/16g N

served destruction during hydrolysis of methionine in amino acid calibration mixtures. Two completely independently prepared sets of fractions were analyzed and the amino acid analyses of each were done in duplicate.

RESULTS & DISCUSSION

A TYPICAL chromatographic separation of the coconut protein by Sephadex G-200 is shown in Figure 1. The second and third peaks contain all but 2% of the protein eluted from the column. The second peak is the major protein component, containing 84% of the total nitrogen (by Kjeldahl analysis) and the third peak 14% (average of several separations). The first peak is caused by light scattering and contains a rather small amount of dark-colored protein. The eluant was 0.95M NaCl, buffered at pH 8.2 with 0.01M Na_2HPO_4.

An estimate of the molecular weight of the protein components was made by Sephadex chromatography. A calibration curve of volume of elution vs. log of molecular weight is shown in Figure 2. The volumes of elution of the two major protein components were 258 cc and 365 cc, indicating approximate molecular weights of 1.5×10^5 and 2.4×10^4, respectively. V_0 was 188 cc. More accurate molecular weight determinations were not undertaken in view of the assumed heterogeneity of the fractions. Spychalski (1930) reported a molecular weight of 1.04×10^5 for the main component of coconut globulin.

The chromatography of the coconut protein on Sephadex G-200 had been undertaken as a means of purifying the coconut protein. However, the neat separation into different molecular weight components prompted us to consider the properties of the components separately.

Some of the properties of the components are summarized in Table 1. The amino acid analyses, given in Table 2, are the averages of duplicate analyses on each of two independently prepared sets of samples.

The solubility data presented in Figure

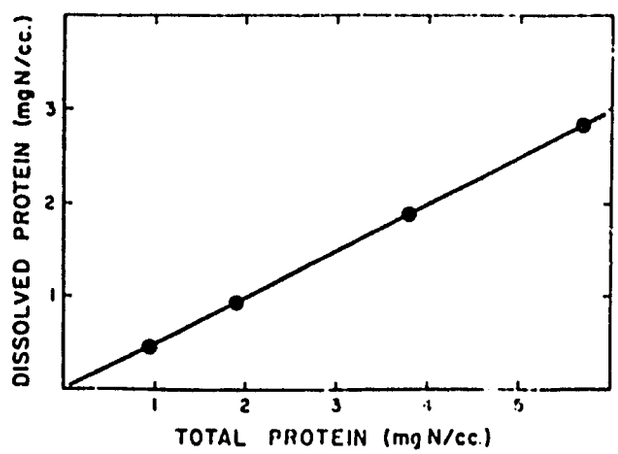

Fig. 3—Dependence of amount of protein dissolved on total amount of large molecular weight component, at pH 6.8, in 0.1M NaCl.

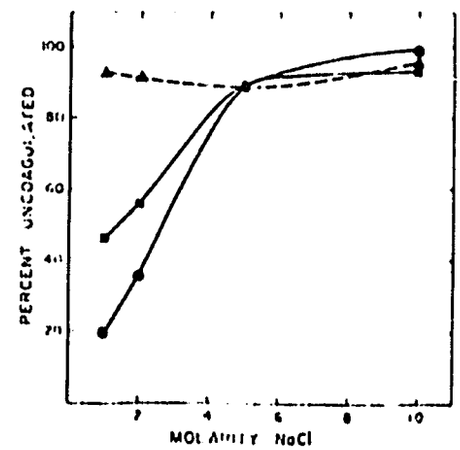

Fig. 4—Dependence of heat coagulation on salt concentration, for the large molecular weight component, at pH 7.4: (•) 100°C; (■) 85°C; (▲) 70°C. The amount coagulated at 0.5M NaCl was the same for all samples.

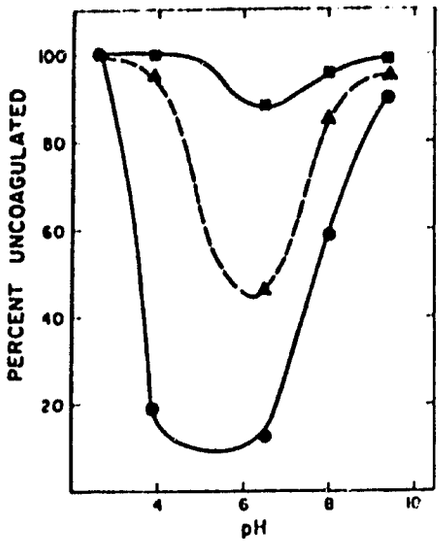

Fig. 5–Dependence of heat coagulation on pH, for the large molecular weight component, in 0.1M NaCl: (•) 100°C; (▲) 80°C; (■) 70°C.

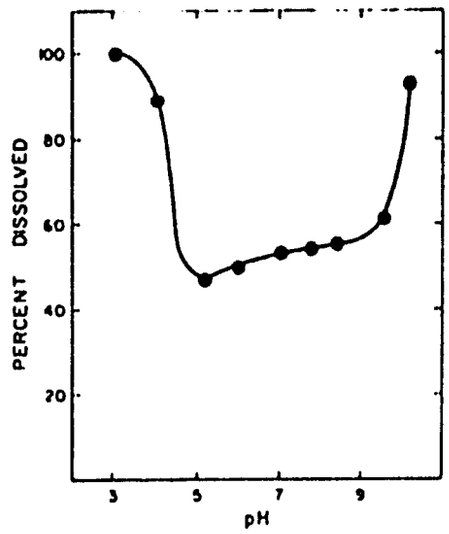

Fig. 6–Dependence of solubility on pH, for the large molecular weight component, in 0.10M NaCl.

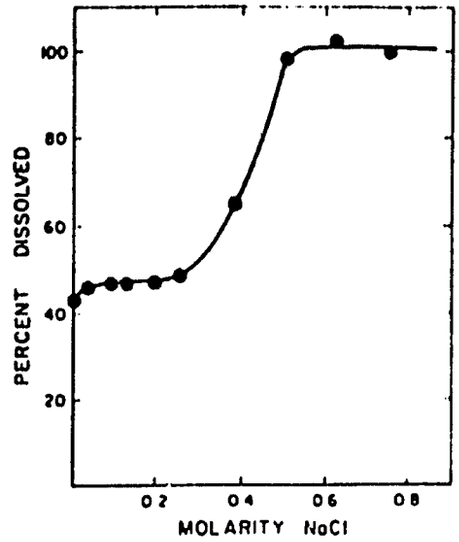

Fig. 7–Dependence of solubility on salt concentration, for the high molecular weight component, at pH 7.0. The lowest salt concentration shown is 0.01M NaCl.

3 indicate that the high molecular weight component is not homogeneous. If it were homogeneous, then the protein should all dissolve until the maximum solubility of the protein is reached–and then no more should dissolve (Taylor, 1953). The straight line dependence of dissolved protein upon total amount of protein present indicates that fraction dissolved in a meaningful parameter of coconut protein solubility. Within the concentration range of 1–5.5 mg N/cc, the solubility of the large molecular weight components seems to be adequately stated by reporting the fraction dissolved, as was done in Figures 6 and 7.

Heat coagulation of the large molecular weight components was at a maximum at neutral pH, low salt concentration and high temperature, as indicated by Figures 4 and 5. Figure 4 contains isotherms at a constant pH (7.4), at different salt concentrations. Figure 5 contains isotherms at a constant salt concentration (0.1M), at different pH values. As might be expected, more protein is coagulated at low salt concentrations and higher temperatures. Also, more coagulation occurs in the pH range 4–7, where it is assumed there is minimum charge on the protein. Peters (1960) reported that the coconut proteins were only heat coagulated in the range pH 3.6–4.1, however he did not specify the solvent used.

The effect of pH and salt on the solubility of the high molecular weight components is shown in Figures 6 and 7. The data show that the point of minimum solubility occurs near pH 5 in 0.1M NaCl. We assume that the isoelectric point is therefore near pH 5. The addition of salt increases the solubility as shown in Figure 7. However, the effect of salt is complicated and varies considerably with pH. At low pH values we have observed a decrease in solubility with addition of salts.

The low molecular weight components were considerably different in their heat coagulation and solubility properties. Under the range of conditions investigated, no significant precipitation or heat coagulation of the small molecular weight components was observed.

The greater heat stability of the low molecular weight proteins could be due to the relatively large amounts of dicarboxylic acids (38.1g/16g N), and basic amino acids (28.1g/16g N), allowing the possibility of many intramolecular salt bridges. Also, the cystine content is higher for the low molecular weight components (3.45 vs. 0.98g/16g N) indicating a larger number of stabilizing disulfide bonds. On the other hand, the greater solubility of the low molecular weight proteins could reflect the usual inverse dependence of solubility on molecular weight of protein (Cohn and Edsall, 1943). The difference in both solubility and heat coagulation of the components might also be attributed to the large differences in aliphatic amino acids, which are present in very small amounts in the low molecular weight components.

The dependence of heat stability on pH and salt concentration means that all of these variables must be considered in finding the maximum safe temperature in the wet processing. However, the data in Figures 4 and 5 indicate that 70°C is safe at nearly all values of pH and salt concentration.

The values for intrinsic viscosity indicate that the coconut proteins have the low viscosity expected of globular proteins. Viscosities were measured in 1.0M NaCl at pH 7, where the protein is all dissolved. Under these conditions the viscosity is very low; however, at lower values of ionic strength, electrostatic factors should cause a somewhat higher viscosity (Tanford and Buzzel, 1956).

For some food uses it is desirable that a protein be soluble at nearly neutral pH values in a solution of low salt concentration. Only half of the high molecular weight proteins are soluble under such conditions. This restricted solubility could have significant implications in considering potential food uses.

In summary, the coconut proteins can be conveniently classified as either high or low molecular weight with the bulk of the proteins being of high molecular weight. The low molecular weight proteins are quite soluble and heat stable. The high molecular weight proteins are heat coagulated at neutral pH values and at low salt concentrations; however, by addition of salt and/or adjustment of pH they may be rendered nonprecipitable by temperatures as high as 100°C.

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Heat Treatment of Coconut Meats and Coconut Meal

by A. S. Samson S.J.*

Food Products Research, Texas Engineering Experiment Station, Texas A & M University, College Station, Texas 77843, U.S.A.

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In attempts to determine the heat tolerance of coconut meats and coconut meal, samples were subjected to several temperatures for varying lengths of time. Properties evaluated were browning (by visual observation), odour, lysine destruction, protein solubility at pH 2, 8 and 10·5, and nitrogen solubility index.

Solubility studies suggest that fresh coconut meats can tolerate up to 100°C air temperature (coconut layer temperature, 78–83°C) without significant loss of protein solubility. At 110°C air temperature, there is loss of solubility at pH 2 and 8, and at 120°C protein solubility significantly decreases under all the pH conditions examined. Data indicate a marked loss of lysine availability upon heating coconut meats with hot air at 120°C.

Coconut meal can tolerate 105°C air for at least 60 min without significant loss of protein solubility. At higher temperatures (120°C for 15 min or longer), protein solubility decreased. Unavailable lysine data indicate that heating at 120°C for 60 min markedly decreases lysine availability.

Introduction

COCONUT protein, only a minor portion of which is presently being utilised for human food consumption, has been shown to be good quality protein, with high nutritional value.[1] Present-day methods of processing coconuts for oil, via copra, however, utilise processes not conducive to producing proteinaceous products fit for human consumption. In the preparation of copra, the water is usually removed by sun- or oven-drying, under conditions liable to cause bacterial and mould growth.[2] The oil is usually recovered by expellers or combinations of expellers and solvent extractors, at temperatures which have been shown to reduce the nutritional value of coconut proteins.[3]

The present studies were undertaken in an attempt to determine the temperature conditions which fresh coconut meats and coconut meal can tolerate without deterioration of protein, as measured by solubility properties and loss of lysine availability. Many studies have been made on the nutritional value of coconut proteins,[1,2,4] using different coconut products processed in a variety of ways. A previous study of Butterworth & Fox made use of solubility and loss of lysine availability as physico-chemical methods of determining protein denaturation.[5] They started, however, with a sun-dried product and, in the solubility tests, employed rather severe conditions (6N-HCl, 0·02N-NaOH, 0·5M-NaCl).

This present work starts with fresh coconut meats and laboratory-prepared coconut meal and makes use of solubility under different pH conditions (acidic, basic and near neutral) and lysine availability as methods of determining protein denaturation.[6,7] It is part of a larger project of developing processes which would make available coconut proteins as an indigenous protein source in coconut-producing countries of the world.

Experimental

Materials

Coconut meats

The coconuts used were food-grade fresh coconuts, obtained from the wholesale market; there were indications that the coconuts originated from Central America. After cracking the nuts and discarding the coconut water, the meats were removed from the shells and manually pared. The pared meats were shredded into smaller fragments using an Urschel mill, Model Comitrol 3600 (cutting head 2-K-020060B)†. The shredded meats contained 47% moisture and volatile materials and 38·5% oil.

Coconut meal

Coconut meal was prepared by processes which were not expected to denature proteins. The fresh coconuts were cracked and the coconut water was discarded. After removal from the shell and washing with water, the meats were sliced into smaller fragments with the Urschel mill (cutting head 2-J-030510B). The shredded meats (which included the testa or parings) were dried under vacuum at 40°C for 24 h to a moisture content of less than 2%. The dried meats were further comminuted by the Urschel mill (cutting head 2-K-020035C), prior to defatting by several extractions with hexane. Residual solvent was removed with dry air. Meal so obtained had the following proximate analysis: protein, 22·2% (N × 6·25); moisture and volatile materials (upon equilibration at room temperature and humidity), 9·9%; oil, 1·1%; ash, 5·2%; and crude fibre, 7·5%.

Methods

Heating of meats

The shredded meats were heated under a draught of air in a box equipped with a heating coil and fan. The temperatures both of the coconut meats (layer thickness 2 cm) and of the ambient air inside the heating box were monitored using a thermocouple and thermometer. To achieve more even heating, the coconut bed was occasionally stirred. Heating was continued to a moisture level of 2–3%, following moisture analysis procedures of A.O.C.S.[8] Prior to further characterisation, the dehydrated meats were defatted by Soxhlet extraction with hexane.

Heating of meal

The coconut meal was heated in a forced draught oven equilibrated to the desired temperature, for a predetermined length of time.

Extraction of nitrogen

Samples (1 g) of dehydrated and defatted coconut were extracted with water at room temperature in a total volume of 20 ml. The desired pH of extraction was maintained with HCl or NaOH. After centrifugation at 7710 × *g* (Sorvall RC2-B Refrigerated Centrifuge), the extracts were filtered through Whatman Filter Paper No. 4 to remove flocculent materials.

* Present address: Department of Chemistry, Ateneo de Manila University, Loyola Heights, Quezon City, Philippines

† Manufactured by the Urschel Laboratories, Inc., Valparaiso, Indiana, U.S.A.

Nitrogen solubility index (*NSI*) was determined according to standard A.O.C.S. methods.[9]

Analytical

Moisture, ash, oil, and crude fibre were determined by standard methods.[9] Nitrogen (protein estimated as N × 6·25) was determined by standard micro-Kjeldahl procedures.[10,11] Amino acid analyses were obtained on Beckman Model 120C Amino Acid Analyzers, following the procedure of Spackman & Moore;[12] unavailable lysine was determined according to the procedure of Roach *et al.*[13]

TABLE 1
Heat treatment of coconut meats

Air temp., °c	Time, min	Thermocouple reading, °c (coconut layer)	Final moisture attained, % sample
60	30	55-60	2·6
70	25	55-60	2·8
80	25	65-70	3·0
90	25	63-68	2·8
100	20	78-83	2·5
110	20	93-98	2·1
120	28	78-83	2·5

Results and Discussion

Fig. 1, giving the solubility of proteins from heated coconut samples, indicates denaturation of coconut proteins when coconut meats were heated with 120°c air, and a partial denaturation at 110°c. At lower temperatures, no such denaturation was observed, as measured by solubility studies. Under acid conditions (pH 2), a marked loss of solubility was observed for coconut meats heated at 110 and 120 °c; at pH 8, there was a significant decrease in solubility for the sample heated at 120°c and a slight decrease for that heated at 110°c. At pH 10·5, only the sample heated at 120°c showed a notable decrease in protein solubility, although the samples heated at 100 and 110 °c were less soluble than the other heat-treated samples.

In terms of the temperatures of the coconut layer itself (Table 1), the solubility data indicate that heating which raises the effective temperature of the meats to higher than 80°c will begin to cause protein denaturation as measured by loss of solubility.

In the case of coconut meal (Figs 2-4), treatment with air heated to 150°c was almost immediately detrimental to protein solubility of coconut proteins. Coconut meal is tolerant to 120°c for a short period of time, but after 15 min heating (Fig. 4), there are indications of loss of protein solubility at pH 10·5. The data also show that coconut meal can tolerate temperatures up to 105°c for as long as 60 min without protein denaturation as measured by protein solubility.

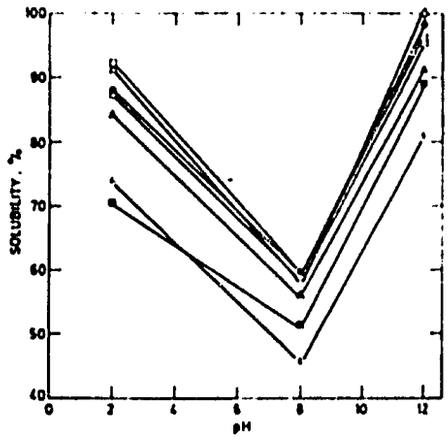

FIG. 1. *Protein solubility of heated coconut meats*
○ 60°; △ 70°; □ 80°; ● 90°; ▲ 100°; ■ 110°; × 120°c

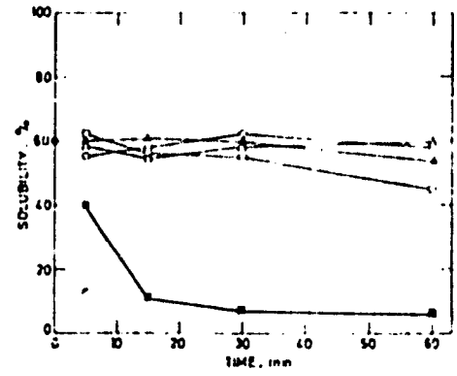

FIG. 3. *Protein solubility of heated coconut meal at pH 8*
● Untreated meal; △ 60°; ○ 90°; ▲ 105°; □ 120°; ■ 150°c

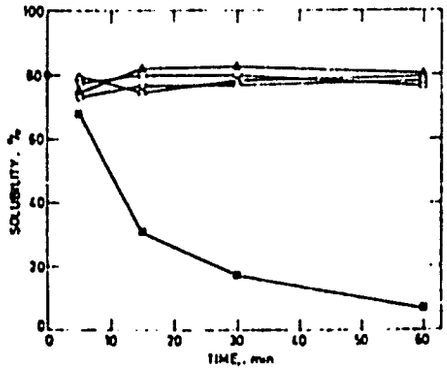

FIG. 2. *Protein solubility of heated coconut meal at pH 2*
● Untreated meal; △ 60°; ○ 90°; ▲ 105°; □ 120°; ■ 150°c

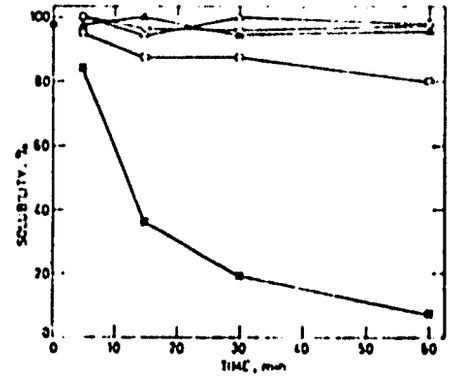

FIG. 4. *Protein solubility of heated coconut meal at pH 10·5*
● Untreated meal; △ 60°; ○ 90°; ▲ 105°; □ 120°; ■ 150°c

TABLE II
Nitrogen solubility index (*NSI*) of heat-treated meats

Air temp., °c	Time, min.	*NSI*, %
60	30	22·1
70	25	23·3
80	25	25·2
90	25	26·8
100	20	20·7
110	20	19·3
120	28	16·9

Table II gives % nitrogen solubility index (*NSI*) results for heated coconut meats. The values obtained, for still unexplained reasons, were invariably lower than those obtained for heated coconut meal. These low values were obtained even for coconut meats heated at lower temperatures. *NSI* values indicate significant denaturation when meats are heated at higher than 110°c air temperature.

Fig. 5, *NSI* of heated coconut meal, confirms that heating coconut meal at 150°c denatures its proteins and, at 120°c, such denaturation is partial but observable.

After heat treatment (and defatting in the case of coconut meats), the appearance (colour) and odour of the coconut samples were organoleptically noted. The heated coconut meats varied from almost white to a light brown colour. Samples heated at air temperatures lower than 100°c (coconut layer not higher than 78–83°c) ranged from almost white to ivory; those heated at 100°c or higher showed a brownish hue which became a definite brown at 120°c.

Each of the coconut meats, after heating, had a characteristic coconut smell. Those heated at 100°c or higher had a slight burned odour. Again, as in the case of colour, this burned smell was much more pronounced prior to defatting.

In the case of coconut meal, visual browning was observed when the meal was heated at 120°c or higher for more than 15 min. Browning was also observed for meal treated at 105°c for 30 min or longer. There was no browning evident in any of the meals treated at 105°c for less than 30 min, or at any of the lower temperatures. A change in odour (burnt as compared with the raw coconut smell of the starting meal) was perceptible in all samples treated at 105°c or higher, with the burnt smell more pronounced with higher temperatures. The slight toasted odour was also observed with samples heated at 90°c for 30 min or longer.

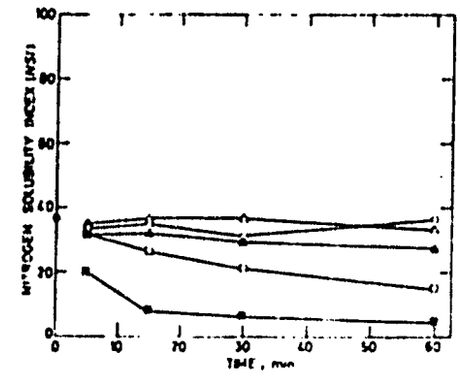

FIG. 5. NSI *of heated coconut meal*
● Untreated meal; △ 60°; ○ 90°; ▲ 105°; □ 120°; ■ 150°c

These observations on odour and colour of the heat-treated samples, in conjunction with the previously discussed solubility data, suggest that visual browning and a burnt odour may be used as early warning signals of incipient loss of protein solubility. When the coconut samples have definitely turned brown and have a toasted odour, protein solubility will be low.

Amino acid analyses of the heated samples are given in Tables III–V. Except for lysine, there were no evident trends in the overall composition of amino acids as a result of the amount of heating employed in this research.

Data on lysine availability[6,7] were more informative. According to Table VI, lysine availability is significantly reduced by heating coconut meats to 120°c; at lower temperatures, no such decrease in lysine availability is observed. Table VII shows that lysine availability was not significantly affected, if at all, by heating coconut meal to 105°c. At 120°c there was a marked loss of available lysine when the meal was heated for 60 min, and some minor loss at 30 min heating. At 150°c, however, both total and available lysine disappeared quite rapidly, a further confirmation of protein denaturation at that temperature.

These results reported for coconut meats and meal prepared in the laboratory can profitably be compared with commercial desiccated coconut and heat-prepared copra. The heating of coconut meats simulated commercial processes of preparing

TABLE III
Amino acid composition of heat-treated coconut meats, % protein

Amino acid	Meal treated at 60°c for 30 min	Meal treated at 70°c for 25 min	Meal treated at 80°c for 25 min	Meal treated at 90°c for 25 min	Meal treated at 100°c for 20 min	Meal treated at 110°c for 20 min	Meal treated at 120°c for 28 min
Lysine	3·8	3·8	3·7	4·0	3·5	3·8	3·1
Histidine	2·1	2·0	2·0	2·0	2·0	2·0	1·9
Arginine	15·7	15·2	15·0	15·7	14·9	15·8	14·0
Aspartic acid	8·0	8·4	8·2	8·0	8·1	8·0	7·9
Threonine	2·9	2·9	2·8	2·9	2·9	3·1	2·9
Serine	4·5	4·1	4·3	4·2	4·4	4·1	4·4
Glutamic acid	19·7	19·9	19·1	19·8	18·9	19·6	19·7
Proline	3·1	3·3	3·1	3·2	3·1	3·3	3·3
Glycine	4·1	4·2	4·1	4·0	4·0	4·0	4·2
Alanine	4·0	3·8	3·8	4·2	3·8	4·6	4·1
Valine	5·1	4·9	4·9	4·6	4·8	4·8	5·0
Methionine	1·8	1·7	1·8	2·1	1·7	1·7	1·9
Isoleucine	3·2	3·1	3·1	3·1	3·1	3·1	3·1
Leucine	6·3	5·6	6·2	6·1	6·1	6·0	6·3
Tyrosine	2·6	2·3	2·6	2·5	2·5	2·5	2·6
Phenylalanine	4·1	3·6	4·3	4·2	4·2	4·2	4·4

TABLE IV

Amino acid composition of heat-treated coconut meal, % protein

Untreated meal and samples treated for 5 and 15 min

Amino acid	Untreated meal	Meal treated at 150 c for 5 min	Meal treated at 60 c for 15 min	Meal treated at 90 c for 15 min	Meal treated at 105°c for 15 min	Meal treated at 120°c for 15 min	Meal treated at 150 c for 15 min
Lysine	3·3	3·4	3·4	3·8	3·5	3·4	2·4
Histidine	1·7	1·8	1·7	1·8	1·7	1·8	1·7
Arginine	12·6	14·5	12·8	13·8	13·9	13·8	13·5
Aspartic acid	8·9	8·1	8·1	8·3	8·4	7·7	7·5
Threonine	2·9	3·0	2·9	3·1	3·0	2·7	2·8
Serine	4·5	4·3	4·1	4·1	4·3	3·8	3·8
Glutamic acid	20·2	19·4	19 0	19·2	20·0	17·8	18·1
Proline	3·6	3·4	3·4	3·1	3·2	3·0	3·1
Glycine	4·2	4·3	4·0	4·1	4·2	3·8	3·8
Alanine	4·5	4·1	4·1	4·1	4·2	3·8	3·6
Valine	5·4	4·9	4·8	5·0	5·0	4·6	4·6
Methionine	1·8	1·7	1·7	1·5	1·5	1·4	1·4
Isoleucine	3·3	3·2	3·1	3·1	3·2	2·9	3·0
Leucine	6·2	6·3	6·0	6·0	6·2	5·6	5·8
Tyrosine	3·0	2·7	2·5	2·7	2·7	2·5	2·4
Phenylalanine	4·3	4·2	4·0	4·0	4·1	3·7	4·0

TABLE V

Amino acid composition of heat-treated coconut meal, % protein

Samples treated for 30 and 60 min

Amino acid	Meal treated at 60 c for 30 min	Meal treated at 90°c for 30 min	Meal treated at 105 c for 30 min	Meal treated at 120 c for 30 min	Meal treated at 150 c for 30 min	Meal treated at 105 c for 60 min	Meal treated at 120 c for 60 min	Meal treated at 150 c for 60 min
Lysine	3·7	3·5	3·6	3·5	2·0	3·8	3·2	1·9
Histidine	1·8	1·8	1·9	1·8	1·7	2·3	1·8	1·4
Arginine	13·8	13·7	14·2	14·7	13·5	14·6	13·8	9·7
Aspartic acid	8·0	7·9	7·9	8·0	8·0	8·3	8·1	7·6
Threonine	2·6	3·1	2·8	2·9	3·4	3·1	2·9	2·7
Serine	3·8	4·1	4·1	4·2	4·2	4·4	4·1	3·9
Glutamic acid	18·1	18·4	18·8	19·3	19·5	19·4	18 9	18 6
Proline	3·2	3·3	3·1	3·5	2·5	4·2	3·3	3·1
Glycine	3·9	4·1	3·9	4·2	4·3	4·4	4·1	4·0
Alanine	3·9	4·1	4·0	4·0	3·9	4·4	4·0	3·6
Valine	4·6	4·7	4·6	4·9	5·1	5·1	5·0	4·8
Methionine	1·5	1·3	1·1	1·6	1·6	1·8	1·6	1·6
Isoleucine	2·8	3·1	3·0	3·3	3·2	3·2	3·1	3·2
Leucine	5·7	6·0	5·9	6·3	6·2	6·4	6·1	6·1
Tyrosine	2·4	2·6	2·4	2·1	2·7	2·7	2·6	2·5
Phenylalanine	3·9	4·0	3·9	4·8	4·2	4·3	4·2	4·1

desiccated coconut, except that the temperatures used in the laboratory were higher than the 60–70°c reported for commercial preparation of desiccated coconut.[14] The copra used was obtained from Surinam and was dried in solar heat-tents.[15]

Fig. 6 summarises the solubility behaviour of commercial desiccated coconut and copra. This copra has a solubility profile like that of unheated coconut meal prepared in the laboratory. The desiccated coconut, however, shows a markedly lower solubility than does copra. The copra gives an *NSI* value of 41·9 and the desiccated coconut of 25·9, confirming loss of protein solubility.

The figures from amino acid analysis, however, indicate that lysine availability has not been significantly reduced for either of the products (Table VIII). The desiccated coconut is perfectly white and does not have any burnt odour. Attempts are being made to find the cause of the anomalous solubility characteristics of the commercial desiccated coconut.

Acknowledgments

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TABLE VI

Available lysine results on heat-treated coconut meats

Temperature, °c		% Protein
60	Total lysine	3·8
	Unavailable lysine	0·3
	Available lysine	3·5
70	Total lysine	3·8
	Unavailable lysine	0·3
	Available lysine	3·5
80	Total lysine	3·7
	Unavailable lysine	0·3
	Available lysine	3·4
90	Total lysine	4·0
	Unavailable lysine	0·3
	Available lysine	3·7
100	Total lysine	3·5
	Unavailable lysine	0·3
	Available lysine	3·2
110	Total lysine	3·8
	Unavailable lysine	0·3
	Available lysine	3·5
120	Total lysine	3·1
	Unavailable lysine	0·5
	Available lysine	2·6
Untreated meal	Total lysine	3·3
	Unavailable lysine	0·3
	Available lysine	3·0

TABLE VII

Available lysine results on heat-treated coconut meal, % protein

Temp. °c		Heat-treatment times 5 min	15 min	30 min	60 min
60	Total lysine	—	3·4	3·7	—
	Unavailable lysine	—	0·2	0·4	—
	Available lysine		3·2	3·3	
90	Total lysine		3·8	3·5	
	Unavailable lysine	—	0·3	0·3	—
	Available lysine		3·5	3·2	
105	Total lysine		3·5	3·6	3·8
	Unavailable lysine	—	0·3	0·3	0·3
	Available lysine		3·2	3·3	3·5
120	Total lysine		3·4	3·5	3·2
	Unavailable lysine	—	0·3	0·4	0·4
	Available lysine		3·1	3·1	2·8
150	Total lysine	3·4	2·4	2·0	1·5
	Unavailable lysine	0·4	0·7	1·0	1·3
	Available lysine	3·0	1·7	1·0	0·2
Untreated meal	Total lysine	3·3			
	Unavailable lysine	0·3			
	Available lysine	3·0			

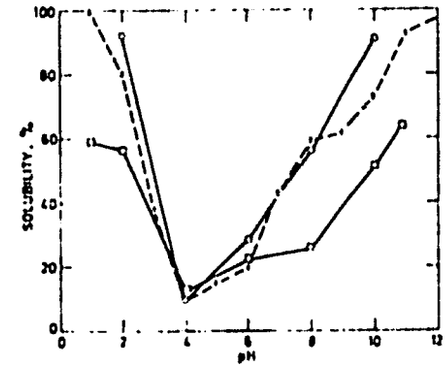

FIG. 6. *Solubility of copra and desiccated coconut*
× Coconut meal; ○ copra; □ desiccated coconut

TABLE VIII

Amino acid composition of copra and desiccated coconut, % protein

Amino acid	Desiccated coconut	Copra
Lysine	4·1	4·1
unavailable lysine	0·3	0·2
available lysine	3·8	3·9
Histidine	2·0	2·0
Arginine	16·0	13·5
Aspartic acid	8·0	7·8
Threonine	3·1	2·9
Serine	4·4	4·3
Glutamic acid	18·9	19·9
Proline	3·3	3·5
Glycine	4·1	4·0
Alanine	4·4	4·4
Valine	4·5	4·7
Methionine	1·4	1·7
Isoleucine	3·2	3·1
Leucine	5·8	6·1
Tyrosine	2·3	2·4
Phenylalanine	3·9	4·2

Preparation and Characterization of Coconut Protein Isolates[1]

A. S. SAMSON S. J.[2], C. M. CATER, and K. F. MATTIL, Texas A&M University, College Station

ABSTRACT

The following protein fractions have been separated from dried, defatted fresh coconut meats: 1) soluble at pH 2, precipitated at pH 3.9; 2) soluble at pH 8, precipitated at pH 3.9; 3) soluble at pH 10.5, precipitated at pH 3.9; 4) extracted sequentially at pH 10.5 from pH 8 residue and subsequently precipitated at pH 3.9; 5) extracted at pH 7 by 1.0M NaCl and precipitated at pH 2, 6) soluble in water at pH 7.0, and 7) proteins remaining in solution at pH 3.9. The recoveries were 1) 45 to 57%; 2) 39 to 53%; 3) 31 to 35%; 4) 14 to 19%; 5) 48 to 54%; 6) 9 to 11%, and 7) 3 to 6%. Hydrochloric was the most efficient precipitating acid of those tested, including sulfuric, phosphoric, acetic, and nitric. Each of the precipitated isolates was dried by lyophilization. While all of the isolates were quite soluble at pH 2, there were substantial differences in solubilities at pH 7, 8, and 10. All isolates were quite insoluble from pH 4 to 6. The amino acid composition of most of the isolates did not vary markedly from that of the original meal. The water solubles were somewhat higher in lysine, arginine, and glutamic acid and lower in the remaining amino acids. The isolate extracted sequentially at pH 10.5 from pH 8 residue was lower in lysine and glutamic acid.

Although fresh coconut meats contain only about 4% protein, they are nevertheless a potentially important source of protein because of the great world production of coconuts, primarily in regions deficient in high protein foods. The world's coconut yield has been estimated from copra data to be 5.6 million metric tons annually (1). These data refer only to coconuts used for copra and do not include those used directly for food. This amount of copra is roughly equivalent to 224 million kg. of protein (basis nitrogen X 6.25), probably none of which is being used for human food consumption.

A 20% protein flour of good quality can be obtained from coconuts by removal of the oil and water. Studies have indicated that the protein efficiency ratio of coconut flour is comparable to that of casein (2). Protein efficiency ratio, biological value, net protein utilization, and digestibility values for coconut products suggest good nutritional quality for coconut proteins (3). Yet, present world practice of handling coconuts through the intermediacy of copra produces a protein meal, after oil removal, which is generally not fit for human consumption (4). Furthermore, the high fiber content of coconut flour (9 to 11%) limits effectively its use as a protein supplement (5).

Because of the low protein content of coconut flour and its high fiber content, even when prepared under conditions which render it suitable for human food use, protein isolates from coconuts take on added significance in attempts to make use of this untapped source of food protein. Isolates, with over 80% protein and very

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[1]Presented at SOS/70, Third International Congress of Food Science and Technology, Washington, D.C., August 1970. Contribution from Food Products Research, Texas Engineering Experiment Station, Texas A&M University, College Station 77843. This work was supported by the Agency for International Development, Contract PIO/T 931-11-190-864, 74-3192010.

[2]Present address: Department of Chemistry, Ateneo de Manila University, Loyola Heights, Quezon City, The Philippines.

low fiber content, could be used more easily as food supplements. Furthermore, like soy proteins or casein, coconut protein isolates may also find added value as the result of any favorable functional properties they may possess.

Previous studies in this laboratory have shown that coconut meal (flour), containing about 20% protein and prepared without excessive heat treatment, displayed suitable solubility for efficient protein extraction in aqueous or salt solutions (6). These investigations showed that it was possible to obtain 80 to 90% protein solubility without resorting to enzymatic extraction conditions, as suggested by Chandrasekaran and King (7).

Peters (8, p. 6) has prepared coconut isolates from a centrifugal wet-process. The National Institute of Science and Technology in The Philippines has operated a pilot plant in which coconut protein concentrates and isolates have been prepared from granulated coconut (9).

Guided by data obtained from preliminary work on the solubility or extractability of coconut proteins, these present studies were undertaken to characterize coconut protein isolates prepared under a variety of extraction conditions.

MATERIALS AND METHODS

Preparation of Coconut Meal

In the production of coconut meal used for the preparation of isolates, care was taken to use steps not expected to denature proteins. Fresh coconuts were cracked and the meats removed from the shells. After shredding through an Urschel mill, the meats were dehydrated in a freeze-dryer, then solvent extracted with hexane. Meal so obtained, after further milling, contained 22.2% protein, 9.9% moisture (after equilibration at room temperature and humidity), 1.1% oil, 5.2% ash, and 7.5% crude fiber (6).

Preparation of Coconut Protein Isolates

Coconut meal was slurried in water or salt solution adjusted to the predetermined pH of extraction, at a 20:1 solvent-to-meal ratio. The mixture was stirred for at least 30 min. on a magnetic stirrer and the pH readjusted, if necessary. This was followed by centrifugation at 5,900 X g for 20 min. (Sorvall RC2-B refrigerated centrifuge) and suction filtration (Whatman filter paper No. 4) to remove flocculent materials from the supernatant extract. The extract was then adjusted to pH 3.9 and centrifuged. After decanting the supernatant layer, the precipitated proteins were washed once with water. Meanwhile, the residue from the initial extraction was re-extracted at the appropriate pH, and the protein separation procedure repeated. The protein isolates were lyophilized and combined. Figure 1 presents a schematic outline of the extraction procedure.

Solubility of Protein Isolates

The protein isolate (400 mg.) was slurried in 40 ml. water (total volume after adjusting pH with HCl or NaOH) and stirred for at least 30 min. at room temperature. After centrifugation at 7,710 X g for 20 min., the extract was filtered to remove flocculent materials. An aliquot of the supernatant was taken for Kjeldahl nitrogen analysis. Percent nitrogen extracted was computed from the total volume of solvent used for the extraction.

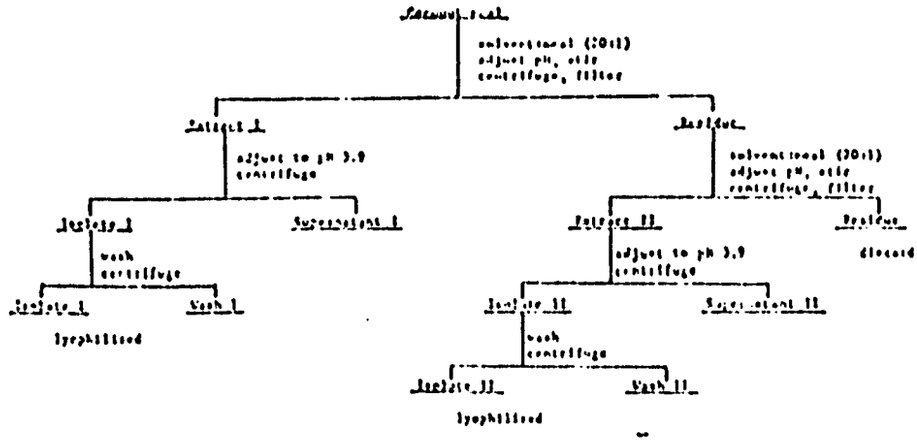

Fig. 1. Extraction procedure for coconut protein isolates.

Analytical Methods

Moisture, oil, ash, and crude fiber were determined by standard methods (10). Nitrogen (protein estimated as nitrogen X 6.25) was determined by standard macro-Kjeldahl (10,11) or micro-Kjeldahl procedures (12,13). Amino acid analyses were obtained on Beckman Model 120C amino acid analyzer, following the procedure of Spackman et al. (14); unavailable lysine was determined according to an unpublished procedure by M. C. Thomas and C. M. Lyman at Texas A&M University.

RESULTS AND DISCUSSION

The nitrogen solubility of the starting coconut meal over the aqueous pH range is given in Fig. 2. From a study of the pH-solubility profile of coconut meal, a

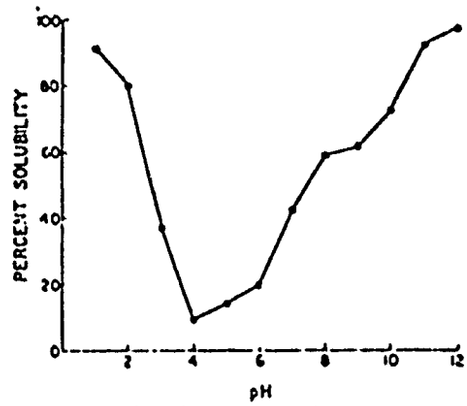

Fig. 2. Solubility profile of the proteins of coconut meal.

determination was made as to the pH conditions both for extracting coconut proteins and for precipitating the proteins from the extracts.

Previous workers have used different pH values for precipitating coconut proteins: pH 3.9 (8, p. 20); 3.4 to 3.5 (15); 4.5 (16). To resolve the differences in the literature values, extractions of coconut meal were made at a series of pH points between pH 3.0 and 5.0. From these it was evident that solubility was minimal from pH 3.6 to 3.9. It was decided to precipitate proteins at pH 3.9.

Guided by the high solubility of coconut proteins on both the acid and alkaline sides (Fig. 2), protein fractions were prepared by extraction under acid (pH 2) and alkaline (pH 10.5) conditions. For the present investigation, stronger acid and alkaline conditions were not employed as a precaution against possible protein denaturation. The small plateau in the solubility profile at about pH 8.0 to 8.5 suggested another set of conditions for preparing protein isolates: extraction at pH 8, followed by a sequential extraction of the residue at pH 10.5. These isolates were made by precipitating the extracts at pH 3.9.

Because of the large percentage of globulins in coconut proteins (62% by the Osborne classification) (6), a salt extract was prepared using 1M NaCl at pH 7, at which pH coconut protein displayed maximum solubility with 1M NaCl solution. Since pH-solubility profiles of coconut meal proteins under salt conditions indicated minimum solubility at acid conditions (6), the salt extract was precipitated at pH 2 by the addition of HCl. To minimize residual NaCl in the isolate, the precipitated extract was dialyzed against water prior to lyophilization.

Two other protein fractions suggested themselves: proteins extracted by water alone (albumins, 31% by Osborne classification tests) and the proteins remaining in solution at the point of minimum solubility, pH 3.9. Both these protein isolate preparations involved dialysis of the extracts against water, prior to air-concentration in the bags, and lyophilization.

Table I gives typical yields of protein isolates, calculated from the nitrogen content of both isolates and starting meal. Table II summarizes nitrogen material balances for the extraction procedure employed in the laboratory.

Table I shows the typical yields of the protein isolates. Each of the isolates was prepared a number of times. The efficiency of protein recovery from meal to isolate was about 50% for preparations under acid (pH 2) and salt (1M NaCl, pH 7) conditions. The total recovery for the step-wise extraction at pH 8 followed by

TABLE I. PREPARATION OF PROTEIN ISOLATES. YIELDS[a]

pH	Isolate I	Isolate II	Total	Protein Content
		% N recovered from meal		%
2	43	10	53	94.6
8[b]	26	13	39	91.6
10.5[c]	19	...	19	90.7
10.5	21	12	33	88.2
7 (salt)	40	13	53	97.6
3.9	3	...	3	50.5
Water	8	...	8	74.8

[a] To be read in conjunction with Fig. 1.

[b] From freshly prepared meal, yield of isolate: 59.7%; protein content: 97.4%.

[c] Sequential from meal extracted at pH 8.

TABLE II. PREPARATION OF PROTEIN ISOLATES: NITROGEN MATERIAL BALANCE[a]

pH	Extract I	Extract II	Total	Supernatant I	Wash I	Isolate I	Supernatant II	Wash II	Isolate II
	% N recovered from meal			% N of extract I			% N of extract II		
2	60.9	14.1	75.0	22.8	5.6	71.2	24.2	2.6	68.9
6	50.6	15.7	66.5	46.1	15.2	51.7	10.5	1.1	82.0
10.5[b]	21.3	...	21.3	4.4	1.0	68.4	...	...	...
10.5	80.6	15.2	95.8	72.1	3.3	25.9	16.0	0.7	76.1
7 (salt)	58.5	23.2	81.8	17.9	...[c]	67.5	19.9	...[c]	55.7
3.9	13.0	2.1	15.1	...	...	...	...	...	...

[a]To be read in conjunction with Fig. 1.

[b]Sequential from meal extracted at pH 8.

[c]Data not taken.

TABLE III. EFFECT OF ACID PRECIPITANT ON PROTEIN YIELD
(proteins extracted at pH 8)

Acid	Protein Content %	Yield of Isolate[a] %
1N HNO_3	93.4	47.5
1N H_2SO_4	93.0	41.8
1M H_3PO_4	89.3	47.6
1N HOAc	93.7	36.2
1N HCl	91.5	52.9

[a]On the basis of protein content of meal and isolate.

TABLE IV. AMINO ACID COMPOSITION OF COCONUT PROTEIN ISOLATES AND FRACTIONS[a]
(expressed as % of total protein)

Amino Acid	Coconut Meal	Extracted at pH 2, Precipitated at pH 3.9	Extracted at pH 7, (1M NaCl) Precipitated at pH 2	Extracted at pH 8, Precipitated at pH 3.9	Extracted at pH 10.5, Precipitated at pH 3.9	Extracted at pH 10.5, Sequented from pH 8, Precipitated at pH 3.9	Water-Solubles
Lysine	3.3	3.4	3.5	3.8	3.5	2.8	5.5
Unavailable lysine	0.3	0.3	0.2	0.3	0.3	0.2	0.3
Available lysine	3.0	3.1	3.3	3.5	3.2	2.6	5.2
Histidine	1.7	2.0	2.0	2.2	2.0	1.4	1.6
Arginine	12.6	12.8	14.3	15.1	14.3	4.2	16.8
Aspartic acid	8.9	9.0	8.6	8.2	9.0	9.1	5.1
Threonine	2.9	3.4	3.2	2.9	3.4	3.8	2.1
Serine	4.5	4.9	4.5	4.2	4.8	5.3	2.5
Glutamic acid	20.2	18.3	16.4	20.1	19.2	6.5	25.4
Proline	3.6	3.5	3.4	3.4	3.7	3.4	2.6
Glycine	4.2	4.4	4.1	4.3	4.5	4.5	3.7
Alanine	4.5	4.3	4.1	3.9	4.2	4.6	2.5
Valine	5.4	5.8	5.6	5.4	5.8	6.1	3.1
Methionine	1.8	2.1	2.2	1.8	2.2	3.4	0.9
Isoleucine	3.3	4.0	3.7	3.4	3.9	4.3	1.9
Leucine	6.2	7.3	6.9	6.9	7.2	7.6	4.5
Tyrosine	3.0	3.1	2.7	2.6	3.0	3.5	2.5
Phenylalanine	4.3	5.4	4.8	4.8	5.2	4.9	2.6

[a]No assays made for tryptophan.

extraction at pH 10.5 was about 60%. For the isolates prepared under alkaline (pH 10.5) and near neutral (pH 8) conditions, the protein yields were under 40%.

These data represent preparations made after the coconut meal had been stored in the laboratory for about 5 months. These protein recoveries were basically the same as those obtained from freshly prepared coconut meal, except in the case of the isolate at pH 8. In Table I, the recovery of proteins extracted at pH 8 was 39%; earlier recoveries, with freshly prepared meal, ranged from 60 to 67%. This suggests that the amount of proteins recoverable from coconut meal changed with age. Material balance data (Table II) indicate that the lower recovery with the aged meal is due to the fact that about half of the extracted proteins failed to precipitate at pH 3.9.

Nitrogen material balances indicated that the nitrogen was extracted at about the levels that were anticipated from the solubility profile. However, some recoveries were lower than anticipated, inasmuch as the protein did not precipitate in the quantities that would be predicted from the solubility profile. This was especially true of the protein which was extracted directly at pH 10.5. While 96% of the available nitrogen was found in the extract, over 70% of the dissolved nitrogen remained in solution at pH 3.9. On the other hand, the protein extracted sequentially at pH 10.5 from the meal previously extracted at pH 8 was 88% insoluble (precipitated) at pH 3.9. These phenomena are under further investigation.

An attempt to maximize the recovery of protein by the use of acids other than hydrochloric for precipitating proteins showed that HCl was the most efficient among the acids tried (Table III).

A further attempt to determine the reason for the increased solubility at pH 3.9 of the extracted proteins (and the yield of precipitated protein isolate much lower than expected) also failed to give a satisfactory answer. From Table II, it should be noted that efficiency of precipitation was usually (except for the pH 2 isolate) much higher in the second and third (for the pH 10.5 sequential isolates) extracts than for the first extract. It appeared possible that the carbohydrates which were soluble under the extraction conditions affected the solubility of the proteins. In the pH-solubility studies, though the carbohydrates were also in solution, the fibrous material and other insoluble solids could still interact with the rest of the mixture; in the case of the protein extracts, however, all insoluble materials have been removed by centrifugation and filtration.

An extraction was performed at pH 8 conditions, and the extracts from the first and second extractions combined. The extract was divided into two portions: the first was precipitated at pH 3.9 and lyophilized; the second was dialyzed against water for 3 days, air-concentrated, and lyophilized. The first portion gave about 10% more protein isolate than the second, showing that carbohydrates and other dissolved substances in the extract did not hinder protein precipitation at pH 3.9.

Characteristics of Protein Isolates

Protein isolates prepared under different pH conditions varied in physical appearance. The isolates obtained at pH 10.5 were brownish in color, those at pH 2 and 8 slightly grayish, whereas the salt isolate was white. The salt isolate was fluffy whereas the other major isolates were more granular.

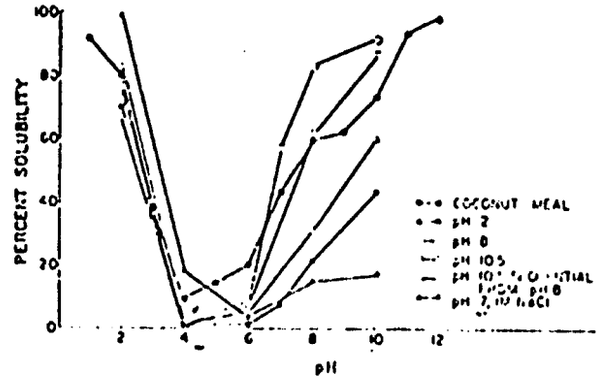

Fig. 3. Solubility profiles of coconut protein isolates extracted under the indicated conditions and precipitated at pH 3.9 (except the salt extract, which was precipitated at pH 2.0).

An important functional property of protein isolates is their solubility at various pH values. In addition to serving as a useful indicator of protein denaturation, the solubility profile may also suggest possible uses. The solubility characteristics of the several coconut protein isolates varied (Fig. 3). All were quite soluble at pH 2, including the salt isolate. The latter is particularly noteworthy, inasmuch as it had been prepared by precipitation at pH 2, although at that time it was in 1M NaCl solution. All the isolates were relatively insoluble from pH 4.0 to 6.0. At pH 8.0 and 10.0, wide differences became apparent. The isolate prepared by salt extraction was surprisingly insoluble under alkaline conditions. Those extracted initially at pH 8 and 10.5 retained good solubility at pH 8 and 10.

Table IV summarizes amino acid analyses for coconut meal and the coconut protein isolates. Except for the water-soluble proteins and the isolate obtained by a sequential extraction at pH 10.5 of the pH 8 residue, the amino acid composition of the isolates does not differ markedly from that of the original meal. The water-solubles were high in lysine, arginine, and glutamic acid and lower in the other amino acids; the pH 10.5 isolate from the sequential extraction was low in lysine and glutamic acid. Available lysine figures were also comparable to the starting meal, indicating that the proteins had not been denatured during their extraction from the meal and preparation into protein isolates.

Further studies are in progress on the other functional properties of the coconut protein isolates.

Acknowledgment

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Critical Unit Operations of the Aqueous Processing of Fresh Coconuts[1]

ROBERT HAGENMAIER, CARL M. CATER and KARL F. MATTIL, Food Protein Research and Development Center, Texas A & M University, College Station, Texas 77843

ABSTRACT

An aqueous process was investigated for the recovery of oil and food grade protein from fresh coconuts. Efficient recovery of oil, which is very important for economical reasons, was related to three critical unit operations: separation of oil from the fiber, destabilization of an oil-in-water emulsion, and recovery of a protein product that is low in oil content. The material balance is reported for a laboratory process that satisfactorily separates oil and breaks the emulsion, and data are shown which has led to a process for recovery of a protein with low oil content.

INTRODUCTION

In the Food Protein Research and Development Center at Texas A&M University, work is in progress on the recovery of oil and food grade protein concentrate from the coconut. We are considering two general approaches to the problem. The first is a conventional processing similar to that currently used for soybeans: the coconut meats are dried and the oil extracted with hexane. The hexane is subsequently distilled from the oil. Protein is separated from the defatted coconut meal by the normal protein isolation procedure: extraction at high pH, followed by precipitation at low pH (1).

Another approach, with which this work is concerned, is the aqueous processing of the coconut. The characteristic feature of aqueous processing is that the coconuts are not subjected to preliminary drying. The fresh coconut meats are ground while still wet, with optional addition of water to facilitate grinding, and the oil and protein are recovered from the resulting emulsion. Various schemes for the aqueous processing of coconuts have been proposed (2). The flow diagram presented in Figure 1 is general enough to incorporate most variations for the aqueous processing of fresh coconuts.

The first step in the process is the grinding of the coconut meats, which results in a slurry of fibrous particles in a milky emulsion. Upon subsequent filtration to remove the suspended solids, the oil and protein are observed to stay in the liquid phase, even though only 30% of the protein is dissolved. The undissolved protein stays with the liquid phase because it is acting as an emulsifying agent for the oil globules, which are small enough to pass through the openings in the filter.

The oil-in-water emulsion, commonly called coconut milk, is centrifuged next, with three phases resulting: the light and heavy liquid phases, and the solids. The composition of the light liquid phase depends on the intensity of centrifugation. Under the very intense centrifugation, as is used by Timmins (unpublished results, 1970) the light phase may be clear oil. Under the conditions of most aqueous processing methods, the light phase is a "cream," which contains a high concentration of protein-emulsified oil globules. The cream-like phase must be demulsified to make possible the separation of clear oil from a solids phase containing the protein. The heavy liquid phase consists of water and water-soluble material, and comprises ca 90% of the total volume of the system. The third phase consists of undissolved solids that are more dense than the aqueous phase. These solids consist of the fiber that survived the filtration operation, together with precipitates formed after the filtration. It should be pointed out that insofar as the centrifugation demulsifies, it forms precipitate, because the protein removed from the oil globules is not dissolved.

A study of the literature has led to the recognition of two elusive goals in the development of an aqueous processing of coconuts. They are: first, the control of conditions so that the protein survives the processing without becoming irreversibly denatured, and so that the oil is recovered in good condition, and secondly, the recovery of at least 95% of the oil. In order to achieve these goals the critical operations seem to be extraction of oil from the fibrous residue, demulsification, and recovery of a protein that does not bind an excessive amount of oil.

Reported processes for the aqueous processing of coconuts apparently fail to achieve these goals. For example in the Krauss-Maffeti process (2,3) the demulsification was accomplished by heating, which irreversibly denatured the

[1]Presented at the 162nd National Meeting of the American Chemical Society.

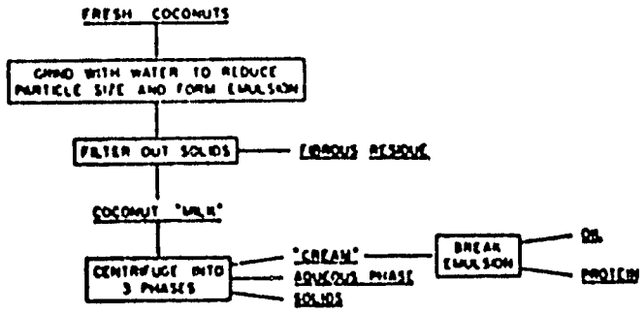

FIG. 1. General procedure for the aqueous processing of fresh coconuts.

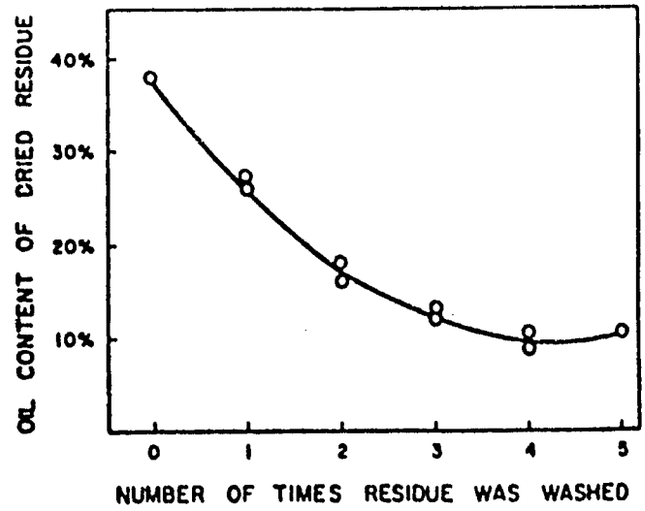

FIG. 2. Removal of oil from residue by repeated washing with water.

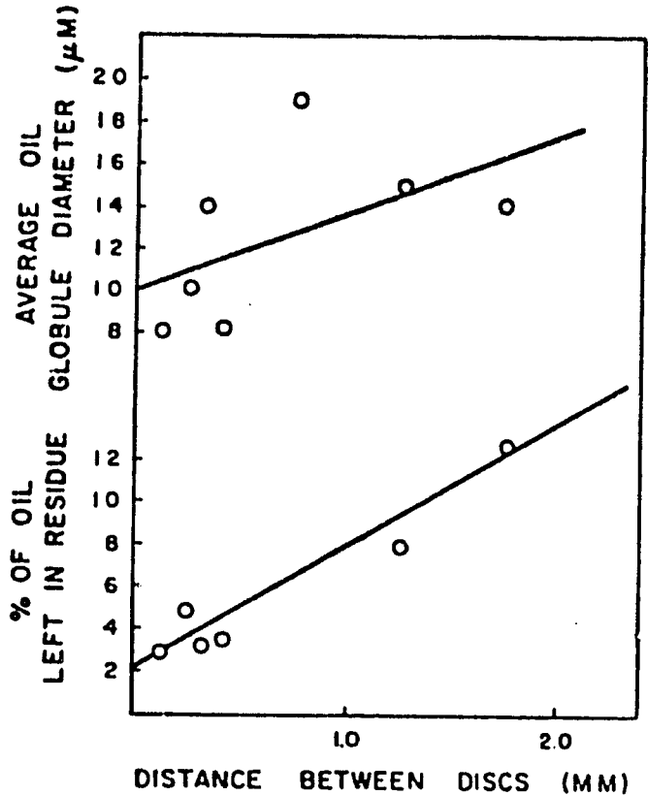

FIG. 3. Effect of disc spacing in Bauer Mill on oil globule size and oil retention of residue.

protein. Furthermore ca. 14% of the oil remained with the residue and ca. 7% with the protein.

Other works on wet processing of coconuts is currently in progress at the Tropical Products Institute in London. The data reported by Timmins (unpublished), indicate that their residue holds ca. 8% of the oil, and that 5-10% of the oil remains with the protein.

METHODS AND EQUIPMENT

Batch centrifugation was performed in the laboratory with a Sorvall Model RC2-B Centrifuge, with a swinging bucket rotor. Centrifugation was at 9000 x g at 30 C, for 10 min. Continuous centrifugation was accomplished with a Westfalia disc type centrifuge (Model SA7-06-476), at 55 C at a flow rate of approximately 100 gal/hr. Wet coconut was ground with a cutting mill (Urschel Laboratories, Comitrol 3600) with cutting spaces of 0.25 mm, followed by milling with water in a disc attrition mill (The Bauer Bros. Co.), normally with a disc spacing of 0.3 mm.

Filtration was accomplished in the laboratory by hand squeezing the ground coconut with water through three layers of cheesecloth. Standard methods were used for the determination of oil, moisture and protein (as nitrogen).

Oil globules were photographed at 440 magnification, and the globule diameter measured in the resulting photographs. Average globule sizes were calculated exactly as one calculates a weight-average molecular weight.

GRINDING

The first critical step in the processing is the wet milling of the coconut. The primary purpose of the grinding is the breakdown of the fibrous meat of the coconut, in order to increase the efficiency of extraction of the oil and protein. However the grinding also has the very important effect of forming a protein stabilized oil-in-water emulsion. Excessive grinding favors cell rupture and removal of oil from the

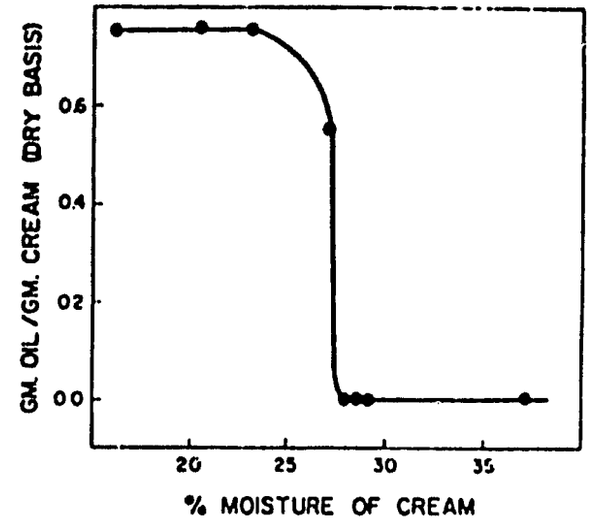

FIG. 4. Effect of moisture content of cream on its inversion by shear, at pH 4.0.

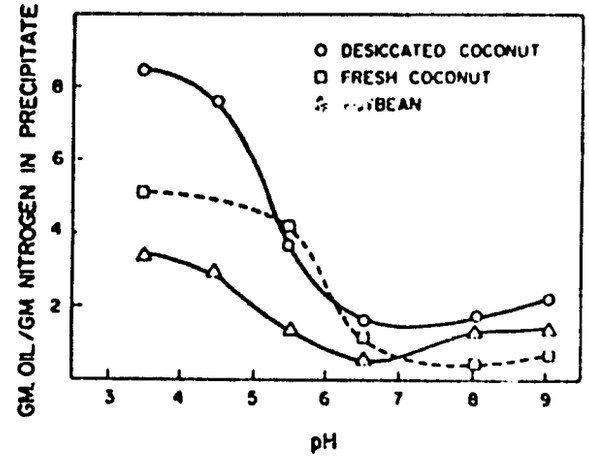

FIG. 5. Effect of pH on ratio of oil and nitrogen in solids obtained when milk is centrifuged.

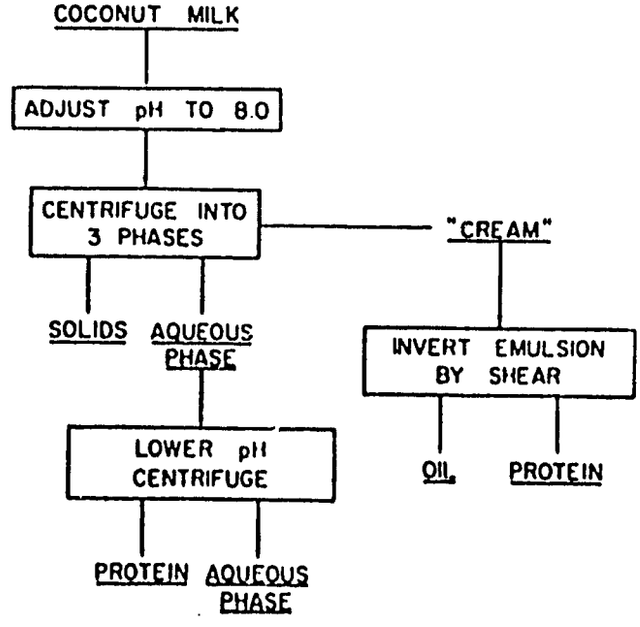

TABLE I

Laboratory Scale Material Balance for the Aqueous Processing of Coconuts at pH 4.0

Material	Dry weight, g	Weight oil, g	Weight protein, g (N x 5.3)
Fibrous residue (washed 3 times)	75	15	1.6
Aqueous phase	45	2	2.1
Solids	7	2.9	2.6
Cream	370	339	21
Oil from cream	306	306	0
Protein from cream	60	30	21
Starting material (1000 kg fresh coconut meats)	550	385	32

makes demulsification more difficult. Insufficient grinding, on the other hand, results in intolerable losses of oil in the residue.

In order to evaluate methods of grinding the coconut, a very simple experiment was conducted. The ground coconut was squeezed through cheesecloth, then the residue was gently mixed with water and squeezed again, and the washing repeated. The idea was that this washing would not break any more cells than were broken in the grinding operation, and therefore allow us to determine the amount of oil present in unbroken cells. Fresh coconut meat was ground in a blender for the experiment reported in Figure 2. The results show that the oil is gradually washed out with successive washes, with two washes being adequate to remove almost all the oil not in unbroken cells. These data, combined with microscopic examination of the wet residue, indicate that oil globules cling to the coconut residue and that washing is an effective method for recovering them. Incomplete removal of these oil globules may be partly responsible for the failure of other workers to efficiently extract oil from the residue.

In order to investigate optimum grinding, coconut-water mixtures were ground in the Bauer mill with varying distances between the grinding surfaces. Fibrous solids were filtered out with cheesecloth and washed three times with water. The resulting average oil globule size was determined for each grinder setting. The results are shown in Figure 3. The disc spacing was varied from 0.1-1.8 mm. The lines were drawn by least squares analysis of the data, and indicate that as the disc spacing increases the globule size increases, and more oil is left in the residue.

It was considered necessary that somewhat less than 5% of the oil remain with the fibrous residue. The data in Figure 3 show that grinding, which conditions the coconut so that less than 5% of the oil remains with the residue, forms oil globules about 10 μm in diameter. An emulsion with oil globules of this size should be expected to be quite stable. To use another protein-stabilized emulsion for comparison, we note that the average globule size of fresh cow's milk is about 10 μm (4). These experiments suggest that a relatively stable emulsion is the price one must pay for efficient wet-extraction of oil from the fresh coconut.

CENTRIFUGATION OF THE COCONUT MILK

Fresh coconut was ground in the Urschel Mill, then in the Bauer Mill with 0.3 mm disc spacing. These grinding conditions leave 4% of the oil in the residue, as evaluated by washing and squeezing through the cheesecloth. It was decided to adjust the coconut milk to pH 4.0 (from its unadjusted value of 6.5), because at this pH the emulsion is reported to be less stable (5). The coconut milk was batch centrifuged, resulting in three phases: solids, aqueous phase and cream. The compositions of these phases are reported in Table I, where each result is the average of at least two experiments.

Note that most of the oil and protein were centrifuged into the cream phase, which contains 66% of the protein and 88% of the oil. The appearance of so much protein in the cream phase results from the fact that the centrifugation step did not efficiently demulsify the milk. The data also show that the aqueous phase at pH 4.0 is very low in both protein and oil. Also only a small amount solid phase was obtained, which was about 42% oil.

A similar experiment was carried out on a large scale with a disc type continuous centrifuge. Here however the results were quite different, with only 14% of the protein appearing in the cream phase instead of the 66% reported for the batch centrifugation. With the disc centrifuge there was much more protein in the solids, indicating that the disc centrifuge was a more effective demulsifier. However in both types of centrifuge the oil content of the solids was rather high, about 40%, which suggests that centrifugation of the coconut milk under these conditions necessarily results in an oily protein precipitate.

EMULSION BREAKAGE

A method was needed for separating the emulsion into clear oil, aqueous phase and solids phase. A criterion for the method is that it avoid irreversibly denaturing the protein, and as always the method must be economically feasible.

The method investigated most thoroughly was the inversion of the emulsion by shear, first suggested by Sugarman (6). The idea is to first concentrate the oil globules of the emulsion into a cream by centrifugation and then to convert the cream into a water-in-oil emulsion by agitation violent enough to break the globules. Subsequent centrifugation separates the continuous phase, which is the oil, from the wet protein precipitate.

The data in Figure 4 show that the use of shear is effective in inverting the coconut emulsion if the water content is below about 28% at pH 4.0. Normally when coconut milk was adjusted to pH 4.0 and centrifuged, the resulting cream layer was about 30% moisture, and agitation of the cream did not invert the emulsion. Therefore it was necessary to reduce further the moisture content of the cream. This reduction in moisture content was accomplished by adding coconut oil to the system before agitating. After agitating and inverting the emulsion, the added oil was recovered together with the original oil that was in the emulsion. Table I indicates the amount of oil and protein recovered from emulsion inversion on the laboratory scale aqueous processing.

One of the problems with the procedure is that the protein precipitate is quite oily. The oil content could only be reduced to 40% with quite severe agitation (3 min agitation at 50 C with a high speed on a blender). An advantage of the method is that the protein recovered was not irreversibly denatured, as judged from the fact that 70% was dissolved at pH 8.0.

There are two products of the pH 4.0 aqueous processing that are high enough in protein content to warrant consideration as food protein. They are the solids obtained when the milk is centrifuged and the solids obtained when the demulsified cream phase is centrifuged, both were 40-45% oil. Although the relative amounts of the two products were different for batch and continuous centrifugation, in both cases about 9% of the oil was retained with the protein solids. That 9% of the total oil represents a loss of marketable oil, which is the principal product of coconut processing from the consideration of economics. Therefore other processing methods were considered.

PROCESSING AT pH 8.0

Oil binding of coconut protein was investigated in an attempt to produce a protein with [illegible]

was prepared by blending coconut or soybean with water and filtering through cheesecloth to remove the solids. The pH values were adjusted, the milky emulsions centrifuged, and the nitrogen and oil contents of the precipitates measured. Figure 5 shows the results. Note that coconut protein binds oil considerably better in the isoelectric pH range, i.e., at pH near 4.0. Soybean protein, shown for comparison, has a similar property. It is not known how the residual oil is bound to the protein; however part of the oil can be removed by rinsing with water, which suggests that part of the oil is bound to the protein as oil globules.

Based on our knowledge of the pH dependence of oil binding, we modified our processing scheme to that shown in Figure 6. The centrifugation of coconut milk is now done at pH 8.0 instead of pH 4.0. This modification was designed to reduce the oil content of the precipitated protein. We expected the protein obtained upon centrifugation of the milk to be low in oil because, as Figure 5 showed, the protein binds less oil at pH 8.0. We further anticipated that the protein precipitated from the aqueous phase would be low in oil, because there is little oil available in the aqueous phase to bind to the protein. As anticipated the results indicate that the protein obtained when the milk is centrifuged at pH 8.0, and that precipitate formed when the aqueous phase is adjusted to low pH, only have about 20% oil. An unexpected result was that the cream phase only contained about 3% of the total protein (for pilot plant centrifugation), as contrasted to 14% observed for identical centrifugation at pH 4.0.

It was decided to determine a material balance for the processing at pH 8.0 on a pilot plant scale, rather than a laboratory scale. We went to the larger scale because on that scale we could use a desludging, continuous-feed centrifuge. As indicated in this report, a continuous centrifuge results in considerably less protein in the cream phase. Consequently a significant effect on the material balance was observed.

The design of the pilot plant equipment, the material balance for processing at pH 8.0 on that scale, and an analysis of the products are under active investigation and will be reported on at a later date.

ACKNOWLEDGMENT

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ROBERT HAGENMAIER, CARL M. CATER and KARL F. MATTIL
Food Protein R&D Center, Texas A & M University, College Station TX 77843

AQUEOUS PROCESSING OF FRESH COCONUTS FOR RECOVERY OF OIL AND COCONUT SKIM MILK

INTRODUCTION

COCONUT PROTEIN represents a substantial source of potentially available food protein in a number of tropical countries. Currently, worldwide growth of coconuts results in about 200,000 metric tons of coconut protein annually (USDA, 1971). This protein is currently not usable as food because of unsanitary processing of copra. Consequently efforts have been undertaken to process fresh coconuts to recover food-grade protein. However, the processes developed have been marked by inefficient recovery of oil and protein (Rajasekharan and Sreenivasan, 1967).

The purpose of this work is to describe an economical method for the pilot plant processing of coconuts. The processing is designed to produce coconut skim milk, a nutritious product currently produced in the kitchen or on a larger scale by rather rudimentary technology (Salon and Manquis, 1969). The pilot plant processing as presented is a development of preliminary work involving bench-scale processing (Hagenmaier et al., 1972).

EXPERIMENTAL

Pilot plant operation

A simplified flow diagram for the process is shown in Figure 1. Pilot plant operation was performed on a scale of approximately 100 lb of coconut meats per day. The coconuts were purchased as husked nuts from a local grocer, shelled manually and the coconut water saved for use in processing. Washed meats were ground in a hammer mill (The Fitzpatrick Co.) with 0.25 in. diam holes in the screen, followed by grinding in a disc attrition mill (Bauer Bros. Co.) with the discs set at minimum clearance.

The ground coconut was briefly mixed with heated coconut water (80°C), or with a corresponding amount of heated tap water, when the coconut water was not used. The mixture was pressed at 50 psi in a pulp press, against a screen with holes 0.01 in. diam. Extraction with hot water, followed by the pressure, was repeated twice more. The two materials resulting from the pressure operation are the liquid filtrate (called coconut milk), and the solids (called residue). The residue came from the press at 70 ± 10% moisture, and was dried in a rotary dryer, in a flow of hot air (inlet air is ca. 100°C). The dried residue is a final product.

The coconut milk was filtered through a vibrating screen (120 mesh) to remove the last traces of residue. The pH of the filtered milk was raised to 7.0 (from 6.3) with sodium hydroxide. The milk was pasturized (ca. 1 hr at 60°C), then centrifuged in a three-phase, desludging type separator (Westfalia). This model develops a centrifugal force of 4,700 × G at the radius of the hole in the disc stack, has a bowl capacity of 3.2 liters and was operated with a feed of 8 liters per minute. The milk was centrifuged until the solids capacity of the bowl was reached, the feed to the separator stopped, and the bowl flushed with aqueous phase for 1 min before desludging. Complete desludging was performed once for every 20 kg of coconut meats used.

Three phases emerge from the separator: (1) The lightest phase, usually called coconut cream, which is ca. 6% oil on a wet basis; (2) The aqueous phase, usually called coconut skim milk, which is a solution of the soluble components of the coconut, with some suspended particles. With counter-current washing of the residue, the aqueous phase would contain ca. 9.5% solids as it comes from the separator, and (3) A solids phase, consisting mostly of insoluble protein, which is dried to give a final product.

The coconut cream is an oil-in-water emulsion with average oil globule size of ca. 10 μm in diameter (Hagenmaier et al., 1972). The emulsion was diluted with oil to reduce the moisture content, then agitated with a high velocity pump to invert the emulsion. Best results were achieved when the temperature was raised to ca. 80°C before the agitation. After inversion of the emulsion, oil and water were separated by first allowing a gravity separation (which took several minutes) into two phases, an aqueous phase and an oil phase. The aqueous phase

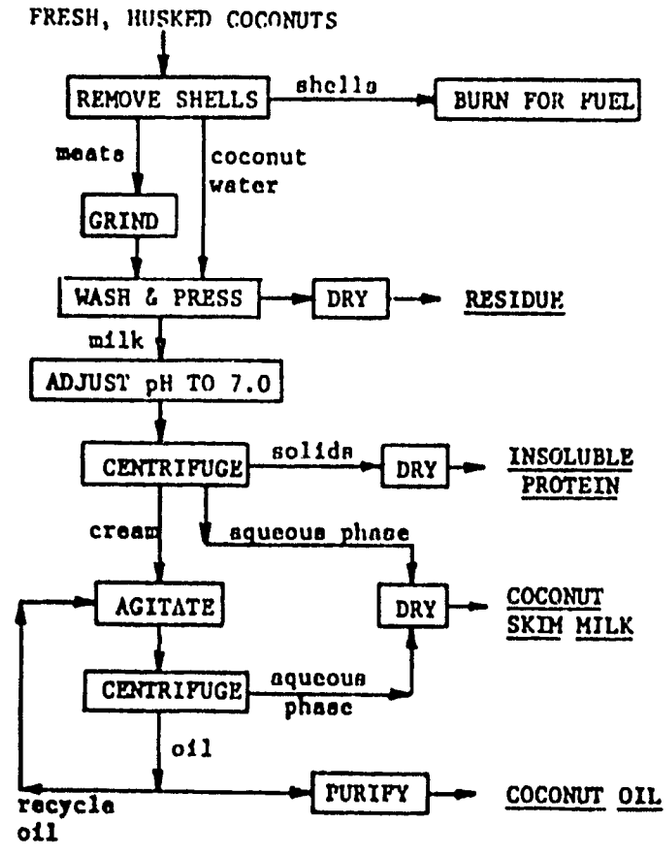

Fig. 1—Simplified flow diagram for the aqueous processing of coconuts. The shells were discarded in our operations, but would be burned for fuel in commerical processing.

after centrifugation, has approximately the same composition as the aqueous layer from the three-phase separation of the milk. The oil phase was purified by centrifugation, heating to drive off residual moisture, and decanting the oil from settled solids.

The insoluble protein recovered from centrifugation of coconut milk was dried by freeze drying. The coconut skim milk was dried by spray drying, with an air outlet temperature of ca. 85°C, and air inlet temperature of ca. 170°C.

Analytical methods

Standard methods of analysis were used for nitrogen and crude fiber. Crude fat was based on ether extraction of dried samples, except for the soluble coconut fraction, which required extraction with a chloroform-methanol mixture (Bligh and Dyer, 1959). Ash analysis followed heating for 2 hr at 600°C, except for the soluble coconut fraction which was ashed overnight at 550°C. Soluble carbohydrate was determined by measurement of reducing sugars in a clarified aqueous extract. The measurement followed inversion for 1 hr at 60°C in 0.7M HCl.

RESULTS & DISCUSSION

Products

The gross analyses of the products are shown in Table 1. All values are the averages of measurements on at least two independently prepared samples. The reliability expressed represents the estimated 95% confidence level for the sum of both analytical errors and variations in analyses due to small changes in processing parameters.

The analysis of coconut oil is not included in Table 1. Analysis of the final oil product showed it to contain 0.4 ± .1% free fatty acids, with an iodine number of 7.5. The oil was centrifuged to clarity in the laboratory and some precipitate was obtained, with the amount of nonoil solids being 0.4 ± .2%. Coconut oil from aqueous processing is therefore superior to oil obtained by crushing copra, which varies widely in analysis. A typical value for free fatty acid content of crude coconut oil from copra would be ca. 5%.

The residue is a fibrous material, which contains small particles of dark testa. Preliminary work shows that the testa can be separated to give a very light-colored product. The protein products are light brown or tan. All products can be obtained almost white if the coconuts are pared.

The products from processing of coconut meats are shown in Table 2. Any product losses that may result from spray drying are not included, because spray-drying losses are expected to be almost negligible in a commercial operation. All results are the averages of at least two independent experiments, performed after many preliminary experiments that were done to determine optimum procedures and operating parameters. The results reflect the product losses resulting from multiple transfer of material between containers and processing equipment.

It is presumed that such losses would be negligible in a continuous process. Therefore a material balance was assumed to calculate the products from the continuous, large-scale aqueous processing of fresh coconuts, with coconut water being used to extract coconut milk from the fiber (by counter-current extraction). Fresh husked nuts are assumed to be 44% meats, 31% water and 25% shells (wet weights). The results of this calculation are shown in Table 3.

These results indicate that 69% of the total protein is in the skim milk fraction, and 99% of the crude fiber is in the residue fraction. This separation of protein from crude fiber is considered one of the principal advantages that aqueous processing has, as compared to dry processing of coconuts.

Similar calculations show that the coconut skim milk also contains 83% of the salts (as estimated from ash analyses) and 97% of the soluble carbohydrates of the coconut. The skim milk fraction therefore contains the bulk of salts, sugars and protein of the coconut.

The preparations of products was performed repeatedly, with slight changes in

Table 1—Typical compositions of dried products

	Residue	Insoluble protein	Skim milk[a]
% Crude protein (N × 6.25)	3.4 ± 0.6	52 ± 10	25 ± 2
% Crude fat	24 ± 3	19 ± 9	5.1 ± 1.5
% Crude fiber	25 ± 4	2.0 ± 0.5	0.03 ± 0.03
% Ash	0.6 ± 0.1	9 ± 2	9 ± 1
% Moisture	5 ± 3	4 ± 2	0.5 ± 0.3
% Carbohydrate, total[b]	60 ± 3	unknown	unknown
% Carbohydrate, soluble	0.3 ± 0.05	7 ± 3	45 ± 3

[a]Composition with coconut water used in processing. See text for composition without use of coconut water. Dehydrated skim milk was stored in desiccator to maintain low moisture content.
[b]By analysis, after digestion in 72% sulfuric acid

Table 2—Experimental recovery of different components in the processing of coconut meats[a]

Component	Fraction of component in product (%)		
Product	Total mass	Oil	Protein
Residue	17[b]	5.8	7.0
Insoluble protein	2.5	0.7	20
Skim milk	16	1.2	65
Oil	59	85	0
Total % recovered	94	93	92

[a]For pilot plant processing of coconut meats only; coconut water was not used in the processing.
[b]The standard deviation for each result is approximately 2% of the value in the table.

Table 3—Products from processing, as calculated from experimental recovery, product compositions and material balance

Product	Mass of product	Protein in product	Oil in product
Residue	43 kg[a]	1.5 kg	10 kg
Insoluble protein	5.7	3.6	0.7
Skim milk	44	11.8	0.6
Oil	142	0.0	142
Totals	234	16.9	153

[a]All results are on a dry weight basis. The starting material is 1000 kg of husked coconuts (wet weight basis).

the processing. The observations considered most significant are the following:

Effect of pH. Coconut milk was centrifuged at pH values from 6.4–8.1. The protein content of the skim milk fraction was observed to be independent of pH over this range. Furthermore, no significant change in composition of the insoluble protein was observed from pH 7.0–8.0.

Use of coconut water. It was observed that only the skim milk fraction was significantly changed in composition if coconut water was not used in the processing. When coconut water was discarded, instead of being used in processing, the skim milk fraction contained 30 ± 2% crude protein, 37 ± 3% soluble carbohydrates, and 9 ± 1% ash (see Table 1).

Temperature. Milk temperatures of 60 ± 5°C were used in the processing. Laboratory results indicate that significant heat coagulation of coconut protein does not occur below 75°C in the solvent system of the aqueous processing (pH 7.0, 8% suspension of the skim milk fraction).

The temperature used during spray drying (air outlet temperature of 85°C) did not reduce protein solubility. Based on four measurements each, it was observed that 89 ± 5% of the nitrogen was dissolved for a spray dried sample, versus 85 ± 5% for a freeze-dried control and 88% for a single measurement on an undried control (at 25°C, pH 7.0, ca. 6% solids in suspension).

Estimation of product costs

In making the cost estimate oil and the skim milk fraction were considered to be the main products of the aqueous processing of coconuts, and were made to carry all costs. Cost of the skim milk fraction was calculated as total costs minus income from oil sales. Total costs were taken as raw material costs plus processing costs. The costs were calculated for a hypothetical processing plant, operating in the Philippines, which would process 125 metric tons per day of husked coconuts.

Purchase prices of fresh, husked coconuts in the Philippines from 1962 to 1971 averaged $34.20 per metric ton, according to information from the United Coconut Association of the Philippines (1971) and Emata (1972). This price includes $0.70 per metric ton estimated as transportation costs. Crude oil prices in the Philippines, also for the period 1962–1971, averaged $0.113 per lb.

Processing costs for the hypothetical processing plant were estimated to total $15.80 per ton of husked coconuts processed. This cost includes labor, equipment depreciation, interest on borrowed capital, maintenance, utilities, administration and other miscellaneous expenses. The manual shelling of coconuts is the largest processing expense, accounting for 22% of total processing costs. The estimated total equipment cost is $650,000. The total installed plant cost is taken as 2.5 times the equipment cost or $1,600,000.

Based on these estimates, which conservatively assume 90% recovery of oil (results in Table 3 indicate 93% oil recovery) and 69% recovery of protein, it was estimated that the dried skim milk fraction would cost $0.16/lb to produce. It should be borne in mind that any income from other products would reduce the estimated cost of the soluble fraction. It might also be pointed out that assumption of zero income from products 1 and 2 has merely been a bookkeeping operation. It is anticipated that products 1 and 2 would, in fact, be marketable commodities.

An alternate process

An alternate process has also been considered, in which the skim milk fraction is sub-fractionated into two components: an acid-precipitated fraction, and an acid-soluble fraction. Our results have indicated that pH 4.3 is optimum for protein precipitation. This agrees well with data of Khaund (1971) on preparation of isolates from hexane-defatted coconut meal.

The pilot plant separation of the two fractions was accomplished by adjustment of the aqueous phase to pH 4.3, followed by centrifugation at ca. 50°C. The freeze-dried, acid precipitated fraction, prepared without using coconut water in the processing, contained 65 ± 6% crude protein, 24 ± 4% oil, 2 ± 1% ash, 0.15 ± .05% crude fiber and 3.5 ± 1% soluble carbohydrates. The acid soluble fraction (also prepared without using coconut water) contains 13 ± 2% crude protein and 13 ± 3% ash. It is estimated that with use of coconut water the acid-precipitated fraction would not change significantly in composition, but the acid soluble fraction would change to a composition of 10% crude protein and 13% ash. From experimental data and material balance considerations, it was estimated that 46% of the total coconut protein would be recovered in an acid-precipitated protein fraction. If 1000 kg of husked coconuts were processed, 12 kg of the acid-precipitated fraction would be recovered, which would contain 7.8 kg of crude protein (see Table 3).

Production costs for this alternate process would be about the same as for the process resulting in the coconut skim milk. If oil and the acid-precipitated protein are taken as the only marketable products, then the cost of producing the acid-precipitated fraction would be $0.59 per pound.

In future reports, detailed chemical analysis of the products will be given, together with some biological evaluation, information on stability and physical properties, and discussion of possible food uses.

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ROBERT D. HAGENMAIER, CARL M. CATER and KARL F. MATTIL
Food Protein Research & Development Center, Texas A&M University, College Station, TX 77843

COCONUT SKIM MILK AS AN INTERMEDIATE MOISTURE PRODUCT

INTRODUCTION

THE AQUEOUS PROCESSING of coconuts consists of comminuting fresh coconuts, making aqueous extraction(s), and centrifuging the resulting oil-in-water emulsion (coconut milk) to obtain an oil phase and an aqueous phase called coconut skim milk. Various aqueous processing schemes have been investigated. In their description of a recently developed process, Dendy and Timmins (1973) give a good set of references on the subject.

The process used to prepare the coconut skim milk used in the present study was developed at the Food Protein Research and Development Center (Hagenmaier et al., 1973). In the Texas A&M process, there is the option of using the coconut water during the extraction step. When the coconut water is used, the composition of the coconut skim milk solids are changed as described by Hagenmaier et al. (1974).

In the process as used, the coconut milk was batch pasteurized (65°C for 15 min), centrifuged, and the coconut skim milk vacuum evaporated at 57°C, after which the moisture content was ca. 60%. The concentrate was either spray dried at 108°C outlet air temperature, or further concentrated with a laboratory rotary evaporator in 43°C water bath. Before pasteurization the pH was raised from ca. 6.2 to ca. 7.8. The purpose of the pH adjustment is to prevent protein coagulation, which is more likely to occur at low values of pH (Hagenmaier et al., 1974).

It has become apparent that elimination of the spray drying step would be economically advantageous, primarily because of the expense of spray drying the small amount (11 tons/day) of coconut skim milk solids produced in a plant of estimated optimum size. Therefore, a concentrated coconut skim milk is being considered as a final product (or possibly as a form suitable for shipment to a central drying facility, where the operation of economies-of-scale would permit lower cost drying).

Gonzales et al. (1971) have previously looked at the stability of canned coconut skim milk concentrates, with 10–15% sugar added as a preservative. Their best preserved samples contained 15% sugar and 20% water, and had good organoleptic characteristics after eight weeks of storage, presumably at ambient temperature.

The purpose of the present work is to examine effects of pH, moisture content, inclusion of coconut water and other variables on the physical properties and storage stability of concentrated coconut skim milk as stored under non-sterile conditions.

MATERIALS & METHODS

THE COCONUTS USED were mature nuts imported from Honduras and Jamaica. The concentrate used for the bulk of the stability data (Tables 2–4, Fig. 3) refers to product rehydrated from spray-dried products. Spray-dried coconut skim milk did not contain the solids from coconut water. Before initiation of the stability experiment the dry (3% moisture) product, pooled from several preparations, had been stored at 23°C for 8 months. At the beginning of the storage experiment, the spray-dried product was reconstituted by mixing with water for ca. 1 hr, with heating in a water bath, during which the temperature of the mixture rose to 60°C. Sodium sulfite added to some batches in processing (in an unsuccessful attempt to produce lighter-colored spray-dried product) would make the pooled spray-dried samples 0.006% in sodium sulfite, if none had been oxidized.

For the storage stability data in Tables 2 and 3, and also Figure 3, the rehydrated concentrate was subdivided, sodium sulfite added as necessary, and the samples sealed in separate glass canning jars. The samples were stored at −20°C or +23°C. Separate jars were opened after 4 and 31 days for analysis. Because of the time required to prepare samples, it was not possible to analyze samples on day zero.

For the data in Tables 5 and 6, and Figures 1 and 2, the concentrate was prepared by direct evaporation. In preparation of product the coconuts were not pared, i.e., the dark testa or seed coat was not separated from the white meats. The one exception is the reference to the lighter color of concentrate made from pared meats.

Viscosities were measured with a Brookfield viscometer, with cylindrical spindles. Three readings were taken over a 5 min interval and the average value used. The observations were made at 3 rpm, with sample temperature of 35°C.

Moisture contents were measured by toluene distillation for 2 hr. Standard plate count conformed to AOAC (1970) methods, except that spread plates were used. Other microbiological methods were also 1970 AOAC methods. Values of pH were measured after mixing 1g of sample with 10g of boiled, distilled water.

Color measurements of reflected light were L, a, b values obtained with a Hunter color-difference meter, standardized on white. The instrument measures whiteness as L value on a 0–100 scale. Positive b value measures amount of blue, and positive a value measures redness. Negative a and b values were not encountered with the coconut samples.

Untrained panelists were presented with four samples each of product as beverage, at 13% solids. The four samples consisted of two caps of each of the two products being compared. Panelists were asked to pair identical samples and give 1–9 hedonic ratings to each pair. Probability of accidental pairing is 33%.

Equilibrium relative humidity was measured according to the method of Fett (1973). Protein solubility was measured at pH 7 and 23°C. Aqueous suspensions at 10% solids were adjusted to pH 7, mixed 30 min and centrifuged 15 min at 1,100 × G. Aqueous layer was decanted and analyzed by the Kjeldahl method.

For the mold growth experiments, unidentified wild mold was scraped off miscellaneous samples of coconut skim milk concentrate. The mold was suspended in lactose broth and plated out on potato dextrose agar. At commencement of the storage experiment one drop of suspension was mixed with 10g of concentrate. The sample jars were sealed to prevent moisture loss, but were opened periodically for a few minutes to maintain aerobic conditions. For all mold growth experiments, the concentrate was prepared by direct evaporation of coconut skim milk.

Measurement of free fatty acid, peroxide value, and nitrogen content conformed to AOAC methods. Titratable acidity was determined by titration to a phenolphthalein endpoint. Reducing sugars were determined as described by Triebold and Aurland (1963). Available lysine was determined by the method of Carpenter (1960).

Each reported observation is the average of at least two measurements.

Observed differences were judged for statistical significance by analysis of average differences and application of student's t. Where appropriate (in Tables 2 and 3) the data were treated as paired comparisons.

RESULTS & DISCUSSION

Physical properties

Concentrated coconut skim milk is a syrup with honey like consistency and appearance at moisture contents of 25–40%. The color of concentrate is light brown with typical L, a, b values of 30, 5 and 11 at 35% moisture both fresh and recon-

stituted samples. Typical color values for spray-dried product (at 3% moisture) are 81, 0.1 and 9. The whiteness of freshly rehydrated spray-dried product is sharply dependent on moisture content, with minimum L value occurring at ca. 35% moisture (data now shown).

Concentrate made from white, pared coconuts was much whiter than the product prepared from coconuts which retained the dark testa. Concentrate from pared coconuts (not spray-dried) had L value of 41 at 35% moisture. All other data in this article refer to products made from unpared coconuts.

The relationship between moisture content and water activity is presented in Figure 1, over a narrow range of moisture content. There is approximate linear dependence of water activity upon moisture content. At given water activity, samples are approximately 6% higher in moisture content if they include the coconut water solids. Higher moisture content of product with coconut water can be accounted for by the deliquescent nature of coconut water, which has 47% moisture at water activity of 0.85. The data presented in Figure 1 are similar to previously reported data (Hayenmaier et al., 1974), but are considered to be more accurate.

Viscosity of intermediate moisture coconut skim milk samples as a function of water-activity is shown in Figure 2, for concentrate with or without coconut water. Coconut water decreases viscosity. Over the narrow range of water activity investigated, the logarithm of viscosity shows approximate linear dependence upon water activity. Viscosity is expressed as a function of water activity, rather than moisture content, in order to emphasize the relationship between these two critical properties. Viscosity of 10,000 cp or water activity of 0.80 are considered limiting conditions because of anticipated processing problems at higher viscosity, and microbiological spoilage at higher moisture.

Viscosity is also quite dependent upon processing temperatures. The data in Table 1 report viscosity as a function of processing heat. Viscosities in Table 1 refer to concentrates with 32.5% moisture, prepared without coconut water. The viscosity in each case was determined from a graph of log viscosity vs. percent moisture. The data indicate that reconstituted spray dried samples have higher viscosity (presumably as a result of drying heat) than concentrate prepared by direct evaporation.

Additional experiments indicated that concentrated with 31–40% moisture (rehydrated from product spray-dried at 98°C) showed an average of 16% increase in viscosity after heating 30 min in a boiling water bath. This heat treatment caused only 0.6% decrease in protein solubility (not statistically significant at 95% level). These data indicate that for coconut skim milk, viscosity is more sensitive than protein solubility to heat effects. Because viscosity is so sensitive to heat effects, the viscosity values reported herein only relate to the present processing conditions.

Gelation was observed after rehydrated, spray-dried product (59% moisture) was placed in the boiling water bath. Gelation did not occur when samples of lower moisture content were heated. Gelled samples gave 30% decrease in protein solubility.

Storage stability

Samples of rehydrated, spray-dried coconut skim milk were prepared as described in the methods section. The experimental design was complete block, with two moisture levels (31% and 37%), two pH values (6.5 and 7.5), two storage temperatures (−20°C and +23°C), and with or without 0.03% added sodium sulfite. In addition, samples at 23°C (no sulfite) were stored in light and darkness. The results of this one experiment are summarized in Tables 2 and 3 and in Figure 3.

The samples stored at −20°C did not change in appearance with 1 month's storage. However, the concentrate stored at 23°C changed color, with a dark brown layer forming on the uppermost 2–3 mm. The data in Table 2 indicate that 23°C storage caused significant decrease in Hunter L, a, b values. The change in pH is also statistically significant, but was small.

No statistically significant differences were noted by taste panel comparisons of fifteen panelists comparing product stored at 23°C for 1 month with fresh or frozen sample; only six correctly paired the stored samples. These six gave average ratings for 6.7 for stored product and 6.3 for control. The taste panel evaluations indicate that no gross changes in flavor occurred during 23°C storage.

The data in Table 2 suggest that no changes of any serious consequence occurred with 1 month's storage at −20°C. Observed changes were either too small to be of much importance or were not statistically significant.

Of particular importance in Table 2 is the data that reflects microbiological activity. The small pH change suggests (but does not prove) that little microbiological activity has occurred. The constant value of total aerobic plate count during 1 month's storage suggests that the product remained wholesome.

The microorganisms present in starting samples were for the most part unidentified. Analysis of initial spray-dried product indicated total aerobic plate count of 20,000/g, total coliforms of 7/g, and negative values for *Salmonella*, *E. coli*, *Staphylococcus aureus*, yeasts and molds. That moisture content is critical to maintaining stable microbiological levels was indicated by an auxiliary experiment: when water content was increased to 42% obvious spoilage occurred.

For further analysis of the variables affecting color change the "total color" change (ΔE) will be used. [$\Delta E^2 = \Delta L^2 + \Delta a^2 + \Delta b^2$ (Hunter, 1973).] The total color change from zero time

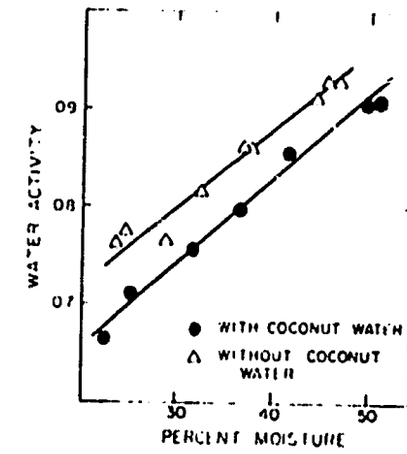

Fig. 1–Water activity as a function of moisture content. Estimated standard deviations are 0.01 unit of water activity and 1% moisture.

Table 1–Viscosity of concentrate as affected by processing heat

Max processing temp	Viscosity[b] (cp)	Protein solubility
Pasteurized at 65°C, but not spray dried	9,000	78%
Pasteurized at 65°C, then spray-dried at 98°C[a]	17,000	76%
Pasteurized at 65°C, then spray-dried at 108°C[a]	87,000	78%

[a] Average outlet air temperature. The spray dried product was reconstituted with water to form the 32.5% moisture product.
[b] At 32.5% moisture and 35°C, measured with Brookfield viscometer

can be examined if an assumption is made. The assumption is that average color values for samples in cold storage will be taken as equal to color of the rehydrated, spray dried coconut skim milk at zero time. This assumption is based on the fact that cold-stored samples showed no significant color change during storage. Zero time is taken as that time after dry product was mixed with water, pH adjusted, portioned into sample jars, and put into cold storage. These activities required ca. 8 hr.

Using averaged values for cold samples as zero time values, total color change at 23°C is indicated in Table 3. These data indicate that neither exposure to light nor added sulfite affected color change. However, color change at 31% moisture was only 34% of the change at 37% moisture, which suggests that the problem of color instability can be lessened by the use of low moisture content.

The effect of pH on color stability is more complex, and is shown in Figure 3. These data indicate that at pH 6.5 the samples darken faster (slope is steeper), although at pH 7.5 the samples were much darker after the 8 hr required for rehydration and sample preparation. The samples with higher pH experienced a big decrease in L value during the rehydration process (which involved heating to 60°C)

In a related experiment, the adverse effect of pH on whiteness was also noted. Solutions of coconut skim milk were adjusted to selected pH values, dried, and color of dry product observed. Samples of coconut skim milk at pH values of 6.1, 6.8 and 7.5 were spray dried to give product with L values of 83.6, 79.3 and 77.3. Freeze drying the samples immediately after pH adjustment gave similar results. These data suggest

Table 2—Changes occurring between 4 and 31 days of storage for paired samples

Parameter observed	Initial value[a]	Observed change[b]	Change required for significance
Stored at −20°C			
Standard plate count (colonies/g)	14,000	+2,000	2,000
Moisture (%)	35.1	−0.1	2.5
Brookfield viscosity (cp)	93,000	+16,000	21,000
Titratable acidity (meq/g)	0.074	+0.002	0.011
pH	7.09	−0.04	0.04
Hunter L value	28.1	−0.2	1.2
Hunter a value	6.0	−0.1	0.17
Hunter b value	9.3	−0.02	0.27
Stored at +23°C			
Standard plate count (colonies/g)	12,500	+800	1,900
Moisture (%)	33.6	−0.2	1.1
Viscosity (cp at 35°C)	89,000	+8,000	10,000
Titratable acidity	0.076	+0.006	0.008
pH	7.05	−0.06	0.02
Hunter L value	24.6	−3.5	1.1
Hunter a value	5.8	−0.2	0.15
Hunter b value	8.2	−0.8	0.3

[a] Each reported initial value is the average of at least eight samples. Each reported change is the average change for at least eight pairs of samples, with one of pair analyzed after 4 days and one after 31 days.

[b] Average change required for significance at 95% level, calculated from t test for paired samples.

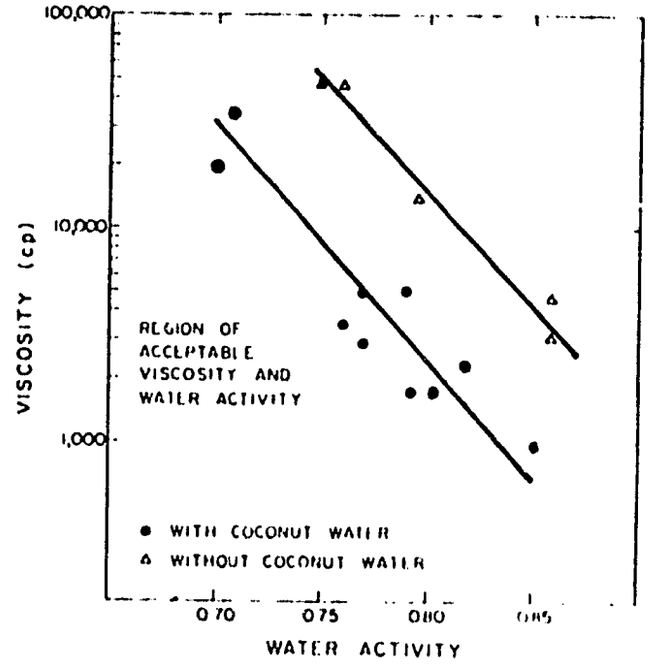

Fig. 2—Viscosity vs water activity. Viscosity and moisture contents were measured, and water activity was read from Figure 1. Samples were prepared by direct evaporation rather than reconstitution of spray dried samples. Viscosity was measured at 35°C, water activity at 23°C.

Table 3—Color change as affected by exposure to light, sulfite addition and moisture content, for paired samples

Treatment	E[a]	Number of paired samples
No added sulfite	3.9	
0.03% Na_2SO_3	3.9	
difference[b]	0.0	4
37% Moisture (a_w = 0.84)	5.9	
31% Moisture (a_w = 0.79)	2.0	
difference[b]	3.9	6
Exposed to light	3.9	
Stored in dark	3.9	
difference[b]	0.0	8

[a] Average total color change after 1 month of storage at 23°C

[b] A difference of less than 0.8 units is not significant at the 95% confidence level.

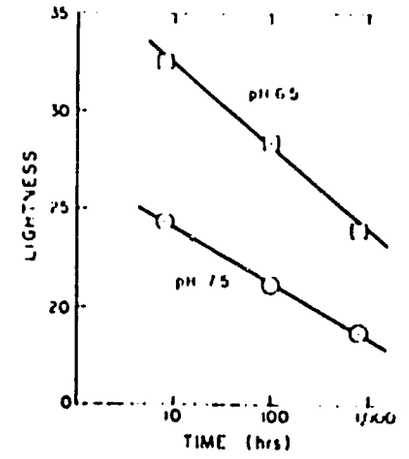

Fig. 3—Decrease in lightness (L) as function of time and pH. Storage was at 23°C. Estimated standard deviation in L value is 1 unit. Each L value is the averaged value for six different samples.

Table 4–Changes occurring during storage of coconut skim milk at 31–37% moisture

Parameter	Avg[a] initial value	Storage	Observed change	Change req for significance[b]
Protein solubility (%)	81	30 days, -20°C	-4	5
Protein solubility (%)	81	30 days, +23°C	-4	3
Reducing sugars, (%)	1.6	3 months, 23°C	+0.2	0.2
Available lysine (g/16g N)	3.9	3 months, 23°C	-0.3	0.4
Peroxide value (milli equivalents/kg)	13	6 months, 23°C	+22	10
Free fatty acids (as % of oil)	3.3	6 months, 23°C	-0.1	0.7

[a] Initial value refers to spray dried coconut skim milk

[b] Average change required for significance at 95% level, calculated from t test for significance, and based on average of at least two observations for both control and stored sample

Table 5–Effect of moisture contents on time elapsed until appearance of visible mold on samples of concentrate[a]

Moisture content	No. of samples	Average time (days)
28%	3	22
29%	3	29
31%	3	14
33%	2	11
35%	3	8
37%	5	8
39%	5	4

[a] Samples stored at 23°C. Concentrate includes coconut water solids and was made by direct evaporation. Mold count after inoculation was 80,000/g.

Table 6–Effects of variables on appearance of visible mold on inoculated samples of concentrate[a]

		Time[b] (days) until appearance of visible mold	
		Control (No sorbic acid)	Samples with 0.1% sorbic acid
35°C Storage			
pH	5.0	7	100+[c]
	5.9	7	100+[c]
	6.5	7	100+[c]
	7.2	7	19
23°C Storage			
pH	5.0	10	100+[c]
	5.9	10	100+[c]
	6.5	10	100+[c]
	7.2	10	16
4°C Storage			
pH	5.0	100+[c]	100+[c]
	5.9	100+[c]	100+[c]
	6.5	100+[c]	100+[c]
	7.2	100+[c]	100+[c]

[a] Coconuts were extracted with coconut water in making the concentrate. Made by direct evaporation (not reconstituted). Mold count after inoculation was 540,000/g.

[b] Each value is average for two samples

[c] No visible mold was observed at termination of experiment, which was after 100 days of storage.

that elevation of pH during processing be avoided if possible. Protein precipitation would best be avoided by avoiding excess heating, rather than by elevation of pH.

Other parameters related to storage stability are reported in Table 4. The observations reported in Table 4, unlike those in Tables 2 and 3, measure differences between spray dried coconut skim milk and stored concentrate. For control observations (Table 4 only) the samples were not rehydrated to form a concentrate before analysis. The stored samples were concentrates made by rehydration.

The data in Table 4 indicate that at 23°C storage reduction in both protein solubility and reducing sugars, and also increase in peroxide value, were significant at the 95% level.

Reduction in available lysine was significant at the 90% level. A reduction in available lysine would suggest that the color change was a Maillard browning.

The rise in peroxide value is considered a problem. Although the samples did not have a rancid odor, the peroxide value is probably approaching the danger point. Use of an antioxidant may be necessary.

Since the changes reported in Tables 2–4 refer to concentrates made by rehydration of spray-dried product, they do not necessarily give quantitative indications of changes that will occur during storage of concentrate made by direct evaporation. However, the data are assumed to give a valid picture of the potential problems to be expected upon storage of intermediate moisture coconut skim milk.

The most serious problem encountered upon storage of coconut skim milk concentrate was the growth of wild, unidentified mold on an occasional sample, although no growth was observed in the samples analyzed during the storage experiment described.

Samples of concentrate at moisture contents of 28 to 39% were inoculated with mold and the samples observed daily for visible mold growth. The results, shown in Table 5, indicate that mold will grow on contaminated samples at 28–39% moisture, although with slower rates at lower moisture content.

Prevention of mold contamination would of course solve the mold growth problem. However, this approach would probably involve use of expensive packaging, which would seem to defeat the purpose of considering an intermediate moisture product (the purpose being to economize).

Therefore, the use of sorbic acid as a mold inhibitor was examined. Because the normal, unadjusted pH of the concentrate (pH 6.0–6.5) is near the upper limit of effectiveness for sorbic acid, samples were prepared with different pH values. The results, shown in Table 6, indicate that at pH 5.0–6.5, sorbic acid (0.1%) is effective in controlling mold growth on concentrated coconut skim milk at 30% moisture. Refrigerated storage is also effective.

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ROBERT HAGENMAIER, KARL F. MATTIL and CARL M. CATER
Food Protein Research & Development Center, Texas A & M University, College Station, TX 77843

DEHYDRATED COCONUT SKIM MILK AS A FOOD PRODUCT: COMPOSITION AND FUNCTIONALITY

INTRODUCTION

"COCONUT MILK" is the name commonly given to the liquid prepared by aqueous extraction of ground-up coconut meats. The oil content of coconut milk differs markedly from that of cow's milk, while cow's milk has about equal amounts of oil and protein, coconut milk has about ten times as much oil as protein. Removal of the excess oil by centrifugation yields a product generally called coconut skim milk.

Coconut skim milk has been previously considered as a high protein food product (Rajasekharan and Sreenivasan, 1967, Salon and Manquis, 1969). However, the product is still not available because of problems encountered in large-scale preparation. The problems center around difficulties with certain unit operations, especially the efficient separation of oil from other components (Hagenmaier et al., 1972a). Oil separation is important because the efficiency of oil recovery determines the cost of the coconut skim milk. Recently, a process has been described which achieves ca. 91% recovery of oil and produces a dried, economical coconut skim milk (Hagenmaier et al., 1973).

The purpose of this work is to present a chemical description of coconut skim milk, describe some physical-chemical properties, and present some taste panel evaluations of reconstituted coconut skim milk as a beverage. It is presumed that the analyses presented will provide a chemical basis for interpretation of the physical, chemical and nutritional characteristics of coconut skim milk.

EXPERIMENTAL

AMINO ACID analyses were performed by standard ion exchange separation of protein hydrolysates, with cysteine analyzed separately as cysteic acid. No corrections were made for destruction of amino acids during hydrolysis. Each reported value is the average of at least three results. Methionine was not analyzed for separately; however, a N_2 flush was used during hydrolysis to prevent oxidation.

Protein solubility was measured by mixing product with distilled water, agitating for ca. 30 min at room temperature, measuring pH, then centrifuging 10 min at 25,000 × G. The supernatant liquid was filtered through Whatman no. 41 filter paper and analyzed for dissolved protein by Kjeldahl analysis, or by the Lowry technique calibrated against Kjeldahl.

The amount of protein precipitated by heat coagulation was determined by heating an aqueous suspension of product for 20 min, then cooling to 25 C. Any water evaporated off during heating was added back, and the protein solubility then measured after centrifuging and filtering.

Oil content was determined after weighing the lipid extract obtained by the chloroform-methanol extraction method of Bligh and Dyer (1959). The extracted oil was analyzed by standard methods.

Reducing sugars were measured by the Munson and Walker method as described by Trebold and Aurand (1963). Reducing sugars after inversion were measured after heating the water extract for 1 hr at 60 C in 0.7M HCl. Sucrose identification was made by matching gas chromatography patterns with standards (trimethylsilyl derivatives of the samples) before and after inversion.

Equilibrium moisture contents were measured after exposure of samples to air of controlled relative humidity until apparent equilibrium was attained (no further weight change). Viscosity was measured with a Brookfield viscometer, with the spindle rotating at 12 rpm.

The low molecular weight nitrogen is taken as the nitrogen that passes through an ultrafiltration membrane with a rated molecular weight cut-off of 1,000. Membranes were checked for passage of larger molecules by ultrafiltration of bovine serum albumin solutions.

Mineral contents were determined by atomic absorption spectrometry, except for chloride and phosphorus. Chloride was determined by volumetric analysis, and phosphorus from weight of a phosphomolybdate complex.

Samples of dehydrated coconut skim milk were prepared with pilot plant equipment. The preparation was as described by Hagenmaier et al. (1973). The preparation consists of water

Table 1—Chemical analysis of spray-dried coconut skim milk

	% Composition[b]		
	Coconut water used in processing	Tap water used in processing	Standard dev
Protein			
Crude Protein (N × 6.25)	25	30	0.8%
Low Molecular Wt. N (as % of N)	8	7	0.5
Fat			
Crude Fat	5	7	1
Free Fatty Acids (as % of Oil)	3.2	1.4	0.3
Non saponifiables (as % of Oil)	3.2		0.5
Iodine Value (as % of Oil)	6.3	–	0.7
Carbohydrates			
Reducing Sugars	2.8	2.0	0.2
Reducing Sugars after inversion	45	37	1.5
Sucrose	33	–	1.5
Crude Fiber	0.03	0.03	0.02
Minerals			
Phosphorus	0.5	0.5	0.05
Calcium	0.17	0.06	0.01
Magnesium	0.26	0.36	0.03
Potassium	3.6	3.3	0.2
Sodium	0.9	1.4	0.3
Chloride	1.6	1.6	0.2
Ash accounted for[a]	8.2	9.3	0.4
Ash (by analysis)	8.8	9.2	0.5

[a] By calculation from stated mineral contents
[b] At level of 3% moisture

extraction of ground coconut meats, removal of solids by pressure filtration, and separation of aqueous phase from oil phase with a centrifuge. The aqueous phase was dried by spray drying at air outlet temperatures of 85-93°C, except where noted.

The water used in pilot plant preparations was tap water which was very soft; a typical composition: 390 ppm bicarbonate, 130 ppm Na, 54 ppm Cl, 1.5 ppm Ca and 0.5 ppm Mg. Distilled water was used in laboratory work.

All results reported in tables are the averaged results of analyses of at least two independently prepared samples.

RESULTS & DISCUSSION

TABLE 1 shows the chemical content of dried coconut skim milk. The values for protein, fat and ash are similar to the values reported by Rao et al. (1967) for coconut skim milk prepared by the Krauss Maffei process. In addition to the results shown in the table, the coconut skim milk made with use of coconut water had the following trace mineral contents: 17 ± 4 ppm of iodine, 70 ± 30 ppm of iron, 50 ± 30 ppm of copper, 15 ± 5 ppm of manganese, 3 ± 2 ppm of chromium and less than 2 ppm of cobalt.

Some of the minerals in the products were contributed by the tap water used in processing. The product made with coconut water as the extracting liquid was made with only about 0.5g tap water per g coconut meats. The product made without use of coconut water was made with 4.5g tap water per g coconut meats. In the latter case, the ions in the tap water would contribute the following calculated ion contents to the dried product: sodium, 0.6%; chloride, 0.2%; calcium, 0.007%; magnesium, 0.002%. Based on these data, it is evident that the increased sodium content of product made without coconut water is due to sodium in the tap water.

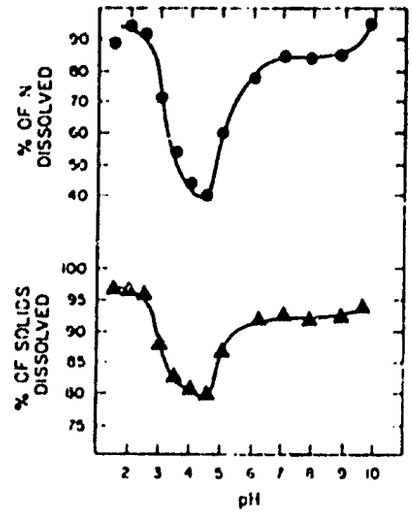

Fig. 1—Solubility as function of pH, for coconut skim milk (at 25°C, 10% solids).

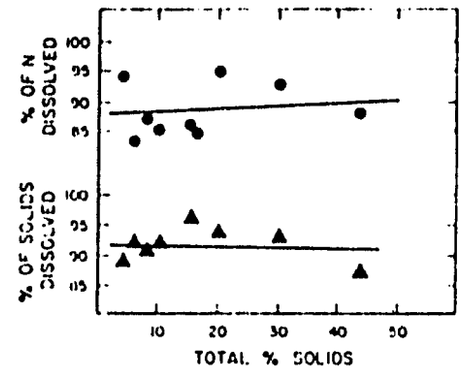

Fig. 2—Solubility as function of concentration for coconut skim milk (at 25°C, pH 7.0).

From the data in Table 1, it is seen that crude protein, plus ash, plus moisture, plus crude fat, plus reducing sugars after inversion equals only 86% for product prepared with or without coconut water. The 14% unaccounted for is currently unidentified and unexplained.

The amino acid content of coconut skim milk was not observably dependent on the amount of coconut water used in the processing. (Not surprising, since the coconut water contains only 3% of the total protein.) The results in Table 2 are averaged amino acid contents of samples prepared with and without coconut water. The amino acid contents in Table 2 are similar to values reported for a similar product prepared by Srinivasan et al. (1964).

The solubility of spray-dried coconut skim milk as a function of pH is shown in Figure 1. The results are averaged from experiments with two independently prepared samples, each prepared with use of coconut water. The product is shown to be highly soluble except in the pH range 3.0-6.0. These data suggest that coconut skim milk might be suitable for a neutral pH beverage.

The effect of concentration on solubility, for the same preparations, is shown in Figure 2. The amount dissolved is shown to be nearly a constant fraction of total solids over the entire concentration range investigated. Approximately 90% of the protein and total solids were observed to be soluble at pH 7.0 and 25°C. Addition-

Table 2—Amino acid composition of spray-dried coconut skim milk

Amino acid	g/16g N[a]	Ratio to egg values[b]	Ratio to FAO pattern[b]
Essential			
Isoleucine	2.6	39%	60%
Leucine	5.4	61%	110%
Lysine	4.6[c]	71%	106%
(Total Aromatic)	(6.1)	(61%)	(106%)
Phenylalanine	3.8	65%	132%
Tyrosine	2.3	55%	80%
(Total Sulfur Containing)	(3.0)	(54%)	(70%)
Cysteine	1.7	71%	84%
Methionine	1.3	41%	56%
Threonine	2.4	47%	83%
Tryptophan	0.9	56%	62%
Valine	4.0	55%	93%
Nonessential			
Histidine	2.2		
Arginine	15.5		
Aspartic Acid	7.1		
Glutamic Acid	22.0		
Serine	3.7		
Proline	3.5		
Alanine	4.1		
Glycine	3.8		
TOTAL	90.9		

[a] Standard deviation is ca. 0.1g/16g N

[b] Each amino acid content was divided by amino acid content of egg or 1957 FAO provisional pattern, for same amount of nitrogen. Egg and FAO values used were as reported in FAO/WHO, 1965, Protein Requirements.

[c] Available lysine was 4.3g/16g N for samples freeze dried or spray dried at air outlet temperatures of 88-105°C, decreasing to 3.8g/16g N at air outlet temperatures of 107-116°C. Available lysine was determined by the method of Carpenter (1960).

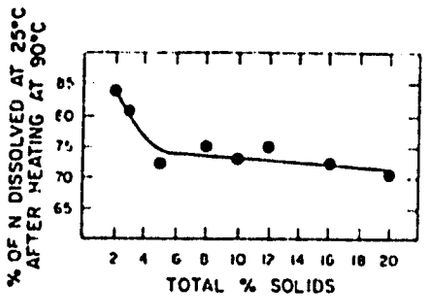

Fig. 3–Protein solubility at 25°C after 90°C heat coagulation, for coconut skim milk (at pH 7.0).

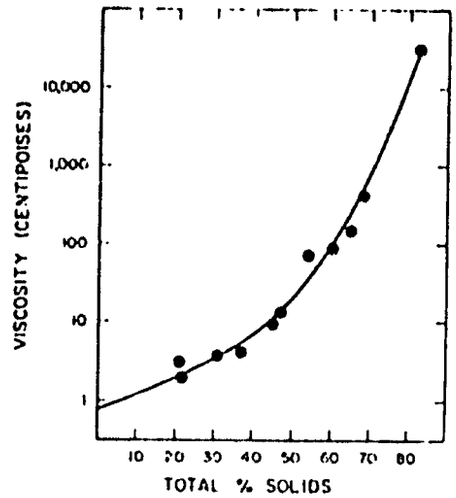

Fig. 4 Viscosity as function of concentration, for coconut skim milk (pH 7.5, 30°C).

al data indicated that solubility continued to be high at 67% solids and 33% water.

The heat coagulation of the proteins in coconut skim milk was also observed. Figure 3 shows the solubility at 25°C and pH 7.0, after heating the samples to 90°C for 20 min. Solubility decreases with increasing concentration. However, increased concentration with no heating did not reduce protein solubility (see Fig. 2). Therefore, the data of Figure 3 indicate that stability to heat coagulation is better at low concentrations (2–3% solids). Although 2–3% solids is below the useful concentration range for economic processing, the heat stability at these low concentrations may be an advantage in some product applications.

The data in Table 3 show the combined effects of pH and temperature on the heat coagulation of the coconut skim milk proteins. The results are averaged from two experiments with independently prepared spray-dried samples (prepared with coconut water). The data indicate that an upward adjustment of pH (from the unadjusted value of 6.0–6.5) will prevent heat coagulation. These results are in agreement with data for purified coconut proteins (Hagenmaier et al., 1972b). The data in Table 3 suggest that coconut skim milk can withstand the heat treatment used to sterilize evaporated milk, without much protein coagulation.

The moisture contents of samples of coconut skim milk solids at regulated values of relative humidity are shown in Table 4. These data show that coconut skim milk is very hygroscopic. The tendency for coconut skim milk to bind water is responsible for the difficulty experienced in drying this product. This difficulty has also been noticed by others (Rajasekharan and Sreenivasan, 1967). The samples lost their powdery character and became glassy or liquid over the entire range of water activity investigated, 0.43–0.91. The hygroscopic nature of this product presents particular problems because tropical coconut producing regions are invariably quite humid. It is assumed that the potassium, sodium and sucrose in the samples (see Table 1) are responsible for most of the water binding. The water binding of isolated coconut protein has been previously reported (Hagenmaier, 1972).

The data in Figure 4 show the viscosity of solutions of coconut skim milk. The viscosities are similar to data for sucrose solutions at the same concentrations over the range 0–65% solids, which is probably due to the high sucrose content of the samples. The solutions used for viscosity measurements were made in the laboratory with coconut water used as an extracting liquid. The viscosity data will be of interest for product applications and in processing work where viscous solutions are handled.

The specific gravity was measured for solutions of coconut skim milk prepared in the laboratory. Data for specific gravity at 30°C was used to develop equation (1), where f_s is taken as the weight fraction of solids.

$$\text{Sp gr} = 1.00 + 0.41 f_s + 0.12 (f_s)^2 \quad (1)$$

Eq. (1) can be used to get a rapid estimate of solids content from measurement of specific gravity over the concentration range 0–80% solids.

For taste panel evaluations, samples of spray-dried coconut skim milk were mixed with tap water and served at room temperature. Preference tests with six-member panels were used to establish best concentration. There was no statistically significant preference over the concentration range 5–15% solids. Therefore, concentration was selected more from nutritional considerations. Selected concentration was 12.5% solids, which would give 3.25% crude protein when formulated with a dry sample containing 30% crude protein.

For a comparison with dairy skim milk, each panelist was simultaneously presented two unidentified samples, one of rehydrated coconut skim milk (12.5% solids) and one of rehydrated NFDM (9.4% solids). With 22 panelists and a hedonic score of 1–9, the coconut skim milk scored 6.2 and the rehydrated NFDM

Table 3–Effect of pH on irreversible heat denaturation of the protein in coconut skim milk. Protein solubility at 25°C after heating for 20 min at specified temperature

	% of Protein dissolved[a]						
pH	25°C	55°C	70°C	80°C	90°C	95°C	125°C[b]
8.0	84	82	83	83	80	80	92
7.5	84	83	85	81	80	76	79
7.0	83	83	83	79	75	70	75
6.5	82	81	80	75	49	44	43

[a] Each sample was 5% solids and 95% water. Standard deviation is ca. 4%.
[b] The samples at 125°C were autoclaved.

Table 4–Equilibrium moisture content of coconut skim milk, at 25°C

	% Moisture after equilibration[a]	
Water activity	Prepared with coconut water	Prepared without coconut water
0.91	51	48
0.84	36	33
0.81	36	33
0.75	24	22
0.43	8	10

[a] Standard deviation is 2.5%.

scored 3.5. The probability (P) that this difference is due to choice was calculated with students' t to be 5% < P < 10%.

Samples of dehydrated coconut skim milk showed no evident deterioration with 6 months storage at 20–30°C as a dry powder in glass containers. Wet samples were stable to microbiological growth at moisture contents of 32% or less.

The data presented in this paper (chemical analysis, functional properties and organoleptic evaluation) suggest that coconut skim milk might be used in the formulation of an acceptable beverage or other food product.

The next stage of our research involves a nutritional evaluation of coconut skim milk and a more detailed look at food uses. Careful consideration is also being given to the construction of a field pilot plant for production of approximately 50 kg per day of dehydrated coconut skim milk. It is assumed that with such a pilot facility a reasonably accurate estimate may be made of production costs.

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ROBERT HAGENMAIER, ROMMANEE LOPITAKWONG and SUKONCHEUN VERASESTAKUL
Food Protein Research & Development Center, Texas A&M University, College Station, TX 77843

A Research Note

NUTRITIVE VALUE AND FOOD USES OF COCONUT SKIM MILK SOLIDS

INTRODUCTION

COCONUT SKIM MILK is a liquid obtained from fresh coconuts by aqueous extraction, followed by gravity (or centrifugal) separation of the oil. Coconut skim milk products have been considered as a food for child feeding (Rajasekharan and Sreenivasan, 1967).

Recently, a process has been developed at Texas A&M University for economical production of oil, concentrated or spray-dried coconut skim milk, insoluble protein, and residue from fresh coconuts by aqueous processing (Hagenmaier et al., 1973). The composition and physical properties of the coconut skim milk so produced have been recently reported (Hagenmaier et al., 1974).

The purpose of the current work is to report on nutritive value and food uses of coconut skim milk products as prepared by the Texas A&M University process.

METHODS & MATERIALS

PROTEIN EFFICIENCY VALUE (PER) determination was determined according to the AOAC (1970) method, by WARF Institute, Inc. The reported values were corrected by multiplying the factor required to adjust the control casein values to 2.5. Each PER result is the average result for one batch of ten rats.

The amino acid analyses were performed by ion exchange separation of protein hydrolysates. The basic amino acids were eluted with 0.35N citrate buffer at pH 5.27, 55°C; acid amino acids with 0.2N citrate buffer at pH 3.30, 55°C; and neutral amino acids with 0.2N citrate buffer at pH 4.25, 55°C. A nitrogen flush was used to prevent methionine oxidation during the acid hydrolysis. Cysteine was analyzed separately as cysteic acid. Tryptophan was measured by the method of Kohler and Palter (1967). All reported amino acid contents are averaged from analyses of at least two independently prepared samples.

Taste panels were conducted by presenting the untrained panelist with two coded samples. The samples were scored on a nine category word scale ranging from "dislike extremely" to "like extremely." For data analysis the categories were graded from one to nine.

The coconut skim milk products were prepared with pilot plant equipment, approximately as described previously (Hagenmaier et al., 1973). For preparation of the concentrate the coconuts were shelled and the testa pared off. The coconuts were comminuted and extracted first with the coconut water and next with tap water. The liquid extract (coconut milk) was centrifuged to give three products: an oily phase called coconut cream, an aqueous phase called coconut skim milk, and a solid phase called insoluble protein. The coconut skim milk was vacuum evaporated at 55°C to give a concentrate. Spray-dried coconut skim milk was prepared similarly except that the testa was not removed, the coconut water was not used as an extractant, and the concentration step was followed by spray drying at 95°C outlet air temperature.

The control beverage was fresh coconut skim milk prepared without heating or evaporation in order to preserve the fresh coconut taste. Fresh mature coconuts were ground, in a hammer mill, extracted with tap water (half the weight of ground coconut), and the mixture squeezed against cheesecloth. The coconut milk was allowed to stand in a separatory funnel for 30 min while the oil globules rose to the surface. After first flushing out solid sediment, the coconut skim milk was drained out. All beverages were served at 15°C.

The coconut pudding consisted of 320g water, 54g spray dried coconut skim milk, 75g brown sugar, 25g corn starch, 25g coconut cream and 1g salt. The coconut cream was prepared with pilot plant equipment as previously described (Hagenmaier et al., 1973) and contained 65% oil and 32% moisture.

The milk and egg custard consisted of 82g egg yolk, 253g whole milk, 74g brown sugar and 0.8g salt. Both desserts were cooked in a water bath in a 177°C oven. The milk and egg custard was cooked for 1 hr and the coconut pudding for 30 min. Both were refrigerated for 3 hr before serving.

RESULTS & DISCUSSION

THE NUTRITIONAL VALUES of coconut skim milk products are reported in Table 1. The concentrated coconut skim milk has slightly lower PER than the coconut meal. Spray-dried coconut skim milk has greater reduction in PER, which was presumably caused by the spray-drying step. It has been previously reported that spray-drying of coconut skim milk at air outlet temperature of 107°C and above causes reduction in available lysine (Hagenmaier et al., 1974). However, it is possible that the higher PER of the concentrate is due to the presence of the coconut water or the absence of the testa or seed coat. Rama Rao et al. (1967) reported a PER (apparently uncorrected) for coconut skim milk to be 1.9, compared to their value of 2.1 for whole coconut protein. The lower PER for coconut skim milk (compared to whole protein) may be accounted for by protein fractionation during processing. The two principal protein fractions obtained from the Texas A&M

Table 1—Protein efficiency ratios

Preparation	Corrected PER
Coconut meal	2.11
Concentrated coconut skim milk	1.98
Spray-dried coconut skim milk	1.67

University process are coconut skim milk, containing 70% of total protein, and the insoluble protein product with 21% of total protein (Hagenmaier et al., 1973).

Previously reported data indicate that the most limiting amino acids of coconut skim milk are isoleucine, methionine and threonine based on egg values, or methionine, isoleucine and tryptophan based on the FAO pattern. All of these limiting amino acids are higher in the insoluble protein, as the data in Table 2 indicate. These data suggest that the reduced PER of concentrated coconut skim milk, as compared to whole coconut protein, is accounted for by protein fractionation, rather than by processing conditions. Based on reported amino acid compositions of large and small molecular weight coconut protein (Hagenmaier et al., 1972), the coconut skim milk seems to contain less of the large molecular weight protein.

Table 2—Amino acid composition of the protein fractions

Amino acid	Coconut skim milk (g/16g N)[a]	Insoluble protein (g/16g N)[b]
Essential		
Isoleucine	2.6	3.7
Leucine	5.4	6.3
Lysine	4.6	2.6
Phenylalanine	3.8	4.1
Tyrosine	2.3	2.9
Cysteine	1.7	1.3
Methionine	1.3	2.2
Threonine	2.4	3.3
Tryptophan	0.9	1.1
Valine	4.0	4.9
Nonessential		
Histidine	2.2	1.4
Arginine	15.5	13.1
Aspartic acid	7.1	8.2
Glutamic acid	22.0	15.2
Serine	3.7	4.6
Proline	3.5	3.6
Alanine	4.1	3.9
Glycine	3.8	3.8
TOTAL	90.9	86.2

[a]Data from Hagenmaier et al., 1974.
[b]Averaged result from two independently prepared samples. Standard deviation is ca 0.1 g/16g N.

Two food uses for coconut skim milk were organoleptically evaluated: a beverage and a custard.

A beverage was made by reconstituting concentrated coconut skim milk with water. This beverage (at 13% solids) was served to panelists, along with a control consisting of a fresh coconut skim milk control, which also contained 13% solids. The fresh coconut skim milk was chosen as comparison because it is a familiar food in coconut-producing countries. The averaged results from 40 panelists was a score of 6.9 for the reconstituted coconut skim milk, whereas the fresh beverage scored 6.2. Upon application of the student's t it was determined that the reconstituted beverage had a higher score at the 98% confidence level. The significance of this experiment is that the taste of coconut skim milk is not impaired by the processing which includes heating and evaporation steps.

Reconstituted, spray-dried coconut skim milk beverage was previously reported to be preferred to reconstituted nonfat dry milk (Hagenmaier et al., 1974).

A dessert pudding was made with spray-dried coconut skim milk. This pudding was presented to a taste panel along with a milk and egg custard. The averaged results of 34 panelists indicated that the coconut skim milk pudding scored 6.7, compared to 5.1 for the milk and egg custard. Upon application of the student's t it was determined that the coconut pudding had a higher score at the 99% confidence level. This result would suggest that coconut skim milk has potential uses in pudding and related dessert foods.

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Coconut Residue: Composition, Properties, Nutritive Value

By Robert D. Hagenmaier, Marie Chittwood and Karl F. Mattil

Food Protein Research and Development Center
Texas A&M University
College Station, Texas 77843

Abstract

Coconut residue is the insoluble material left after water extraction of ground coconut endosperm. Coconut residue made from pared coconuts contains ca. 21% crude fiber on moisture and oil free basis. The carbohydrates in the residue hydrolyze to give 74% mannose, 21% dextrose and are 5% unidentified.

Dried residue readily absorbs and holds water. Rat feeding studies indicate that coconut residue has no nutritive value. Consumption of residue contributes significantly to amount of feces. The data suggest use of coconut residue as bulking agent or as a source of dietary fiber.

Introduction

Fresh, wet coconut meats can be comminuted and extracted with water to give coconut milk and an insoluble, fibrous product which will here be called "coconut residue" after Rajasekharan (1964), and Dendy and Timmins (1973). Other names used for the residue have been "coconut meal" (Velasco et al, 1972); and "coconut press-cake" by Cancel (1970).

The residue presumably consists chiefly of cell walls. It has generally been viewed as a useless by-product because of its high fiber content, and in a recent economic analysis zero value was assigned to residue product (Hagenmaier et al, 1975). However, recent concerns about the low fiber content of the Western diet (Burkitt et al, 1974; Scala, 1974; Spiller and Amen, 1974) have raised the possibility that coconut residue might be useful as a source of dietary fiber. The purpose of the research was to evaluate coconut fiber as a potential food product.

Samples of coconut residue made from coconuts that include the dark seed coat (testa) have been prepared and the proximate analysis reported by Rajasekharan (1964) and by Hagenmaier et al (1973), with similar results. The oil content of dried residue is determined by efficiency of oil extraction and is quite dependent on processing conditions (Hagenmaier et al, 1972; Dendy and Timmins, 1973). Typical oil content of 24% was reported for residue prepared by triple extraction of comminuted coconut with water (Hagenmaier et al, 1973).

The proximate analyses indicate that coconut residue, on moisture and oil free basis, is ca. 92% carbohydrate (by difference). Mukherjee and Rao (1962) and Rao et al (1961) have reported water-insoluble mannan and galactomannan from coconut, plus highly branched water-soluble galactomannan. Dendy and Timmins (1974) report that 50% of coconut carbohydrates are cellulose, which would presumably be concentrated in the residue (the water insoluble fraction). Caray (1924) reports copra carbohydrate to contain 36% cellulose, 5.5% galactose, 1.1% galactan, but no mannose nor mannans. Nozawa et al (1969) reported mannan in coconuts. Based on these reports the carbohydrate composition of coconut is somewhat uncertain.

Materials and Methods

Residue preparation: Processing was approximately as described by Hagenmaier et al (1973). Fresh, mature coconuts were manually shelled, and the testa optionally pared off. The meats were comminuted with a hammer mill and a disc attrition mill, and then extracted in two steps: first with coconut water, then with tap water. The mixtures were passed through a screw press (pulp press) to separate wet residue (65% moisture) from coconut milk. The wet residue was dried to ca. 7% moisture in a vibrating fluid bed dryer. Dry residue was hexane extracted and ground to a flour.

With one exception, reported results are the average of at least one observation each on two independently prepared samples. The exception was determination of nutritive value of fiber, for which

only one sample was used.

Water retention was measured after rehydrating dry residue in water at 50°C for 15 minutes, then cooling to room temperature. The mixture was placed in a centrifugal filter fitted with a cellulose ester membrane (Millipore, 5μ pore size) , centrifuged 10 minutes at 2,000 xg and the wet sample weighed. Water retention was determined from weight uptake, without correction for solubles. For comparison purposes purified alpha cellulose (microcrystalline cellulose from Applied Science Labs, Inc.) was used.

The biocaloric assay was performed by Wisconsin Alumni Research Foundation by an FDA modification of the method of Rice et al (1957). This method involves feeding young rats a high protein basal diet with varying amounts of residue supplement, with ten rats per group. The residue sample used for the nutritive evaluations did not have the testa removed. After ten days on the diet the rats were weighed, killed, gutted, and the parts weighed. In addition the feces were collected for analysis during the last four days. The rats were also checked for abnormalities, but none were found.

Analyses for moisture, nitrogen, crude fiber and ash were by standard AOAC methods. Oil analyses used four hours extraction with boiling petroleum ether. Mineral analysis were determined by atomic absorption spectrometry, except for phosphorus, which was determined from weight of phospho-molybdate complex.

Carbohydrates were determined by hydrolysis, derivatization

and gas chromatography analysis. Defatted residue samples were acid hydrolyzed as described by Easterwood and Huff (1969). The carbohydrate content (as dextrose equivalent) was determined by analysis of reducing sugars of a sample of neutralized filtrate (Triebold and Aurand, 1963). Neutralized filtrate was freeze-dried.

The trimethyl silyl derivatives were made from freeze-dried filtrate (or dried sugar standards) and then analyzed by gas chromatography approximately as described by Richardson et al (1971), except that the column material was 3% SE-30 80/100 chromosorb W, and temperature was programmed (140°C for 2.5 minutes, followed by increase of 4°/minute for 30 minutes). Peak areas and retention times were measured and recorded with a Hewlett-Packard 3373B integrator-recorder. Zero retention time was taken as time of injection.

Residue was incubated with amylase to check for possible presence of starch. Crude hog pancreas amylase (100 mg) was incubated with 5 g sample in 100 ml buffer for one hour at 25°C. The buffer was 0.05 M phosphate buffer with 0.01 M NaCl at pH 6.9. Substrate samples (starch or residue) were boiled and cooled to gelatinize starch before incubation.

Results and Discussion

Coconut residue contains many dark particles unless the testa is removed before grinding and extracting. Lightness (Hunter L value) was 91 for white residue from pared coconuts, but only 66 for

the residue from unpared nuts. The residue prepared from unpared nuts is probably too dark for food uses.

The particle size of residue is of some importance in its food applications. The dried residue as normally recovered from aqueous processing has its particle size determined by degree of grinding of the wet coconut meats before water extraction. This residue had a gritty mouthfeel and only 30% passed through a No. 40 screen (0.42 mm holes). Further size reduction was indicated.

However, preliminary trials indicated that further size reduction of dried residue was difficult if oil content exceeded ca. 15%, which it was after the two extraction steps employed (oil content was ca. 32%).

Hexane extraction of residue for ca. 30 minutes at 45°C reduced oil content to ca. 14%. The last remaining oil was difficult to remove, even by hexane extraction, probably indicating that it is enclosed in unbroken cell walls. Extracted residue (at 14% oil) was successfully ground in a pin mill (12,000 rpm) to give a residue flour. The hexane extraction step is considered important for two reasons: it results in higher yield of coconut oil and it prepares the residue for further size reduction necessary to remove gritty mouthfeel. Some properties of residue flour from pared coconuts are shown in Table 1, including typical oil and moisture contents.

The chemical compositions of residue, with and without testa, are shown in Table 2. These data, on moisture and oil free basis,

indicate that residue prepared from unpared coconuts (with testa) contains more crude fiber and less protein.

A readily apparent property of coconut residue is its sponge-like tendency to take up water, which would presumably be a property of some importance to food systems. Preliminary experiments indicated that water retention decreased with increasing centrifugal force, but nearly leveled off at 2,000 g, which was therefore chosen. Water retention values are shown in Table 3, with alpha cellulose samples used for comparison. The data indicate that water retention of residue on oil free basis is ca. 1.6 g water/g solids at oil content of 7-36%. The alpha cellulose holds significantly less water.

The properties of coconut residue are determined by its composition, which Table 2 data indicate is 80-90% carbohydrate. The carbohydrate content of hydrolyzed residue was determined by gas chromatography, with the typical chromatographic pattern being as shown in Figure 1. All residue samples gave four major peaks, which are designated A, B, C, D in Figure 1.

The retention times for the four major peaks are shown in Table 4, together with retention times of standards. The retention time of mannose was determined with mixed alpha and beta anomers. The alpha and beta assignments were made on the basis of published results (Holligan and Drew, 1971). The similarities in retention times between standard and sample suggest that the principal carbohydrates are mannose (peaks A and C) and glucose (peaks B and D). Based on analyses of other

standards (not shown) it was concluded that peaks A, B, C and D could not be caused by ribose, xylose, arabinose, mannose or levulose. The internal standard was chosen because its retention time did not coincide with any detectable sample peaks.

The peak areas and retention times indicate that the carbohydrate in hydrolyzed residue are ca. 74% mannose, 21% dextrose and 5% unidentified, based on analysis of data from ten samples of white residue. Similar results were obtained from analysis of carbohydrate of residue containing the testa or seed coat.

The data suggest that the carbohydrate in coconut residue is either gluco-mannan or a mixture of mannan and glucan (cellulose or starch). Samples of residue were incubated with amylase. The results, shown in Table 5, suggest that coconut residue contains no starch. Rao (1969) has reported that incubation of residue with cellulase causes up to 80% decomposition of fiber, which result does not seem consistent with the small amount of glucose we found in the coconut residue, unless the cellulase preparation used catalyzes hydrolysis of mannose as well as cellulose.

Residue was analyzed for nutritive value by a biocaloric availability study and also by fecal analysis. The residue sample used was exhaustively extracted with hexane to reduce the oil content to 2%, but was not subjected to further size reduction. The biocaloric value is therefore minimally influenced by oil content. The results, shown in Table 6, indicate that no total weight change

can be attributed to coconut residue, although eviscerated weight is significantly less (at 95% confidence) for 3 g daily of residue, and cecum weight was significantly greater for all levels of residue. The weight gains for groups with sucrose supplement are shown to give an indication of sensitivity of the method.

The data in Table 7 indicate that supplementation of the diet with residue caused a large increase in fecal excretion for the same group of rats. The dependence of feces weight on residue intake was linear with one gram residue intake resulting in 0.81 $\pm$ 0.05 g of additional feces. From the digestibility data it is estimated that 47% of the coconut crude fiber is digested. However, the energy obtained from digestion of the residue is about cancelled out by the lowered digestibility of other nutrients, presumably because they are soaked up by the residue and held in the digestive tract. Coconut residue may, therefore, be considered a low calorie filler.

All nutritional data refer to residue made from unpared coconuts (testa not removed). Different results may have been obtained from pared coconuts (testa removed).

Other workers have reported nutritive evaluations of diets high in coconut fiber eg, Rao, et al (1965) examined diet which included coconut meal instead of coconut residue. They report increased fecal bulk and lower protein digestibility for eleven year old boys, with coconut meal as the source of protein. Fecal bulk for coarsely ground meal was considerably higher than

for finely ground meal. More recent work (Lachance and Molina, 1974) indicates that a diet with 4.85% crude fiber (all from coconut) depressed the nutritional value of coconut protein in rat-feeding tests.

The results in general point suggest that coconut fiber decreases nutrient digestibility and increases amount of feces. The increased feces bulk is presumably caused by undigested coconut mannan. However, it should be pointed out that at least one type of mannan is completely digestible by man (Inoue, 1947).

The data suggest that coconut residue might be incorporated into food systems to increase dietary fiber or to act as a bulking agent. Residue may also have uses as a bulking agent in uses other than as a food product.

Specific food applications of coconut residue have been investigated, are promising, and will be reported separately.

Table 1 -- Typical Properties of White Residue Flour (prepared from pared coconuts).

Oil Content		14%
Moisture		5%
Bulk Density		0.16 g/cc
Amount passing through No. 80 sieve		65%
Reflected Color (Hunter)		
	L	92
	a	-0.5
	b	+5

Table 2 -- Composition of Residue (moisture free and oil free basis).

	Made from Coconuts		
Proximate Analyses	With Testa (%)	Without Testa (%)	S.D. of Ave. (%)[a]
Crude Fiber	30	22	3
Crude Protein	5	8	0.6
Ash	0.9	1.4	0.2
Other			
Carbohydrates (as dextrose)[b]	80	85	5
Potassium	0.5	0.5	0.07
Phosphorus	0.12	0.16	0.02
Sodium	0.1	0.1	0.03
Magnesium	0.07	0.08	0.01
Calcium	0.05	0.07	0.01

[a] Standard deviation of the average result reported. Crude fiber and protein contents are based on averages of at least five samples. Other results based on analysis of at least two samples.

[b] Carbohydrates were determined by analysis, and not by difference.

Table 3 -- Water Retention by Coconut Residue in Centrifugal field of 2,000 xg.

Sample	Oil Content (%, dry basis)[a]	Water Absorbed (g water / g solids)[a]	Water Absorbed (g water / g non-oil solids)[b]
Residue with testa	34	1.1	1.6
Residue with testa	7	1.5	1.6
Residue without testa	36	1.0	1.5
Residue without testa	9	1.4	1.6
Alpha cellulose control	0	0.9	0.9

a) Each value is average of at least two observations.

b) Result calculated from first two columns. Honestly significant difference is 0.4. Water absorption values were not corrected for dissolved solids. Independent experiments indicated that the residue contained 10 $\pm$ 3% soluble solids.

Table 4 -- Relative Retention Times for Major Peaks from Gas Chromatography of Carbohydrates.

Standard	Relative Retention Time[a]
α - Mannose	2.74
α - Glucose	3.18
β - Mannose	3.29
β - Glucose	3.80
Residue Sample	
Peak A	2.73
B	3.17
C	3.28
D	3.78

[a] The largest peak of the internal standard was assigned value of 1. Reported result is average of four values, and standard deviation of average is 0.01.

Table 5 -- Digestion of Residue with Amylase.

	% Reducing Sugars (as dextrose) after Incubation	
	With Amylase Added	Without Amylase
Residue (unpared)	0.2%	0.5%
Residue (pared)	0.0%	0.3%
Starch	35%	0.2%

Table 6--Incremental[a] Final Weight Gains for Rats.

Daily diet supplement	Incremental final weight gain[b] (g)	Incremental final eviscerated weight gain[c] (g)	Incremental cecum weight gain[d] (g)
1 g residue[e]	4.4[b]	+1.9[c]	0.61
2 g residue	1.8	-1.6	0.73
3 g residue	-1.3	-5.4	1.16
1 g sucrose	11.9	+8.5	0.15
2 g sucrose	22.1	+16.0	0.41
3 g sucrose	25.7	+20.4	0.35

[a] "Incremental" final weight here means ten day rat weight for basal diet plus supplement minus ten day weight for basal diet. Average initial weight of rats was 54.1 g for all diets. Final weights for rats on basal diet were 69.6 g total, 52.8 g eviscerated, and 1.19 g for cecum.

[b] 95% confidence interval is value $\pm$ 4.6 g

[c] 95% confidence interval is value $\pm$ 4.5 g

[d] 95% confidence interval is value $\pm$ 0.44 g

[e] The residue sample used for the nutritional evaluation (Table 3 data also) was made from unpared coconuts. This residue sample had the following analysis: 3% moisture, 2% fat, 1.0% nitrogen and 23% crude fiber.

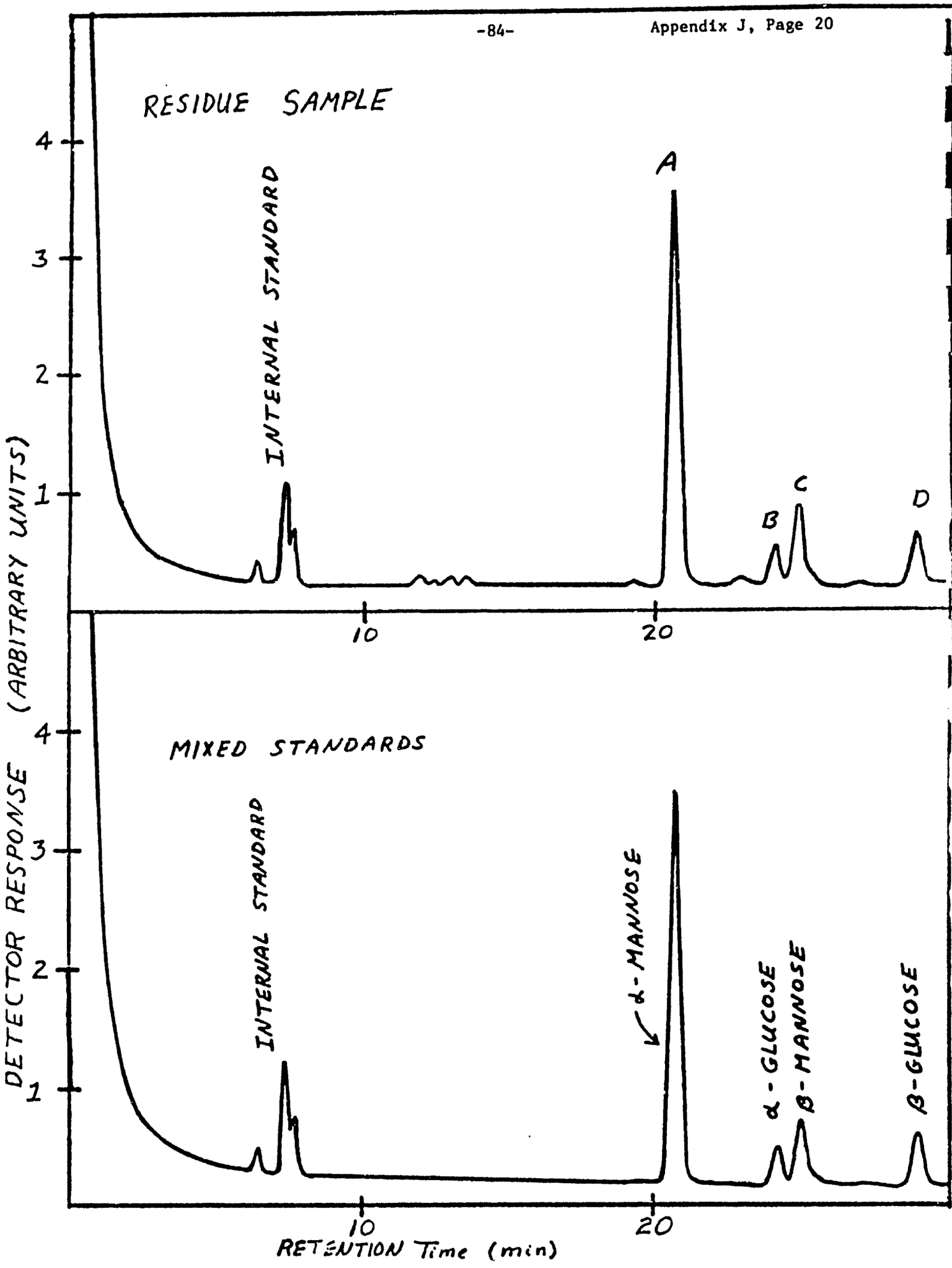
Table 7 -Digestibility and Fecal Data (Based on last four days).

Diet supplement	Digestibility (%)				Fecal wt. g/day	Feces Moisture (%)
	Protein	Fat	Ash	Fiber		
none	97	96	76	7	0.26	10
1 g residue	88	95	68	42	1.03	11
2 g residue	83	88	58	42	1.85	12
3 g residue	77	88	50	49	2.70	18
1 g sucrose	98	96	82	19	0.21	9
2 g sucrose	97	94	85	7	0.23	9
3 g sucrose	97	95	86	11	0.22	9

No statistical data. Each value is based on one measurement on pooled sample.

Caption for Figure

Figure 1 - Chromatographic patterns for residue and mixture of standards. The internal standard is the trimethylsilyl derivative of 2-Deoxy-D-Ribose. The residue sample was prepared from pared coconuts.


RESIDUE SAMPLE
INTERNAL STANDARD
A
B
C
D
MIXED STANDARDS
INTERNAL STANDARD
α-MANNOSE
α-GLUCOSE
β-MANNOSE
β-GLUCOSE
DETECTOR RESPONSE (ARBITRARY UNITS)
1
2
3
4
10
20
RETENTION Time (min)

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Aqueous Processing of Coconuts: Economic Analysis

ROBERT D. HAGENMAIER, CARL M. CATER, KARL F. MATTIL, Food Protein Research and Development Center, Texas A&M University, College Station, Texas 77843

ABSTRACT

A process previously has been developed at Texas A&M University for aqueous processing of coconuts to produce oil and a concentrated skim milk. This paper details the costs for that process and concludes that 20% of rate-of-return can be realized if the market price of coconut skim milk solids are assigned the value of $0.50/kg (bulk price).

INTRODUCTION

The aqueous processing of fresh coconuts to recover oil and dehydrated coconut skim milk has been described recently (1). A more recent publication has been devoted to the description of the coconut skim milk so obtained (2), and a flow diagram is presented in Figure 1. The purpose of the present article is to provide information for eventual commercialization by an estimate of processing costs. The Philippines, which is the largest producer of coconuts, was selected as the site for the hypothetical plant.

An economic evaluation was made by Edmonds, et al., (3) for an aqueous coconut process developed by Tropical Products Institute (TPI, London, England), and described recently by Dendy and Timmins (4,5). The proposed TPI process consists of aqueous extraction of ground coconuts, followed by acidification of the milk and gravity creaming. The resulting cream phase is centrifuged to give oil, an aqueous phase, and protein solids. The protein solids are vacuum dried and extracted with alcohol to yield an isolate containing 80% protein. Edmonds, et al., concluded that the TPI process would be uneconomic in Malaysia or Sri Lanka. In their evaluation, the protein isolate was valued at only $0.29/kg.

Aqueous processing of coconuts is not currently commercial. In discussions with industry people, it has become apparent that aqueous coconut processing would be considered a high risk venture, demanding a high return on investment and short expected project life. Consequently, some assumptions were made which minimized investment, although at the price of increasing processing costs. (A) fuel and electricity would be purchased, (B) by products (residue and insoluble protein) would be disposed of wet at the factory door rather than dried, packaged, and sold, and (C) no processing equipment would be added to recover additional oil from the residue by hexane extraction.

Alternate assumptions would operate if the project was to be considered a low risk venture. Shells would be burned for fuel, by-products would be dried and packaged, and additional oil would be recovered from the residue by hexane extraction.

METHODS

Estimates of equipment were obtained by contacting equipment manufacturers. All prices were updated to mid 1974 by using indices of inflation published monthly in *Chemical Engineering*.

Estimates of local costs in the Philippines, including costs of building, utilities, transportation, and wages, were made on the basis of unpublished data from P. Quintio and also from San Miguel Corp., Manila, Philippines. Data regarding Philippine prices of fresh coconuts and coconut oil were provided by United Coconut Association of the Philippines (UCAP) via unpublished data and from *Coconut Statistics* (6).

Estimate of total installed plant cost was based upon an approach similar to that used by Weaver, et al., (7) and by Hackney (8). In estimation of total installed plant cost, the costs of equipment and buildings were estimated directly, and other contributions to fixed costs were estimated with factors.

The pattern used for economic analysis was the rate-of-return pattern as described by DeGarmo (9), which is recommended for cases with a single investment followed by constant revenue and cost data for the duration of the project. In using this method, the salvage value of fixed capital was assumed to be negligible, which is a reasonable assumption for equipment situated in an economically underdeveloped country. The rate-of-return pattern calculates return to the investor in terms of interest paid annually, while allowing for pay-back of capital invested at project's end. For purposes of comparison, the accounted cash flow pattern (also described by DeGarmo [9]) was applied to the simple case but not, in general, used for sensitivity analysis.

The return on investment is reported to the nearest tenth percentage to show more accurately the change in return caused by different assumptions. However, no pretense is made for 0.1% accuracy.

DESCRIPTION OF PLANT

Preliminary estimates of processing costs for different sized plants led to selection of a plant which processes 250 m tons/day of dehusked coconuts. The capacities for the plant are given in Table 1, with yields from Hagenmaier et al., (1) and from more recent data of the authors (unpublished).

ESTIMATION OF FIXED CAPITAL

The fixed capital investment for the plant was estimated to be $1,910,000, with working capital of $800,000. The

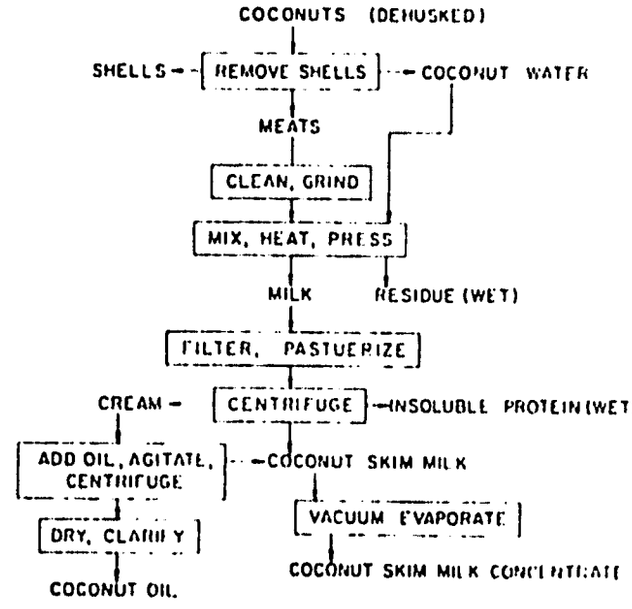

FIG. 1. Flow diagram for processing plant.

TABLE I

Capacity of Processing Plant

Material	Capacity in m tons		
	Per year	Per day[a]	Per hr[b]
Input			
Coconuts (weighed without husk)	62,500	250	12.5
Copra equivalent (at 8% moisture)	14,900	59.7	3.0
Intermediate products (wt undried)			
Shells	15,600	62.5	3.1
Coconut water	19,400	77.5	3.9
Meats[d]	27,500	110	5.5
Milk	37,000	152	7.6
Cream phase (at 65% oil)	13,500	54	2.7
Coconut skim milk (before evaporation)	28,200	113	5.6
Final products			
Coconut oil (91% recovery) (dry)	8,700	34.8	1.74
Coconut skim milk, as produced (30% moisture)[e]	3,930	15.7	0.79
Dry wt basis	2,750	11.0	0.55
Residue, as produced (68% moisture)	8,100	33.9	1.8
Dry wt basis	2,690	10.7	0.54
Insoluble protein, as produced (68% moisture)	523	3.1	0.23
Dry wt basis	356	1.4	0.071

[a]Assuming 250 working days/year.

[b]Assuming 20 working hr/day.

[c]Represents 75,000,000 nuts year. If coconuts were purchases with husks, they would weigh 50% more.

[d]Includes seed coat or testa, which comprises 12% of wt of meats.

[e]Yield is based upon process that uses coconut water, with 60% of coconut water solids accumulating in the coconut skim milk, which contains 25% protein (N x 6.25) on a 3% moisture basis.

TABLE II

Estimation of Equipment Costs and Energy Requirements

Processing steps	Equipment cost ($)	Electrical energy (kwh/hr)	Steam (m tons/hr)
Cleaning of meats and coconut water[a]	38,000	11	0.3
Grinding of meats	38,000	125	0
Heating and mixing prior to pressing	36,000	16	0.5
Counter-current pressing to separate residue from milk	55,000	90	0
Filtration, pasteurization of milk[a]	32,000	4	0.2
Centrifuge	46,000	20	0
Break emulsion and separate oil	53,000	20	0.1
Clarify and dry oil	29,000	9	0.1
Evaporate aqueous phase to 45% solids[a]	94,000	24	1.8
Evaporate aqueous phase to 70% solids and package[a]	30,000	12	0.2
By-product drying and packaging[b]	0	0	0
Finished product storage	30,000	0	0
Pumps and conveyors	37,000	12	0
Boiler	20,000	1	0
Energy required for ventilation, lighting and conveying	...	95	0
Totals	538,000	440	3.2

[a]Prices include appropriate holding tanks.

[b]For flash drying of residue and packaging, estimated values are $35,000, 20 kw, 2.0 m tons/hr. For drum drying and packaging of insoluble protein, values are $39,000, 12 kw, 0.2 m tons/hr.

bases for these estimates are detailed in Tables II and III.

ESTIMATION OF CASH FLOW FOR 1974 OPERATIONS

The cash flow is given in Table IV. Of critical importance to the cash flow are prices paid for coconuts and finished products, and these prices now will be discussed in some detail.

Average monthly domestic prices for crude coconut oil and copra at 8% moisture are published by UCAP (6), and the prices from January 1967-December 1973 were analyzed by linear regression. The copra and oil prices had a correlation coefficient of 0.995, with a regression equation of:

$$\text{copra price (\$/m ton)} = (0.5906 \pm 0.013)(\text{crude oil price} - 234.5) + (136.0 \pm 1.5). \quad [I]$$

where original data in pesos had been converted to U.S. dollars by the conversion $1.00 = ₱6.70, where 0.013 and 1.5 represent 95% confidence intervals, and 234.5 and 136.0 represent average crude oil and copra prices, respectively.

According to unpublished data from UCAP, the price paid for fresh coconuts, exclusive of handling and transportation charges, equals 22% of the Manila (domestic) price of copra at 8% moisture. The price of fresh coconuts exclusive of handling and transportation will, henceforth, be referred to as "farm" price for fresh coconuts. From substitution into equation I:

$$\text{farm price for coconuts (\$/ton)} = 0.130(\text{crude oil price} - 234.5) + 29.92 \quad [II]$$

To calculate coconut prices, it is next necessary to

TABLE III

Estimation of Total Capital Outlay

Capital	Cost ($)	Percent of total
Fixed capital (FC)		
Equipment (f.o.b. manf)	538,000	28
Overseas shipment of equipment (16.7% of E.)	90,000	5
Import duties and taxes (20% of E.)[a]	110,000	6
Buildings (1,500 m²) and land	340,000	18
Equipment installation		
Materials (including instruments)	180,000	9
Labor	72,000	4
Distribution of product, power, water, steam		
Materials	200,000	10
Labor	110,000	6
Yard work (materials and labor)	18,000	1
Engineering and overhead	180,000	9
Miscellaneous	72,000	4
Total FC	1,910,000	100
Working capital (WC)		
Raw materials accounts payable (1 month)	200,000	
Labor and utilities accounts payable (2 weeks)	20,000	
Inventory (1 month' supplies, 2 weeks' product)	190,000	
Finished product accounts receivable (1 month)	390,000	
Total WC	800,000	
Total capital investment FC + WC	$2,710,000	

[a]See ref. 10.

TABLE IV

Operating Expenses and Revenue (1974 Operations)

Expenses and Revenue	Dollars
Expenses	
Personal services[a]	
Direct	
Shelling (300)	150,000
Other operating (90)	52,000
Supervisory (25)	29,000
Repair (6)	6,000
Indirect	
Administrative (11)	35,000
Technical (5)	15,000
Sales and purchasing (11)	35,000
General (drivers, watchmen) (10)	7,000
Subtotal	329,000
Materials, supplies, utilities, transportation	
Coconuts (62,500 tons at $35.38)	2,205,000
Transportation of coconuts to factory[b]	221,000
Packaging ($0.02/kg for skim milk concentrate)	78,000
Maintenance supplies (2% of fixed capital)	38,000
Fuel (at 0.08/liter, 0.85 thermal efficiency)	82,000
Electricity (0.017/kwh)	42,000
Miscellaneous	50,000
Subtotal	2,716,000
Insurance and property taxes (3% of fixed capital)	57,000
Total operating expenses	3,102,000
Revenue	
Oil (8,700 tons at $275.1)	2,393,000
Coconut skim milk (2,750 tons dwb at $500)	1,375,000
Total revenue	3,768,000

[a]The numbers in parentheses indicate the number of personnel employed. Sample annual rates used (including fringe benefits) were $500 for unskilled labor, $700 for skilled, $1000 for foreman, $4000 for second level staff, and $6500 for manager.

[b]Assuming average distance hauled is 31 km, at cost of $0.09/km-ton, plus $0.75/ton handling costs.

choose a value for 1974 crude oil prices. Regression analysis was performed with yearly average exported oil prices from 1964-1973. Export prices were used in this case to get prices in U.S. dollars (domestic prices were published in pesos only), with all data from UCAP (6). The regression equation calculated was:

$$\text{crude oil price } (\$/\text{m ton}) = (1.41 \pm 12.4)(\text{time} - 1969.0) + (267.9 \pm 35.7) \quad [111]$$

TABLE V

Sensitivity Analysis for Rate-of-Return

Assumption varied	Values chosen	Rate-of-return
Plant capacity (dehusked coconuts, m tons/day)	100	10.5
	250[a]	19.7
	400	21.9
Price of Coconut skim milk solids ($/kg)	0.70	40.0
	0.50[a]	19.7
	0.35	4.4
Quantity of oil produced (efficiency of recovery)	100%	28.4
	91%[a]	19.7
Project life (years)	5	12.5
	10[a]	19.7
	15	22.0
Price of crude coconut oil ($/m ton)	500	24.3
	275.7[a]	19.7
	200	18.1
Quality of road network (with average distance coconuts hauled)	Ideal (11 km)	23.8
	Medium (31 km)[a]	19.7
	Poor (51 km)	15.5
Fixed capital (U.S.,$)	1,610,000	24.2
	1,910,000	19.7
	2,210,000	15.5
Days/year plant operated	300	25.7
	250[a]	19.7
	200	13.7
Inflation	None[a]	(22.1)[b]
	As described in text	(24.6)

[a]Value for assumed parameter that was used in Tables II, III, IV, and VI.

[b]The numbers in parentheses were calculated as discounted return on investment, rather than rate-of-return.

TABLE VI

Sample Calculation of Pre-Income Tax Rate-of-Return

Item	Amount
Amount paid out	
Operating expenses (from Table IV)	$3,102,000
Depreciation (8% sinking fund, 10 years)	$ 132,000
Subtotal	$3,234,000
Income	
(from Table IV)	$3,768,000
Net return	$ 534,000
Rate of return[a]	19.7%

[a]Net return ÷ total capital, or $534,000 ÷ 2,710,000.

where "time" indicates data (e.g. mid-1974 is 1974.5) and where 267.9 gives the average oil price. The low correlation coefficient of 0.09 indicates that oil prices are quite independent of time, and, therefore, the high oil prices of today do not lead to the conclusion that oil prices will be high in the future. Standard deviation about the repression line was $49/ton.

The mid-1974 price used for crude coconut oil was calculated from equation III to be $275.7/m ton, which is considerably lower than the actual market price prevailing in mid-1974. The sensitivity analysis in Table V indicates that the prevailing oil price would give a higher estimate of return than that estimated from the calculated mid-1974 oil price.

The price paid in mid-1974 for fresh coconuts was next calculated by equation II to be $35.28/m ton, which is the value used in Table IV.

The income from oil next must be related to crude oil prices. The oil from aqueous processing has been found to contain only ca. 0.2% free fatty acids. The price premium for oil is assumed to be the same as for copra. Premium of 0.5% for each 1% of free fatty acid below 3.5%, down to 1% free fatty acid, plus 0.75% for each 1% of free fatty acids below 1%, according to 1969 trading rules as cited by Woodroof (11). The calculated premium is 1.85% of selling price. In the Philippines there is a "millers tax" for production of coconut oil, equal to 2% of the value of the product. Therefore, the oil income will be:

$$\text{oil income (\$/m ton)} = 1.0185 \times 0.98 \times \text{crude oil price} = 0.998 \times \text{crude oil price} \quad [IV]$$

For crude oil price of $275.7/ton, the oil income would, therefore, be $275.1/ton, which is the value used in Table IV.

The next important point is market price of coconut skim milk solids, which have composition and properties intermediate between those of nonfat dry milk and whey solids. The mid-1974 market prices for non-fat dry milk and cheese whey solids were $1.36/kg and $0.26/kg, respectively (for bulk quantities, free on board factory, U.S. prices). Based upon these observations, the market price of the coconut skim milk solids was assigned the value of $0.50/kg.

Calculation of Rate of Return

The rate of return was calculated according to the procedure outlined in Table VI, and was found to be 19.7% (pre-income tax).

To express the results in terms of the often used discounted cash flow pattern (internal rate of return pattern), the return on investment (pre-income tax) was so calculated and found to be 22.1%. The explanation for the difference between the two results is that in the rate of return pattern, 8% interest was assumed for the sinking fund for depreciation, whereas in discounted cash flow no separate funds were set aside for depreciation.

Sensitivity Analysis

It is considered important to know the effect upon return caused by variation in the assumptions made in the cost estimate to determine which assumptions are most critical. The effect upon return of variation in certain

assumptions is shown in Table V.

For analysis of different plant capacities, it was assumed that fixed capital cost and indirect labor were proportional to square root of plant capacity and that working capital and direct labor were proportional to capacity. The average distance coconuts were transported was estimated to be 22, 31, and 45 km for the 3 plant sizes.

For variation in efficiency of oil recovery, no change in costs were assumed for processing the different amounts of oil. For change in market value of oil and coconut skim milk, no changes in working capital were assumed.

For the quality of road network, two situations were visualized. In the ideal case, all coconuts are transported by straight line from tree to processing plant. In case of poor roads, the plant was assumed to be located on a main road, only accessible by 2 km long feeder roads spaced 1 km apart. The compromise case is an average of the two extreme cases. The land surrounding the processing plants was assumed to be planted 20% in coconuts (average for Philippines) for a yield of 77 m tons of husked nuts/year/square km. Transportation costs were taken as $0.09/km/ton plus $0.75/ton handling and were calculated to be $1.71/ton, $3.53/ton, and $5.33/ton for the ideal, compromise, and poor road network, respectively.

To consider the effect of inflation, both income from oil and cost of fresh coconuts were assumed to inflate at an annual rate of 1% (based upon equation III). Labor rates were assumed to inflate at 5% annually, based upon Philippine labor rate indices. Market price of coconut skim milk also was assumed to inflate at 5%. All other costs also were inflated at the 5% rate. Working capital also was increased by 5% annually.

The sensitivity analysis indicates that, of the parameters examined, selling price of the coconut milk solids is the most critical. Also of considerable importance are plant capacity and quantity of coconut oil produced. Other variables have a lesser effect upon return.

Not included in Table V is effect upon return of spray drying. If the product were spray-dried instead of being evaporated to a syrup, the added equipment cost would be $120,000, and added annual operating costs would be $71,000. With spray-drying, the rate of return would drop from 19.7% to 14.7%. This decrease in return is a reflection of the fact that spray-drying is uneconomical for such a small plant. If product specifications called for spray-drying, it would seem to be more economical to spray-dry in a central facility that services a group of 250 ton/day plants.

For the processing plant described in Tables I-IV, the foregoing analysis has resulted in an estimated 20% annual rate of return. Based upon discussions with industry people, 20% is ca. the minimum acceptable return for new ventures. Therefore, the profitability of the venture would depend rather critically upon assumptions made in the cost estimate, and, in particular, upon cost of coconut skim milk solids. In addition, realization of income from by-products would improve the profitability of the process. The coconut residue is of particular interest as a by-product. Its quantity is equal to that of the coconut skim milk solids. The residue is characterized by its large crude fiber content, and as such might be of interest in increasing the fiber content of the diet.

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Coconut Flour: Technology and Cost of Manufacture

R.D. HAGENMAIER, P.H. QUINITIO, and S.P. CLARK, Food Protein Research and Development Center, Texas A & M University, College Station, Texas 77843

ABSTRACT

Processing operations are described for production of oil and coconut flour from fresh coconuts. The nuts first are made into white desiccated coconuts in drying plants, and then prepressed, flaked, and hexane extracted in a central facility. White coconut flour that is produced contains 25% protein and only 0.5% oil. The process would be commercially feasible in the Philippines if a coconut flour price of $315 per metric ton were assumed.

INTRODUCTION

Coconut flour is the name commonly applied to the food-grade product made from the coconut endosperm by drying to make desiccated coconut, followed by removal of oil by pressing and/or solvent extraction to give coconut meal. The coconut meal is finally milled to give the flour. A bench scale preparation is described by Claudio, et al. (1). In many cases, the brown seed coat, or testa, is pared off to give white coconut meats before the endosperm is dried.

The composition of coconut meal, which includes the brown seed coat or testa, has been reported by Samson, et al., (2) to be 25% protein (Nitrogen x 6.25), 7.7% crude fiber, and 4.7% ash, moisture and oil-free basis, and average values for 3 preparations.

Coconut flour has good nutritive value and contains no known substances present at toxic levels, as do some other oilseeds. Miranda, et al., (3) reported corrected rat assay protein efficiency ratio (PER) at 10% diet protein level, true digestibility (TD), and net protein utilization (NPU) values. For coconut flour without testa the values of PER, TD, and NPU were 2.8, 81, and 52 respectively. However, the 2 samples were prepared from different coconut sources by different methods. Butterworth and Fox (4) report TD of 78 and NPU of 46 for meal containing testa which was prepared with maximum processing temperature of 40 C. However, they reported lower nutritive value for samples of meal heated to 90 C or above. Rao, et al., (5) reported TD of 83 and biological value of 70 with human subjects (children) with coconut meal containing testa.

Food preparations containing coconut flour have been developed by the National Institute of Science and Technology (Manila). These foods include noodles, snacks, weaning foods, and some native recipes (6). In addition, coconut flour has been used at the 3-5% level in large scale preparation of the Nutribun, a bread-like product (7).

The production of coconut flour is viewed as an alternative to copra manufacture, which results in a protein product, copra meal, that is dirty, unsanitary, scorched, and, in general, unfit for human consumption. Potential world production of coconut flour without testa, at 5% moisture is equal to 40% of world production of coconut oil, which in recent years has been over 2 million metric tons annually.

Despite published information concerning nutritive value and food applications, the current commercial production of coconut flour is zero. Commercial production appears to be contingent upon more food applications research, and also on knowledge of costs and technology of production. The purpose of this paper is to present information on cost and technology of production of low fat coconut flour, with the goal of developing coconut flour as a food product.

referred to contains only ca. 0.5% oil. Coconut flour with 15-20% oil has sometimes been referred to as low fat flour in the literature (3). In discussions with knowledgeable persons, it has become apparent that coconut flour with 15-20% oil would have somewhat less market potential.

METHODS AND MATERIALS

Most of the coconut samples used were supplied by the Franklin Baker Company of the Philippines (Manila, Philippines), manufacturers of desiccated coconut. The samples were produced in the plant whose operations were described in some detail by Woodroof (8). In normal production, the coconuts are manually shelled and pared, washed with water, shredded, steam blanched, and dried in a conveyor dryer. Besides samples of commercial type desiccated coconut, Franklin Baker Company supplied desiccated coconut samples prepared without the blanching step. Samples of both blanched and unblanched coconut were dried at each of 2 different temperatures, a) constant air inlet temperature of ca. 85 C, or b) commercial drying conditions, which consist of initial air inlet temperature of 110 C followed by adjustment to ca. 85 C.

A sample of presscake also was supplied. This consisted of white unblanched coconut that was dried, then pressed in a commercial Anderson screw expeller to give a presscake with oil content of 17%. In the pressing operation, the press was cooled and the feed rate controlled in order to limit temperature of presscake to 80 C as it was exiting from the expeller.

Samples from the Philippines were shipped to Texas A & M University for further processing and analysis, which took place at the Food Protein Research and Development Center of Texas A & M University, and also at test facilities of Crown Iron Works Company (Minneapolis, MN).

Presscake was reduced in size by cracking rolls or hammer mill. The presscake particles then were adjusted to 10% moisture and formed into flakes of 0.3 mm thickness with one pair high flaking rolls. The flakes (bulk density of 0.29 gm/cc) were extracted with hexane at 60 C in a Crown Iron Works Company pilot plant extractor with residence time of 15-60 min, which reduced oil content to 0.4-0.7. The extracted flakes were desolventized in a steam jacketed ribbon conveyor and finally ground with an impact stud mill to give a white flour, of which 85% passed through a No. 80 sieve.

The desiccated coconut used for comparative solvent extraction was white and unblanched with a moisture content of 5%. It was not flaked, but ground to a small particle size so that 92% passed through a No. 20 screen. The flaked presscake was as described.

The samples were column extracted for 15 min at 50 C, using 0.9 liters of sample and 4 liters of hexane, with recirculation, except that the first liter collected was not recirculated. In addition, one liter of fresh hexane was poured through the column just before draining and air desolventizing. For time dependence of extraction, the data in Figure 1, 0.2 liters of sample were column extracted with 0.5 liters of 50 C hexane, without recirculation. The column used measured 4.8 cm inside diameter (ID) by 60 cm tall. The bottom was fitted with perforated plate and sealed on funnel connected to tygon tubing. Oil contents are reported on a dry wt basis.

Protein solubilities of coconut samples were determined by mixing 5 g hexane extracted meal with 100 g water. The

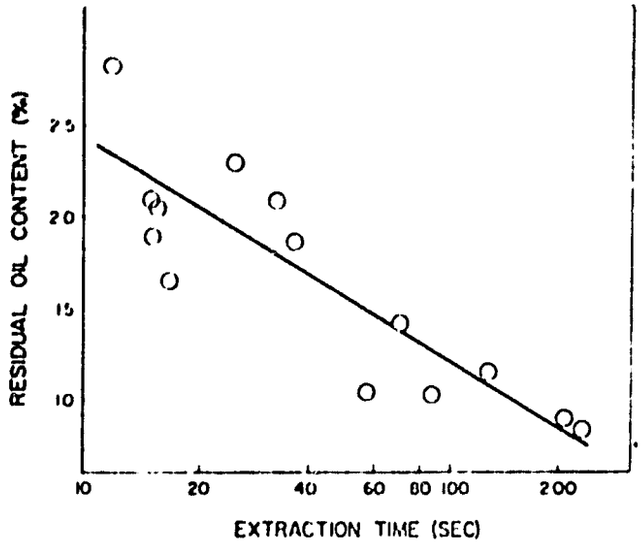

FIG. 1. Extraction of oil from flaked presscake. Initial oil content was 17%. Samples were extracted with fresh hexane at 50 C for time indicated. Each point represents one observation.

TABLE I

Composition and Properties of White Coconut Flour[a]

	Average value	Range
Crude protein (N x 6.25) (%)	25	21-28
Crude fiber (%)	9	7-11
Oil (%)	0.5	0.1-0.7
Ash (%)	5	4-6
Bulk density (g/cc)	0.33	0.30-0.36
Hunter color values[b]		
L	91	90-93
a	-0.6	-0.7 to 0.5
b	7	5-8

[a]Based on 5% moisture content, 3 samples, each analyzed twice.

[b]L = Measure of whiteness, from 0 (black) to 100 (white). a = measure of redness (plus), greenness (minus). b = measure of yellowness (plus), blueness (minus).

periodic readjustment of pH with NaOH solutions. Samples were centrifuged 15 min at 16,000 g, then filtered. Nitrogen content of filtrate was determined by Kjeldahl analysis. Fraction of nitrogen extracted was assumed equal to fraction of protein extracted.

Laboratory analyses followed 1970 Association of Official Analytical Chemists (AOAC) methods. For oil content determination, samples were extracted 2 hours, ground, then extracted 2 more hours. Color measurements were made with a Hunter color difference meter. Reported results are the average of at least 2 observations, except where noted.

Equipment manufacturers were contacted to determine suitability, prices, capacity, and energy consumption for the major processing steps of grinding, drying, transporting dried coconut, pressing, preparing flakes, extracting with solvent, recovering solvent, and milling to a flour. The dryers are Proctor and Schwartz, Model SCF Conveyor Dryers, which are those commonly used for manufacture of desiccated coconut.

The proposed plant is assumed to be located in the Philippines because of the quantity of coconuts produced there. Local costs in the Philippines were estimated with the help of information from San Miguel Corporation (Manila, Philippines), Franklin Baker Company (Manila, Philippines), and others. The prices of buildings were estimated directly; however, costs of shipment, import duties, and installation were estimated with factors (9,10). Factors and equipment prices were based on price trends and observed correlation between prices of fresh coconuts and coconut

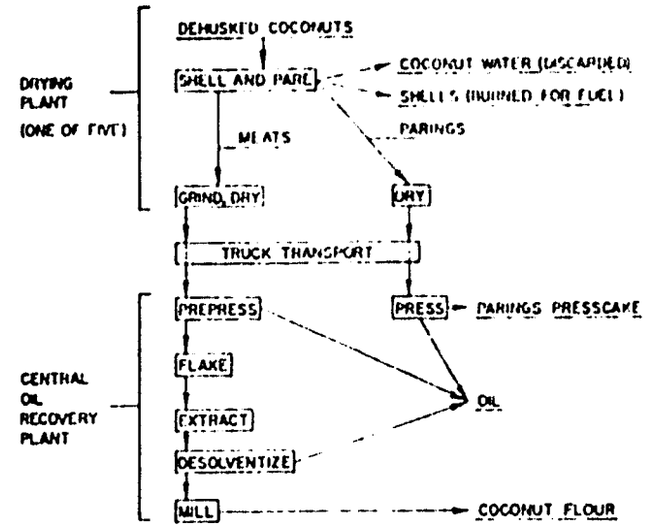

FIG. 2. Flow diagram for proposed process.

oil, as previously computed (11). All prices and costs are on a mid-1974 basis. Large taxes recently imposed on coconut processors in the Philippines have been ignored in this analysis in an attempt to free the estimates from local tax laws, and hopefully make the estimates applicable to processing in other countries as well.

For purposes of estimating production, it was assumed that the coconuts processed contain 25% shells, 31% coconut water, and 44% meats with testa. The meats with testa are 50% moisture and contain 69% oil on a dry wt basis. The relative wts of meats, shells, and water are quite variable for different types of coconuts (12); however, the values used are average values for Philippine coconuts (13).

RESULTS AND DISCUSSION

The process as described consists of unit operations well known in the oilseeds processing industry, for which standard equipment is available. The drying step is patterned after desiccated coconut operations and the extraction step is patterned after prepress solvent extraction of copra. The process here proposed consists in combining these operations so as to produce a white, low-fat, nutritious coconut flour, with high protein solubility, if possible. Coconut flour with high protein solubility could be used for further processing to produce isolates. In addition, flour with high protein solubility may prove to have superior functional properties, although this never has been demonstrated.

The normal desiccated coconut process includes 2 heating steps considered critical to product properties. These are the blanching and drying steps. The effect of blanching was determined by comparing samples dried with and without blanching. With 85 C air inlet temperatures or with commercial drying conditions the results were the same. The protein in blanched samples was 26% soluble at pH 7.5, whereas, the protein in unblanched samples was 58% soluble. The unblanched samples had protein solubility equal to that previously reported for coconut dried at 40 C (2), which indicated that protein solubility was not reduced when coconut was desiccated commercially.

However, blanching caused a large decrease in protein solubility. If product specifications for coconut flour require high protein solubility, it presumably will be necessary to replace the blanching step with some other microbiological kill step. Two possibilities are milder heat treatment or use of chemical bacteriocide.

Another critical unit operation in the oil recovery plant is the pressing operation. It is a well known fact that severe pressing can produce oilseed cakes that have dark color and

TABLE II

Anticipated Plant Capacities in Metric Tons

	Each Plant		All 5 Plants
	Tons/hr	Tons/day	Tons/yr
Drying Plants[a]			
Input			
Coconuts, without husk	10.4	228	285,000
Intermediate products			
Shells	2.6	57	71,000
White meats (50% moisture)	4.0	87	109,000
Parings (50% moisture)	0.6	13	16,000
Output			
Coconut water	3.2	71	88,400
Dried white meats (5% moisture)	2.1	46	57,000
Dried parings (5% moisture)	0.3	6.7	8,400
Extraction plant[b]			
Input			
Dried white meats	8.0	191	57,400
Dried parings	1.2	28	8,400
Intermediate product			
Prepressed white meats (5% moisture, 17% oil)	3.0	72	21,700
Output			
Oil[c]	6.0	143	42,900
White flour (5% moisture, 0.5% oil)	2.5	60	17,900
Parings presscake (5% moisture, 10% oil)	0.4	10	2,900

[a]Assuming 250 days/year x 22 hr/day.

[b]Assuming 300 days/year x 24 hr/day.

[c]Oil from 3 sources: 75% from prepressing white meats, 20% from pressing of parings, and 5% from extraction.

TABLE III

Estimated Equipment Costs and Energy Consumption

	Equipment costs (dollars)	Electricity consumption (kw)
Drying plants (per plant)		
Prebreaker and cutting mills	52,000	35
Dryers (conveyor)[d]	220,000	115
Boiler 7m tons steam/hr[b]	40,000	0
Miscellaneous[c]	70,000	50
Total equipment	382,000	200
Buildings (2,500m^2) and land	180,000	
Central oil recovery plant		
Screw prepresses[d]	260,000	160
Screw press for dried parings[d]	60,000	420
Cracking and flaking mills	57,000	70
Solvent extractor[e]	230,000	20
Flour mill	35,000	50
Oil storage tanks (10 days output)	140,000	0
Boiler (3m tons steam/hr)[b]	30,000	0
Miscellaneous	100,000	75
Total	912,000	795
Buildings (6000m^2) and land	450,000	
Transportation equipment		
Trucks[f]	470,000	0

[a]Assuming separate dryers for white meats and parings.

[b]The boiler burns coconut shells for fuel

[c]Includes equipment for conveying, laboratory, shop, and offices, and electricity for lighting and ventilation

[d]Includes conditioning equipment, conveyors, and oil handling equipment.

[e]Includes solvent recovery system and meal desolventizer.

[f]Ten hopper bottom pressurized bulk trucks, capacity 28 m^3 (7m tons) each.

low protein solubility. Work (S. Verasestakul, private communication, 1973) has shown that processing can reduce coconut protein solubility without markedly affecting color.

Presscake with 17% oil content, prepared as described, was observed to be white and have protein solubility of 94% at pH 10.5, 56% at pH 8.4, and 42% at pH 6.0. These data indicate good protein solubility when compared to published data for solubility of coconut meal prepared without excess heat (2). The data suggest that commercial type screw prepresses be used to give presscake with ca.

Therefore, if blanching were eliminated and pressing were controlled, coconut flour with high protein solubility could be prepared by minimizing processing heat. However, the effect of protein solubility on food applications has not been determined. No evidence is available that high protein solubility is superior to low solubility.

Efficient extraction of oil is primarily of importance from the viewpoint of economics, oil being the principal product. However efficient extraction is also necessary for the production of low fat flour.

TABLE IV

Estimation of Total Invested Capital

Fixed Capital	Drying[a] plants (drs x 10^3)	Central oil recovery plant (drs x 10^3)	Transportation of dried coconut (drs x 10^3)	Total (drs x 10^3)
Equipment (fob cost)	1,910	912	370	3,292
Shipment and import duties	325[b]	155[b]	80	560
Buildings and land	900	450	0	1,350
Equipment installation	764[b]	547	0	1,311
Distribution — power, water, steam	382[b]	274	0	656
Miscellaneous	382[b]	182	0	564
Engineering and overhead	669[b]	365	47	1,081
Subtotal	5,332	2,885	597	8,814
Working capital				
Raw materials, accounts payable (1 month)				900
Labor, utilities, and supplies (2 weeks)				70
Inventory and accounts receivable for products (1 month production				1,300
Subtotal				2,270
Total capital (fixed capital and working capital				11,084

[a]Cost of six identical plants.

[b]Calculated as percentage of equipment cost

TABLE V

Annual Operating Expenses (1974)

Personal services for 5 plants[a]	Amount ($)	Personal services for oil recovery plant[a]	Amount ($)
Direct		**Direct**	
Shelling (1430)	700,000	Operating labor (70)	40,000
Paring (1430)	700,000	Supervisors (10)	10,000
Other operating (300)	175,000	Maintenance (20)	15,000
Supervisors (75)	90,000		
Maintenance (30)	23,000	**Indirect**	
Indirect		Administrative (30)	100,000
Administrative (35)	110,000	Technical (15)	45,000
Technical (20)	60,000	Sales and purchasing (30)	100,000
Purchasing (11)	35,000	General (30)	20,000
General (50)	35,000		
Other		**Other**	
Coconuts ($38.28/ton)	10,055,000	Flour packaging material ($0.01/kg)	175,000
Transportation to factory[b]	984,000	Hexane losses ($0.20/liter)	106,000
Maintenance supplies	106,000	Maintenance supplies[c]	60,000
Electricity ($0.037/kw hr)	200,000	Electricity ($0.18/kw hr)	210,000
Miscellaneous	200,000	Diesel fuel for trucks ($0.13/liter)	65,000
Insurance and property taxes[d]	160,000	Insurance and property taxes[d]	87,000
		Miscellaneous	100,000
Subtotal	13,633,000	Subtotal	1,133,000

[a]Includes cost of transporting dried coconut and fringe benefits. Numbers in parentheses indicate number of personnel.

[b]Assuming average distance hauled of 30 km, at $0.09/km ton, plus $0.75/ton handling costs.

[c]2% of fixed capital.

[d]1% of fixed capital.

as described, the oil content of the desiccated coconut was reduced to 10%; whereas, the oil content of presscake was lowered to 0.6%. These were averages of 4 observations with a standard deviation of oil content of 10% of the reported result.

The hexane flow through the flaked presscake was 0.4 ± 0.1 liters/min, which was only 35% of the flow through desiccated coconut. However, the flow through the flaked presscake was fast enough for the commerical type solvent extractor used.

The results shown in Figure 1 indicate that only ca. 2 min of extraction were required to reduce residual oil in presscake flakes to 1%. In contrast, desiccated coconut had residual oil of ca. 10% after 15 min extraction. These data suggested that prepressing and/or flaking resulted in sufficient cell disruption to facilitate hexane extraction of remaining oil.

Attempts to flake desiccated coconut without prepressing resulted in failure. The flaking rolls became covered with oil. However, in early work, a fine powder was produced by successively passing desiccated coconut through 2 pairs of roller mills, spaced at 0.3 mm and 0.05 mm. This material, when extracted with hexane, had residual oil content of 0.8%. For the extraction of this finely ground material, the sample was slurried with hexane for 30 min, poured onto a fine mesh screen, and the solids washed with fresh hexane.

The slow percolation of hexane through the roller milled desiccated coconut, and also the passage of fines into the hexane filtrate, raised some doubts about the feasibility of processing without the prepressing operation. For these reasons the prepressing step was included in the proposed process.

The data in Table I give analysis of coconut flour without testa. These and other data, not shown, suggested that composition was somewhat variable. The factors responsible for this variability seemed to be maturity and variety of coconut processed. The oil produced is a premium oil with-

out the high free fatty acid content of coconut oil derived from normal copra. Although this article emphasizes the coconut flour product, the coconut oil was the principal product of coconut processing, both in quantity and commercial value. The operation described is a vegetable oil plant, which produces coconut flour as a by product.

The process costed out consists of a combination of the described unit operations as depicted in Figure 2. The hypothetical arrangement consists of five identical drying plants and one centralized oil recovery plant. The drying operations are dispersed for two reasons, to reduce cost of hauling fresh coconuts and to permit utilization of laborers over a wider geographical area.

The plant capacities, shown in Table II, are based on typical coconut compositions and assume no losses of material in processing. Coconuts rejected as spoiled were expected to constitute ca. 1% of the total. It was assumed that spoiled coconuts are dried and sold as copra, with the income from copra buying the fresh nuts. This being an equal trade off, it was not entered in the cash flow.

The figures shown in Table III are direct estimates of equipment costs, energy consumption, and building costs. Equipment costs were based on FOB US prices of mid-1974. However, inflation rates undoubtedly will make these numbers obsolete in a short time, and local costs will vary with location.

Total invested capital was estimated as shown in Table IV. Annual operating expenses are shown in Table V, and annual cash flow in Table VI. The selling price of coconut flour was adjusted to give 20% pre-income tax rate of return, which was the same rate of return calculated for aqueous processing of coconuts (11), also calculated for mid-1974.

The selling price of coconut flour so calculated was $315/m ton. This compared to the price of $500/m ton calculated from coconut skim milk solids obtained from aqueous processing of coconuts (11). However, this comparison was somewhat misleading because no income was assumed from sale of the high fiber product obtained by aqueous processing. On the other hand, in the prepress solvent extraction process, the fiber is sold as one of the components of coconut flour. A more meaningful comparison of the 2 processes would seem to be value added, calculated as income from oil and protein product minus farm price of coconuts. In both processes the value added was ca. $25/mT of husked coconuts. However, it should be noted that this comparison is highly dependent on market value of the protein products, which have not yet been determined.

The economic data pertinent to coconut processing are dependent on inflation rates, taxes, and other local costs, especially labor. These are all highly variable and can have important effects on actual return on investment. However, the estimates as presented are sufficiently general to give an indication of the major expenses anticipated for processing in the typical coconut producing country.

One of the largest direct costs is transportation of the fresh coconuts to the drying plants. Furthermore, the costs of the drying plants represent 60% of the total fixed capital, and, as such, contribute substantially to overhead costs.

A system in which coconuts were dried with smaller drying units positioned nearer the coconut trees would be expected to reduce transportation and drying costs substantially, albeit at the expense of more difficult sanitary and quality control, particularly if the meats were pared to make a white product. Such a system, for making food grade copra, is currently under investigation at Texas A & M University. Coconut flour made from food grade copra would be expected to require much lower price for price a comparable return on investment.

TABLE VI

Calculation of Rate of Return

	Amount ($)
Annual revenue	
Oil (42,900 tons at $275.4[a])	11,802,000
Coconut Flour (17,900 tons at $314[a])	5,619,000
Parings meal (2,900 tons at $60[a])	175,000
Total revenue	17,616,000
Annual expenses	
Operating expenses	
Drying plants (includes coconuts)	13,613,000
Oil recovery plant	1,133,000
Depreciation (8% sinking fund)	
Drying plants (30 years)	168,000
Oil recovery plant (30 years)	199,000
Trucks (5 years)	102,000
Total expenses	15,415,000
Net return	2,201,000
Rate of return (pre income tax)[b]	20%

[a]Bulk prices, fob plant.

[b]Net return plus total capital.

Of all the economic data presented, that with the highest uncertainty is the market value of the protein product, namely white coconut flour. For the process as analyzed with large drying plants, the flour price of $315/metric ton is about the minimum value to permit a reasonable return on investment. However, the real market value of coconut flour can only be determined after more samples are made and evaluated.

Most evaluations of coconut flour to date have been limited to nutritional analysis. This paper has been devoted to technology and cost of production. More information is needed now regarding food uses and market value of the coconut flour.

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I. Title: Development of a Process for Preparation of Coconut Protein Products for Use in Foods. (AID Contract csd/2804)

II. Subtitle: Biochemical and Structural Changes in Maturing Coconut Solid Endosperm and Related Tissues of the Fruit Important to Processing.

III. Principal Investigator: Julius W. Dieckert

IV. Summary:

The growth and development of the coconut seed and its content of solid endosperm is a complex well-ordered process. Once the spathe opens and the female flowers are pollinated, the fruit grow rapidly. The ovular cavity, limited by the seed coat, enlarges for about 240 days after the spathe opens. The endocarp hardens from the distal end toward the proximal end. This process is complete shortly after the ovule ceases to enlarge. The growth of the solid endosperm lags behind the growth of the ovule. Under favorable conditions only about 20 % or less of the maximum growth of the endosperm is completed after 90 % or more of the growth of the ovule is achieved. The increase in weight of the endosperm ends about 365 days after spathe opening.

Ultrastructural analysis shows that, except for a few cell layers adjacent to the seed coat, the cells of the mature endosperm are necrotic. Practically all of the intracellular substance that stains like protein is located in watery vacuoles with a single unit membrane similar to the tonoplast. The protein in the aleurone vacuole consists of a crystalline core surrounded by an amorphous layer. The lipids are non-osmiophilic and are located in fat droplets, the spherosomes, in the cell and are separate from the aleurone vacuoles. What few plastids, mitochondria, nuclei and

other cell organelles that remain are necrotic and obviously nonfunctional. The cell walls are thick and woody.

In the early stages of growth the endosperm is gelatinous. The cells have active mitochondria, plastids, dictyosomes and nuclei. The cell walls are very thin, which explains the gelatinous quality of the tissue. The rough endoplasmic reticulum isn't obvious, and there are no amorphous or crystalline protein deposits in the vacuoles. The gelatinous endosperm grows over the surface of the inner seed coat from the distal to the proximal end of the ovule. It takes roughly 31 days for the endosperm to cover the inner surface of the seed coat, once the rapid growth of the endosperm starts.

After the endosperm is established on a given region of seed coat, it differentiates. The cell walls thicken, the rough endoplasmic reticulum proliferates, the dictyosomal vesicles develop material that stains like protein, and biphasic protein deposits similar to those in the aleurone vacuoles of mature endosperm appear. The latter signals the beginning of the process of synthesis and sequestration of the principal reserve aleurins of the mature seed. The endosperm increases in thickness and characteristic changes occur in the consistency of the tissue. Approximately 105 days are required for the conversion of the gelatinous endosperm to hard endosperm in a given region. Ultrastructural analysis suggests that the synthesis of reserve aleurins continues from the cooked egg white stage that follows the gelatinous phase of endosperm differentiation to the early hard phase of differentiation. The process continues for about 112 days in a given region. The synthesis of the reserve aleurins begins about 200 to 210 days post spathe opening in the distal region of the ovule and ends in

the proximal region at about 330 to 340 days post spathe opening. Relating ovular growth to reserve aleurin synthesis, the ovule is formed first; then, the vacuolar proteins are synthesized and packaged. There is some time overlap in the two processes.

The ultrastructural pattern of the endosperm producing reserve aleurins is similar to that of embryos of dicotyledonous seeds producing aleurins. Classical aleurone grains do not form in mature coconut endosperm, however, since the latter tissue does not desiccate with maturation. A comparison of SDS gel electrophoretograms of crude protein extracts of coconut endosperm in the cooked egg white and hard phases show the presence of the principal coconut seed proteins in each. It appears that the principal reserve proteins are synthesized and sequestered by coconut endosperm in essentially the same way as in developing embryos of the dicotyledons. It is proposed that the aleurins of the coconut endosperm are synthesized by polyribosomes attached to the outer surface of the endoplasmic reticulum, transported to the dictyosomes, concentrated and/or modified by the dictyosomes and deposited in the aleurone vacuoles by a process of membrane fusion. Such a model is an aid in understanding the coconut seed proteins and the effects of environmental stress on the deposition of these commercially valuable proteins.

The bulk aleurins of seeds are thought to function as a source of organic nitrogen for the germinating embryo. In most seeds that have been studied carefully, the number of individual reserve aleurins is small. As pointed out earlier, ultrastructural analysis confirms that the principal proteins of mature coconut solid endosperm are aleurins. There is little doubt that they serve as a nutritional reserve for the germinating coconut

embryo. Analysis of the crude proteins of mature coconut endosperm by SDS gel electrophoresis shows a relatively simple pattern. Most of the protein is in three molecular weight classes: 51,500, 30,000, and 20,500 daltons. Careful salt fractionation resolves the native proteins into one fraction bearing the 51,500 subunit plus impurities and another containing the 30,000 and 20,500 molecular weight classes. The latter isolate was fractionated into the two molecular weight classes by a new system of preparative SDS gel electrophoresis of our invention. The amino acid composition of each subunit and the starting material was determined. The amino acid composition of the starting material could be accounted for satisfactorily by the amino acid compositions of the two subunits and the assumption that the starting material contained equimolar quantities of each. Presumably, the native protein is of the form $(P_{20} \cdot P_{31})_n$. The Metzger index was used to compare the amino acid composition of $P_{20} \cdot P_{31}$ with legumin type proteins from peanuts, beans and cotton seed. Low values of the Metzger index (D.I.< 15) are presumptive evidence that two proteins are homologous (i.e., arise from genes with a common ancestral gene).

By the Metzger criterion the following aleurins are homologous proteins: $P_{20} \cdot P_{31}$ (C. nucifera); AB (legumin, Vicia sativa); AC (legumin, V. sativa); αβ (legumin, Vicia faba); glycinin (Glycine max); P_{67} (arachin, Arachis hypogaea); and, P_{40} (acalin B, Gossypium hirsutum). The difference indices range from 9.04 to 12.45. The amino acid composition of P_{20} and P_{31} were also compared with the individual subunits of the legumin type proteins. Two potential homologous series are apparent. One consists of P_{20}, A (V. sativa), β (V. faba) and P_{24}

(A. hypogaea); and, the other series consists of P_{31}, B (V. sativa), C (V. sativa), α (V. faba) and P_{43} (A. hypogaea). In the first series the difference indices ranged from 13.37 to 13.67. In the second series they ranged from 9.84 to 12.40. No data were available for the individual reduced subunits of P_{40} from acalin B. These values of the difference index are respectably low when the phylogenetic distance between the coconut palm and the other species is considered. The apparent homology between P_{20} · P_{31} and the legumin type aleurins lends additional support to the concept that all are produced and sequestered by a common process.

A natural experiment on the effects of water stress on the production of solid endosperm is repeated yearly on the coconut palms on St. John Island. A considerable body of circumstantial evidence is adduced supporting the hypothesis that water stress at key times during flowering and fruit development severely limits the production of solid endosperm. The most sensitive phases are related to periods of rapid growth. Water stress during periods of spathe enlargement increases the time elapsed between the opening of successive spathes, thereby reducing the number of clusters of coconuts produced per year per tree. The interval between spathe openings can be lengthened from 26 days to 47 days, depending upon the severity of the water stress. Such an effect can cause serious economic losses. When water stress occurs during the period of rapid ovular growth, the size of the ovule at maturity is greatly reduced. Consequently, the quantity of endosperm produced per mature seed is lowered correspondingly. As much as a 60 % reduction in the fresh weight of the endosperm per seed at maturity was noted for palms suffering water

stress during the period of maximum ovular growth. Most of the growth of solid endosperm and the synthesis and segregation of the coconut seed protein occurs after the ovule reaches maximum size. Water stress during this interval reduces the growth rate of the solid endosperm and probably reduces the rate of synthesis and deposition of the vacuolar proteins. The effect of water stress on the growth rate of the solid endosperm is less dramatic than on the growth of the ovule.

V. Experimental:

Ultrastructural Analysis of Solid Coconut Endosperm

As reported earlier, the ultrastructural analysis shows that, except for a few cell layers bordering the seed coat, the cell organelles of the mature endosperm are necrotic and clearly non-operational. Practically all of the intracellular substance that stains like protein is located in watery vacuoles of the aleurone type. The proteins of the vacuole are present in two forms, a central crystal surrounded by an amorphous layer. The lipids are non-osmiophilic and are located in spherical droplets (spherosomes) that are separate from the aleurone vacuoles. The cell walls are thick and woody and penetrated in certain directions by plasmodesmata.

At the other extreme is the gelatinous endosperm. These cells have active mitochondria, plastids, dictyosomes and nuclei. The cell walls are very thin, which explains the jelly-like character of the tissue. The rough endoplasmic reticulum is not obvious and there are no crystalline or amorphous protein deposits in the vacuoles.

Once the endosperm is established on the inner surface of the seed coat, it differentiates. The cell walls thicken and the tissue changes

consistency from the jelly-like phase through a "cooked egg white" phase, progressing through a rubbery phase and finally achieving the hard stage characteristic of mature coconut meat. At the cooked egg white stage a new system of cell organelles appears. Vacuoles appear with the biphasic protein deposits that are characteristic of the mature endosperm (Figure 1a). That the crystalline deposits are protein is confirmed by cytochemistry. Alternate serial sections were digested with a protease from Streptomyces griseus (Sigma Chemical Co.) and compared with the alternate untreated sections by electron microscopy. Figure 2a , 2b show the results of such an experiment. Clearly, the protease removed the crystalline core. The removal of the amorphous layer surrounding the crystal is not so clear cut.

The tissue which was at the cooked egg white stage was fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in epoxy resin. Serial sections were cut, each section was treated with hydrogen peroxide to remove the osmium, and alternate sections were digested with the proteolytic enzyme -- protease from Streptomyces griseus. All sections were stained for electron microscopy, mounted on carbon stabilized formvar coated copper slot or hole grids and viewed with an Hitachi HU11A electron microscope.

Tissue at the cooked egg white stage also shows well developed rough endoplasmic reticulum (Figure 3) and dictyosomes with vesicles containing material that stains like protein (Figure 4). Similarly active dictyosomes are present at the rubbery stage (Figures 5, 6, 7). That the dictyosomal vesicles contain protein is verified by cytochemical experiments with the protease as shown in Figures 8a and 8b. An electron micrograph of protein crystals at the rubbery stage is given in Figure 1b.

The ultrastructural pattern for the cooked egg white through rubbery stages is similar to that observed for developing plant embryos that are actively producing and segregating the vacuolar seed proteins, the aleurins. A detailed account of the ultrastructure of the process in developing embryos of cotton, peanut and shepherd's purse is given elsewhere (Dieckert and Dieckert, 1975). Dieckert and Dieckert (1972) proposed that the aleurins are synthesized by polyribosomes attached to the outer surface of the endoplasmic reticulum, transported to the dictyosomes, packaged and/or modified by the dictyosomes and sequestered in vacuoles bounded by a single cytomembrane. Since the pattern is similar in developing coconut endosperm, it is now proposed that the vacuolar proteins of the mature coconut endosperm are produced and deposited in vacuoles by a similar process. Unlike the seeds of the aforementioned species, coconut seeds do not desiccate at maturity. The practical result of this difference is that classical aleurone grains do not form. Instead, the aleurins remain in watery vacuoles at maturity. The total body of data available for all seeds makes the hypothesis attractive. The hypothesis is useful here because it helps in understanding the nature of the economically important coconut seed proteins and the effects of environmental stress on the overall process of their production by the palm.

The Coconut Aleurins

The seed proteins located in vacuoles are called aleurins. The principal aleurins appear to function primarily as a revserve of organic nitrogen for use by the germinating embryo. In earlier reports it was shown that most of the protein of mature solid coconut endosperm is confined to vacuoles of the aleurone type where it is present in crystalline and

amorphic forms. Therefore the ultrastructural evidence indicates that the economically important coconut seed proteins are aleurins. In an earlier section electron micrographs were presented that show similar protein deposits in vacuoles at the cooked egg white stage of endosperm maturation. An analysis of the crude protein extracted from mature endosperm and endosperm containing tissue of the cooked egg white stage by SDS gel electrophoresis confirmed that the principal polypeptides of mature coconut endosperm are a subset of those from the immature tissue.

To obtain a better insight into the nature of the principal coconut seed aleurins, the mature proteins of a crude extract of solid coconut endosperm was fractionated by standard salt fractionation techniques. Two principal cuts were obtained. SDS gel electrophoresis showed that the first contained subunits of ∿20,000 and 30,500 molecular weights with a minor contaminant of higher molecular weight. The other contained a polypeptide of 51,500 molecular weight plus some impurities of other molecular weights. This result confirms the work of Dr. Robin Khaund (1971) reported earlier. Isolate 1 was fractionated into its subunits by a method of preparative SDS gel electrophoresis developed in my laboratory. The results are shown in Figure 9. From the densitometric measurement of the protein content of the two principal bands and a knowledge of the molecular weights of the two polypeptides, it is concluded that P_{20} and P_{30} are present in about equimolar quantities. This suggests that isolate 1 has the stoichiometry $(P_{20}, P_{30})_n$. Other data suggest that n is probably 4.

The amino acid composition of the starting material and each of the two subunits was determined on samples hydrolyzed with methane sulfonic acid according to the method of Liu and Chang (1971). Samples of each preparation

were hydrolyzed for approximately 24 hr, 48 hr, and 72 hr and the data used to assess degradative losses and losses due to incomplete hydrolysis. The corrected values were used to determine the mole % of each amino acid. The results are given in Table 1. Assuming the starting material was an equimolar mixture of the two principal subunit classes, the composition of the starting material was estimated from the data for P_{20} and P_{30}. The values obtained are given in the column marked P_{51} Reconst. of Table 1. The results show that the individual subunits have a different amino acid composition and that the reconstructed values for the starting material are similar but not identical with the "reconstructed" values.

Probably the best way to compare proteins for suspected identity or homology is to compare their amino acid sequences. A less satisfactory procedure is to compare their amino acid compositions by the "difference index" method of Metzger et al. (1968). The essence of this procedure is given in Table 2. The Metzger procedure was employed to compare the "reconstructed" P_{51} with the starting material and, more importantly, to compare P_{51} with aleurins from other species.

To provide insight for the use of the difference index, difference indices for selected systems of proteins are cited in Table 3. Even carefully done analyses seldom agree exactly. The first entry in Table 3 compares corrected values of the amino acid composition for two samples of the same isolate of protein done by the same experimenter. Clearly, the difference index should have been zero. That it is not reflects the experimental errors we experienced. Calculation shows that if two proteins contained no amino acid in common, the difference index would be 100. Metzger et al. point out that functionally unrelated proteins usually show difference indices of 15 - 40. Proteins that are homologous, i.e.,

have structural genes with a common ancestor, often have similar amino acid composition and correspondingly low values of the difference index. Examples of homologous pairs of proteins are given in Table 3. Although Metzger et al. noted that homologous proteins usually give values of 15 or less, some homologous pairs give values larger than 15 for the difference index. Porcine insulin : porcine proinsulin are clearly homologous, but a large value of the difference index is observed because the C-peptide is removed from the proinsulin molecule as a post ribosomal modification. Hen egg lysozyme and bovine lactalbumin provide another such instance. In this case, gene duplication and divergent evolution to different functions provides the explanation for the large difference index observed.

If the amino acid composition of P_{51} is correctly reconstructed from the amino acid compositions of the subunits and the assumption of equimolar ratio is correct, then the difference index should be small for P_{51} : P_{51}Reconst. This is the case, as shown in Table 4, and is added confirmation of the proposed stoichiometry for P_{51}.

As reported elsewhere (Dieckert and Dieckert, 1975; Dieckert and Dieckert, In Press) apparently only a few solutions to the problem of storage of organic nitrogen by developing seeds have survived the evolutionary selection process. It is of interest, therefore, to see if P_{51} is homologous to aleurins from other species of higher plants. The results of comparing the amino acid composition of P_{51} with selected aleurins from other species of higher plants is given in Table 4. Clearly, P_{51} is similar in amino acid composition to legumin from V. sativa and V. faba, glycinin from G. max, arachin from A. hypogaea and two unnamed aleurins from cottonseed.

Legumin, glycinin, arachin and P_{51} seem to be of the $(A \cdot B)_n$ type stoichiometry (Dieckert and Dieckert, In Press). A pairwise comparison of P_{20} and P_{30} from coconut endosperm with the subunits of the legumin type protein is interesting (Tables 5 and 6). By the Metzger criterion for homology P_{20} is homologous to A (V. sativa), β (V. faba) and P_{23} (A. hypogaea). Similarly, P_{30} is homologous to B and C (V. sativa), α (V. faba) and P_{43} (A. hypogaea).

The similarities in the ultrastructural patterns associated with production and segregation of the storage proteins in a range of species of plants that are phylogenetically quite different, and the similarities in the amino acid composition of key aleurins from each, lend additional support to the present interpretation of coconut seed proteins and their biosynthesis and sequestration in vacuoles.

Effects of Water Stress

A natural experiment on the effects of water stress on the production of solid endosperm is repeated yearly on the coconut palms on St. John Island. The hypothesis is made that water stress at key times in the flower and fruit development cycle severely limits the production of solid endosperm. The most sensitive phases are related to periods of maximum active growth of a tissue or organ. Relevant data presented in earlier reports and newly acquired data are pooled and presented here in support of the water stress hypothesis.

First, some preliminary matters must be considered. Three coconut palms located in environmentally different sites on St. John Island, American Virgin Islands, received detailed attention over a relatively long period of time. Palm 18 and Palm 42 grew under essentially natural conditions and did not receive any cultural attention. Palm 18 is located just above the

high tide mark on a narrow coral sand beach at Little Cinnamon Bay. Its roots are shallow and have ready access to water from the Atlantic Ocean. Fresh water to the roots comes from direct rainfall or run-off from the mountainside above it. A modest stream of surface or subsurface water pours through the root zone after heavy rains. The action of tide and waves probably modifies the salinity of the water in the root zone. Palm 42 is located at the 80' contour on the mountainside above the beach at Little Cinnamon Bay. Its roots are partly covered by an asphalt driveway and the earthen fill beneath it. Rainwater retained by the soil under the driveway and neighboring areas seems to be the main source of moisture for the palm. The hillside is steep, thus any water run-off is rapidly lost. Caneel Bay Palm 2 is located about 1 3/4 miles to the west of Little Cinnamon Bay on the grounds of the Caneel Bay Plantation. This palm grows at the margin of a reclaimed salt pond. An experienced horticulturist takes care of the tree. It receives water, fertilizer and protection from insect attack. When the soil in the root zone becomes waterlogged, a sump pump is used to help remove excess water. All three palms are of about the same age and produce inflorescences regularly.

Tagging records for the inflorescences on Palm 18 and 42 showed large variations in the interval of time between the opening of the spathes of successive inflorescences. Each palm produced a new inflorescence for each new leaf, as indicated by the constant angular spacing of about 144° between the inflorescences. The average time elapsed between the opening of successive spathes for Caneel Bay Palm 2 from day 164, 1974, to day 300, 1974, was 23 days. The interval was 42 days for palm 42 for about the same period. It might be thought that the two palms inherently produce inflorescences at quite different rates, but this is not the case. A long

series of observations were made on the average length of time between the opening of successive inflorescences on Palm 42 and Palm 18. The results are shown in Table 7. The average time elapsed between spathe openings varies from 28 to 42 days for Palm 42 and from 26 to 47 days for Palm 18. The average intervals for Palm 18 and Palm 42 are similar for a given calendar period of time.

Early in the project it was shown that the size of the mature coconut and the quantity of solid endosperm per mature seed varied greatly from inflorescence to inflorescence for individual palms. The weight of endosperm per seed ranged from about 125 g to 326 g. The variation within coconuts taken from a given inflorescence was much less. The variation was so great that efforts to obtain growth curves for the solid endosperm were frustrated. An equation was discovered that adequately relates size parameters of the coconut seed to the quantity of endosperm produced at maturity by a given seed. The equation was established from data gathered for Palm 18. The agreement between the observed values and the calculated values for Palm 18 is illustrated in Figure 10. The solid line is for perfect correlation. Each data point was for a single coconut and no coconuts taken were excluded unless they were clearly defective. One coconut was excluded because the endocarp failed to close at the proximal end (a split) and a mushy hyperplasia of the endosperm developed. Coconuts from 12 inflorescences of Palm 18 with spathe opening dates from 316, 1970, through 79, 1972, are represented. The same equation was applied to coconuts from Palm 42 and Caneel Bay Palm 2. The comparison of the observed values and calculated values is given in Figure 11. The correspondence is very good, but Caneel Bay Palm 2 produced slightly more endosperm per seed than expected, and Palm 42 seemed to produce slightly less.

As pointed out earlier in this report, the growth of the ovule is almost completed by the time the solid endosperm is in the rapid growth phase, and the shape parameters of the ovule are fixed. From these facts, and the equation mentioned in the last paragraph, normalized growth curves were constructed for the endosperm of Palm 18, Palm 42 and Caneel Bay Palm 2. The ratio of the observed fresh weight of solid endosperm to the calculated value for the mature seed was computed for each coconut. The average value, E_o/E_c, for each inflorescence was then plotted as a function of the age of the inflorescence as measured from the estimated calendar day of spathe opening. The resulting normalized growth curves for the endosperm of Caneel Bay Palm 2, Palm 18 and Palm 42 are given in Figures 12, 13 and 14, respectively.

The normalized growth curves for the endosperm of the three palms do not superimpose and in the case of the graphs for Palms 18 and 42 the values for the ratio E_o/E_c do not cluster tightly about the graph. The graph for Caneel Bay Palm 2 is based on coconuts collected May-June, 1974, and November-December, 1974. Three sets of data collected in February, 1972, September-October, 1972, and April, 1973, were used for the growth curve for Palm 18. And, two sets of data collected in October-November, 1973, and May-June, 1974, were used to develop the growth curve for the endosperm of Palm 42. The important observation here is that the calendar time period of major endosperm growth for coconuts of a given inflorescence is variable for each palm. That is, depending upon when the spathe opened, the endosperm from the associated coconuts grows maximally at different seasons of the year. One might expect that environmental factors that vary with the seasons will affect the growth of the solid endosperm. It is interesting that the growth curve is shifted to left for the managed palm,

Caneel Bay Palm 2. The normalized growth curves indicate that the endosperm achieves maximum fresh weight about 365 days post spathe opening.

A periodic function is obtained if the average of the calculated weights of the endosperm produced per seed for a given inflorescence is plotted as a function of the day the spathe opened (Figure 15 for Palm 18, Figure 16 for Palm 42). Averages of the observed mature weight of endosperm per seed for a given inflorescence follows the same pattern. The calculated values were used so that most of the immature coconuts could also be used for the study. The graphs tend to repeat themselves on a calendar year basis, but not exactly. This type of response suggests a seasonal environmental variable that does not repeat itself very well. The fact that the effect was largely abolished for Caneel Bay Palm 2, which received water regularly, suggests that rainfall is the variable of primary importance.

The rainfall in the American Virgin Islands is known to be unevenly distributed over the year. January through May tend to be dry and June through December tend to be wet. Figures 17 through 21 give the monthly rainfall records for St. John Island for the period of the present study. Each value is the average of the monthly rainfall reported for Cruz Bay and Caneel Bay Plantation. The former is maintained by the Virgin Islands National Park and the latter by the Caneel Bay Plantation. A comparison of the rainfall distributions shows that the general pattern of rainfall was as I have indicated -- dry the first five months and wet the last seven months. However, the variations in the distribution for 1970 through 1974 were great.

It is of interest to see if the observed variations in time interval between the opening of successive spathes, variations in the size of the

mature ovule and, consequently, the quantity of endosperm produced per seed at maturity, and the variations in the normalized growth curves of the solid endosperm can be correlated with variations in rainfall.

It was not feasible to measure the rainfall received by each palm directly. Instead, the rainfall for the area was estimated from daily records maintained by the National Park Service for Lameshur Bay and Cruz Bay and by the Caneel Bay Plantation at the Plantation. The records for Lameshur Bay are incomplete but did serve to verify the evenness of the rainfall for the island on a day by day basis. The time interval covered by the study was divided into consecutive 10 day intervals, and the rainfall for each 10 day interval was determined for the Cruz Bay and Caneel Bay Stations. The averages of the corresponding values for each station were plotted as functions of the corresponding calendar intervals to give the rainfall histograms shown in Figures 22 through 26. These were used to estimate the rainfall during suspected sensitive growth periods.

As shown in Table 8 the interval of time between the opening of successive spathes was shortest during the periods when the average 10-day rainfall was the highest. The average of 26 and 28 days for Palms 18 and 42 compare favorably with the observed average of 23 days for Caneel Bay Palm 2, which received supplemental water during dry periods. There is an anomaly for the period 85, 1973 to 294, 1973. The average rainfall improved somewhat, but the average interval between spathe openings did not decrease. The significance of the observation is not clear.

As stated earlier, the average calculated weight of fresh endosperm per seed at maturity per inflorescence is a periodic function of the estimated day of spathe opening for the inflorescence (See Figures 15 and 16).

The function does not repeat itself exactly year by year. There is, however, a correlation between the total rainfall for the estimated period of maximum growth of the ovule and the average fresh weight of endosperm per seed produced at maturity for the corresponding inflorescence, as shown in Tables 9 and 10. The calendar period when the ovules of a given inflorescence grew from 10 % to 90 % of maximum, the central 100 days, was estimated (Figure 11) to begin about 84 days after the spathe opened and to end 100 days later. The estimated total rainfall for the central 100 days for each inflorescence was obtained by summing the 10 day rainfall values of the rainfall histograms for the nearest corresponding period. These are the rainfall values reported in Tables 9 and 10.

A family of fortuitous experiments were in effect for Palms 18 and 42. The data are grouped so that inflorescences that opened on about the same calendar day, but in different years, are near neighbors in the tables. Except for a few cases the higher the rainfall for the central 100 days of ovular growth, the higher the average endosperm weight calculated for the coconuts of the inflorescence. At first glance this is rather surprising, since no account is taken of the distribution of rainfall within the central 100 days of growth or of the period preceding it. When the rainfall is not evenly distributed over the central 100 days or the time immediately preceding it, anomalous growth is expected. The apparent anomalies marked in the two tables can be explained qualitatively on this basis.

The inflorescence of Palm 18 that opened 231 1972, produced smaller coconuts than expected. The 84 days preceding the central 100 days was much drier than the corresponding period for the inflorescence opening 236, 1971. The dry preperiod for the inflorescence opening 231, 1972,

probably resulted in a dilution of the fresh water lens of Palm 18 with salt water from the Atlantic Ocean, thus reducing the water quality of Palm 18 during the early phase of fruit development. In addition, over three inches of rain, occurring in the central 100 day growth period of the ovules, fell in one 10 day period. The rainfall was more evenly distributed for the central 100 day period for the inflorescence opening 236, 1971. The last effect caused the effective rate of rainfall for the period to be smaller than the total would indicate. A similar argument can explain the second anomalous result for Palm 18. Long dry periods seem to deplete the lens of fresh water feeding the roots of Palm 18, and heavy rains accompanied by extensive run-off seem to augment it. The latter was observed to happen for Palm 18 during the heavy rains of November, 1974. A small stream that flowed for several weeks bathed the root zone of the palm during this time.

The coconuts of Palm 42 showed a similar correlation between the rainfall during the central 100 days of growth of the ovule and the average value of the fresh weight of the endosperm calculated for the fruit of the corresponding inflorescence (Table 10). Two of the inflorescences that opened in 1974 produced less endosperm than expected based on rainfall for the central 100 day growth period. Drought was severe during the first 84 days preceding the central 100 day period, for five of the 10-day intervals registered essentially no rain. Most of the rainfall for the central 100 day growth period came in the last half of the period. There was a better distribution of rainfall for the inflorescence opening 88, 1973, but here the rainfall for the 84 preceding days was erratic. Almost 50 days in this interval went without significant rain. The rainfall

distribution for the critical growth period for coconuts was better, and so was the average calculated value for the frest weight of the endosperm at maturity than the one opening 50, 1973. A similar rainfall pattern prevailed for the inflorescence opening 121, 1974. The first 40 days of the central growth period and most of the preceding 84 days were under drought conditions. Most of the rain fell in the last 60 days of the central growth period of the ovule. The totality of circumstantial evidence clearly shows that water stress during or immediately preceding the central 100 day growth period of the ovule will severely reduce the size of the ovule and, consequently, the fresh weight of the solid endosperm produced per seed. This conclusion is strengthened by the observation that Caneel Bay Palm 2, which received supplemental water during dry periods, did not show the effect.

The data on the direct effect of water stress on the growth of the solid endosperm is limited but unequivocal. As shown in Table 11, the percentage of the expected growth of the endosperm achieved is less during periods when the rainfall for the interval of active growth of the endosperm is low than when it is high. This explains the failure of the data points to cluster more closely about the normalized growth curve for Palm 42 and the apparent shift to the right of the normalized growth curve of Palm 42 with respect to that for Caneel Bay Palm 2. Endosperm from coconuts from Palm 42 grew at almost the same rate as that for Caneel Bay Palm 2 when the average rainfall per 10 day period was the highest. The normalization process does not take into account the effect of water stress on the growth rate of the solid endosperm. This effect probably accounts for some of the variation between the observed and calculated values of the fresh weight of solid endosperm achieved at maturity, as noted in Figures

10 and 11. Since the coconut seed proteins are synthesized during the period of active growth of the solid endosperm, the quantity of protein per unit of fresh weight of endosperm will almost certainly be affected adversely by water stress during this period.

Evidence of the same kind shows that the overall growth of the fruit is affected similarly by water stress. Taken all together the evidence shows that water stress greatly reduces the productivity of the coconut palm when it occurs during periods of active growth of tissues associated with endosperm production. The time elapsed between the opening of successive inflorescences (time of spathe opening) increases with water stress. The ovule and other tissues of the fruit are stunted when drought occurs during the time they are growing rapidly.

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Figure 1.

Crystalline protein deposits in coconut endosperm.

a. Vacuolar protein crystals (PC) in cell of coconut endosperm at cooked egg white stage. X 10,000.

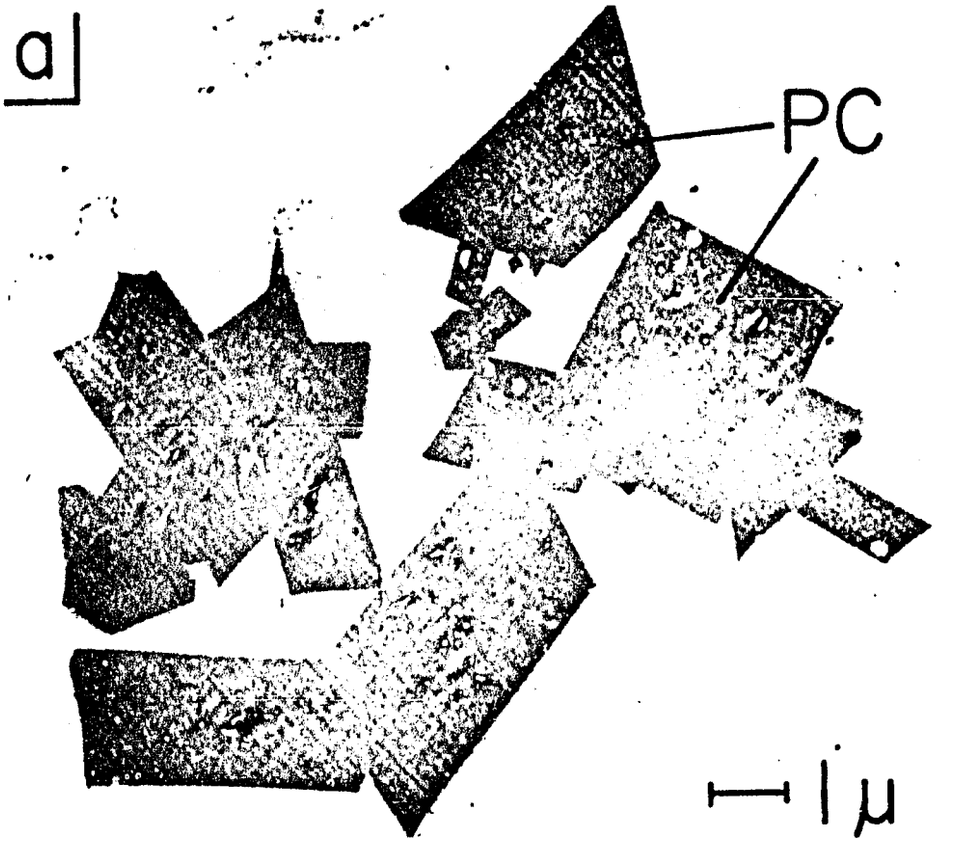
b. Biphasic protein deposits, showing protein crystals (PC) bounded by amorphous protein, present in vacuole of cell of rubbery endosperm. X 15,750.

Figure 2.

Protein digestion experiment.

a. Coconut cell at cooked egg white stage containing protein crystal (PC) which has not been treated with the protease.

b. Serial section of the same cell which shows the results of the protease treatment on the protein crystal. X 17,500.


a
PC
1 μ

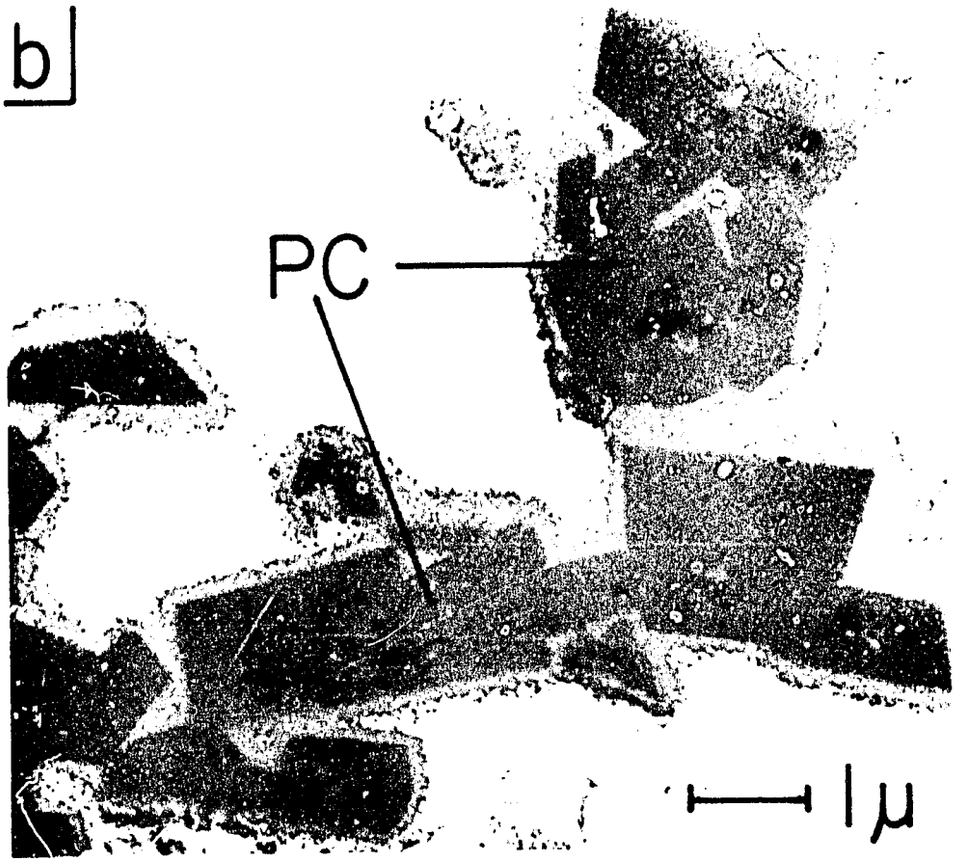
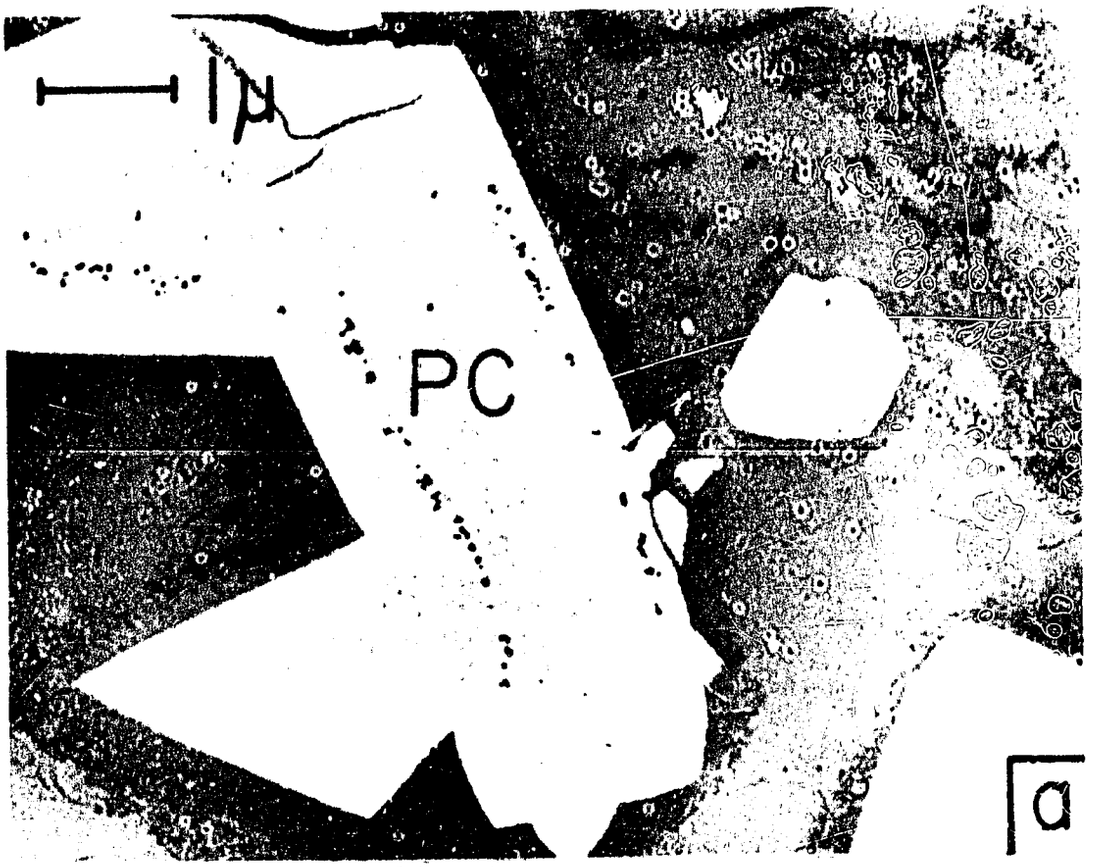

b
PC
1 μ

Figure 3.

Cooked egg white stage of coconut endosperm showing linear array of ribosomes characteristic of the rough endoplasmic reticulum (RER). X 69,875.


1μ
PC
a

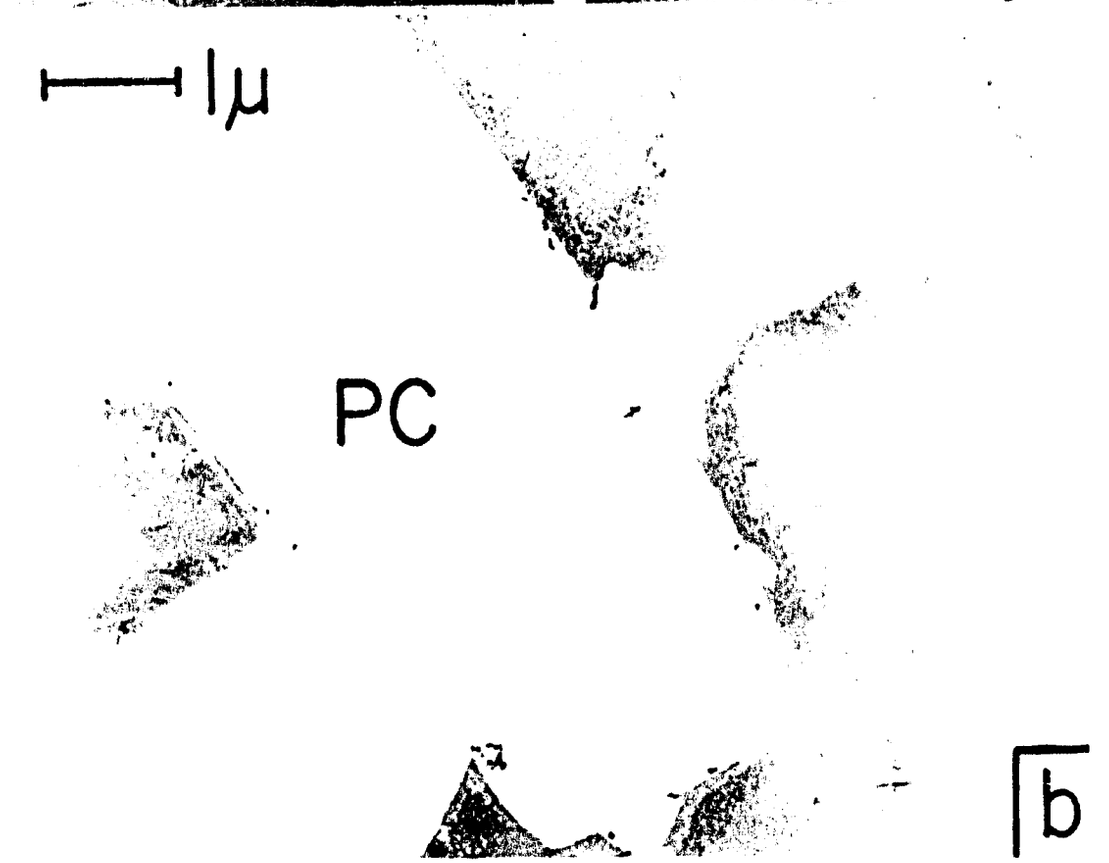

1μ
PC
b

Figure 4.

Coconut endosperm; "cooked egg white" stage.

This portion of a cell from the cooked egg white stage of coconut endosperm shows a dictyosome (D) actively engaged in packaging the protein into Golgi vesicles (GV) for transport to the vacuole, where it will be deposited as vacuolar protein (VP). X 127,500.

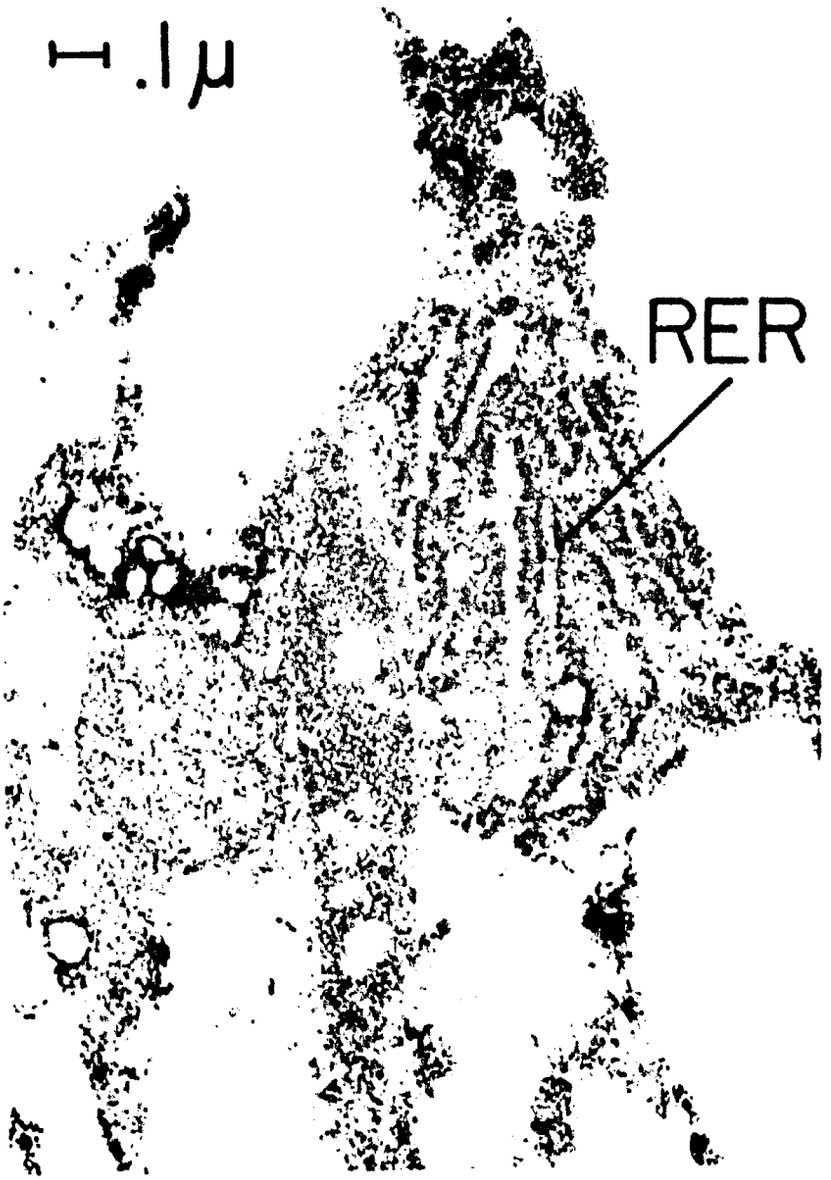

.1μ
RER

Figure 5.

Rubbery endosperm of coconut, showing a dictyosome (D) with protein in its flattened vesicles, also protein-containing granules (G).

X 107,500.

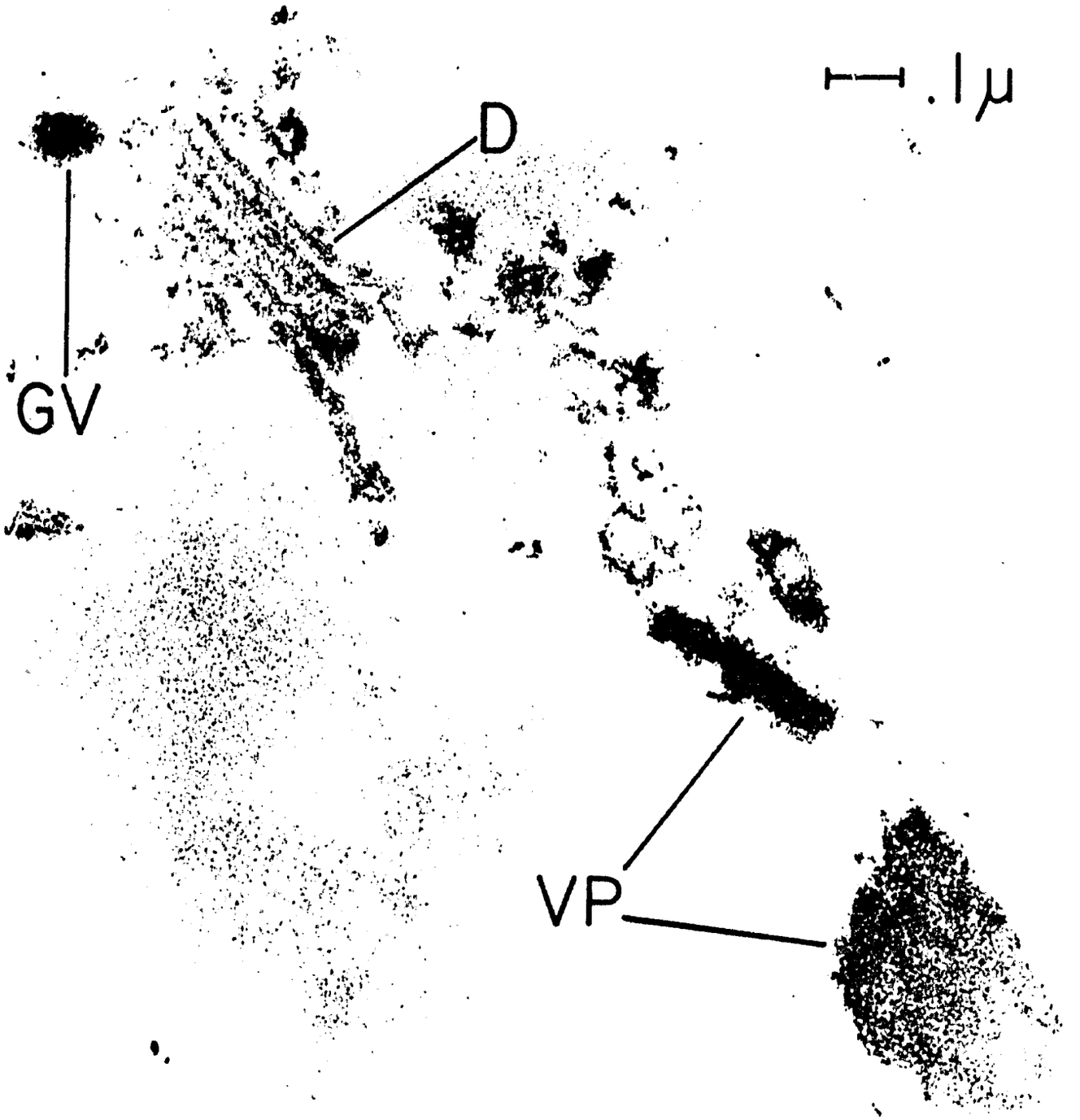

.1μ
D
GV
VP

Figure 6.

Coconut endosperm at the rubbery stage.

This electron micrograph shows a dictyosome (D) containing protein.

X 192,500.

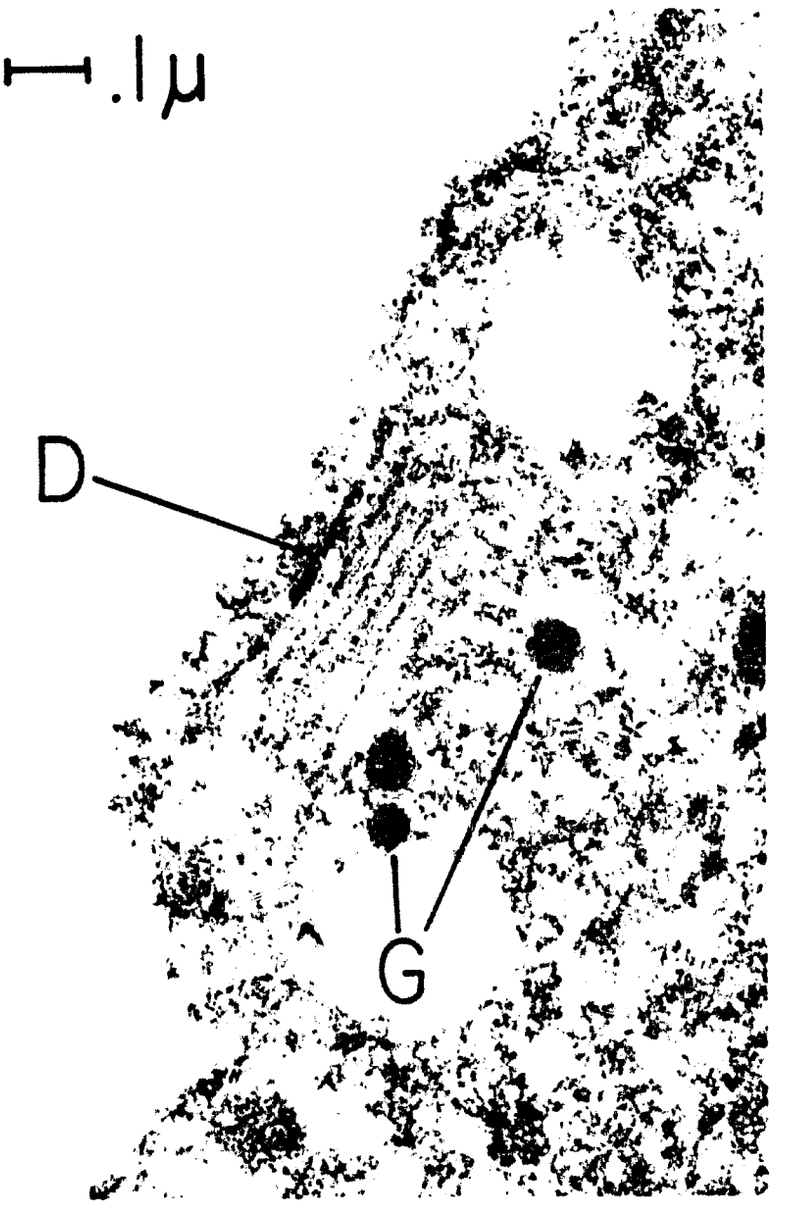

.1μ
D
G

Figure 7.

Coconut endosperm: rubbery stage.

This enlarged view of a dictyosome (D) shows protein within some of its Golgi vesicles (GV). X 107,500.

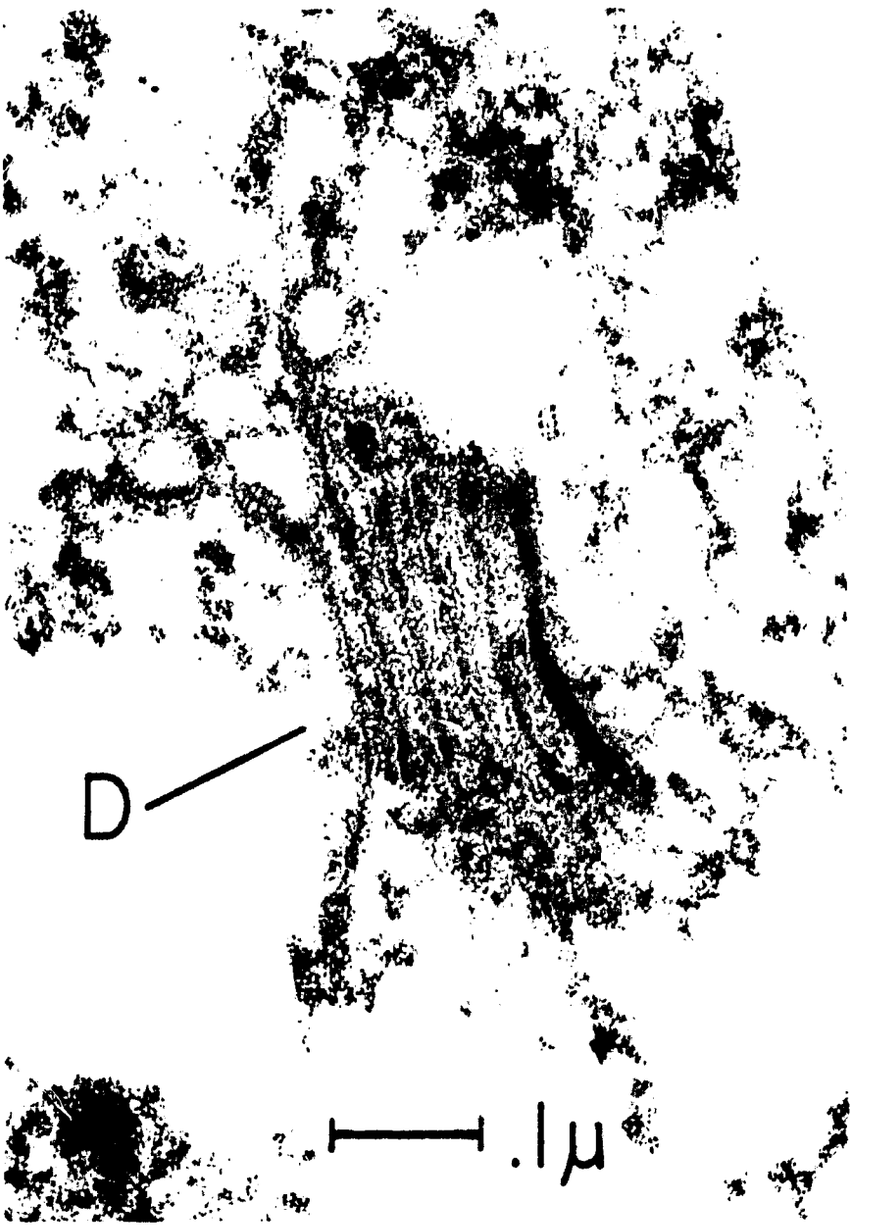

D
.1μ

Figure 8.

Cooked egg white stage of coconut endosperm, demonstrating results of protease experiment. Serial sections.

a. Portion of endosperm cell which has not been treated with protease, showing presence of vacuolar protein (VP), a dictyosome (D) and granules (G) containing protein. X 45,500.

b. Serial section of same cell which has been treated with protease, showing the digestion of the protein from the dictyosome (D), granules (G), and the vacuoles (VP). X 45,500.

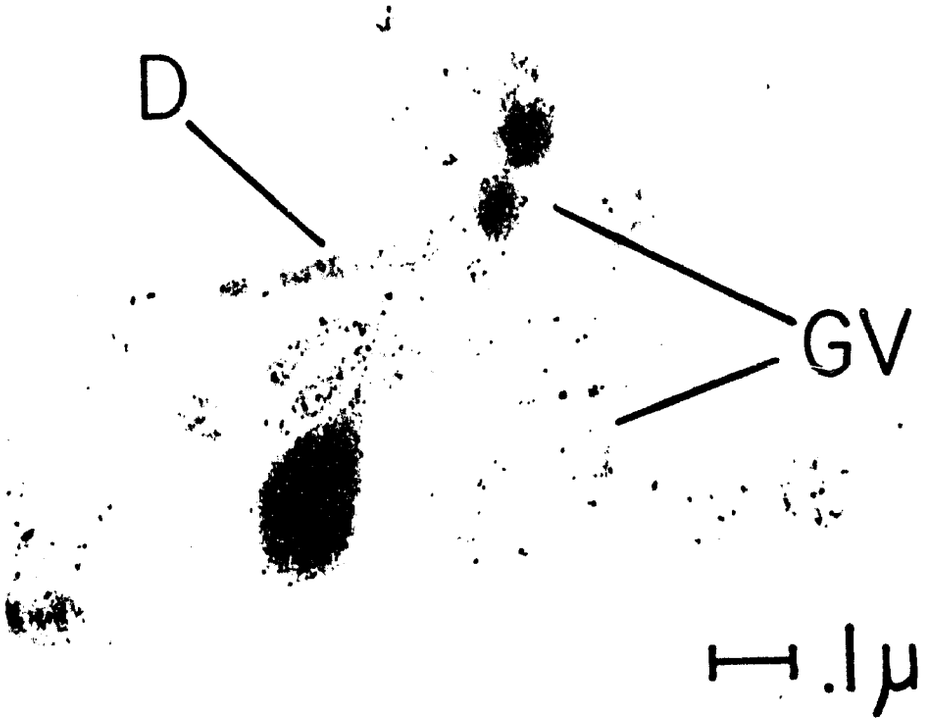
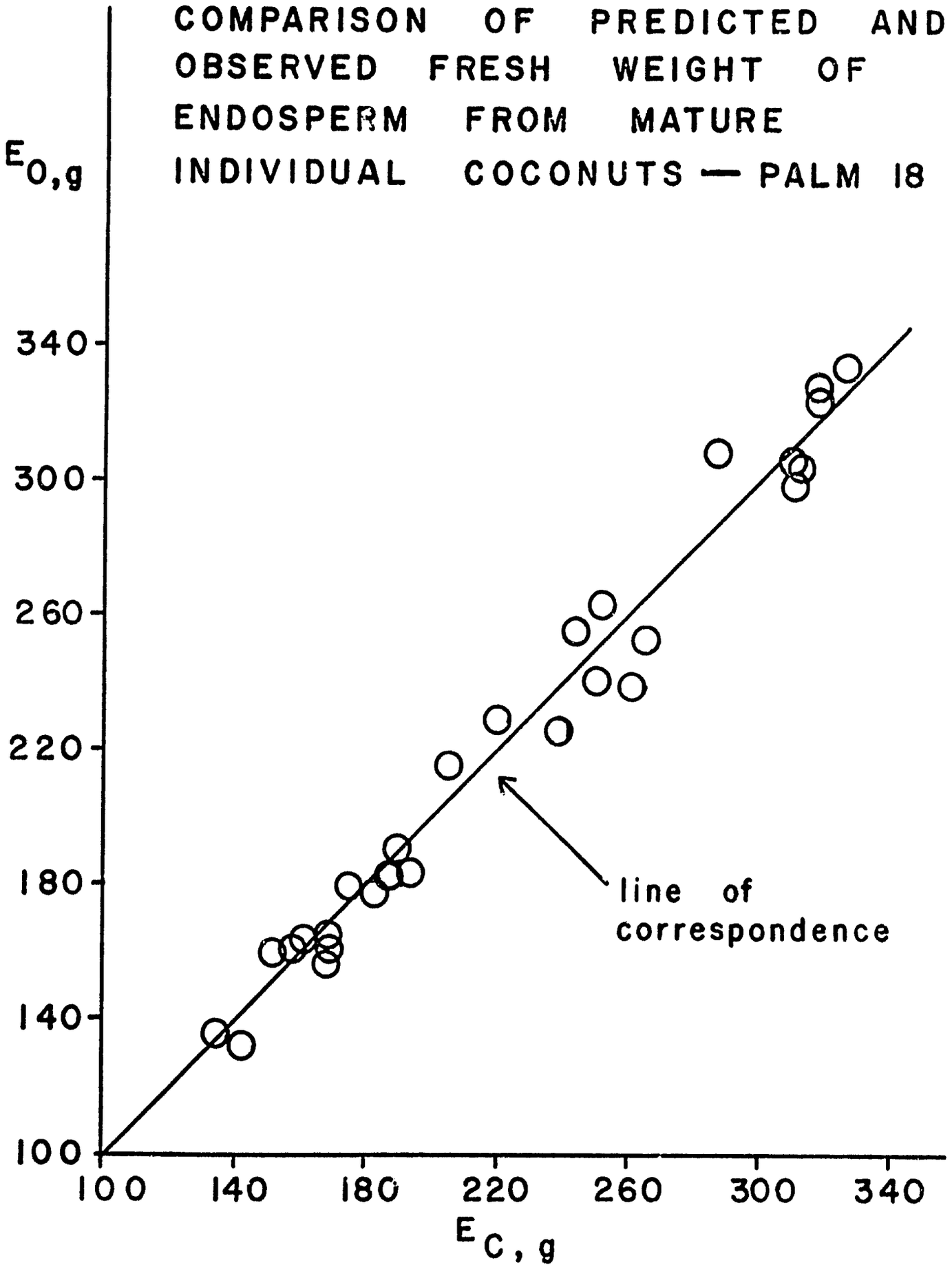

D
GV
.1μ

Figure 10


COMPARISON OF PREDICTED AND OBSERVED FRESH WEIGHT OF ENDOSPERM FROM MATURE INDIVIDUAL COCONUTS — PALM 18
$E_{O,g}$
340
300
260
220
180
140
100
100
140
180
220
260
300
340
$E_{C,g}$
line of correspondence

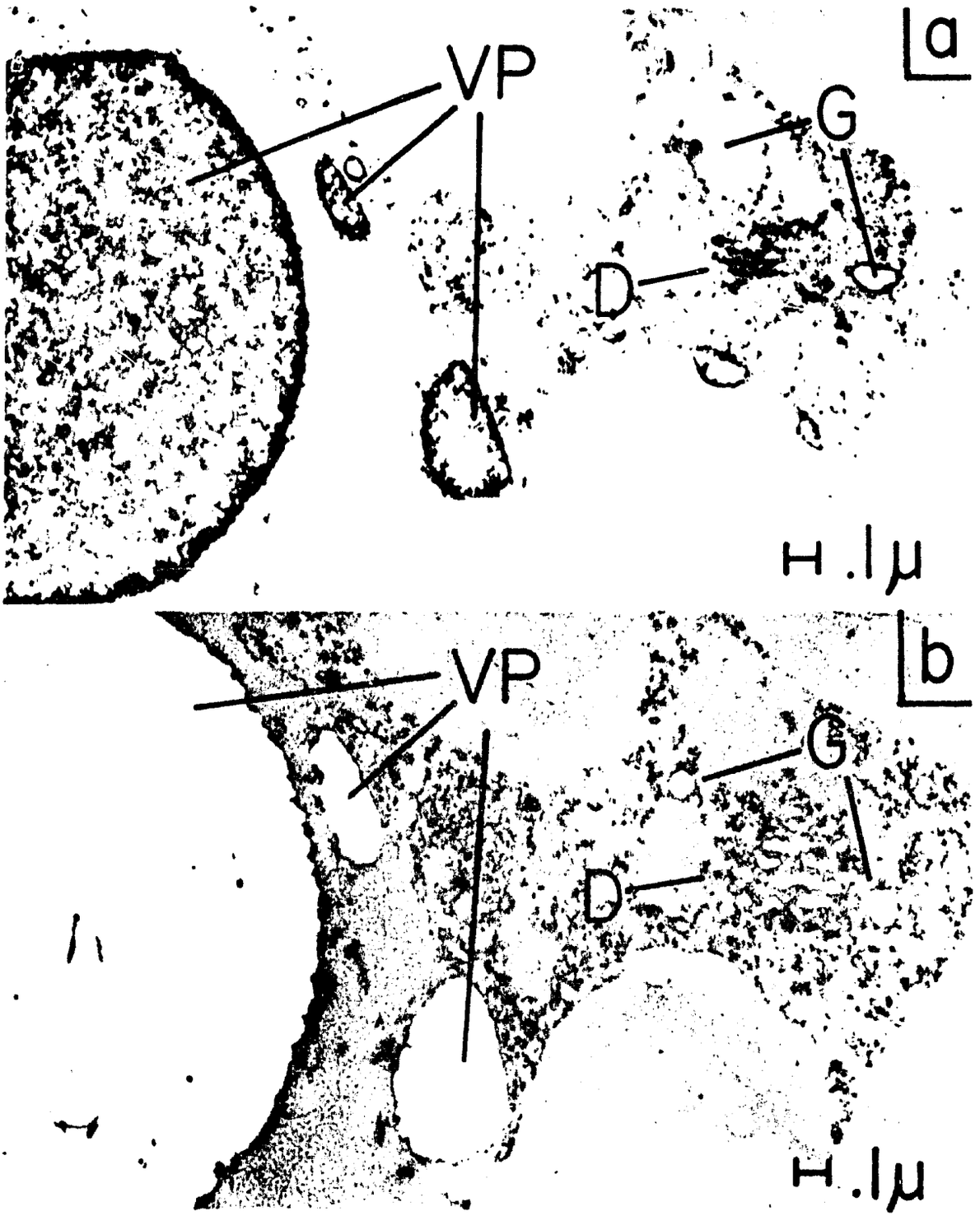

a
VP
G
D
H .1μ
b
VP
G
D
H .1μ

Figure 11

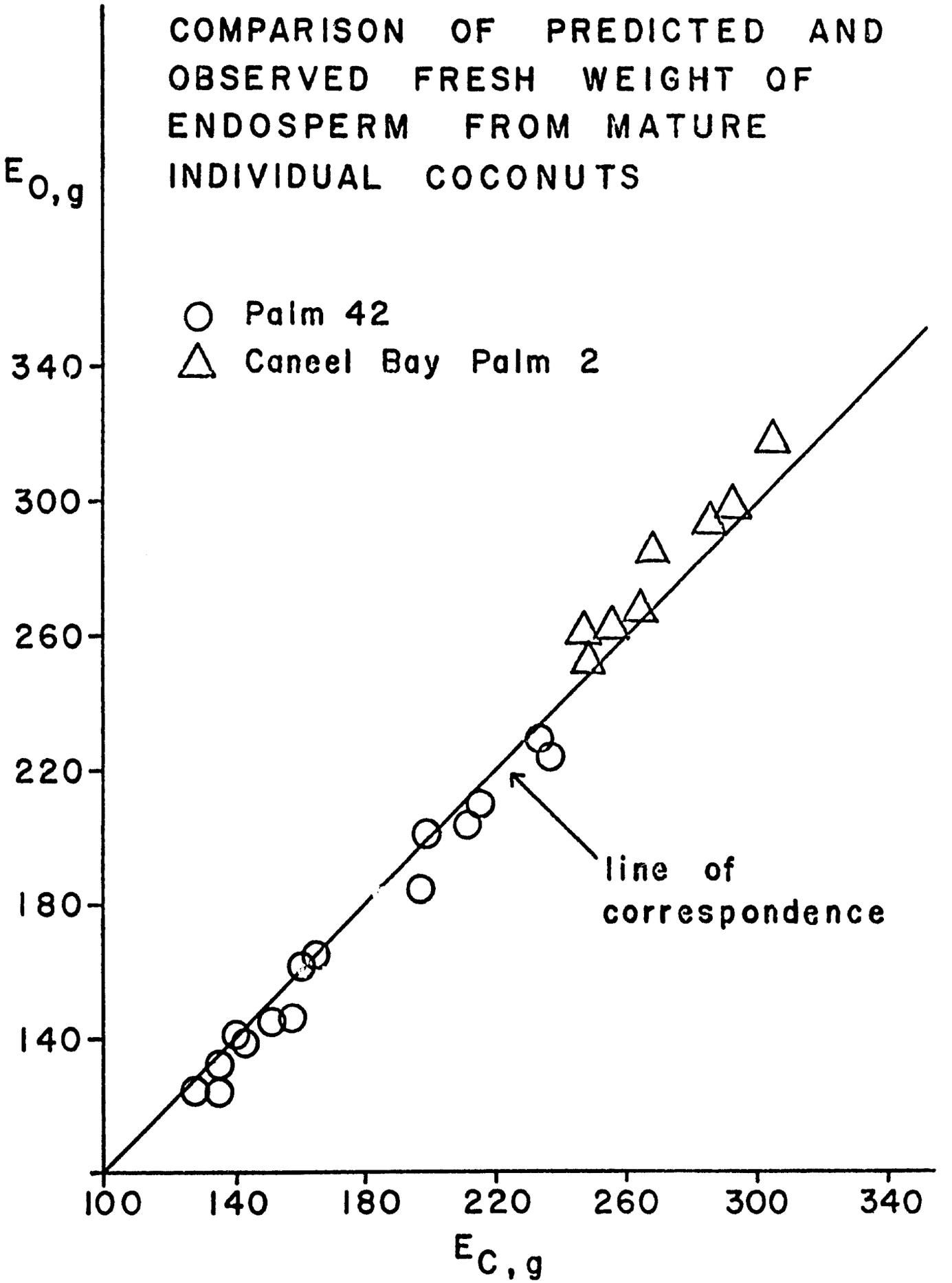

COMPARISON OF PREDICTED AND OBSERVED FRESH WEIGHT OF ENDOSPERM FROM MATURE INDIVIDUAL COCONUTS
$E_{O,g}$
Palm 42
Caneel Bay Palm 2
340
300
260
220
180
140
line of correspondence
100
140
180
220
260
300
340
$E_{C,g}$

Figure 12

NORMALIZED GROWTH CURVE FOR SOLID ENDOSPERM OF CANEEL BAY PALM 2

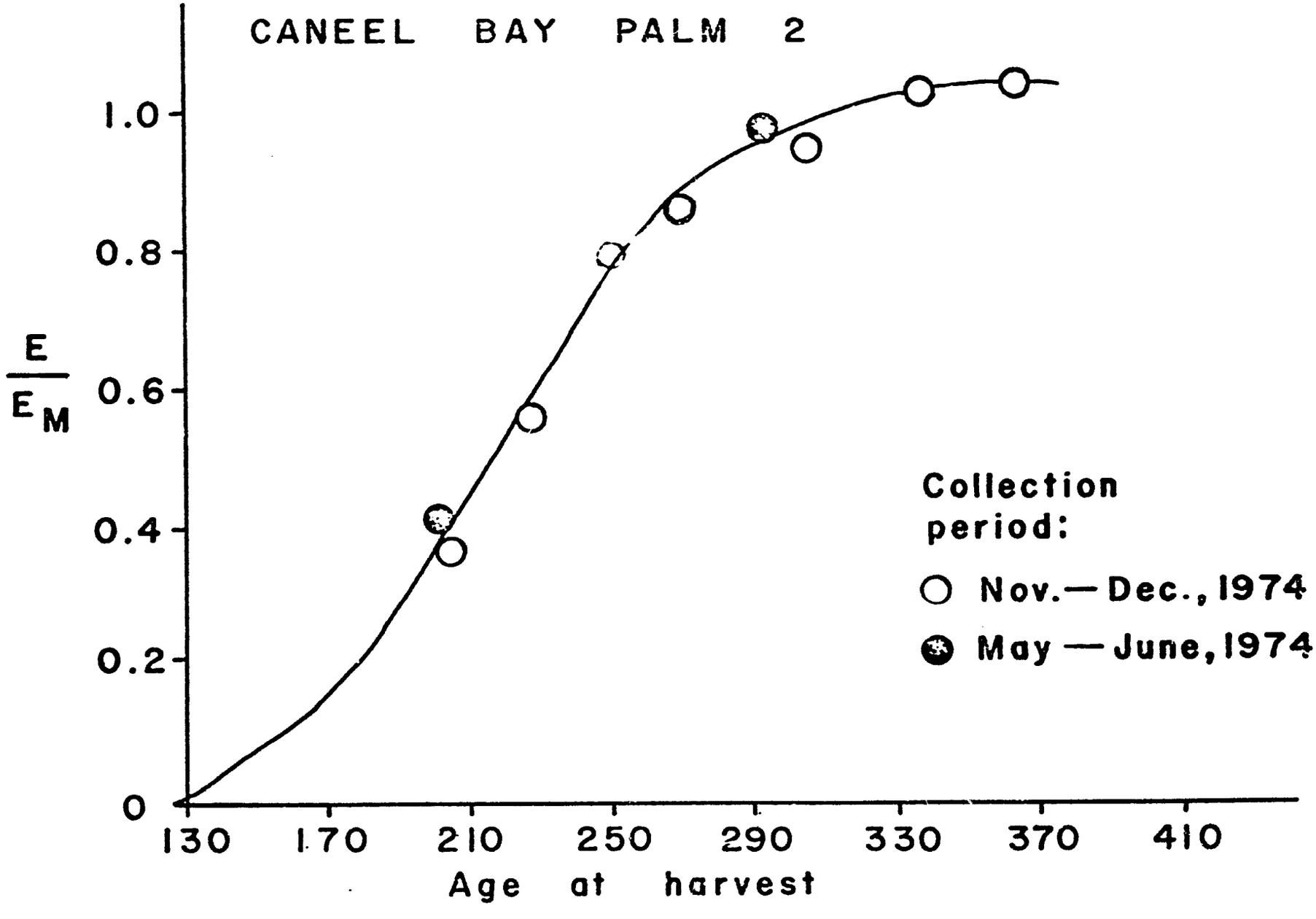


Figure 13

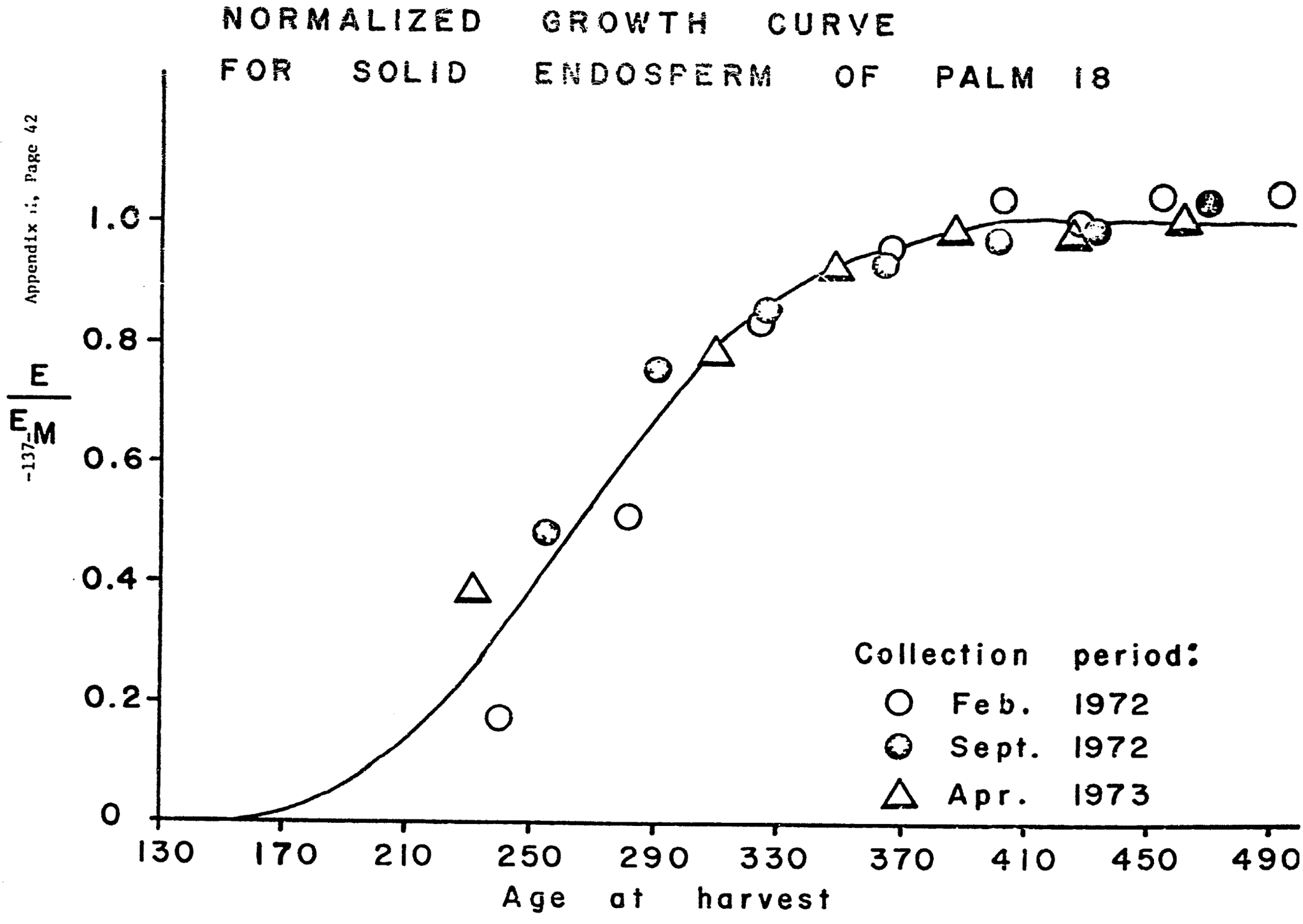

NORMALIZED GROWTH CURVE
FOR SOLID ENDOSPERM OF PALM 18
E / E_M
1.0
0.8
0.6
0.4
0.2
0
130
170
210
250
290
330
370
410
450
490
Age at harvest
Collection period:
Feb. 1972
Sept. 1972
Apr. 1973

Figure 14

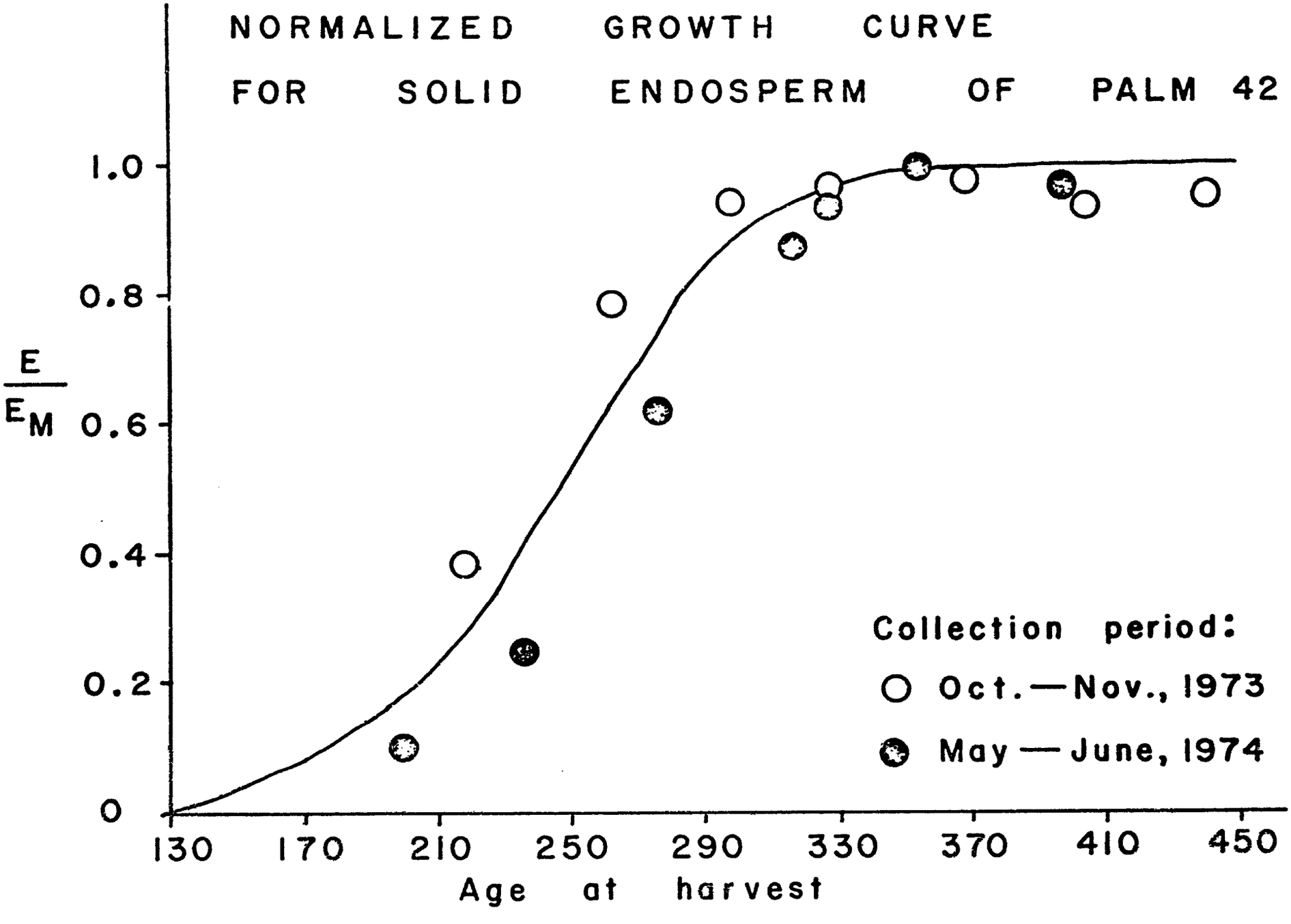

NORMALIZED GROWTH CURVE
FOR SOLID ENDOSPERM OF PALM 42
E / EM
1.0
0.8
0.6
0.4
0.2
0
130
170
210
250
290
330
370
410
450
Age at harvest
Collection period:
Oct.—Nov., 1973
May — June, 1974

Figure 15

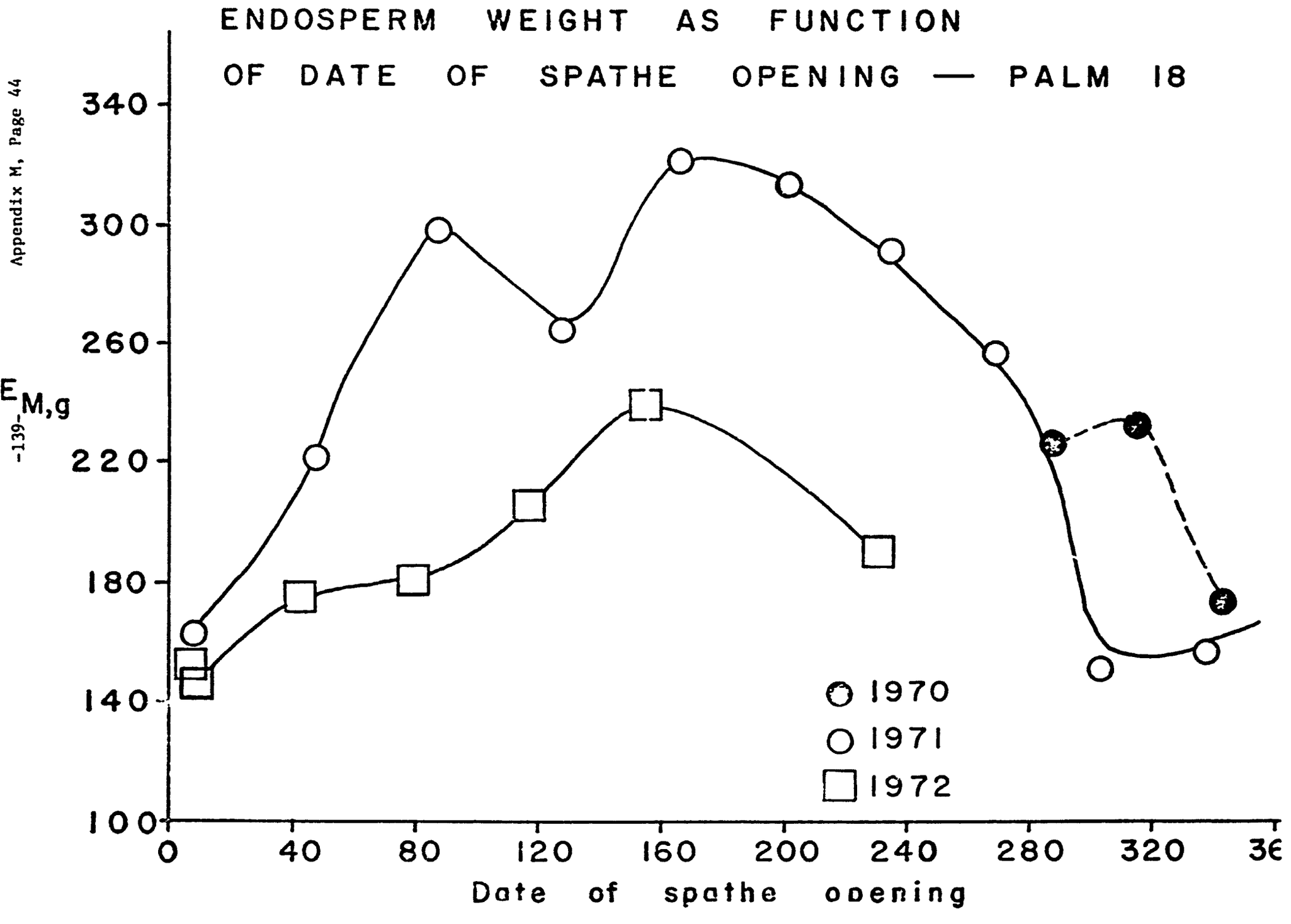

ENDOSPERM WEIGHT AS FUNCTION
OF DATE OF SPATHE OPENING — PALM 18
$E_{M,g}$
340
300
260
220
180
140
100
0
40
80
120
160
200
240
280
320
36
Date of spathe opening
1970
1971
1972

Figure 1

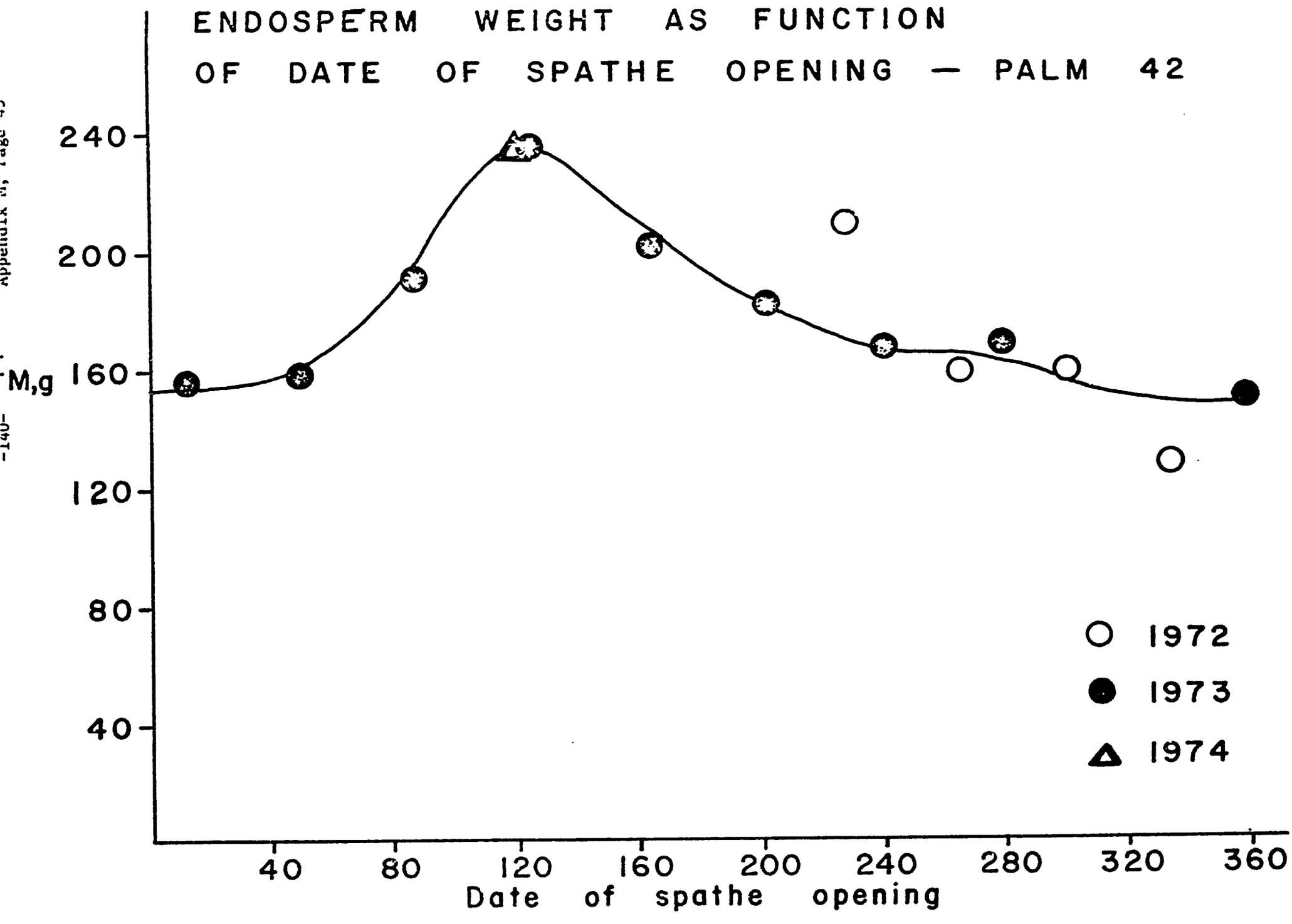

ENDOSPERM WEIGHT AS FUNCTION
OF DATE OF SPATHE OPENING — PALM 42
M,g
240
200
160
120
80
40
40
80
120
160
200
240
280
320
360
Date of spathe opening
1972
1973
1974

Figure 17

Appendix M, Page 46

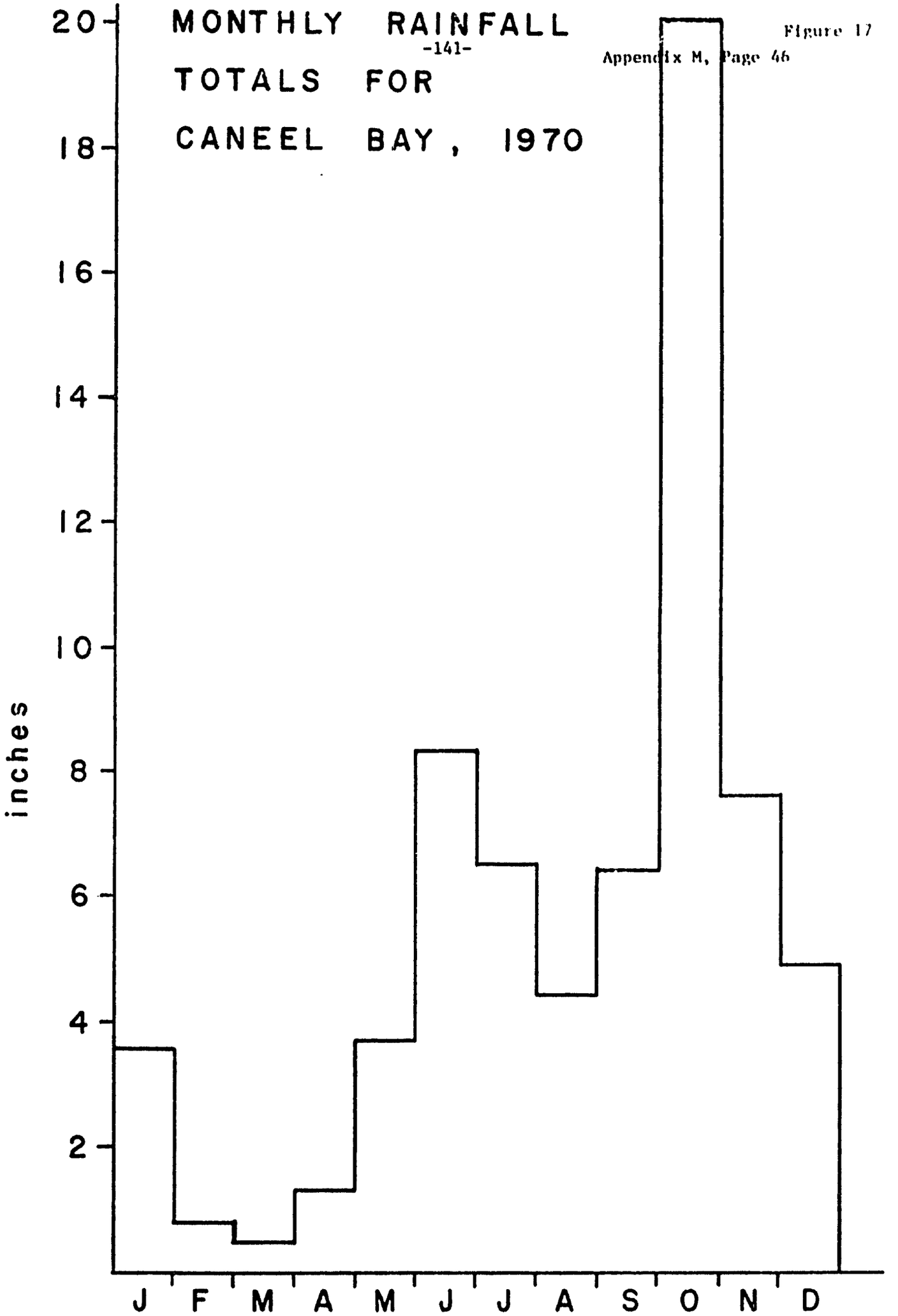


Figure 18

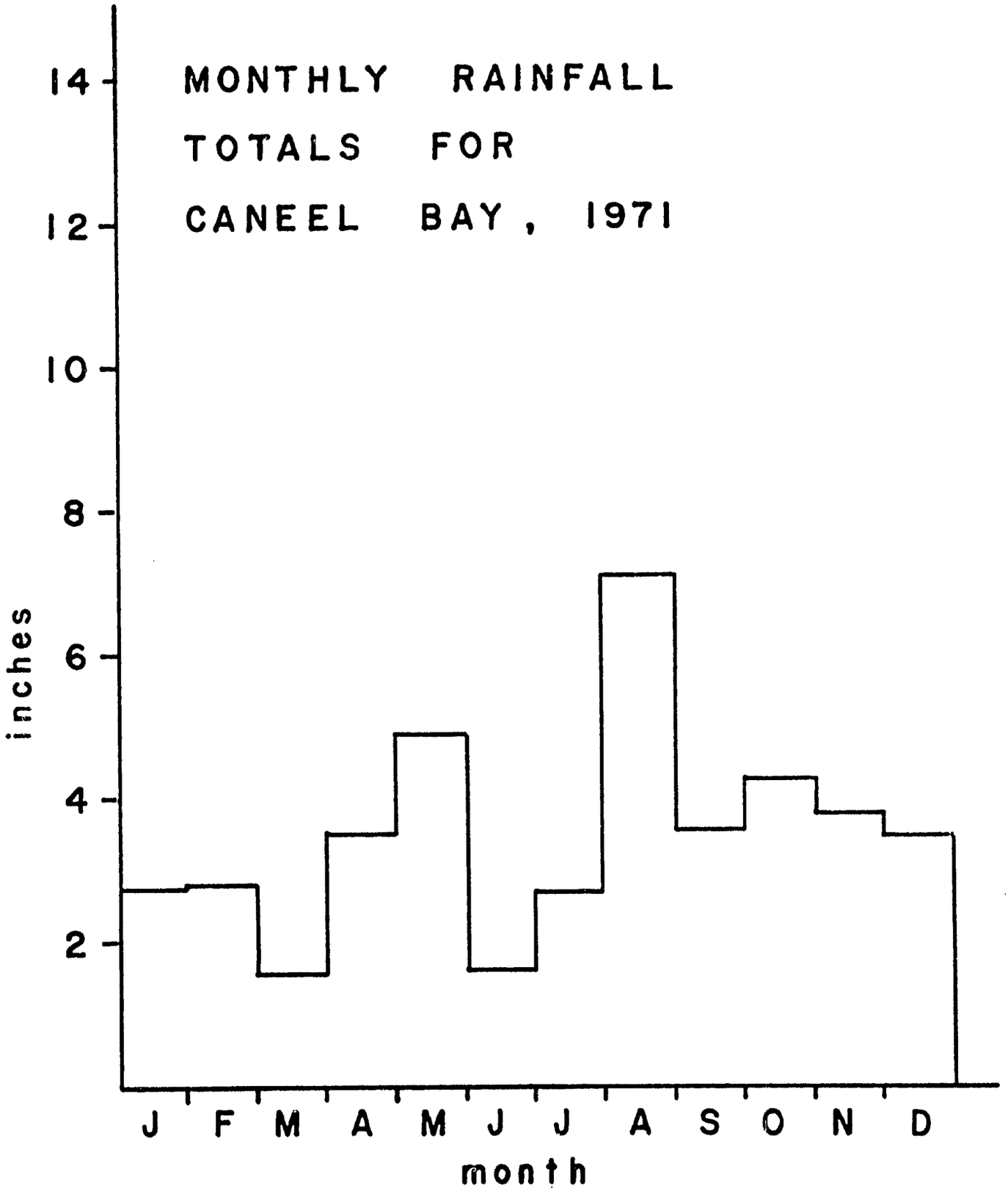

MONTHLY RAINFALL
TOTALS FOR
CANEEL BAY, 1971
14
12
10
8
6
4
2
inches
J F M A M J J A S O N D
month

Figure 19

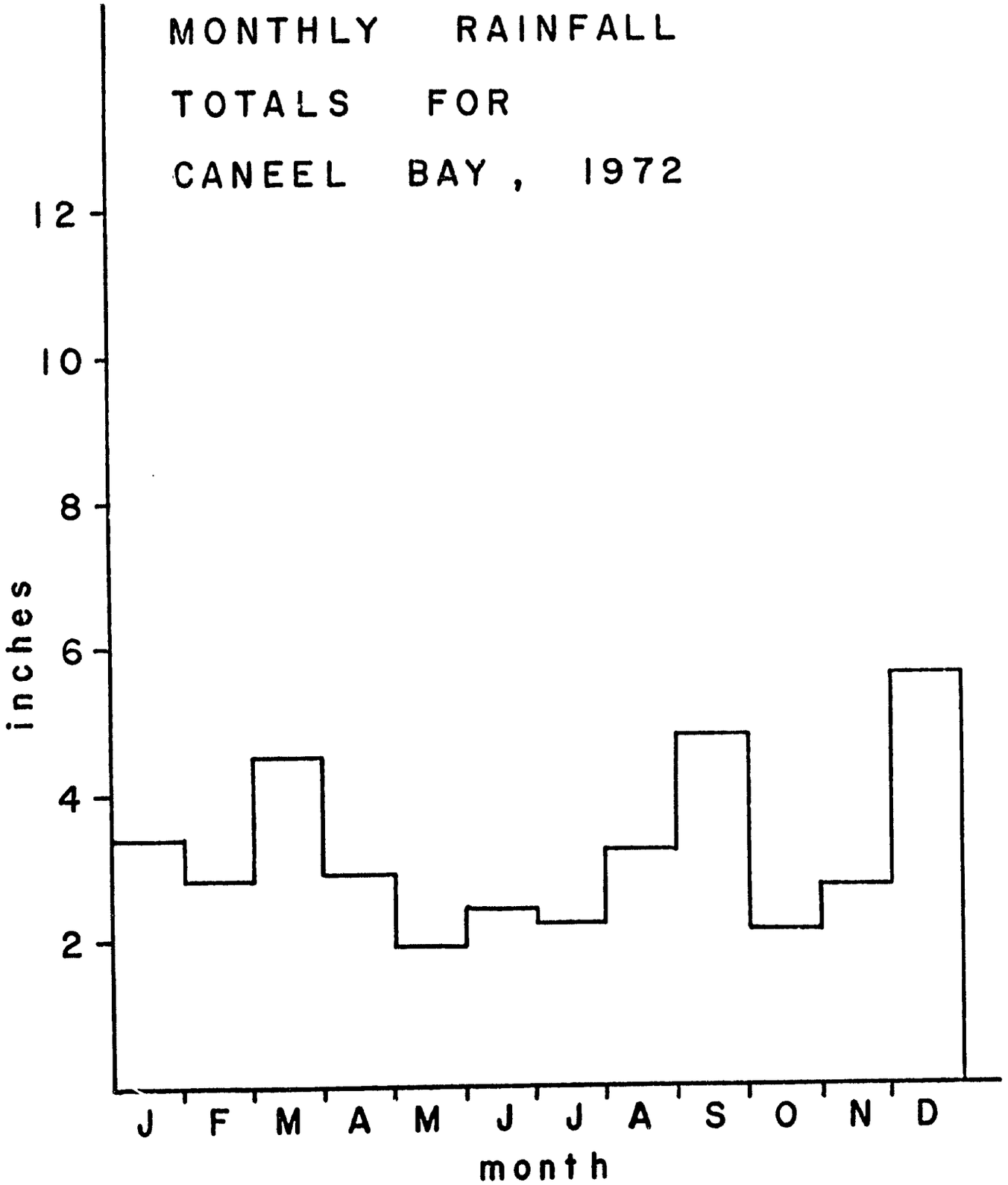

MONTHLY RAINFALL
TOTALS FOR
CANEEL BAY, 1972
12
10
8
6
4
2
inches
J F M A M J J A S O N D
month

Figure 20

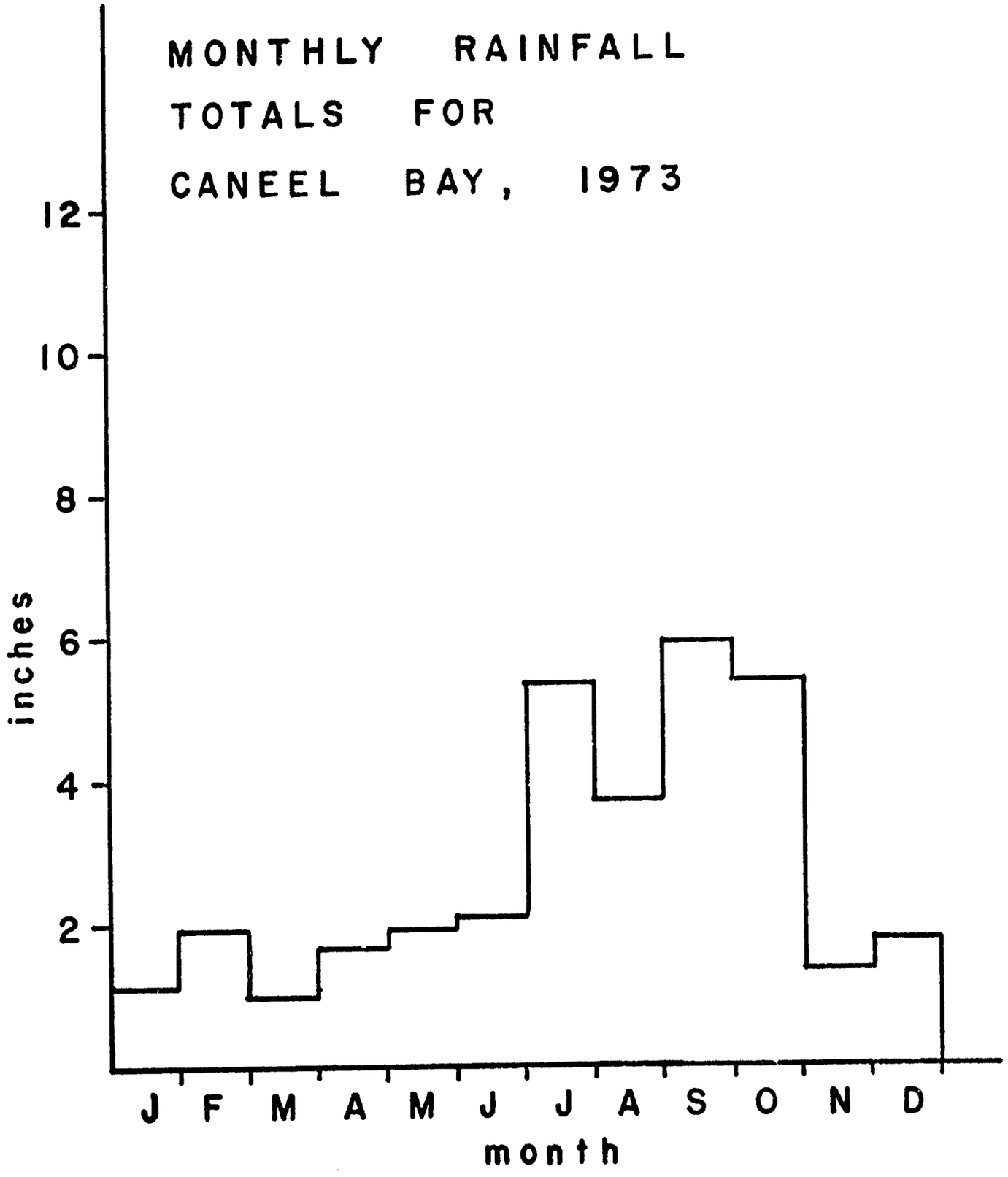

MONTHLY RAINFALL
TOTALS FOR
CANEEL BAY, 1973
12
10
8
6
4
2
inches
J F M A M J J A S O N D
month

Figure 21

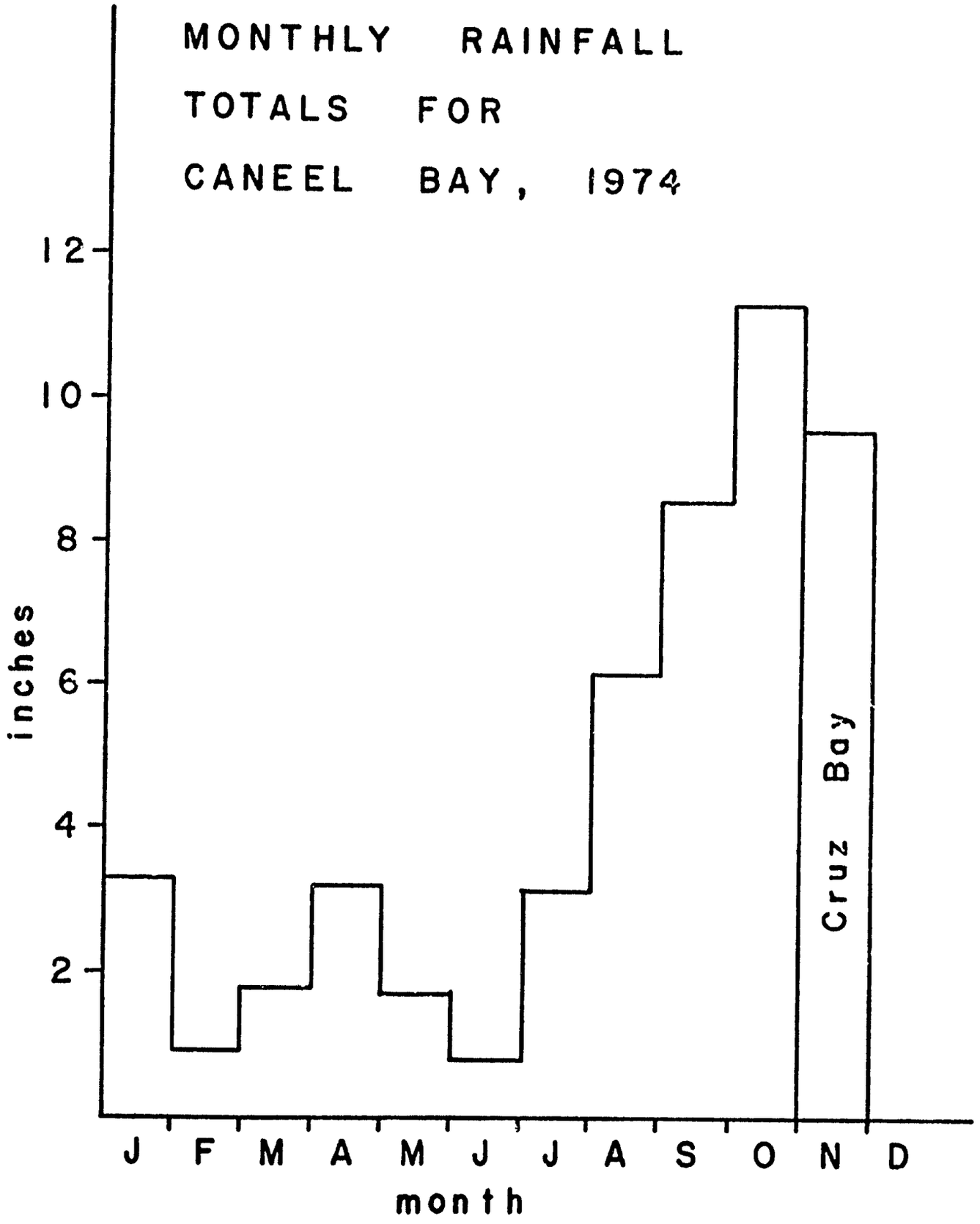

MONTHLY RAINFALL
TOTALS FOR
CANEEL BAY, 1974
12
10
8
6
4
2
inches
Cruz Bay
J F M A M J J A S O N D
month

Figure 22

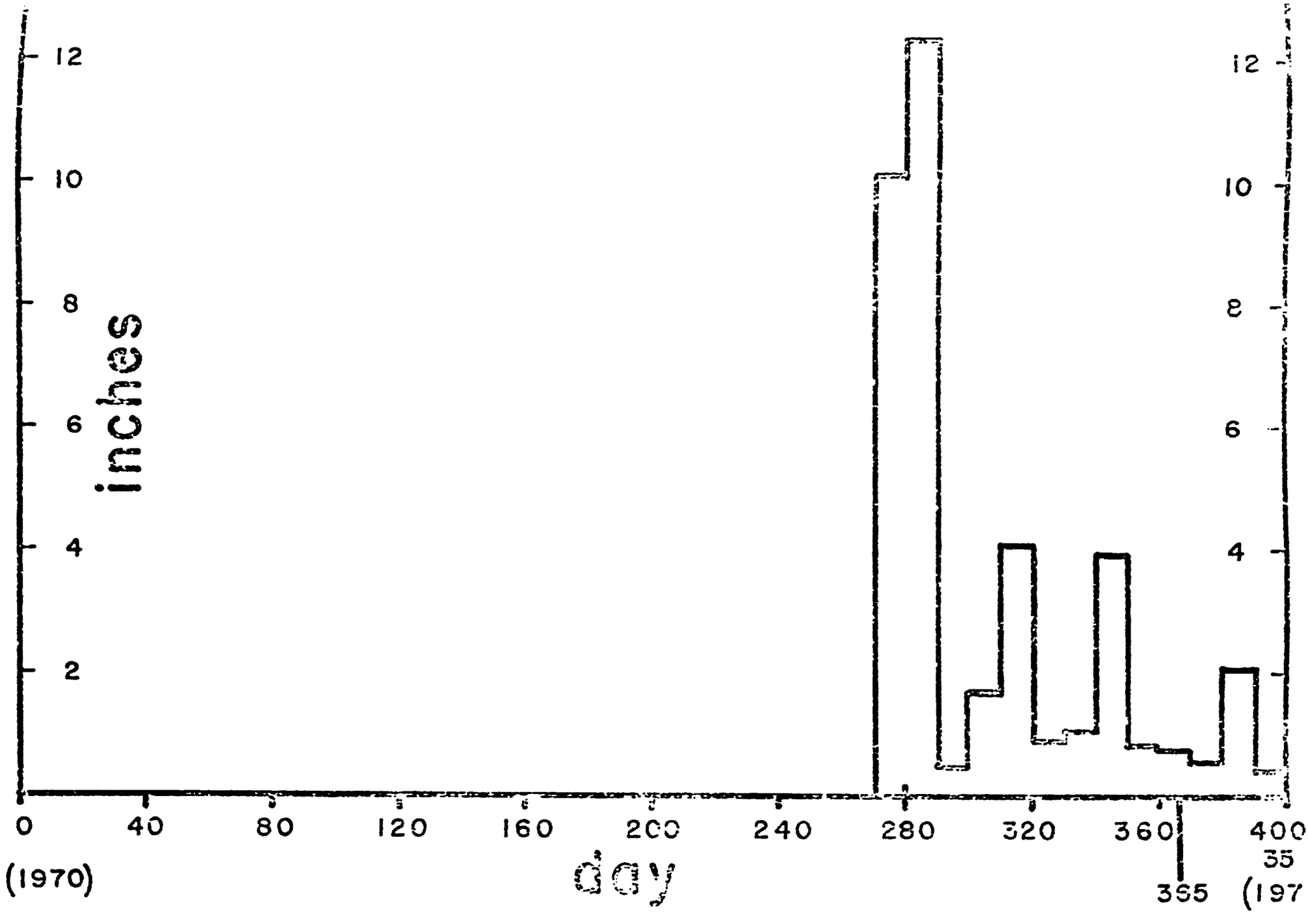

inches
12
10
8
6
4
2
0
40
80
120
160
200
240
280
320
360
400
(1970)
day
365
35
(197

Figure 23

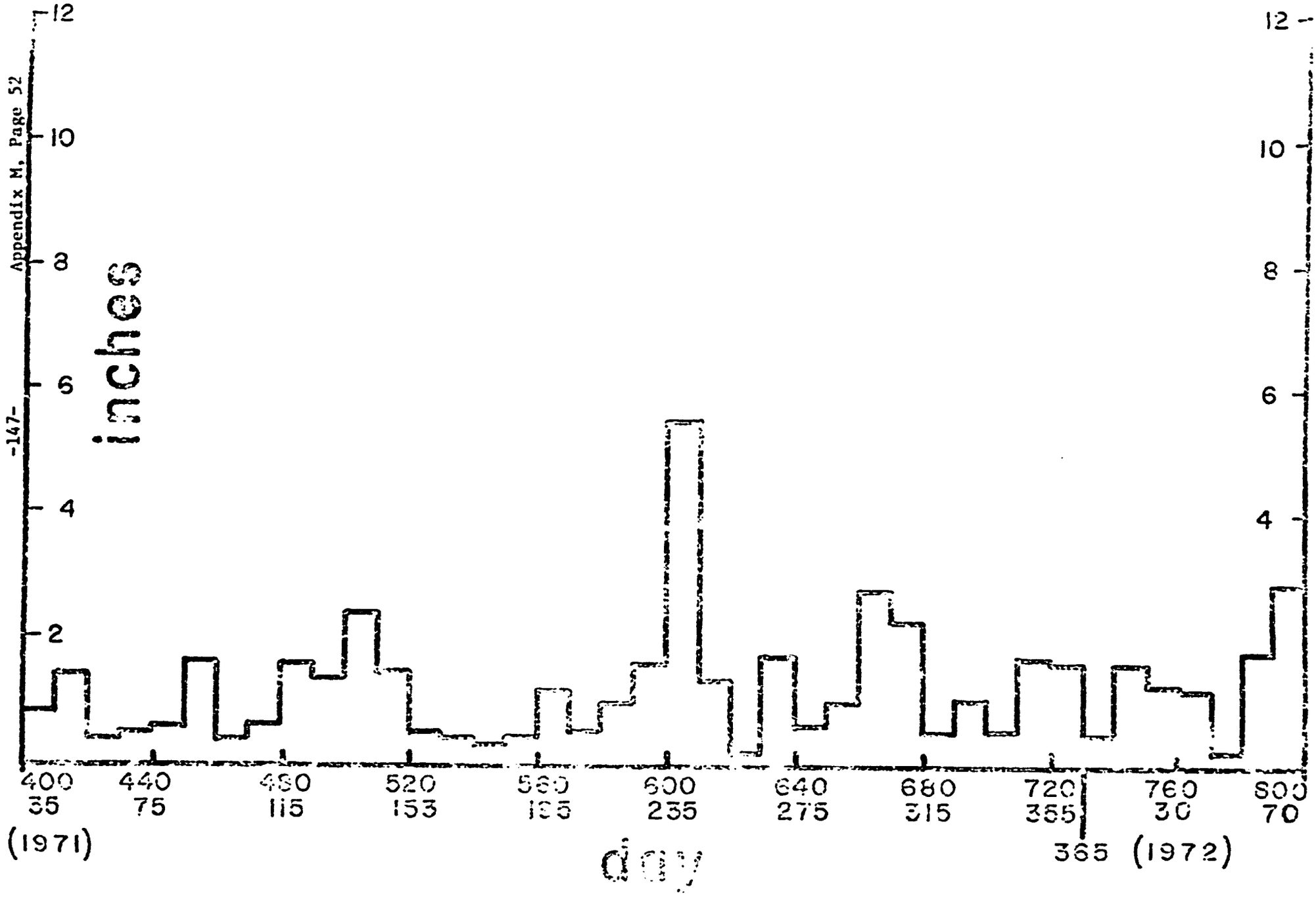

inches
12
10
8
6
4
2
400
35
(1971)
440
75
480
115
520
153
560
195
600
235
640
275
680
315
720
355
365 (1972)
760
30
800
70
day

Figure 4

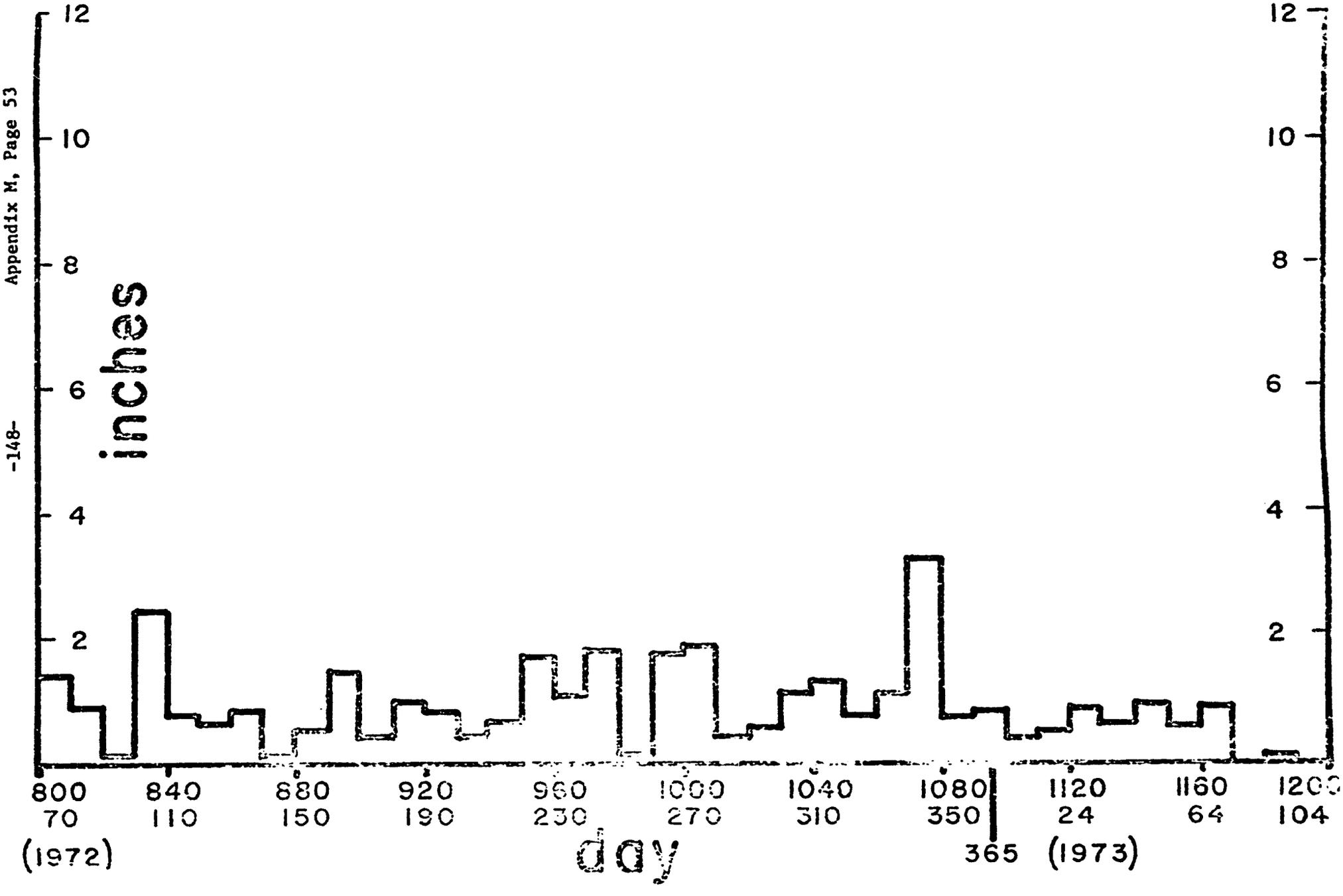

inches
12
10
8
6
4
2
800
70
(1972)
840
110
880
150
920
190
960
230
1000
270
1040
310
1080
350
365
(1973)
1120
24
1160
64
1200
104
day

Figure 25

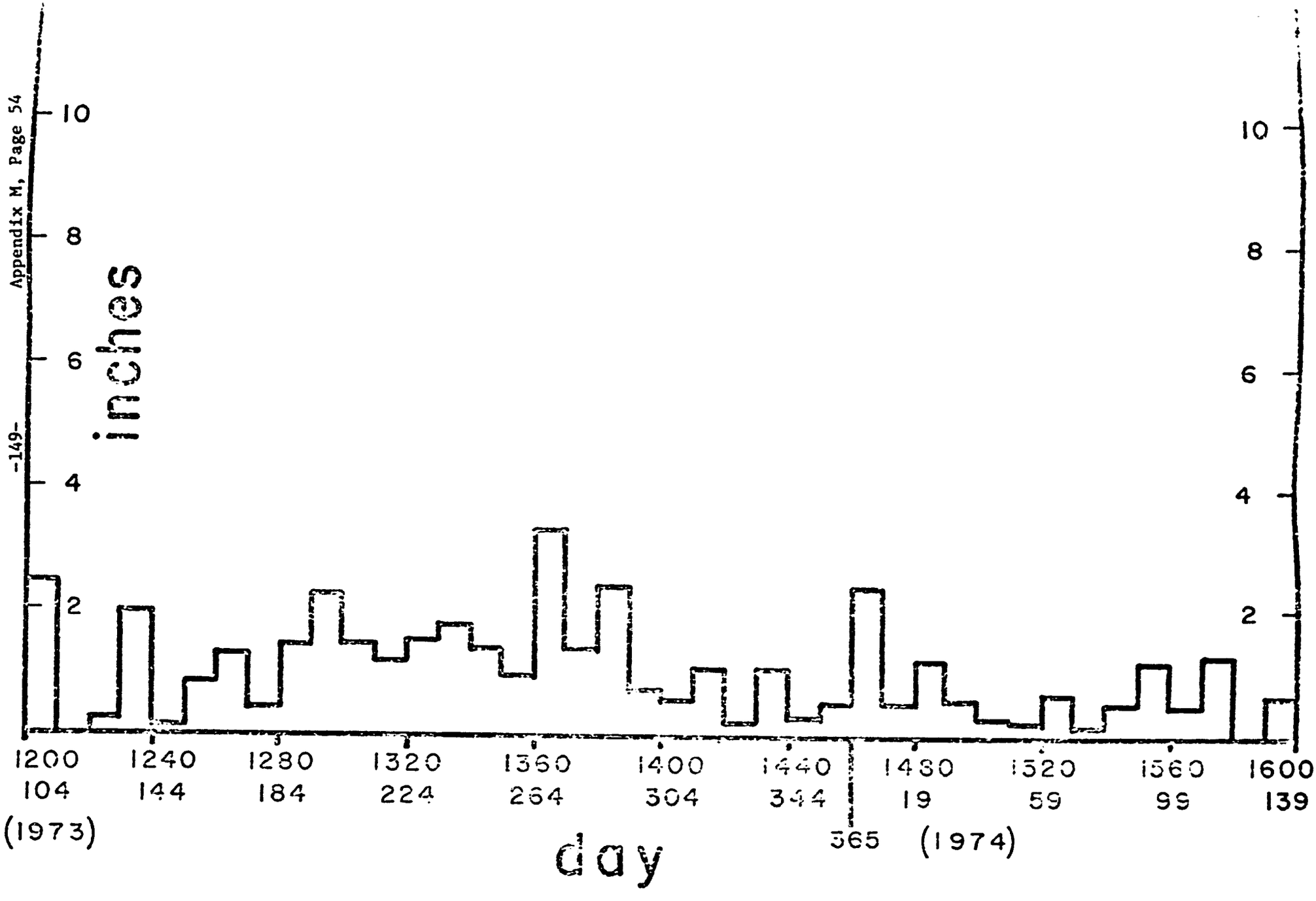

inches
10
8
6
4
2
1200
104
(1973)
1240
144
1280
184
1320
224
1360
264
1400
304
1440
344
365
(1974)
1480
19
1520
59
1560
99
1600
139
day

Figure 2b

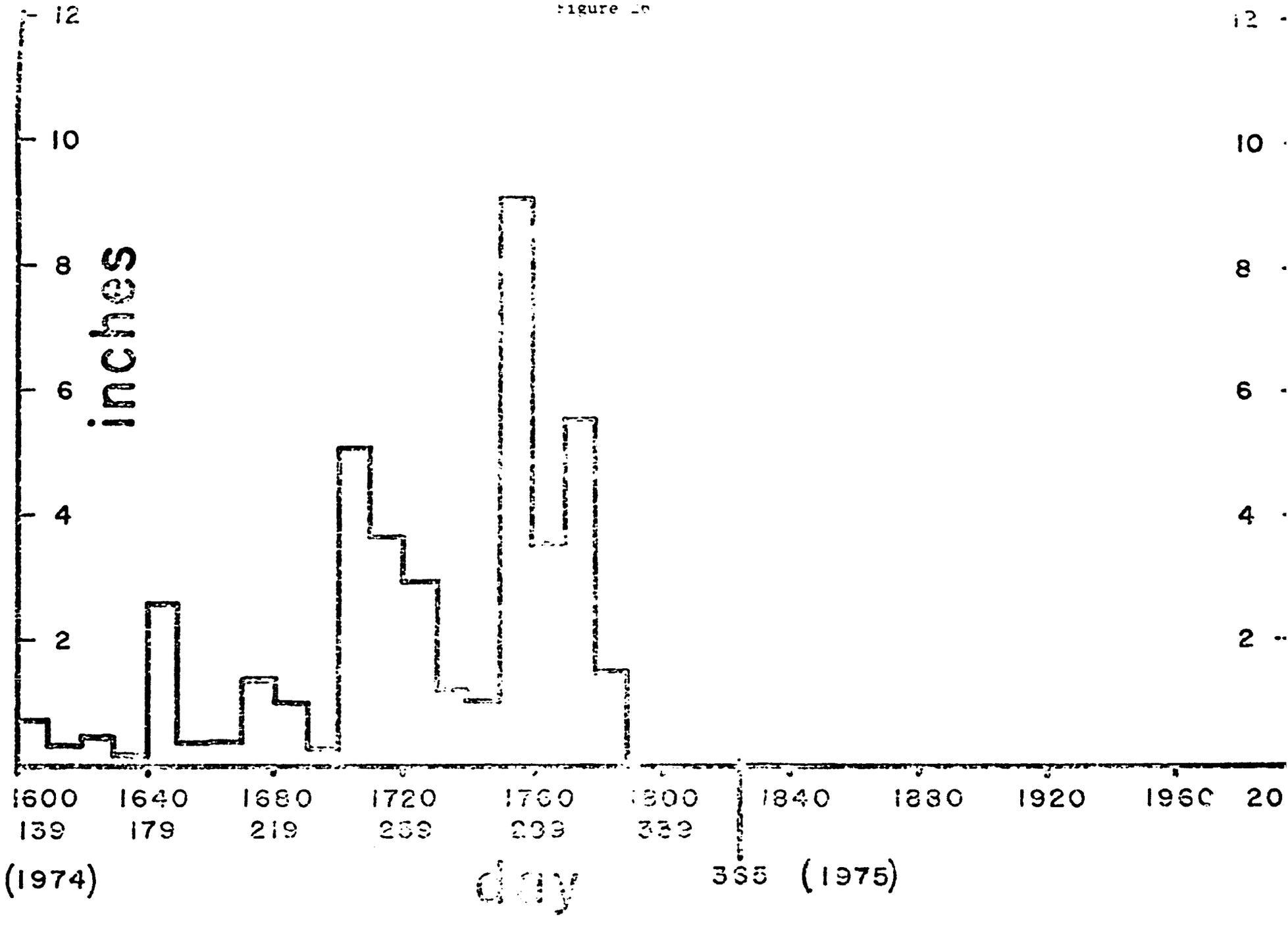

12
10
8
6
4
2
inches
1600
139
1640
179
1680
219
1720
259
1760
299
1800
339
1840
1880
1920
1960
20
(1974)
day
365 (1975)

Table 1

AMINO ACID COMPOSITION[a] OF P_{51} AND SUBUNITS P_{20} AND P_{30}

Amino Acid	Mole % [b]			
	P_{51}	P_{20}	P_{30}	P_{51} reconst.
Lys	2.53	4.08	2.21	2.96
His	1.22	1.09	1.28	1.20
Arg	11.97	11.37	12.19	11.86
Trp	0.45	0.36	0.41	0.39
Asp	9.24	9.57	9.72	9.66
Thr	4.38	4.96	3.71	4.21
Ser	7.29	7.47	7.76	7.64
Glu	16.54	12.82	19.59	16.88
Pro	4.42	4.26	4.99	4.70
Gly	7.95	8.41	7.93	8.12
Ala	6.30	7.12	5.74	6.29
1/2 Cys	1.47	0.39	0.92	0.71
Val	6.74	7.61	6.04	6.67
Met	2.15	2.15	0.97	1.44
Ile	4.11	5.42	3.14	4.05
Leu	6.89	6.86	7.20	7.06
Tyr	2.62	2.63	2.39	2.49
Phe	3.62	3.41	3.80	3.64

[a] Analysis by the method of Liu and Chang, J. Biol. Chem. 246: 2842-2848, 1971.

[b] All values are corrected for degradative losses and incomplete hydrolysis.

Table 2

METZGER INDEX FOR COMPARING PROTEINS

$$\text{Difference Index (D.I.)} = \frac{\sum_{i=1}^{18} \left| A_i - B_i \right|}{2}$$

A_i and B_i = The mole % of the $i^{\underline{th}}$ amino acid in Protein A and B.

D. I. = 0 if A and B are identical and 100 if A and B have no amino acid in common.

Reference: Metzger et. al.
Nature 219 : 1166, 1968.

TABLE 3

DIFFERENCE INDICES FOR SELECTED SYSTEMS OF PROTEINS

SYSTEM	D.I.
P_{68} : P_{68} [a]	1.03
FUNCTIONALLY UNRELATED[b]	15-40
TRIOSEPHOSPHATE, DEHYDROGENASE, MAN : E. COLI[b]	7.5
LIGHT CHAIN : HEAVY CHAIN, I_G[b]	7.4-11.5
BOVINE CROTONASE : CLOSTRIDIAL CROTONASE[c]	8.4
TRYPSINOGEN : CHYMOTRYPSINOGEN[b]	13.8
α-CHAIN : β-CHAIN, HEMOGLOBIN[b]	13.9
PORCINE INSULIN : PORCINE PROINSULIN[d]	19.3
HEN EGG LYSOZYME : BOVINE LACTALBUMIN[b]	22.6

Data drawn from: [a]Dieckert and Yu (Unpublished); [b]Metzger *et al.*, 1968; [c]Waterson *et al.*, 1972; [d]Chance *et al.*, 1968.

TABLE

DIFFERENCE INDICES FOR SUSPECTED HOMOLOGUES OF COCOSIN P_{51} FROM COCOS NUCIFERA L.

SYSTEM	D.I.
P_{51} (51,100) COCOSIN[a]: $\alpha\beta$(60,800) VICIA FABA[b]	9.04
P_{51} (51,100) COCOSIN : A B (61,900) VICIA SATIVA[c]	10.03
P_{51} (51,100) COCOSIN : A C (56,300) VICIA SATIVA[c]	10.05
P_{51} (51,100) COCOSIN : GLYCININ (59,500) GLYCINE MAX[d]	11.76
P_{51} (51,100) COCOSIN : P_{68} (66,700) ARACHIS HYPOGAEA[e]	12.45
P_{51} (51,100) COCOSIN : P_{40} (39,842) GOSSYPIUM HIRSUTUM[a]	9.68
P_{51} (51,100) COCOSIN : P_{54} (53,500) GOSSYPIUM HIRSUTUM[a]	11.28
P_{51} (51,100) COCOSIN : COCOSIN RECONSTRUCTED (51,125)[a]	2.06

Raw data from: [a]Wallace and Dieckert (Unpublished); [b]Wright and Boulter, 1974; [c]Weintraub and Tuen, 1971; [d]Catsimpoolas *et al.*, 1971; [e]Yu and Dieckert (Unpublished).

Table 5

COMPARISON OF P_{20} OF COCOSIN FROM COCOS NUCIFERA L. WITH SUBUNITS OF LEGUMIN-TYPE PROTEINS OF OTHER SPECIES

SYSTEM	D.I.
P_{20} (20,300) COCOSIN[a]: A (24,300) VICIA SATIVA[b]	13.37
P_{20} (20,300) COCOSIN : B (37,600) VICIA SATIVA	14.55
P_{20} (20,300) COCOSIN : C (32,000) VICIA SATIVA	18.24
P_{20} (20,300) COCOSIN : β (23,800) VICIA FABA[c]	13.41
P_{20} (20,300) COCOSIN : α (37,000) VICIA FABA	15.90
P_{20} (20,300) COCOSIN : P_{23} (23,600) ARACHIS HYPOGAEA[d]	13.67
P_{20} (20,300) COCOSIN : P_{43} (43,100) ARACHIS HYPOGAEA	21.05

Raw data from: [a]Wallace and Dieckert (Unpublished); [b]Weintraub and Tuen, 1971; [c]Wright and Boulter, 1974; [d]Yu and Dieckert (Unpublished).

Table 6

COMPARISON OF P_{30} OF COCOSIN FROM COCOS NUCIFERA L. WITH SUBUNITS OF LEGUMIN-TYPE PROTEINS OF OTHER SPECIES

SYSTEM	D.I.
P_{30} (30,900) COCOSIN[a]: B (37,600) VICIA SATIVA[b]	9.84
P_{30} (30,900) COCOSIN : C (32,000) VICIA SATIVA	13.22
P_{30} (30,900) COCOSIN : A (24,300) VICIA SATIVA	17.86
P_{30} (30,900) COCOSIN : α (37,000) VICIA FABA[c]	10.99
P_{30} (30,900) COCOSIN : β (23,800) VICIA FABA	18.04
P_{30} (30,900) COCOSIN : P_{43} (43,100) ARACHIS HYPOGAEA[d]	12.40
P_{30} (30,900) COCOSIN : P_{23} (23,600) ARACHIS HYPOGAEA	15.97
P_{30} (30,900) COCOSIN : P_{20} (20,300) COCOSIN	10.27

Raw data from: [a]Wallace and Dieckert (Unpublished); [b]Weintraub and Tuen, 1971; [c]Wright and Boulter, 1974; [d]Yu and Dieckert (Unpublished).

Table 7

AVERAGE TIME ELAPSED BETWEEN OPENING OF SUCCESSIVE SPATHES

Period	Days	
	Palm 18	Palm 42
200(1970)-1(1971)		28
219(1970)-7(1971)	26	
1(1971)-182(1971)		36
7(1971)-168(1971)	40	
182(1971)-8(1972		32
168(1971)-48(1972)	35	
8(1972)-262(1972)		36
48(1972)-272(1972)	37	
262(1972)-88(1973)		38
272(1972)-100(1973)	39	
88(1973)-280(1973)		38
100(1973)-287(1973)	47	
280(1973)-121(1974)		42
287(1973)-123(1974)	40	
121(1974)-330(1974)		42

Table 8

RAINFALL AND THE ELAPSED TIME BETWEEN THE OPENING OF SUCCESSIVE SPATHES

Period	Av. rainfall 10 days, in.	Av. interval, days	
		Palm 18	Palm 42
201(1970)-15(1971)	2.68	26	28
361(1970)-186(1971)	0.94	40	36
166(1971)-50(1972)	1.21	35	32
1(1972)-280(1972)	1.06	37	36
261(1972)-104(1973)	0.85	39	38
85(1973)-294(1973)	1.21	47	38 ←--
275(1973)-129(1974)	0.83	40	42

←-- Apparent anomaly

Table 9

ENDOSPERM PRODUCTION AND RAINFALL DURING THE PERIOD OF MAXIMUM GROWTH OF OVULE FOR PALM 18

Spathe Open		E_M, g [1]	Rainfall, inches [2]	
Day	Year		10 to 90 % [3]	
7	1971	163	9.29	
7	1972	149	8.16	
41	1972	175	6.80	
47	1971	221	9.62	
79	1972	180	9.36	
88	1971	298	12.24	
117	1972	206	10.24	
128	1971	263	15.97	
157	1972	240	10.62	
168	1971	320	15.97	
202	1971	314	13.31	
231	1972	190	9.99	←--
236	1971	291	9.88	
270	1971	257	13.14	
288	1970	227	8.54	
304	1971	151	12.74	←--
316	1970	232	8.99	
338	1971	157	11.74	
344	1970	174	11.14	

1. Average for the inflorescence.

2. Average for Cruz Bay and Caneel Bay. Values for corresponding 10-day periods for each station were averaged.

3. The 10-day rainfall increments were summed for the period during which the ovule was estimated to grow from 10 to 90 % (central 100 days) of maximum growth.

←-- Apparent anomaly.

Table 10

ENDOSPERM PRODUCTION AND RAINFALL DURING THE PERIOD OF MAXIMUM GROWTH OF OVULE FOR PALM 42

Spathe Open		E_M, g [1]	Rainfall, inches [2]	
Day	Year		10 - 90 % [3]	
12	1973	156	7.93	
50	1973	158	12.06	←--
88	1973	191	15.50	←--
121	(1974)	234	25.94	←--
126	1973	235	15.85	
164	1973	201	12.83	
202	1973	181	9.28	
226	1972	209	9.99	
242	1973	167	7.08	
262	1972	158	5.95	
280	1973	168	8.91	
301	1972	158	6.24	
335	1972	126	5.64	
360	1973	148	6.76	

1. Average for the inflorescence.

2. Average values for Cruz Bay and Cancel Bay. Values for corresponding 10-day periods for each station were averaged.

3. The 10-day rainfall increments were summed for the period during which the ovule was estimated to grow from 10 to 90% (central 100 days) of maximum growth.

← Apparent anomaly.

Table 11

RAINFALL RATE AND ENDOSPERM GROWTH FOR PALM 42

Day spathe open	Growth interval days[a]	$(E_o/E_c)_{ob}$	$(E_o/E_c)_{exp}$[b]	f %[c]	RF/10 day
88 (1973)	85	0.38	0.50	76	1.59
280 (1973)	103	0.25	0.64	39	0.68
50 (1973)	133	0.78	0.85	92	1.46
242 (1973)	144	0.62	0.91	68	0.77
12 (1973)	167	0.94	0.97	97	1.36
203 (1973)	186	0.88	1.02	86	0.71

[a] Growth interval: collection date - date spathe open - 130 days.

[b] $(E_o/E_c)_{expected}$: E_o/E_c read from normalized growth curve for the endosperm determined for Caneel Bay Palm 2 (Figure).

[c] $f\ \% = \frac{(E_o/E_c)_{ob} \times 100}{(E_o/E_c)_{exp}}$

ISOLATION OF COCONUT STORAGE PROTEINS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

Robert W. Wallace[1] and Julius W. Dieckert[2]

Running Title: Preparative Scale PAGE

Subject Area: Protein Structure

Department of Biochemistry and Biophysics
Texas Agricultural Experiment Station
The Texas A&M University System
College Station, Texas 77843

[1] To be included as a portion of a dissertation for the Doctor of Philosophy degree from Texas A&M University.

[2] Author to whom correspondence should be addressed.

Abstract

By combining SDS-gel electrophoresis with a new chilling technique for visualization of protein-SDS complexes in polyacrylamide gels, a process has been developed which will permit the isolation of milligram quantities of pure polypeptides. Using this technique, we have isolated two molecular weight classes of polypeptides from coconut storage globulins and determined the amino acid composition of each. When the two amino acid compositions were summed on a molar basis, the result agreed reasonably well with the amino acid composition of the starting material with the exception of cystine. Apparently, some contaminant from the polyacrylamide caused its destruction to be accelerated during hydrolysis.

Introduction

Since the introduction of electrophoresis of proteins after denaturation with SDS (1), the technique has been recognized as a powerful analytical tool and is used extensively to determine the molecular weight of polypeptides (2,3). Denaturation and wrapping of a protein with SDS, while often necessary when working with proteins insoluble in dilute buffers, severely limits the possibilities for further fractionation. Two possibilities are hydroxylapatite chromatography (4) and procedures which fractionate on the basis of size such as gel permeation chromatography and gel electrophoresis. The high resolving power of gel electrophoresis makes the technique attractive as a preparative procedure. Disadvantages of the technique are low capacity and a problem of removing ultraviolet absorbing, nondializable contaminants from the acrylamide extracts.

Previous attempts at preparative electrophoresis (5) have resulted in the isolation of nanomole quantities of pure polypeptides. This is sufficient for characterization studies if microprocedures are used for N-terminal analysis, amino acid analysis, and tryptic mapping. Another approach (6) is to cut out stained bands from polyacrylamide gels and hydrolyze gel, dye, and protein to determine the amino acid composition. The very large quantity of ammonia released during hydrolysis interferes with amino acid analyzers which use ninhydrin. This often results in spreading of the ammonia peak to a degree which prevents the quantitation of the adjacent histidine peak. High concentrations of ammonia will also form a precipitate with ninhydrin and stop the flow through the heating coils of the amino acid analyzer. In addition, the presence of gel components during hydrolysis may alter the hydrolysis rate or cause the destruction of various amino acids.

The techniques described in this paper permit the isolation of milligram quantities of polypeptides which allows many characterization studies to be conducted without resorting to microprocedures. The protein is eluted from the gel. Consequently, most of the gel components have been removed prior to hydrolysis. The technique involves electrophoresis on (10 x 0.4 x 6.5mm) polyacrylamide slabs, chilling of the slabs to visualize the protein bands as described previously (7), cutting the bands from the slab, and extraction of the protein-SDS complexes.

Materials

Coconuts were grown in Jamaica and donated by the Texas A&M Food Protein Research and Development Center. Bromphenol blue and reagent grade potassium persulfate were obtained from J. T. Baker Chemical Co. Acrylamide, N,N'-methylene-bis-acrylamide (Bis), and Comassie brilliant blue R-250 were electrophoresis grade from Bio-Rad Laboratories. 2-mercaptoethanol and N,N,N',N' tetramethylethylenediamine (TEMED) were purchased from Eastman Organic Chemicals. Sodium dodecyl sulfate (SDS) and 4N methanesulfonic acid with 0.2% 3-(2-aminoethyl)-indole were sequanal grade from Pierce Chemical Co. Ammonium sulfate was enzyme grade from Schwartz-Mann. Preparative electrophoresis was performed in an Ortec model 2100 electrophoresis apparatus.

Methods

Preparation of Coconut Storage Proteins

Isolation of the S2-45-45 coconut isolate is summarized in figure I. The white coconut endosperm from mature coconuts was first extracted by mixing in a Waring blender with 0.1 M NaCl, pH 7.0, 50 mM sodium phosphate buffer which was 0.02% sodium azide (buffer A). After centrifugation, three layers were present; a top layer of solid coconut fat, a middle layer of solvent with dissolved coconut protein, and a precipitate on the bottom. The top two layers were removed, and the precipitate was extracted in a Waring blender with 1.0 M NaCl pH 7.0, 50 mM sodium phosphate buffer (buffer B). The slurry was centrifuged, and the supernatant was brought to 45% saturation with ammonium sulfate. After stirring for two hours at room temperature, the slurry was centrifuged and the precipitate redissolved in buffer B. The protein solution was again brought to 45% saturation with ammonium sulfate. The resulting precipitate was centrifuged, dissolved in buffer B, and dialized 48 hours against the same buffer at 9°C. This protein isolate was labeled S2-45-45 and is the starting material for preparative electrophoresis.

Electrophoresis

The electrophoresis system is essentially the same as that described by Weber and Osborn (2) with a few modifications. The S2-45-45 protein solution was combined with sufficient 10% SDS in water to provide five times as much SDS as protein on a weight basis. The mixture was immediately placed in a 100°C water bath and incubated for five minutes. It was then transferred to a dialysis bag and dialized against distilled water for 48 hours. The desalted protein solution was freeze dried and dissolved in sufficient 10 mM sodium phosphate buffer, pH 7.2, to give a 2% protein solution. A portion of this solution was subjected to analytical SDS electrophoresis and the molecular weights of the peptides determined according to the procedure described by Weber and Osborn (2). The relative proportion of each polypeptide was determined by scanning the Coomassie blue stained gel at 650 nM in a Gilford model 240 spectrophotometer.

The acrylamide gels for preparative work were acrylamide of which 3% was Bis (7%T, 3%C). They were prepared by combini 12.5 ml 28%T, 3%C acrylamide stock solution, 25 ml gel buffer (0.2M sodium phosphate buffer, pH 7.2, 1.0% SDS, 0.04% sodium azide), 0.075ml TEMED, and 10ml of water. The solution is mixed well in an ice bath and then 2.5 ml of potassium persulfate solution

(10 mg/ml) is added. 24 ml of the acrylamide solution is delivered into each of two Ortec slab gel molds. The acrylamide solution is layered with sufficient isobutyl alcohol as described by Neville (8) to provide a flat interface between the acrylamide and the alcohol. The acrylamide should polymerize within 10 to 15 minutes. Then the isobutyl alcohol is removed, the gel surface washed with distilled water, and layered with a 1:1 dilution of gel buffer and water. The gels should be allowed to polymerize for one to two hours before they are used for electrophoresis. They may be stored for several weeks at 4° C without noticable changes in their electrophoretic properties.

The amount of protein applied to the gel will vary depending upon the degree of separation of the protein bands of interest. The two predominant polypeptides in the S2-45-45 coconut isolate are sufficiently separated to allow 5 mg of protein to be applied to the gel. 0.25 ml of the 2% S2-45-45 protein - SDS solution is combined with seven to eight crystals of sucrose, 10μl of a 0.5% solution of bromphenol blue tracking dye, and 10 μl of 2-mercaptoethanol. The solution is incubated for five minutes at 100°C. We have found that it is best to fill both the upper and lower reservoir buffers of the electrophoresis apparatus before the protein is applied. The protein solution can then be layered on top of the acrylamide gels with a pasteur pipet.

Electrophoresis was for four to five hours with a current of 20 mA per cm^2 of gel surface area (163 mA for the ortec system) with the anode in the bottom chamber. During electrophoresis the upper chamber pH rises rapidly. This problem can be controlled by changing the upper electrode buffer twice during the three to four hour electrophoresis period. The lower electrode buffer is routinely used for three runs before it is discarded. In preparative work it is important not to mix the upper and lower reservoir buffers since charged impurities, which are removed from the gel during the initial electrophoresis, will be reintroduced into subsequent gels after the buffers are mixed.

After electrophoresis, the protein bands can be visualized by chilling as described previously (7). The gels are removed from the casting molds and placed on a glass chromatography plate. They are covered with a layer of Saran wrap and chilled overnight at 4° C or for several hours on cracked ice. The protein bands appear as white opaque areas in the gel. They may then be removed by cutting the gel with a razor blade, and the gel containing the

desired protein band is macerated by the action of a glass stirring rod on the side of a beaker containing the gel. Twenty or more volumes of 0.01% SDS in water is applied and the slurry is stirred overnight at room temperature. The slurry is filtered through Whatman #41 filter paper and then through a 0.45μm millipore filter. The supernatant is concentrated by freeze drying.

The protein extracted from the acrylamide gels was analyzed qualitatively by Weber and Osborn's procedure of analytical gel electrophoresis (2). Quantitative analysis for protein was by the procedure of Lowry (9) after the protein had been precipitated with ten volumes of 10% perchloric acid, 1% phosphotungstic acid.

If the product is not of desired quality, it may be recycled through the electrophoresis procedure as shown in figure II. We have been able to purify proteins which have very similar mobilities with a maximum of two cycles of electrophoresis. With the S2-45-45 coconut isolate only one cycle of electrophoresis was necessary.

Amino Acid Analysis

Hydrolysis of the polypeptides was with methanesulfonic acid according to a modification of the procedure of Liu and Chang (10).

1) A protein sample containing a minimum of 3.6 mg of protein dissolved in SDS is dialized overnight against distilled water.

2) The protein solution is removed from the dialysis bag and precipitated by the addition of 10 volumes of acetone. Then it is redissolved in sufficient quantity of 10% SDS in water to give a concentration of 8-15 mg/ml.

3) A portion of this solution containing 0.8-1.5 mg of protein is transferred with a 100μl Hamilton syringe to each of three 1 ml ampules.

4) Sufficient acetone is added to the ampule to make the total volume 1 ml. The ampule is placed in an ice bath for 1 hour and then centrifuged with a clinical centrifuge. The white protein precipitate packs well on the bottom of the ampule and the supernatant can be removed with a pasteur pipet. The precipitate is dried thoroughly over a stream of nitrogen.

5) 0.5 ml of 4N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)-indole is added to the ampule.

6) The ampule is alternately evacuated with a water aspirator and flushed with nitrogen. This is continued for four to five cycles. Finally, the ampule is evacuated for ten minutes and sealed under vacuum with an oxygen torch.

7) The ampules were heated for periods of 24, 48 and 72 hours at 110° C.

8) After hydrolysis, the content of each ampule is removed quantitatively, combined with 0.5 ml of 3.5N NaOH, and diluted to a final volume of 1.5 ml.

9) Duplicate aliquots of 0.25 ml are applied to the long and short columns of a Beckman 121 C amino acid analyzer.

10) The amino acid data is plotted as a function of hydrolysis time. The average values for three hydrolysis times, the values obtained by extrapolation to zero hydrolysis time, or the 72 hour data was used as considered appropriate.

11) The amino acid composition data is compared by calculating the difference indicies as described by Metzger et. al. (11) and Dieckert and Dieckert (12).

Results and Discussion

The procedure for the isolation of the S2-45-45 coconut protein is similar to that for cocosin as described by Khaund (13) and most likely contains the same proteins. The SDS-electrophoresis pattern and quantitative scan of the gel is shown in figure III and the molecular weight calibration curve in figure IV. The isolate is dominated by two molecular weight classes of polypeptides with molecular weights of 30,000 and 19,000 as determined by SDS gel electrophoresis. In addition, a small amount of polypeptide with a molecular weight of 51,000 was present. The polypeptides were labeled P_{30}, P_{19} and P_{51}, respectively. The S2-45-45 isolate accounts for 27.6% of the total protein extracted from the coconut.

It is tempting to speculate that the P_{30} and P_{19} polypeptides combine to form a 49,000 dalton subunit in the native molecule. Integration of the quantitative scan of the Coomassie blue stained gel gives the relative proportions of P_{19}:P_{30} to be 1:1.26. If they were present in exactly equal molar amounts, the result should be 1:1.58. The difference in the observed and expected relative proportions may be due to the inaccuracies inherent in the molecular weight estimates, due to difficulties in scanning Coomassie blue stained gels, (14) or there might be a small amount of contaminating protein which has a molecular weight of approximately 19,000.

During preparative electrophoresis, the pH of the upper electrode buffer, as well as the gel, will rise to as high as 10.5 to 11.0 if the upper reservoir buffer is not changed during the run. This was first observed when a strong smell of ammonia was detected from the gel after a run. Apparently, the pH had risen high enough to hydrolyze the amide groups of the polyacrylamide. In addition to this problem, disulfide interchange was observed in certain polypeptides with cystine residues. It seems likely that breaking of certain peptide bonds which are highly susceptible to basic hydrolysis could also take place. The increase in pH would also be expected to cleave some of the amides in the asparagine and glutamine residues of the proteins. This would introduce charge heterogenity and complex patterns if the purified polypeptides were later checked for purity by a procedure such as isoelectric focusing. Changing the upper reservoir buffer solves the problem but a better solution would be to design an electrophoresis apparatus which has a large buffer reservoir at both the upper and lower electrode. The Ortec design provides a 3 liter lower electrode buffer chamber, but the upper chamber will hold only 0.75 liter of buffer.

The P_{19} and P_{30} bands are well separated with 5 mg of protein placed on the gel. Even the P_{51} polypeptide is visible after chilling. There was no problem in cutting the polyacrylamide slabs before the SDS-protein complexes redissolved and disappeared. However, if a large number of bands are being removed from a complex pattern, this may become a problem. It is possible to visualize the protein bands again by rechilling the gels without noticable changes in the patterns, provided the gel has not been left at room temperature for a long period of time.

Table I summarizes the quantitative data from four electrophoretic isolation experiments. One of the major problems of the preparative electrophoresis technique is the extraction of the protein from the gel. In these experiments, the recovery was 53-73%, and the yield decreased as the amount of protein applied to the gel increased. Figure V shows the result of the qualitative analysis of experiment IV.

The amino acid composition of the S2-45-45 isolate, P_{30}, and P_{19} are shown in Table II. The $P_{19} + P_{30}$ data was computed by summing the analysis of P_{19} and P_{30} on a molar basis. Calculation of the difference index (D.I.) provides a means of comparing the amino acid composition of two proteins (11,12). The procedure is to express the amino acid composition of the two proteins as mole percent (M%). Then use the following formula to calculate the difference index.

$$D.I. = 1/2 \sum (\Delta M\%)$$

If the two amino acid compositions are identical, the difference index will be zero. However, if the two proteins have no amino acids in common, the result will be 100. Our experience has indicated that a difference index as high as 1.5 or 2.0 may be obtained due to experimental error when duplicate analysis of the same proteins are conducted. When the $P_{19} + P_{30}$ values are compared with the amino acid composition of the S2-45-45 isolate, a difference index of 2.31 was obtained. Upon further inspection, it is noted that the largest deviation for any amino acid is for cystine with a $\Delta M\%$ of 1.16. If the cystine data is subtracted, the difference index drops to 1.73. One possible explanation for the large deviation in the cystine values is that a contaminant from the polyacrylamide gels increased the rate of destruction of cystine during hydrolysis.

When the proteins which had been extracted from acrylamide gels were hydrolyzed and analyzed with the amino acid analyzer, a very large ammonia peak was present. However, the procedure described resulted in an ammonia peak which

remained on scale and did not result in peak spreading or the formation of a precipitate in the heating coils of the amino acid analyzer. Apparently soluble acrylamide from the gel was responsible for the ammonia peak. We are now experimenting with a number of possible procedures for the removal of this material from the extracted protein prior to hydrolysis.

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Table I

Data on four preparative electrophoresis experiments.

TABLE I

EXPERIMENT	TOTAL PROTEIN ELECTROPHORESED	TOTAL PROTEIN EXTRACTED FROM GEL			RECOVERY
		P_{51}	P_{30}	P_{19}	
I	1 mg/slab X 2 slabs	———	0.63 mg	0.32 mg	73 %
II	2 mg/slab X 2 slabs	———	1.07 mg	0.63 mg	65 %
III	3 mg/slab X 2 slabs	0.244 mg	1.31 mg	0.97 mg	63.5 %
IV	5 mg/slab X 2 slabs	0.439 mg	2.32 mg	1.54 mg	52.9 %

Table II

Amino acid composition of the S2-45-45 coconut isolate and the 19,000 and 30,000 dalton polypeptides. Difference index is calculated according to Metzger (11).

TABLE II

AMINO ACID COMPOSITION OF VARIOUS ISOLATES IN MOLE %

AMINO ACID	PROTEIN ISOLATE				
	P_{19}	P_{30}	$P_{19} + P_{30}$	S2-45-45	Δ MOLE %
LYS	4.10	2.09	2.91	2.48	0.43
HIS	1.04	1.14	1.10	1.22	0.12
ARG	11.35	12.37	11.96	11.89	0.07
TRP	0.363	0.427	0.401	0.407	0.006
ASP	9.41	9.70	9.59	9.24	0.35
THR	5.04	3.83	4.32	4.46	0.14
SER	7.48	8.01	7.80	7.24	0.56
GLU	12.89	19.59	16.88	16.56	0.32
PRO	4.27	4.37	4.33	4.43	0.10
GLY	8.44	7.89	8.11	7.98	0.13
ALA	7.18	5.84	6.38	6.30	0.08
1/2 CYS	0.265	0.803	0.565	1.72	1.16
VAL	7.65	6.18	6.77	6.74	0.03
MET	2.13	0.994	1.45	2.15	0.70
ILE	5.45	3.21	4.12	4.11	0.01
LEU	6.90	7.31	7.15	6.91	0.24
TYR	2.62	2.44	2.51	2.55	0.04
PHE	3.43	3.81	3.66	3.53	0.13
				D.I. =	2.31

Figure I

ISOLATION OF THE S2-45-45 FRACTION OF COCONUT GLOBULINS

120 gm fresh coconut endosperm mixed in blender with 300 ml of 0.1 M NaCl, 50 mM sodium phosphate buffer, pH 7.0

- Supernatant (S1)
- Precipitate

extract with 400 ml 1.0 M NaCl, 50 mM sodium phosphate buffer, pH 7.0

- Supernatant (S2)
- Precipitate

45% saturation with $(NH_4)_2SO_4$

- Supernatant
- Precipitate (S2-45)

– dissolve in 100 ml 1.0 M NaCl, 50 mM sodium phosphate buffer, pH 7.0

– 45% saturation with $(NH_4)_2SO_4$

- Supernatant
- Precipitate (S2-45-45)

Figure II

STRATEGY FOR ISOLATION OF SDS-PEPTIDES BY PREPARATIVE ELECTROPHORESIS

Denaturation of protein with 5x SDS, 1% 2-mercapto-ethanol, incubate 5 min. 100° C

↓

Electrophoresis on slabs (102mm x 3mm x 90mm)

↓

Visualization of protein bands by chilling
Remove protein band and grind into small pieces

↓

Extract with 10 volumes of 0.01% SDS in H_2O

↓

Filter slurry and lyophilize filtrate

↓

Qualitative analysis by analytical SDS gel electrophoresis

Quantitative Lowry Analysis

Store frozen at -10° C

Figure III

Quantitative scan of S2-45-45 coconut isolate. Optical density measured at 650 millimicrons on a Gilford model 240 spectrophotometer.

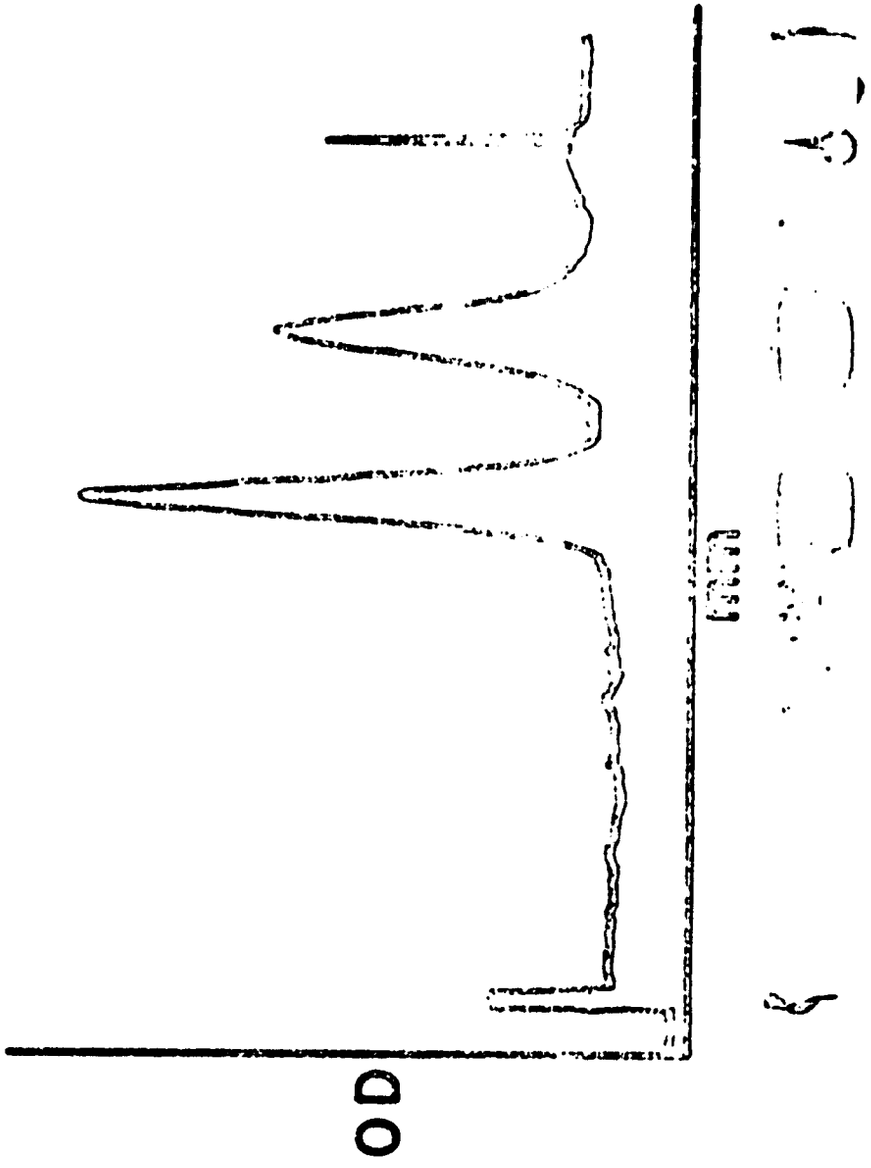

OD

Figure IV

Molecular weight calibration curve for SDS gel electrophoresis.

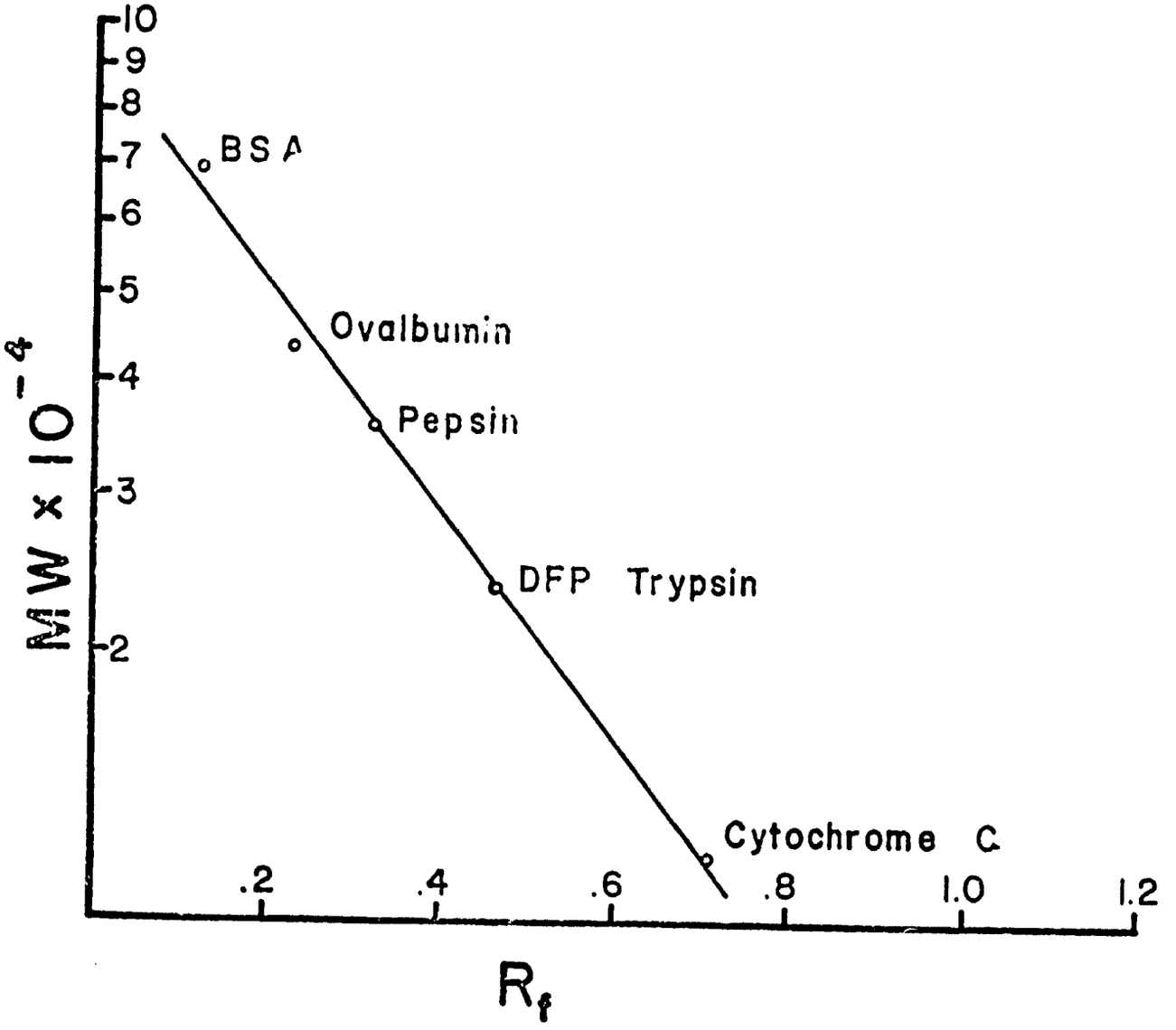
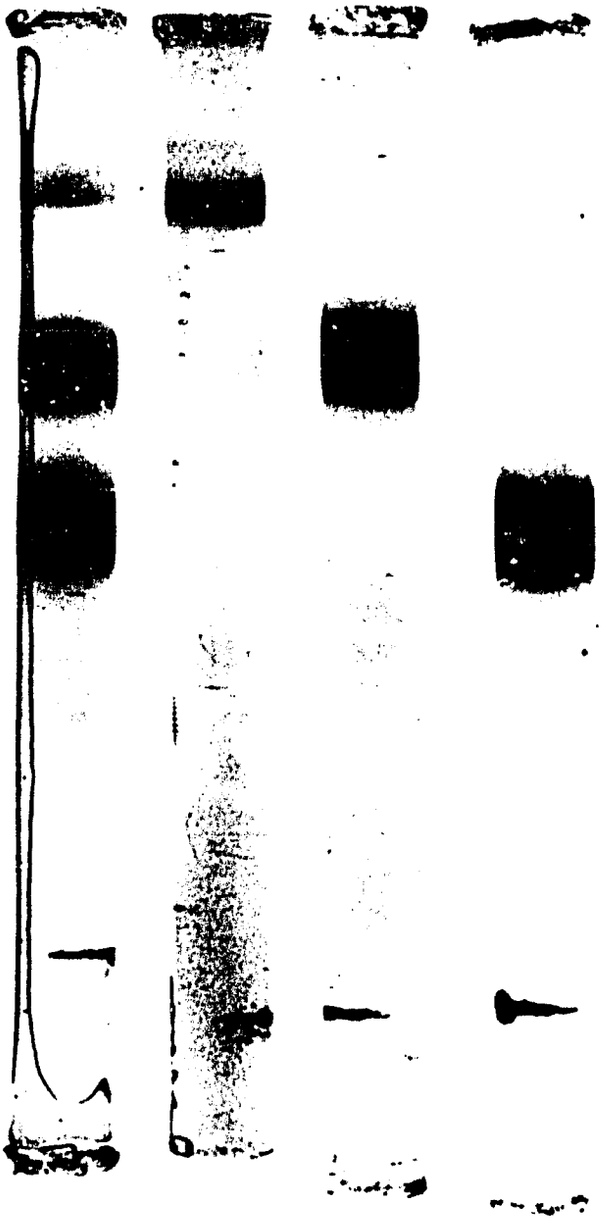

10
9
8
7
6
5
4
3
2
MW x 10^-4
BSA
Ovalbumin
Pepsin
DFP Trypsin
Cytochrome C
.2
.4
.6
.8
1.0
1.2
Rf

FIGURE V.

Qualitative analysis of preparative electrophoresis experiment IV. Starting material is the S2-45-45 Coconut isolate.

FRACTIONATION OF P_{51} BY PREPARATIVE SDS GEL ELECTROPHORESIS



starting material | P_{52} | P_{30} | P_{20}

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