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PHYSICO-CHEMICAL CONSIDERATIONS IN THE STORAGE OF PROCESSED COWPEA PRODUCTS

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ABSTRACT

PHYSICO-CHEMICAL CONSIDERATIONS IN THE STORAGE OF PROCESSED COWPEA PRODUCTS

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Raw and drum-dried cooked cowpea powders were prepared and stored for six months at water activities (A_w's) of 0.11, 0.33 and 0.75 at a constant temperature of 25° C and at 5°, 25° and 40° C at a constant A_w of 0.75.

Physico-chemical evaluations of these powders revealed that lipid oxidation decreased when the A_w was increased from 0.11 to 0.33 and increased when the A_w was increased from 0.33 to 0.75. Temperature increases had the effect of increasing lipid oxidation. Although the raw samples had lower oxidation status immediately after processing, they had oxidized more than the drum-dried samples at the end of storage. Refrigeration temperature effectively minimized lipid oxidation in all the samples.

Unsaturated fatty acids decreased in the course of storage. The saturated/unsaturated fatty acid ratio was about unity at the beginning of storage, but this ratio increased at all temperatures, save 5° C, and at all A_w's, as the length of storage increased. The more

highly unsaturated fatty acids oxidized faster than the less unsaturated ones.

Lipid free-amino groups decreased in storage with increases in A_w and temperature.

Powder whiteness decreased with increased A_w and temperature.

Losses of reducing sugar and available lysine followed expected trends vis-a-vis browning trends with losses recorded at higher A_w and temperature.

The loss of soluble protein in the drum-dried samples followed losses of both reducing sugar and available lysine and therefore appeared to be due to Maillard browning. In the raw samples, the first three months were marked by higher soluble protein contents as the A_w was increased, after which the reverse was true, as would be expected from Maillard browning.

Changes in pH, although following color changes, were minimal over a six month storage. Temperature of 40° C appeared to have led to more marked decreases in pH than all the other storage conditions.

There was no measurable ascorbic acid in the samples; while riboflavin and thiamine were relatively high in these powders. Increasing A_w led to increasing losses of both vitamins in the drum-dried samples. The opposite was true in the raw samples.

Mark E. Ukhun

Examination of the relationships between the various cowpea quality parameters studied indicated that:

a. Lipid oxidation may account for some of the losses of riboflavin and thiamine
b. the lipid free-amino groups may have been lost in storage as a result of both oxidation and carbonyl-amine reactions, as in browning
c. the decrease in the pH of the powders during storage may be partly accounted for by the loss of the basic amino groups of the lipid free-amino groups and the lysine
d. the loss of reducing sugar and of the available lysine was, at least, partly due to carbonyl-amine reactions as in browning
e. the browning process was probably responsible for the loss of protein solubility during storage.

Drum drying caused minimal losses in the nutritional parameters of these powders but, in storage, drum-drying, in fact, reduced the rates of losses in the nutritional qualities.

Proximate analyses of the products revealed high protein content, low total lipid, high carbohydrate and fair amounts of crude fiber and ash contents.

Some of the problems observed in storage were cakiness at A_w 0.75 at all temperatures, and mold growth

was noticed after five months of storage at an A_w of 0.75 at a temperature of 25° C.

Based on the results obtained in this work, it is apparent that the optimum storage conditions for these powders are an A_w of 0.11 and temperature of 5° C.

DEDICATION

The facts and truths of Science testify to the facts and truths of the existence and omnipotence of God. A belief in Science is not incompatible with a belief in God. To Him and to all the members of my family who represent part of His wonderful creation, this work is dedicated.

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TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

FIGURES PAGE

FIGURES PAGE

INTRODUCTION

Cooked dry bean, pea and lentil powders were developed by Morris (1961) in response to requests from growers and processors for a dried product in convenience form. These powders are adaptable for use in a variety of dry mixes, such as soup, bean leaf or patty, and dips for crackers. All of the new products have excellent quality immediately after processing and evaluations have indicated that they have a satisfactory shelf-life when appropriately packaged (Boggs, et al. 1964).

Powders prepared from legume seeds may represent just another convenience food, but it has another potential which might even be more significant. It is increasingly being realized that the world's protein supply is becoming more and more critical especially in view of the ever increasing world population. In the so-called developing countries of the world where, in fact, populations are rising faster than agricultural output and protein-food production, the protein problem is particularly serious. When compared to other plant sources, the legumes as a group are high in protein. This, combined with the fact that they are consumed by large sections of people in the third world countries, have

increased speculations and raised hopes that one possible way to meet the world's protein problem is by supplying cheap, stable protein foods from legumes. A 1966 estimate by Altschul puts the annual protein production from legumes at 8.5M tons, while according to Bressani et al. (1963), about 20-30% of the protein intake of the economically poor in Latin America comes from legumes.

Cowpea (Vigna unguiculata) is an example of a legume with high protein content. Analysis by Bressani et al. (1961) puts the value at about 24%. Another analysis by Oyenuga (1968) shows the protein to be well balanced with respect to the essential amino-acids. It is high in lysine but rather deficient in methionine, as is true of most other legumes (Silbernagel, 1971).

Cowpea is a legume crop which grows prolifically in the warm and humid tropical conditions of Nigeria. It is consumed by a large section of the population—rich and poor—in the form of various indigenous Nigerian dishes such as "moin-moin" and "akara". As a member of the so-called developing countries of the world, Nigeria stands to gain by increased utilization of the legume in meeting the protein needs of its population. It is also true, however, that growing incomes and urbanization have created a real potential for convenience foods prepared from the cowpeas. Such convenience foods should grow in importance in a country like Nigeria where expectations are running higher everyday while they should also be

readily marketable in a country like the U.S.A. where expectations have always been high.

With that in mind, the objective of this work was to prepare cowpea powders by drum-drying and simple milling operations and to study physico-chemical changes in these powders during a six month storage period at water activity of 0.11, 0.33 and 0.75 at a constant temperature of 25° C (room temperature) and at temperatures of 5° C, 25° C and 40° C with the water activity (A_w) held constant at 0.75.

The A_w's of 0.11, 0.33 and 0.75 were chosen to reflect the conditions which might exist in foods stored in the three main climatic belts of Nigeria. These are 0.11 for the Northern Belt, 0.33 for the Middle Belt, and 0.75 for the Southern Belt. Incidentally, the A_w's of 0.11, 0.33 and 0.75 also represent, generally, the three main zones of A_w described by Labuza (1971) at which different reactions occur at varying rates in dehydrated foods.

5° C was chosen to represent refrigeration temperature, 25° C for ambient temperature and 40° C to represent accelerated storage condition. Moreover, there are some sections of Nigeria (Northern) where 40° C temperatures occur at certain times of the year.

Drum-drying, rather than freeze-drying or spray drying, was chosen as the dehydration technique purely for reasons of cost. It was thought that the powders

should be affordable by the average consumer by lowering production cost which usually translates to lower prices. This would be important particularly in a country like Nigeria.

The physico-chemical studies undertaken in the research included: lipid oxidation as measured by changes in diene conjugation and changes in the fatty-acid content, reducing sugar, soluble protein, available lysine, color, pH, and ascorbic acid, riboflavin and thiamine contents. These parameters were measured on a monthly basis over a six month storage period.

To obtain a base value against which storage changes were assessed, all the above measurements were made on the fresh products immediately after processing.

Observations were also made from time to time to monitor certain physical characteristics while the effects of the type of processing were examined by comparing the drum-dried samples with the raw samples.

It was hoped that at the end of the studies, it would be possible to arrive at a picture of the type of changes one can expect in dried cowpea products and, on the basis of this, be able to recommend optimum storage conditions for them.

LITERATURE REVIEW

Cowpeas (Vigna unguiculata) have always constituted an important part of the diets of the peoples of West Africa. Lewicki (1974) noted that even as early as the middle ages cowpeas were very important in the foods of the West African peoples. Oyenuga (1968) has described vividly the importance of this legume crop in the diets of modern-day Nigerians.

Cowpeas, belong to the family Leguminosae which consists of three subfamilies, Caesalpiniaceae, Papilionaceae and Mimosaceae. Vigna unguiculata is a warmth-loving herbaceous annual sensitive to cold and severe frost.

Chemical analysis by Oyenuga (1968) of cowpeas grown in Nigeria revealed that:

a. The seeds are high in protein and soluble carbohydrate, low in crude fiber and oil, and contain a fair amount of minerals.

b. They contain all the essential amino-acids in amounts sufficient for growth.

c. The amino-acid pattern of cowpeas is similar to that of whole hen's eggs and is of high biological value.

d. Cowpeas contain fair amounts of thiamine, niacin and riboflavin and some carotene but are deficient in ascorbic acid.

e. Like many legumes, the seeds are rich in phosphorus with only fair amounts of calcium and iron.

f. The major and trace elements are well distributed in the cowpea seed.

g. They contain only about 2.5 percent fat with 52 percent of the fatty acids being unsaturated while some 40 percent occurs as the saturated palmitic and stearic acids.

Such composition in combination with a high degree of acceptability have made cowpeas highly desirable as ingredients in soups, gravies, stews and other food products such as "moin-moin" and "akara" which are very popular in Nigeria.

The legumes as a group represent a valueable source of protein for the ever-increasing population of the world especially in the so-called developing countries of the world. According to Altschul (1966) more than 8.5M tons of protein are obtained from legumes annually. Another estimate by Bressani et al. (1963) showed that about 20-30% of the protein intake of the poor in Latin America countries is derived from legumes.

However, despite the well documented high protein content of legumes, their nutritional value is limited by their low content of the sulphur amino-acids methionine

and cystine. Working with navy beans, Silbernagel (1971) reported that although the average protein content of this legume was about 25%, its real nutritional value was limited to about 8%. He attributed this to the low content of the sulphur amino-acids and suggested supplementation with these amino-acids to improve their nutritive quality.

Another reason why the nutritive value of a legume might be lower than expected would be its content of antinutritional factors such as hemagglutinins, saponins and antitrypsin. Ricin, a hemagglutinin was the first toxic plant protein to be isolated (Liener, 1966). It is known that relatively mild heat treatment is sufficient to inactivate these antinutritional factors. Kakade and Evans (1963) destroyed the antitrypsin factors in navy beans by autoclaving for 5 minutes at a temperature of 121° C.

Processing of Legumes

One popular processing method for cowpeas in Nigeria involves, among other things, cooking the whole legume seeds in water, the aim being to detoxify the product, soften the seeds and eliminate the coat. According to Bressani and Elias (1974), a minimum of two hours is required for cooking soaked dry beans at atmospheric pressure while Dovlo et al. (1975) noted that the cooking time varies with the variety of cowpea used, with cooking time

at atmospheric pressure ranging from about 1½ hours to about 2½ hours.

Wet and dry methods are available for the dehulling of legume seeds if dehulling prior to cooking is desired. A wet method of dehulling involves soaking the seeds overnight followed by gentle rubbing to separate the seed coats from the cotyledons. Because the seed coats have lower density than the cotyledons, they rise to the surface and float when water is added. A dry method described by Dovlo (1975) involves pounding the dry beans with suitable equipment such as a mortar and pestle followed by winnowing to remove the seed coats.

Attempts have been made at applying intermediate technology to the processing of legumes in Nigeria. Siegel and Fawcett (1976) in an investigative work with three dehulling units—an attrition-type mill, a barley pearler and a grain thresher—have found that the George O. Hill thresher was effective in mechanically dehulling brown cowpeas. Earlier, Reichert et al. (1974) had reported that flour obtained from such dehulled cowpea seeds were as good as those dehulled by traditional methods of pounding and winnowing.

The processes of soaking and dehulling cowpea seeds have both good and bad effects. Small losses of important nutrients are incurred with the dehulling process while water soluble components may be lost as a result of soaking.

For example, Ogunmodede (1972) has reported that the soaking process leads to losses of thiamine and niacin. On the other hand, Kakade and Evans (1966) have reported that soaking Phaseolus vulgaris for four days led to a 75% reduction in the activity of hemagglutinin and a 28% reduction in the activity of the trypsin inhibitor. Again, dehulling may also result in improved digestibility of the bean.

Because of the long cooking times required for legume seeds, several processes aimed at overcoming this problem have been described in the literature. One such process described by Rockland and Metzler (1967) for lima beans involves soaking the beans in solutions of inorganic salts for about an hour during which the beans are given a vacuum treatment by the Hydravac Process. The beans are then allowed to soak in the salt solution for another six hours after which they are rinsed and dried. The process was thought to yield a quick cooking legume product, first by loosening the seed coats from the cotyledons such that the latter is able to imbibe the sale solution. Once imbibed, the cotyledon proteins are dispersed and solubilized by the hydrating medium which is comprised of 2.5% sodium chloride, 1.0% sodium tripolyphosphate, 0.75% sodium bicarbonate and 0.25% sodium carbonate. According to Rockland and Metzler (1967), the polyphosphates act as metal chelating agents which aid in breaking metal salt-protein bonds. In a later report by Rockland (1974), it was

stated that it is possible to reduce the cooking time of dry beans by as much as 80%.

Steinkraus et al. (1964) have also described a process for the preparation of quick cooking dry beans. Essentially, the method involved soaking the beans in water for about 15 minutes, cooking with steam, dipping in a 20% sucrose solution at 160° F and finally dehydrating.

Recently, the processing of legumes has taken a new dimension with the introduction of spray and drum-drying techniques. These methods produce instant legume powders or ready-to-eat products which are projected to gain a wider share of the market for legumes in view of the reported decline in the demand for canned whole beans. Morris (1961) was the first to introduce the concept of instant legume powders while Bakker-Arkema et al. (1966), developed the idea of spray drying cooked legume powders. Later, in 1967, they also applied the drum-dehydration technique in preparing legume powders. In evaluating both products, Bakker-Arkema et al. (1967) pointed out that the drum-dried products had a lower free starch index which translated to better rehydration properties than the spray dried products. Moreover, Bakker-Arkema et al. (1967) also indicated that the drum-drying process saved about 2500 BTU per pound of beans when compared to the spray drying technique. The elimination of the pureeing process in the drum-drying operation accounted for the saving in energy.

More recently,other workers have applied the drum-drying technique to the production of ready-to-eat legume products. Onayemi and Potter (1976) and Kon et al. (1974) have prepared cowpea powders by using the drum-drier.

Storage of Legumes

Many chemical and physical changes occur in both fresh and dry legume seeds when they are stored.

Worthington and Burns (1971) noted that, when fresh cowpea seeds were stored at 110° F for 12 hours, the firmness of the beans increased significantly.

Hoover (1955) indicated that cowpeas are a good source of ascorbic acid only if they are harvested early and are utilized soon after harvest or stored at about 40° F. Temperatures appreciably higher than 40° F or long storage time had the effect of decreasing the ascorbic acid content of fresh cowpeas.

According to Burr (1973), prolonged storage of dry beans may lead to undesirable changes in their cooking characteristics which are dependent on the moisture content, time and temperature of storage.

In a study by Dexter et al. (1955), holding dry beans at temperatures below 38° C decreased the water holding capacities of the beans. Bean discoloration and rancidity were associated with high moisture content.

Working with varieties of Phaseolus vulgaris, Morris and Wood (1956) reported that samples with the

highest moisture contents also suffered the greatest quality changes as judged by lipid acid values, texture and color. Similarly, Burr et al. (1968) observed that high moisture contents were associated with losses in bean quality as measured by the cooking time. Moisture contents higher than 10% were observed to increase the cooking time of beans.

In his work on the quick cooking characteristics of dry lima beans, Rockland (1966) reported increases in cooking times after 3 months storage at 38 and 70° F. Flavor, color and odor problems were also reported.

Obviously, the nutritional quality of legumes change during storage. When Mitchell and Beadles (1949) stored soybeans at room temperature, the nitrogen digestibility of the beans was reported to decrease from about 85% to about 75%. The corresponding loss in the nutritive value of the protein was about 28%. Supplementation with methionine helped increase the biological value of the beans.

Bressani et al. (1961) hinted that the limiting amino-acid in beans is methionine. However, in studies by Molina et al. (1975), the methionine and lysine contents of raw beans were reported to increase during storage suggesting an increase in the protein quality of the beans during storage. Results from protein efficiency ratio (PER) measurements did not indicate this. Earlier, Evans et al. (1974) had reported that the PER values correlated well

with methionine and lysine availability. It was therefore reasoned from the results of Molina et al. (1975), that processing and storage may adversely affect the availability of lysine and the sulfur amino acids. Indeed, when Ben-Gera and Zimmerman (1964) stored raw defatted soybean flakes of 9.1% moisture content at 30° C for 12 weeks, the available lysine content decreased by 18%. This agreed with the later findings of Yannai and Zimmerman (1970) in which the available lysine content of stored soybean was reported to decrease.

According to Harrington (1963), dry legume seeds may also lose their ability to germinate if stored under conditions of high relative humidity. Increased respiration rates and loss of catalase and phosphatase activities were reported by Morris and Wood (1956) to occur in stored dry legume seeds with high moisture contents.

The vitamin content of legume seeds is also affected by storage conditions. Although Chitre et al. (1955) did not find statistically significant losses of niacin and thiamine in pulses stored for six mohths, Dawson et al. (1952) reported that pea beans, stored at 24° C and at relative humidities ranging from 67 to 95%, lost about 20% of thiamine, even at low moisture content.

Microbial attack is an important occurence in stored legumes. Heavy attack by Cladosporium of stored dry beans was reported by Swanson et al. (1970). Similarly, when Watson and Morris (1954) stored beans at a high

relative humidity of 98%, mold growth was reported after six weeks of storage. Christenson and Kaufman (1969) have hinted that if the temperature of storage is too high and if the moisture content of stored legume seeds was high enough, storage fungi could attack the product to cause mustiness, germ damage, caking and bin-burning.

With the advent of preprocessed legume powders, some work has been reported on the storage stability of these products. The work by Burr et al. (1969) with dry instant legume powders showed that a moisture content of 4%, a temperature of 22° C and a nitrogen atmosphere were effective in maintaining product quality as judged by lipid oxidation and taste panel assessments. They were also able to demonstrate the effectiveness of the anti-oxidant butylatedhydroxytoluene in extending the shelf life of these products. Counter (1969) also reported that a nitrogen atmosphere could be used to extent the shelf-life of instant navy bean powder.

Storage and the Protein Quality of Foods

The concept of food protein quality is so important that many works have been published on this topic.

Hodson and Miller (1957) reported marked browning in stored milk proteins and that this browning was accompanied by significant loss of lysine. In milk casein samples stored in air, Hodson and Kruger (1947) reported losses of some amino acids particularly lysine, methionine,

arginine and histidine. Nitrogen atmosphere storage helped to reduce these losses. Stevens and McGinnis (1947) also observed decreased lysine availability in studies with stored milk protein.

Jones and Gersdorff (1941) indicated that the protein quality factors adversely affected by increasing length of storage of stored wheat flour were digestibility, solubility and true protein content. The report of Mitchell and Beadles (1949) also noted that decreased in-vitro protein digestibility associated with decreased protein solubility occurred in stored wheat and corn. Schultz and Thomas (1949) mentioned lysine losses in stored corn.

Attempts have been made by various authors to explain the loss of protein quality in stored foods. According to Melnick and Oser (1949), the loss of protein quality in stored foods may occur as a result of the formation of new and enzymatically resistant peptide linkages. They pointed out that the direct destruction of essential amino-acids of the proteins was not as important as the formation of these new and resistant linkages. This agrees with the reasoning of Mitchell and Block (1946) who stated that the formation of unusual cross-linkages between proteins and or peptides may lead to reduced protein digestibility. According to Seegers and Matill (1935), enzyme action on these unusual linkages produce unusual peptides which resist absorption and fecal elimination and

which are neither absorbed nor excreted in the urine. Similarly, Henry and Kon (1948) have stated that browning, due to the long term storage of a food, may act to alter the digestibility of the food protein. Clark and Tannenbaum (1970) did isolate products of the Maillard reaction which have been described by Henry and Kon (1948) to be resistant to enzymatic action. In their work, McInroy et al. (1945) were able to correlate browning with the formation of enzyme and even acid resistant linkages which correspondingly led to decreased protein value. The report of Hill and Patton (1947) showed that apart from lysine, the amount of other essential amino acids of a protein may also be affected by storage browning. The indole group of tryptophan and the imidazole group of histidine, for example, may be involved in these reactions. As shown clearly by Ben-Gera and Zimmerman (1964), storage or heat browning lead to loss of available lysine by a blocking of its epsilon amino group by reaction with reducing sugars or by reaction with the free carboxyl groups of aspartic and glutamic acid.

Lipid Content and Quality of Stored Protein in Foods

There are many reports which have correlated the protein quality of stored foods with their degrees of lipid oxidation, or with other reactions.

Ponting, et al. (1964) mentioned lipid oxidation as contributing to the protin quality of stored vegetable

foods by reaction between the products of lipid oxidation which supply carbonyl functions and proteins which supply amino functions in the non-enzymatic browning process. Similarly, according to Carpenter et al. (1962) in their work with stored fish meal, secondary products of lipid oxidation were able to react with the epsilon amino group of lysine in the non-enzymatic browning process to cause deterioration of the fish meal protein.

By forming resistant complexes with leaf protein concentrates, the products of lipid oxidation helped reduce the enzyme digestibility of the stored leaf protein (Buchanan, 1969). The greater the storage time and the moisture content of the leaf protein concentrates, the greater was the reported loss in digestibility. When Lea et al. (1960) stored herring meal for a year at 20° C and with a moisture content of about 6%, lipid oxidation was demonstrated to lower the nutritive value of the product by damaging the protein constituent of the stored herring meal. Work by Olley and Duncan (1965) with frozen stored fish muscle was able to link the degree of fatty-acid release from these samples with lower protein quality accruing from a denaturation phenomenon.

According to Koch (1962), lipid oxidation during the freeze-drying of foods causes changes in the rehydration properties, texture and flavor, and thereby limits the shelf-life of these foods, because of the interaction between the products of lipid oxidation and proteins.

Solubility changes and the loss of protein amino-acids were reported by Roubal and Tappel (1966a) to be the result of enzyme and protein damage from peroxidizing lipids resulting in polymerization of proteins with poly-unsaturated lipids as oxidation progresses. Radical attack rather than aldehyde attack, on protein is mainly responsible for protein damage in a lipid-protein system. (Roubal and Tappel, 1966b).

The exact mechanism of the protein-lipid interactions in food systems which lead to the deterioration of stored foods have not yet been fully established. Possibly, the binding of lipids to proteins or vice versa may depend on such factors as configuration, multiple attachment sites, and/or the matching of polarity between opposing groups in much the same manner as for the combination of enzyme and substrate.

Chapman (1969) referred to metal ions and electrostatic and hydrophobic binding as being important in the interaction between lipids and proteins. In consonance with this, Gurd (1960) suggested that the interactions might be between similar types of functional groups in the two classes of compounds such as between non-polar hydrophobic residues of the fatty-acid moieties of lipids and the similar residue of certain of the side chain groups of proteins; while other interactions may involve polar or charged groups. Primary covalent linkages such as ester bonds were regarded as being of little importance.

In their studies on a gelatin-linoleate system in the dry state, Zirlin and Karel (1960) observed that gelatin-lipid reactions lead to the scission of protein coupled with cross-linking. Narayan and Kummerow (1958) have referred to complex formation in the presence of oxidized lipids; with the reaction being environment dependent and different proteins responding differently. Complex formation was attributed to secondary bonds such as hydrogen bonding.

The isolation of fluroescent compounds with -C=N- functional groups from an oxidizing system consisting of methyl linoleate and coho salmon myosin and the observation that the epsilon amino groups of myosin were destroyed led Braddock and Dugan (1973) to speculate a cross linking reaction between amino groups and the products of fatty-acid oxidation. Lysine, methionine and histidine were found to be most susceptible to such destruction.

Working with carbonyl compunds, Schwenke (1975) stated that blocking of the L-epsilon-amino groups alters the iso-electric point, electrophoretic properties, solubility and precipitation characteristics of proteins. Accordingly, he speculated that radicals from oxidizing lipids play a role in protein destruction with the possibility that carbonyl-amino reactions, as in Maillard browning, form Schiff bases and secondary products.

Jarenback and Liljemark (1975) reported that linoleic acid hydroperoxides were much more effective than

linoleic acid in reducing the amount of protein in a KCl-extract. They therefore suggested more extensive binding between the linoleic acid hydroperoxides and protein than between the linoleic acid and protein.

Crawford *et al.* (1967) have implicated malonaldehyde in protein destruction in food systems. They stated that malonaldehyde's reactivity is enhanced by resonance stabilization. As such they react, with free amino-acids in proteins by nucleophilic attack *via* an SN-2 mechanism to produce enamines; the epsilon-NH_2 group of lysine and the N-terminal, γ -NH_2 groups of aspartic acid being involved in these reactions. Similarly, Chio and Tappel (1969) observed that malonaldehyde produced from oxidizing lipids reacted with enzymes to produce yellow fluorescent compounds with a consequent loss of enzyme activity. In addition, -SH groups of the proteins, tyrosine, lysine and histidine were destroyed by the malonaldehyde. Then, work by Fletcher and Tappel (1971) using fatty-acids bound to horse serum albumin showed too that polyunsaturated fatty-acids undergoing oxidation reacted with amino-acids to yield Schiff base compounds which fluoresced.

Ganage and Matsushita (1973) have, however, hinted that both radical and non-radical products of oxidizing lipids can polymerize with proteins. This accounts for the observation by Roubal (1971) that free radical attack on food proteins rather than aldehyde reactions were mainly responsible for amino-acid losses.

Phospholipids are apparently one type of lipid that are involved in these protein destroying reactions. According to Bidlack and Tappel (1973a), fluorescent materials were produced in systems composed of microsomal membranes and oxidizing lipids. In the course of time, the amounts of extractable phosphatidyl ethanolamine decreased as was the number of reactive amino groups in the phosphatidyl ethanolamine. Obviously, carbonyl compounds from oxidizing lipids, including malonaldehyde were involved in Schiff base formation reaction with the phospholipid free amine. A similar explanation was advanced for the results of Dillard and Tappel (1973) in which fluorescent products with an excitation at 360 nm and emission at 430-460 nm also led to lower phospholipid free amine. Another work by Reiss and Tappel (1973) using a system composed of deoxyribonucleic acid and polyunsaturated fatty-acid indicated the production of such fluorescent compounds. Again, Bidlack and Tappel (1973b) showed that during the oxidation of phosphatidyl serine and phosphatidyl ethanolamine, fluorescent compounds were produced and that the fluorescent intensity increased with increasing peroxidation. Along the same lines, Corliss and Dugan (1970) indicated a link between phosphatidyl ethanolamine oxidation and browning.

It would appear that the destructive effects to proteins in some protein-lipid food systems is matched by a stabilizing effect on the lipid component of the system.

Bishov and Henick (1972) reported an antioxidant effect of autolyzed yeast protein (AYP) and hydrolyzed vegetable protein (HVP) in a freeze-dried model system of lipid, carboxymethyl cellulose, the protein and distilled water. Earlier work by Bishov et al. (1967) had indicated improved fat stability in dehydrated proteinaceous food mixes of chicken flavored soup and gravy mix. The universality of synergism between protein hydrolyzate and phenolic antioxidants in stabilizing lipids was also demonstrated by Bishov and Henick (1975).

The ability of protein to impart stability in such a system was also found to vary with the type of protein, method of preparation, pH, and experimental condition. For example, in their work reported above, differences in the stabilizing effect between the AYP and HVP were attributed to the milder conditions of autolysis in comparison to those of acid induced hydrolysis, which gave better stabilizing potential to the former. HVP is obtained by hydrolyzing soy protein with an acid followed by alkaline neutralization. The salt which may be thus formed is also thought to lower its antioxidant effectiveness. AYP, on the other hand, is obtained with minimum heat treatment since the yeast cells are self-digesting.

In consonance with the above, Hayes et al. (1977) has hinted, with respect to soy flour antioxidant, that there is no clear cut, generalized relationship between concentration of added soy flour and the degree of

stability obtained. They added that the nature of other product ingredients, the character of the fat or oil involved and whether the soy flour is full fat or defatted all probably complicate the outcome.

The antioxidant activity of soy flour has been ascribed to its natural components: such as isoflavones which Walz (1931) isolated. Amino acids and peptides are others. Soybeans normally contain small quantities of peptides and amino acids which are present as a result of incomplete protein synthesis or possibly because of some protein degradation (Smith and Circle, 1972a). Another class of compounds associated with the antioxidant activity of soy flour has been the phospholipids and Smith and Circle (1972a) have indicated that only part of the phospholipids are removed in commercial extraction of soybean flakes.

In their studies with methyl linoleate in freeze-dried model systems, Karel et al. (1966) found that certain amino acids including histidine, α-amino-butyric acid, lysine and cysteine had substantial antioxidant activity and that the nature of this activity was different from that observed with propyl gallate since the main effect of the amino compounds was to prolong the induction period and to affect the initial rate of oxidation. No effect was present in the more rapid, bimolecular phase of oxidation, whereas propyl gallate had an inhibiting effect in the latter stage.

As a follow-up to this, Tjho and Karel (1969) reported that histidine in low concentration has an antioxidant effect in the very early stages of oxidation followed by a slight pro-oxidant effect in the later stages.

Working with herring oil, Marcuse (1960) observed that while histidine had an antioxidant effect, cysteine was pro-oxidant. Marcuse (1961) also reported on a study of the antioxidant effects of several amino acids added to aqueous solutions of linoleate at pH 7.5 and concluded that:

1. Histidine, alanine, methionine, and lysine reduced oxygan absorption by linoleate by as much as 50-80%.

2. Each amino acid had an optimum concentration for the antioxidant activity and at high concentrations showed an activity inversion; becoming pro-oxidant rather than antioxidant in its action.

3. The antioxidant activity depended on pH, the presence of other antioxidants or synergists, and the state of oxidation of linoleate.

4. Since antioxidant properties were shown in the absence of tocopherol and other antioxidants, amino acids may act as primary antioxidants.

Then in 1962, Marcuse observed that the antioxidant effect is enhanced, and the pro-oxidant effect is lowered by addition of phosphate.

Working on the stability of amine salts of linoleic acid, Dorlores and Lopieks (1971) suggested that

the carboxylic acid group of the fatty acid form a salt with one of the amino groups of the basic amino acids, leaving the other amino group available to affect the double bond area of the fatty acid. If the latter interaction is a charge-transfer complex, it is a very weak one involving only partial donation of electrons and little bond distortion. It would appear that the protective effect is specifically associated with the solid state.

The rigid crystalline lattice of lysinium linoleate could act as a barrier to the diffusion of oxygen and oxidizer intermediates, and to the propagation of chain reaction. On the other hand, studies of the oxidation in solution by Koch et al. (1971) suggest that the salt complex also prolongs the induction period and retards the rate of autoxidation. Therefore, one might speculate that, initially the solid complex physically prevents oxygen from entering the reaction. After the oxygen succeeds in attacking the organic substrate, the oxidation would be terminated by a mechanism in which the basic amino acid acts as an oxygen scavenger or a free-radical chain terminator (Dorlores and Lopieks, 1971).

Water Activity and the Quality of Stored Foods

Water activity is defined by the ratio $P/_{P_O}$ where P is the partial pressure of water in a sample and P_o is

the vapor pressure of pure water at the same temperature (Ayerst, 1965).

Water with full activity does not exist except in the pure state. The remaining types of water are present in biological matter and can best be visualized by considering the changes in water activity that occur during a process of drying or freezing (also a drying process with the removed water deposited internally in the form of pure ice-crystals). This approach is greatly facilitated by considering plots of water activity versus moisture content (Fennema, 1976). According to Rockland (1969), when foods are stored under conditions of differing relative humidities they either take in or give up moisture to attain a moisture content which represents the equilibrum moisture content. It is this moisture content that is actually employed in the plots referred to by Fennema (1976) and which is known as a "moisture sorption isotherm".

Water activity may indicate both the structure of the material and the manner in which the water is found (Heldman, 1972). The first water removed, Type III, has an activity slightly less than that of pure water. This type of water which represents the majority of water in plant and animal food tissues is easily removable and is readily available for growth of micro-organisms and chemical reactions. As it is removed, the remaining water gradually assumes a lower activity. When all of the Type

III water has been removed, the moisture content is about 12-25% and the water activity is about 0.8 depending on the type of product and the temperature. Type II water is substantially more difficult to remove than Type III water, and removal of a given increment of Type II water results in a much greater reduction of the remaining water's activity than occurs when a like increment of Type III water is removed. Partial removal of Type II water eliminates the last possibility of microbial growth and greatly reduces most kinds of chemical reactions. Complete or near complete removal of Type II water 3-7% moisture level, depending on the product and temperature, corresponds approximately to optimum stability of dry products that contain significant amounts of oxidizable lipids. Partial removal of Type I water can be accomplished by conventional dehydration but not by freezing. Type I water is very tightly bound and is what some authors refer to as "true bound water". The degree of binding is such that any reactions depending on solvation are so slow as to be unmeasurable (Fennema, 1976).

Other authors have used different terms while agreeing in their characterization of the three types of water described by Fennema above. Wolf et al. (1973) referred to Type I water as constituting the "monolayer region" in which water is held on polar sites and bound with relatively high energy in a single layer distribution over the surfaces of the food. The region, according to them

involves water activity up to 0.3. In describing Type II water, Sitt (1958) indicated that it is water found in the 0.3-0.7 A_w region of the isotherm and that here water exists in multiple layers held by forces weaker than those in the monolayer, i.e. Van der Waal forces and similar forces. Brunauer et al. (1938) in propounding their multimolecular adsorption theory described the Type III water as that occupying the zone above 0.7 A_w in the sorption isotherm. In this zone, water is thought to exist relatively free and macro capillary forces are at work here.

Ayerst (1965) hinted that the stability of foods is related to their water activity. Since then, several authors have attempted to relate water activity to the stability of low moisture foods. The descriptions by Labuza (1968), Rockland (1969) and Labuza et al. (1970) tell the general story.

In the monolayer region, one sees an acceleration of lipid phase reactions such as oxidative rancidity. According to Labuza (1971), the small amount of water that is needed to inhibit oxidative rancidity in the monolayer region apparently functions by facilitating destruction of free radicals, hydrogren bonding to hydroperoxides and slowing the rate of their conversion to other products, and hydrating or reacting with metals and thereby reducing their abilities to catalyze oxidation. As the water content is increased above the optimum level into the multilayer region, the lipid oxidation increases,

presumably, because of increased catalyst mobility and because new catalyst surfaces are exposed as the food matrix swells.

Non-enzymatic browning is accelerated if Type II water is present since the reactants are water soluble. At high water activity, however, dilution effect sets in to lower the rate. Because of increased enzymatic activity in this region, enzymatic processes such as enzymatic browning would be expected to increase too.

The region above 0.7 A_w in the sorption isotherm is marked by mold, yeast and bacterial activity.

It is known that desorption isotherms and resorption isotherms usually do not coincide, i.e. they exhibit hysteresis. At any given moisture content, the A_w during desorption is less than the A_w during resorption and at any given A_w the moisture content during desorption is greater than that during resorption. The cause is apparently related to interactions of nonaqueous constituents during desorption so that some adsorption sites are irreversibly lost, and differences in water vapor pressure needed to fill and empty capillaries of irregular shape (Rao, 1941). The lower moisture contents of samples during resorption results in their having greater viscosities than samples of the same A_w during desorption and one consequence, according to Labuza *et al.* (1972), is that oxidation at a given A_w proceeds more slowly in resorption

samples. Wolf et al. (1972) have related the extent of hysteresis to the quality of foods in storage.

Water activity may also be important in the quality of stored foods by the type of interactions that can occur between water and proteins. In most proteins, about 40% of the total amino-acids have non-polar side chains, such as the methyl group of alanine, the benzyl group of phenylalanine, the isopropyl group of valine, the mercaptomethyl group of cysteine and the secondary butyl and isobutyl groups of leucines. There is good agreement that these non-polar groups have a structure-forming action on adjacent water and that the interaction between water and the non-polar groups has an important influence on the reactivity of the protein and on its native tertiary conformation (Schackman, 1963).

The mechanisms of interaction between water and non-polar groups, however, is a subject of disagreement. According to Kauzmann (1959) and Scherage et al. (1962) the mechanism involves hydrophobic association in which, after a protein is placed in an aqueous environment, an alteration in tertiary structure occurs so that hydrophobic, non-polar groups can associate forming, in essence, an intramolecular micelle and thereby minimizing contact of these groups with water. The net effect is that a protein with a large number of exposed non-polar groups would have the greatest likelihood of aggregation and precipitation.

Klotz (1958) proposed a different mechanism. He noted that the non-polar side chains of certain amino-acids are analogous to compounds known to form crystalline clathrate hydrates. Since a large number of these groups exist along the protein macromolecule, Klotz has reasoned that they act cooperatively to induce a "stabilized arrangement of water in a microscopically crystalline array" with a structure similar to clathrate hydrates. Hydrophobic groups according to this view, would be abundant on the exterior of the molecule.

The water structure may also be enhanced by such non-polar compounds as alcohols, fatty acids and free amino-acids.

Stability of Lipids

Dugan (1976) has described stability as the capability of a fat, oil, or fatty food to maintain a fresh taste and odor during storage and use and that it is related to composition of the lipid moiety, the nature and degree of stress on the system, the presence or absence of prooxidants and antioxidants, and the effectiveness of packaging. He added that fats with substantial unsaturation in the fatty acids are usually unstable or moderately unstable and foods containing them reflect this instability; but that vegetable oils usually tend to be more stable than some of the animal fats, such as lard, even

though the total unsaturation of the vegetable oils may be greater because natural antioxidants are usually present in the vegetable oils. Smith and Dunkley (1962) and Waters (1971) have also referred to the promoting effects of light, especially of short wave length, heat and metal catalysts, especially iron and copper, in the autoxidation of the lipid components of foods.

The stability of the lipid or fat component of a food is, of course, related and relatable to the level of deterioration of that food. Lipolysis is a term which refers to the hydrolysis of ester linkages of lipids resulting from enzymes, from thermal stress, or from chemical action. A major cause of food deterioration is also attributable to fat rancidity as a result of oxidation—"oxidative rancidity". Lipolytic rancidity is thought to pose less of a flavor problem than oxidative rancidity because the former develops off-flavor only in those fats which contain short-chain fatty acids (less than C_{14}).

Evaluation of the State of Oxidation

Ultraviolet (UV) Absorption Method

The ultraviolet spectrum is particularly useful in composition studies on fats and in the analysis and identification of fatty materials. Spectral examination has become indispensable in studying and following chemical reactions of fatty materials, especially cis-trans and position isomerization, oxidation and polymerization. The spectra of fats can indicate whether they have been mishandled, since oxidation and polymerization products have characteristically different spectra from those of fresh materials (Swern, 1964). He has also stated that unsaturated fatty materials absorb light in the ultraviolet region of the spectrum. This region is between 200-400 nm. In the case of non-conjugated and saturated materials, the absorption is weak and general and cannot be used for analytical purposes.

In describing the application of UV absorption to oxidation studies, Mehlenbacher (1960) also indicated that the oxidation of polyunsaturated fatty acids produces peroxides and the position of the double bond shifts to a conjugated form. Conjugated linkages give rise to characteristic and intense absorption bands within the spectral range of 200-400 nm, while the absorption of isolated double bonds within the same region is very weak. This, he

added is the basis for the ultraviolet absorption method for determining the state of oxidation.

However, many workers including Rusoff et al.(1945), and Pitt and Morton (1957) have reported ultraviolet spectral data on numerous non-conjugated compounds.

In addition, when ultraviolet absorption methods indicate the presence of small quantities of conjugated compounds in natural fats, the results must be carefully interpreted because non-conjugated polyunsaturated components may have undergone conjugation as a result of autoxidation or other mishandling (Swern, 1964). He had earlier reported (1961) that the oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption and that the magnitude of change is not easily related to the degree of oxidation because the effects upon the various unsaturated fatty acids are different in quality and magnitude as depicted in the analysis of a sample containing dienoic, trienoic, tetraenoic and pentaenoic groups where the total diene content was due not only to linoleic acid, but also to each of the more unsaturated acids. Therefore, according to Swern, the spectral change for a given substance should be used as a relative measure of oxidation, rather than its measurement.

In a study on the shelf-life stability of peanut butters during long and short-term storage, Angelo et al. (1975) found good correlation between the .

spectrophotometric determination of conjugated diene hydroperoxides and the peroxide value determinations, over four and twelve week periods of storage. The spectrophotometric estimation of conjugated hydroperoxides was stated to require smaller samples, to be quicker, more accurate and simpler than the method of peroxide value determination. The non-requirement of additional reagents and non-dependence upon a chemical reaction or color development were also stated as obvious advantages over the method of peroxide value determination. Vitamin A, which has a characteristic maximum at 325 nm can be determined in fats and in Vitamin A concentrates by absorption in the ultraviolet region of the electromagnetic spectrum. β -unsaturated carbonyl compounds can be detected in autoxidized fats. Ultraviolet absorption has been used fairly extensively in studying the autoxidation of drying oils since the conjugation of polyunsaturated components parallels oxygen absorption (Swern, 1964).

Evaluation of Protein Quality

One method for assessing the quality of a protein material involves measurement of protein efficiency ratio (PER). It is a ratio of the weight gained in grams by test animals, usually rats, to the weight in grams of the protein consumed. In short, PER measures protein quality by assessing its ability to support the growth of test animals. The method was developed by Osborne et al. (1919). Onayemi and Potter (1976) used the PER procedure to evaluate the protein quality of drum-dried cowpea powders in storage. They found that the PER of stored powders increased from 1.64 without methionine supplementation to 2.65 at the 0.6% addition level.

Another method of protein evaluation was developed by Mitchell (1924) which measures the nitrogen retention of test animals over a period of time, when they are fed first a diet devoid of protein and then fed the protein material.

Instead of using rats, Elliot (1963) used the meadow vole (Microtus pennsylvanicus) over a six-day test period to assess protein quality. Later, Iriarte (1969) adopted the procedure to measure the PER value of navy bean powder. Results from the study agreed with those obtained later by Miller et al. (1973) and Bressani et al. (1963).

Methods involving the use of micro-organisms have been developed (Ford, 1960).

Protein quality may also be evaluated by methods based on its digestibility by animals as was done by Chen et al. (1962).

When measuring protein quality using laboratory animals, age, species, strain and general condition of the animals must be taken into account (Ford, 1962).

The limitations imposed by age, species, strain and the time required to perform biological evaluations of protein quality have led to the development of chemical methods. Oser (1951) used essential amino acid index as a measure of protein quality. Moore et al. (1958) used essential amino-acid analyses while the chemical score was used by Mitchell and Block (1946).

It is known that deficiency of any of the essential amino-acid in a protein will prevent the utilization of the protein, Said et al. (1974). So, if direct destruction of essential amino-acids have occurred as might happen in processing and storage the chemical methods referred to above may be a good assessment of protein quality.

However, it is known that chemical analyses might reveal the presence of an essential amino-acid in a protein sample even when this amino-acid is not available to the animal. Based on this knowledge, many workers have used in-vitro enzymatic digestibility procedures to measure protein quality (Melnick et al., 1946; Eldred and

Rodney, 1945; Clandinin, 1949; Clandinin et al., 1951; Jones et al., 1942; Evans and St. Johns, 1945; Evans and Butts, 1948; Jones and Gersdorff, 1941; and Carpenter, 1958).

Based on the pepsin digest residue index (PDRI) of Sheffner et al. (1956), Akeson and Stahman (1964) used a double enzyme system of pepsin and pancreatin in measuring protein quality.

Other workers have attempted to measure protein quality simply by measuring chemically available lysine. Carpenter et al. (1957, 1958 and 1960) reported good agreement between results obtained biologically and those obtained by measuring chemically available lysine. Similarly, Buttersworth and Fox (1963) established a relationship between protein solubility and chemically available lysine. Mattu and Mauron (1967) also obtained good agreement between in-vivo and in-vitro evaluations of protein quality based on available lysine measurements.

Although chemical assessments of protein quality have the advantages of simplicity, lower cost, and rapidity, the specificity of proteases (Byers, 1967) and ionic environment (Saunders, et al., 1973) must be taken into account.

Browning Reactions

"Browning reactions are a part of the natural processes of decay; e.g. the drying and decomposition of vegetation of humus, peat, and coal. When ordered, life-supporting substances are deprived of the protection of water, they become oxidized and/or chemically dehydrated to reactive intermediates; these polymerize into highly ordered dull-brown humic substances. The dull-brown color itself indicates a disordered array of red and yellow chromophores and a diffuse light absorption spectrum. Browning, therefore, consists of a series of "downhill" exothermic chemical reactions that proceed spontaneously after the "uphill" activation of energy-filled molecules" (Hodge and Osman 1976).

The conventional thing has been to classify browning reactions according to the catalysts involved. Hodge and Osman (1976) however, believe that since the initial reactions are either oxidative or non-oxidative in which catalysis by enzymes may or may not be involved, browning reactions should be classified as oxidative and non-oxidative, not enzymic or non-enzymic as is now done.

When enzymes are involved in oxidative browning, they catalyze only the first step in the process. For example, as a first step only, catecholase or catechol oxidase, ascorbate oxidase, or lipoxidase will catalyze the conversion, respectively, of phenol, enediol, and/or

conjugated diene functional groups to reactive carbonyl compounds. The reactions that follow this transformation are non-enzymic. Even the initial oxidation reaction can occur, although much more slowly, in the absence of the oxidases. For example, the conversion of ascorbic acid to the vicinal tricarbonyl compound dehydroascorbic acid can occur enzymically or non-enzymically. Once formed, dehydroascorbic acid can react non-enzymically with amino-acids to produce red and brown polymers (Ranganna and Setty, 1974).

As indicated by Phillips (1963) and by Weitzel et al. (1957), oxidations and subsequent degradations with browning are induced in carbohydrates by ultra-violet and ionizing radiations. Alditols are oxidized to aldoses, aldoses are oxidized to osones (glycosuloses) and aldonic acids, aldonic acids are oxidized to 2-keto aldonic acids (which enolize to ascrobic acids) and car-bonyl groups are introduced into polysaccharides to in-itiate their degradation by β -elimination reactions.

Non-Oxidative Browning

Non-oxidative browning may also involve enzymes in the initial step. For example, in the supposedly non-enzymatic browning process in natural products, the first step involving the release of reactive reducing sugars from their conjugates is catalyzed by the glycosyl hydro-lase enzyme. Hodge (1967) indicated that this was an

important step in color and flavor development in dates, honey, maple syrup, chocolate, and vanilla.

After the sugars have been released enzymatically or non-enzymatically, they go through ring opening, enolization, dehydration, and fragmentation reactions to produce more reactive hydroxycarbonyl, α-dicarbonyl and $\alpha\beta$-unsaturated carbonyl compounds. These carbonylic intermediates then react to produce brown polymers and flavor compounds, as in caramelization of sugars. Browning may, however, be accelerated greatly by condensation with amines or amino-acids. When this happens, according to Hodge and Osman (1976), nitrogen is found covalently bonded in some of the simple end products such as pyrrole aldehydes, alkyl pyrazines and imidazoles and in the more complex ones like the melanoidin polymers of foods.

Caramelization: The essential reactions in the caramelization process are (a) inversion of sucrose to D-glucose and D-fructose (b) equilibration of anomeric and ring forms (c) condensation, intermolecular, i.e.; acid-catalyzed reversion of starch sugars to di, tri- and higher oligosaccharides, (d) condensation, intramolecular, i.e.; formation of glycosans and difrustose dianhydrides, (e) isomerization of aldoses to ketoses (f) dehydration reactions and (g) browning (formation of unsaturated polymers) (Hodge and Osman, 1976).

Sugar-Amine or Maillard Browning: The name "Maillard Browning" derives from the fact that Maillard (1912) first attempted to characterize the sequence of reactions involved in the process by studying the reactions between the carbonyls of reducing sugars and free amino-acids. However, it has to be noted that, as has been demonstrated by many workers, the carbonyl function could come also from lipid oxidation-products rather than from reducing sugars (Jones and Gersdorff, 1941; Tappel, 1955; Lea, 1958; Burton et al., 1962; Hoffman, 1962; Koch, 1962; Narayan and Kummerow, 1958; Crawford et al., 1967).

Hodge (1953) made a review of the chemistry of the Maillard browning but a more recent description by Hodge and Osman (1976) is presented below.

A. Initial state (Colorless; no absorption in near-ultraviolet).
   Reactions involve condensation, enolization, Amadori rearrangement. With proteins, glucose and free amino groups combine in 1:1 ratio. Properties include increase in the reducing power in alkaline solution. Storage of a colorless 1:1 glucose-protein product produces browning and insolubility.

B. Intermediate stage (buff yellow; strong absorption in near-ultraviolet range).
   Reactions involve sugar dehydration to 3-deoxyglucosone and its -3, 4- ene, HMF, and

2- (hydroxyacetyl) furan; sugar fragmentation; formation of 1-dicarbonyl compounds, reductones, pigments. Properties include the fact that addition of sulfite decolorizes; reducing power in acidic solution develops, pH decreases; sugars disappear faster than amino acids. With proteins, acid hydrolysis fails to regenerate the sugar (D-glucose). Positive Elson-Morgan test for amino sugars (Amadori compounds).

C. Final stages (red-brown and dark brown color) Reactions involve aldol condensation; polymerization; Strecker degradation of α-amino acids to aldehydes and N-heterocyclics at elevated temperatures. CO_2 evolves. Properties include acidity, caramel-like and roasted aromas develop; colloidal and insoluble melanoidins form; fluorescences; reductone reducing power in acid solution; addition of sulfite does not decolorize.

α-Amino acids, peptides, and proteins offer basic amino groups when the pH of the adjacent medium rises above the iso-electric point of the amino compound. An exposed epsilon-amino group of lysine residues in proteins reacts like a separate amine. Some reactive non-protein amino compounds present in foods include 4-amino butyric acid, thiamine, and piperidine-2-carboxylic acid. If spoilage

has begun, ammonia, methylamines, and histamines could also be present to react.

According to Overend, et al. (1961) the initial rate of browning of a reducing sugar with an amino compound is dependent on the rate at which the sugar's ring opens to its reducible form. Pentoses and 2-deoxy-D-ribose were reported to undergo browning faster than 2-deoxy hexoses which in turn brown more readily than hexoses. Among the hexoses, D-galactose, D-mannose, and D-glucose show decreasing browning rates (Lea and Liau, 1972).

Hodge (1967) referred to the Strecker degradation of amino-acids. When temperatures are high enough α-dicarbonyl compounds such as 3-deoxyglucosone, pyruvaldehyde, glyoxal, or dehydroascorbic acid will cause the degradation of an α-amino acid to the next lower aldehyde. This has been demonstrated by Fujiimaki et al. (1969) and Wang et al. (1969).

Some melanoidins are soluble in water and aqueous alcohol while some are insoluble. As hinted by Kato et al. (1968) and by Kirigaya (1968), they usually exhibit reductone reducing power, even after dialysis and antioxidant activity toward linoleic acid and in strongly browned foods and dark-beers.

The lowering of food quality which normally accompanies Maillard browning have previously been discussed. In the report of Fragne and Adrian (1971), lysine, methionine, and the N-terminal amino-acids of proteins were

indicated to react most strongly with pentoses, less strongly with D-galactose and D-mannose, less with D-glucose, still less with D-fructose and lactose, and least with sucrose. Sucrose was reported to react very strongly with lactalbumin, although not with lacto-globulin.

"The losses of lysine and methionine that have been demonstrated in model systems of proteins and sugars were obtained under rather drastic conditions that do not occur during normal processing of high protein foods. However, losses of these amino-acids in roasted and flaked cereals (e.g.; breakfast foods) may be significant because they are not abundant at the outset. More important are the off-flavors and dark colors that develop from the Maillard reaction in dehydrated products. These sensory defects may cause consumer rejection of otherwise nutritious foods." (Hodge 1976).

MATERIALS

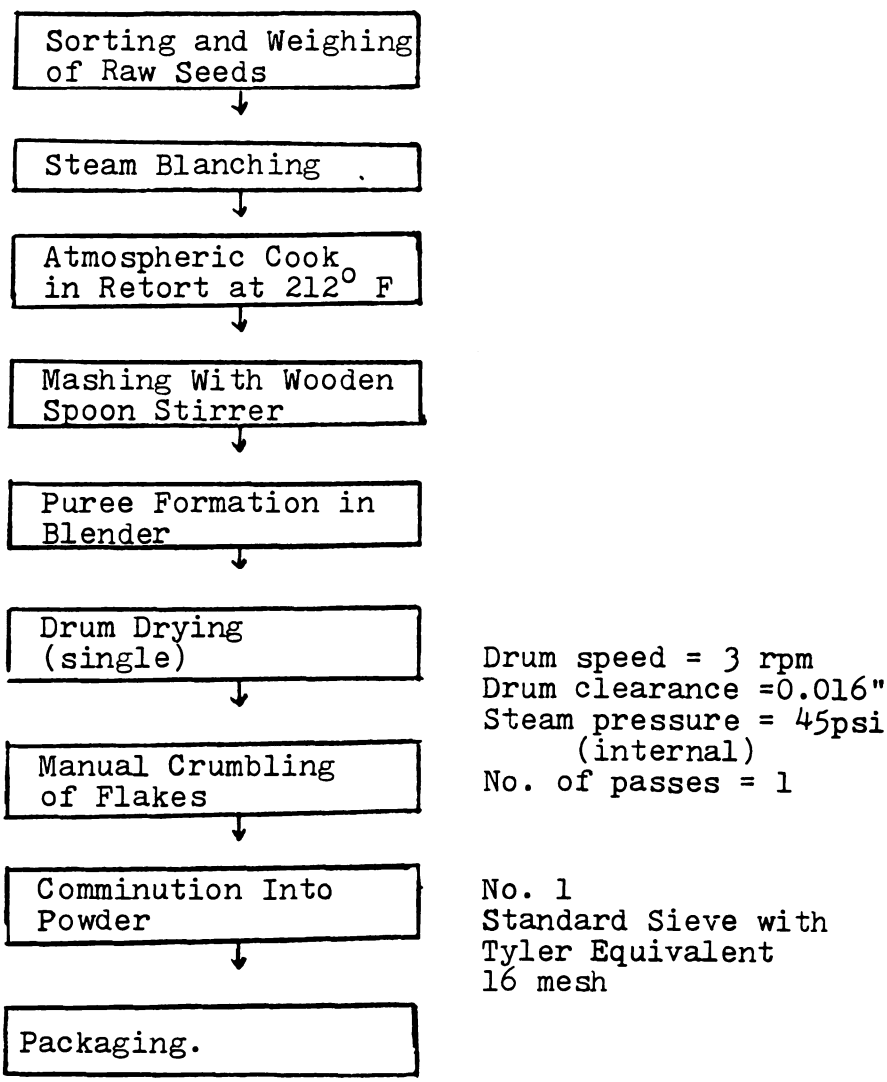
Cowpea (Vigna unguiculata) was obtained from National Seed Co. in New Orleans.

Reagents and Solvents: All reagents and solvents were of analytical grade, except those for HPLC and spectrophotometric measurements which were of spectro grade.

All reagents and solvents when not in use were stored in accordance with the specifications of the manufacturers.

Preparation of Drum-Dried Cowpea Powders

The drum-dried cowpea powders were prepared from the cowpea seeds obtained from a seed company in New Orleans as outlined in the flow diagram below.



Preparation of Raw Cowpea Powders

Raw cowpea powders were prepared from the raw cowpea seeds by using the mill (Hobart Mfg. Co., Model #3430) located in the Animal Science Department Building at Michigan State University. Powders able to pass through No. 1 Standard Sieve with Tyler Equivalent of 16 mesh were thus obtained and packaged ready for storage studies.

Experimental Set Up

Drum-dried and raw cowpea powders obtained as described above were employed in the storage studies.

400 g of samples were weighed into 500 ml beakers and stored in desiccators where water-activities of 0.11, 0.33 and 0.75 were obtained according to the method described by Rockland (1960) and at a uniform temperature of 25° C.

Similar samples were stored at three different temperatures of 5° C, 25° C and 40° C all at a uniform water activity of 0.75.

Samples were analyzed on a monthly basis for the various physical and chemical parameters of quality.

METHODS

Extraction of Total Lipids

Total lipids were extracted by the method described by Folch *et al*. (1957). This method makes use of a 2:1 v/v chloroform-methanol solvent.

50 grams of sample were homogenized at high speed in a Vortex homogenizer with 300 ml of the 2:1 v/v chloroform-methanal solvent for three minutes. The homogenized sample was then filtered through a Buchner funnel fitted with a #1 Whatman filter paper after which the resulting filtrate was transferred to a 500 ml separatory funnel. The residual cake was re-extracted with 100 ml of additional solvent for one minute.

The crude lipid extract so obtained was washed with 0.2 its volume with 0.74% KCl solution and allowed to stand overnight at refrigeration temperature to facilitate its separation. The chloroform layer was then collected by passing the solution through a glass funnel containing about 20 grams of anhydrous sodium sulfate into a glass-stoppered round bottomed flask.

After this step, the solvent was evaporated at 20° C by using a rotary vacuum evaporator (Rinco Instrument Co.). A stream of nitrogen was used to evaporate traces of chloroform.

Lipid so obtained was stored at refrigeration temperature when not in immediate use.

Measurement of Lipid Diene Conjugation

Lipid oxidation in the stored samples was followed by taking lipid diene conjugation readings on a monthly basis over a six-month storage period.

10 mg of lipid obtained as already described was placed into 30 ml test-tube into which 10 ml of iso-octane (2,2,4-trimethylpentane) was poured and mixed thoroughly in a Fisher mini shaker. The mixture was then filtered through a Whatman No. 1 filter paper and absorbance values taken in duplicate on a Beckman DU spectrophotometer at 233 nm using iso-octane as blank.

Gas-Liquid Chromatography

Fatty acid contents of cowpea lipids were determined by gas-liquid chromatography.

Preparation of Fatty-Acid Methyl Esters

A rapid method described by Metcalfe *et al*. (1966) for preparing methyl esters from lipids was used to prepare these esters from the lipid extract obtained from stored samples.

4 ml of 0.5N methanolic NaOH was added to 150 mg of lipid extract in a 50 ml volumetric flask and the mixture heated on a steam bath until the lipid globules were in solution (about 5 minutes). This was then followed by boiling the soaps with 5 ml of BF_3-methanol for 2 minutes to give a quantitative conversion of the fatty-acids to methyl esters.

Enough of a saturated solution of sodium chloride was then added to the flask to float the methyl esters up into the narrow neck of the flask where they were readily withdrawn with a syringe.

Gas-Liquid Chromatography of Methyl Esters

Gas-liquid chromatography was carried out on a 5830 A Gas Chromatograph (Hewlett-Packard) equipped with a computerized integrator for peak area and percentage fatty-acid quantifications.

The column was of glass (6' x ¼" o.d.) packed with 15% DEGS on 80/100 mesh Chromosorb W. The operating conditions used were:

Injection temperature	220° C
FID Temp.	250° C
Oven Temp.	190° C
Chart speed	1.00
Carrier gas flow rate	27 ml/min

Extraction of Reducing Sugars

The Official Method of Analysis of the Association of Official Analytical Chemists (AOAC) (1970) was used to

extract sugars from the samples and to determine the reducing sugar content of the stored powders.

Determination of Available Lysine

A. Spectrophotometric Method

The reagent, 1-fluoro-2,4-dinitrobenzene (FDNB) has been used by Sanger (1945) and Porter and Sanger (1948) to determine the free epsilon-amino groups of proteins. To study the functional role of epsilon-amino groups in the biological activity of proteins, Massey and Hartley (1956), Ikenaka (1959) and Blass and Raymond (1960) also used the reagent.

Carpenter (1960) developed a method involving the use of FDNB to determine the available lysine content of protein foodstuffs and observed a good correlation between the available lysine content and the biological value of the protein (Boyne et al. 1961; Carpenter and March, 1961). Other investigators such as Kakade and Evans (1966) and Boctor and Harper (1968) reached the same conclusion. However, the FDNB method is laborious and time consuming for the routine analysis of proteins.

To overcome this limitation, Kakade and Liener (1969) developed a method using the reagent 2,4,6-trinitrobenzesulfonic acid (TNBS) which specifically reacts with primary amino groups. (Okumaya and Satake, 1960; Satake, et al., 1960; and Kotaki and Satake, 1964).

The method has the added advantage of differentiating between the free epsilon (ε)-amino and N-terminal amino groups of proteins. The method was used in this project to determine the available lysine content of the cowpea powder samples.

B. High Performance Liquid Chromatography (HPLC) Method

Peterson and Warthesen (1979) have described a method which employs a high performance liquid chromatography technique. Advantages claimed for this technique include speed of resolution and the avoidance of interference from some products formed during sample hydrolysis.

This method was used in this study to compare it with the spectrophotometric method described by Kakade and Liener (1969).

Measurement of Browning Pigments

The procedure of Choi *et al*. (1949) as modified by Karel and Labuza (1968) was used to measure the intensity of browning in the stored samples.

Color Measurements By The Hunter Colorimeter

The color of the stored samples were also measured on a monthly basis by using the Hunter Colorimeter (Hunter Associates Laboratory, Inc., VA).

Enough amount of sample (40 gms) was added to the colorimeter cup to cover the bottom of the cup such that there were no transparent areas. After standardizing the equipment with a white plate standard supplied with the equipment, "L" values which measure the degree of whiteness of the samples, were determined.

Determination of Soluble Protein

The first thing that was done in this determination was to extract the soluble protein from the cowpea samples.

5.0 g of cowpea powder was weighed accurately into 50 ml centrifuge tube containing 10 ml 8 M urea: 1 N sodium hydroxide (1:1 v/v). The cowpea powder was allowed to be in contact with the extracting solvent for 40 minutes after which the extracts were clarified by centrifugation at 10,000 g for 25 minutes. Love (1975) used a 15 minute centrifugation period but experience gained in course of this research revealed that at least a 25 minute centrifugation was necessary to obtain a truly clear extract. This is a crucial step in this determination because cloudly extracts can lead to gross overestimation of soluble protein content of food stuffs like cowpea powder. A model HR-1, IEC centrifuge was used in this study.

The clear aliquots so obtained were used in the determination of the soluble protein content according to

the procedure described by Leggett-Bailey, (1969) and Gornall, (1949).

First, a standard curve using bovine serum albumin (BSA) was prepared as described below.

5.0 ml of clear BSA solution (10 gms in 100 ml solution were pipetted into a stoppered graduated cylinder, and diluted to 50 ml with 0.9 percent sodium chloride and mixed.

In duplicate, a series of nine cuvettes (or test-tubes) were prepared. Into them 2.0, 1.85, 1.70, 1.55, 1.40, 1.20, 1.00, 0.80, and 0.60 of 0.9 percent saline were successively pipetted. Then in the same order, 0, 0.15, 0.30, 0.45, 0.60, 0.80, 1.00, 1.20, and 1.40 ml of the diluted serum was added.

8 ml of Biuret reagent (1.50 g cupric sulfate + 6.0 g potassium tartrate dissolved in 500 ml of water. With swirling, 300 ml of 10 percent sodium hydroxide and made to volume with water) were then added, mixed and absorbance read after 30 minutes at 540 mu. Absorbance at this wavelength using a digital spectrophotometer was plotted against the calculated mg BSA to obtain a standard curve whose linearity extended up to 25 mg BSA. Values for the sample were read off within this range of linearity. The cuvettescontaining 2.0 ml saline i.e. 22.6 percent sodium sulfate solution served as blank.

To 2 ml of the cowpea powder extract, was added 8.0 ml of the Biuret reagent and mixed and its absorbance read

off at 540 mu after 30 minutes. The corresponding amount of soluble protein was read off from the standard curve.

Determination of pH

The pH of the samples were determined by weighing accurately 5.00 g of material into a 100 ml beaker after which 50 ml of deionized distilled water were added. The sample was stirred into the water with a glass-rod and left to stand for 30 minutes. This allowed the aqueous phase to separate from the solid phase, while allowing for acid extraction.

pH readings were obtained on a digital pH meter (Leeds and Northrup Model #7421) by allowing the pH electrode to dip down into the sample slurry which was stirred with a glass rod just before electrode immersion.

Before use, the pH meter was standardized with buffer solutions of pH 4.01 and 7.0.

Determination of Lipid Free-Amino Groups

DeKoning (1966), Lea and Rhodes (1955) and Magee *et al*. (1960), all have used the reaction with ninhydrin for the detection and analysis of lipids containing free amino groups.

Ninhydrin reagents, however, are difficult to use since reproducible color yields are obtained only with very careful control of all conditions and color yields

vary widely with different substances. In contrast, reactions with trinitrobenzene sulfonic acid (TNBS) yield uniformly reproducible and stable intensities for free amino groups of a variety of substances both lipid and non-lipid.

The procedure described by Satake (1960) for amino-acids cannot be applied to lipids because of their hydrophobic nature. Accordingly, Siakotos (1967) has described a rapid method for the determination of lipids containing free amino groups with TNBS. This method was used in this work.

Determination of Ascorbic Acid

The titrimetric method described by the Association of Vitamin Chemists (1966) was used to determine the ascorbic acid content of the cowpea powders.

Fluorometric Determination of Riboflavin

The Association of Vitamin Chemists (1966) fluorometric procedure was used to determine the riboflavin content of the stored cowpea powder samples. Riboflavin fluoresces in radiation of wavelength 440 to 500 nm with intensity of fluorescence proportional to the concentration of riboflavin in dilute solutions. The riboflavin is measured in terms of the difference between the fluorescence before and after chemical reduction.

Since riboflavin is light-sensitive and is most readily destroyed by light in the blue and violet regions, it was necessary to perform all operations in this study in the absence of strong light.

Fluorometric Determination of Thiamine

The method described by the Association of Vitamin Chemists (1966) was used to determine the thiamine content of the stored cowpea products.

Proximate Analysis of Cowpea Powders

Proximate analyses of both the drum-dried and raw milled cowpea powders involving moisture, ash, protein, fiber and lipid determinations were made. Except for fiber which was determined according to the procedure described by Whitehouse *et al*. (1945) all other determinations were made in accordance with the procedure stipulated in the AOAC (1970).

RESULTS AND DISCUSSION

Proximate Analyses of Raw and Drum-Dried Cowpea Powders

The proximate analyses for both the raw and drum-dried cowpea powders are shown in Table 1. Both samples are high in crude protein content. This, in combination with the reported good profile of the essential amino-acids particularly lysine (Bressani *et al.*, 1961), makes cowpea products a nutritionally valuable source of protein.

Drum-drying of the cooked beans did not appreciably alter the crude protein content. The slight decrease in the crude protein content of the drum-dried powder may have been due to losses during the thermal treatments of blanching, atmospheric cooking and the drum dehydration process itself.

TABLE 1 PROXIMATE ANALYSIS OF COWPEAS

Parameter[a]	Drum-Dried Cooked Sample	Raw Un-Processed Sample
Moisture, %	3.26	10.17
Crude Fibre, %	2.19	2.32
Total Lipid, %	1.97	2.08
Crude Protein, %	25.22	26.15
Total Ash, %	3.48	3.44
Carbohydrate, % (by difference)	67.14	66.01

a = (i) values are the means of duplicate readings

(ii) values, except for moisture, are based on the % dry matter content.

The powders had low lipid contents just like most legumes. Many flavor problems of foods are associated with their lipid content which are subject to hydrolytic or oxidative rancidity. Although it is also true that the lipid content of foods may also contribute positively to their flavor, the low lipid content reported for these powders may be important in maintaining their wholesomeness during storage.

The very small difference between the total lipid content of the raw and drum-dried powders may also have been due to losses during the thermal treatments associated with the whole drum-drying operation.

Both powders were low in crude fiber content which agrees with the results of Bressani et al. (1961). Drum-drying and cooking had virtually no effect on the crude fiber content. Apparently the conditions employed were not severe enough to cause changes in the amount of crude fiber which normally constitutes one of the more stable components of plant materials. In addition to this, the dehulling step which the few workers who have prepared drum-dried powders from cowpeas, such as Onayemi and Potter (1976) and Kon et al. (1974), have included in their procedure, was omitted in this study. First, the inclusion of the seed hulls did not impart any objectionable color to the product as long as the cooked beans were mashed and blended properly and uniformly before the drum-drying

step. Second, many food nutrients especially minerals and vitamins are lost when the hulls are removed. Finally, the increasingly documented speculation of the importance of food fibers in promoting gut peristalsis, food energy dilution, and in preventing certain forms of colonic cancer demanded that the seed hulls be retained in the preparation of the cowpea powders used in this study.

The low ash contents of both samples also agree with literature values (Oyenuga, 1968) and Bressani, et al. (1961).

The only marked difference between the raw and drum-dried cooked powders occurred in the moisture content values. As the table shows, the drum-drying process was very effective in reducing the moisture content of the powders.

Lipid Oxidation As Measured by Diene Conjugation

Figures 1 and 2 and Table 2 show the effects of varying the water activities, temperature, and the storage time on lipid oxidation as measured by lipid diene conjugation absorbance at 233 nm., for both the raw and drum-dried cowpea powders.

It is seen that an increase of water-activitiy (A_w) from 0.11 to 0.33 led to a decrease in the lipid oxidation of each product when the samples were stored at a constant temperature of 25° C. However, when the

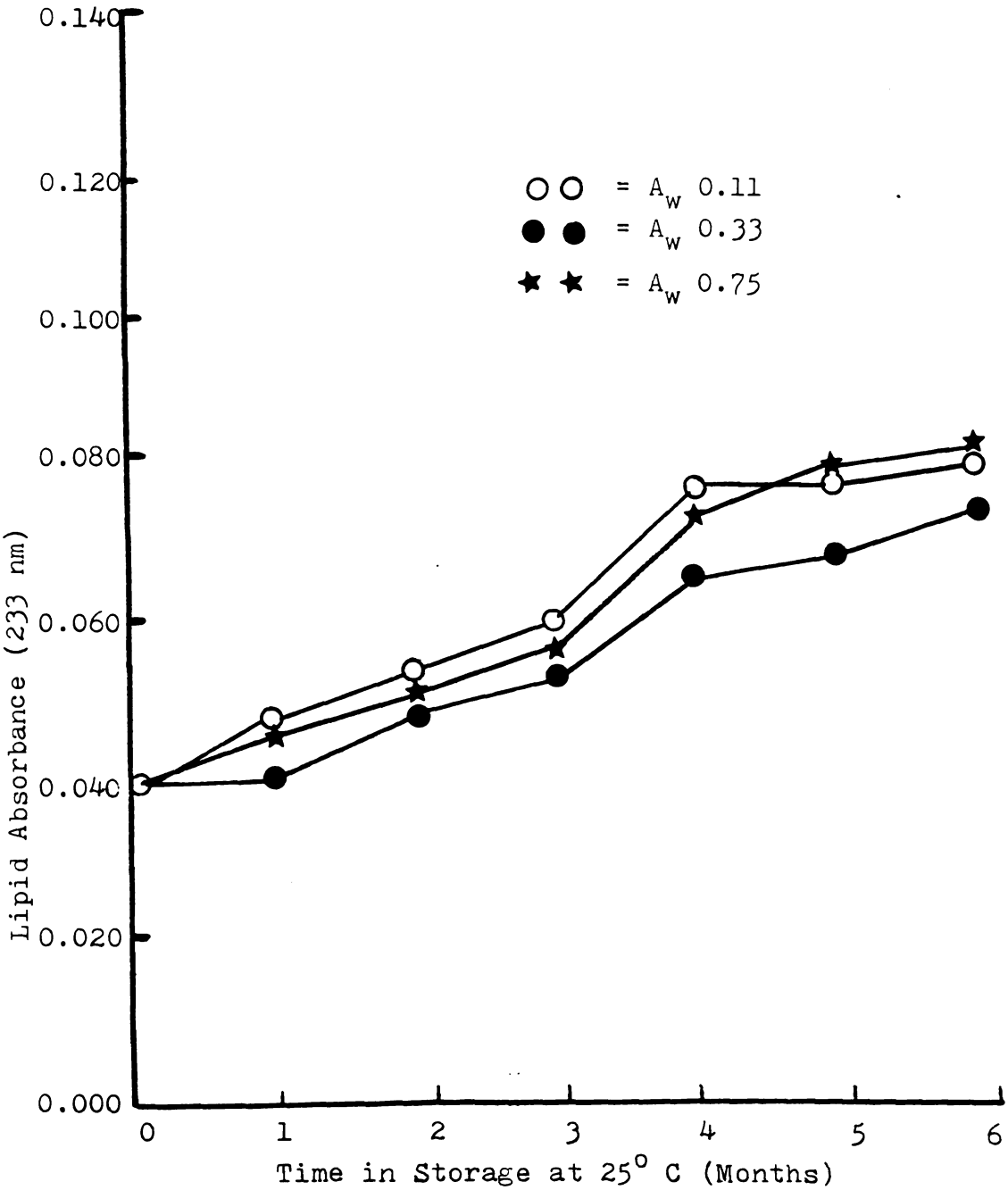


FIGURE 1. Effect of A_w on Lipid Absorbance at 233 nm In Drum-Dried Cowpea Powders During Storage

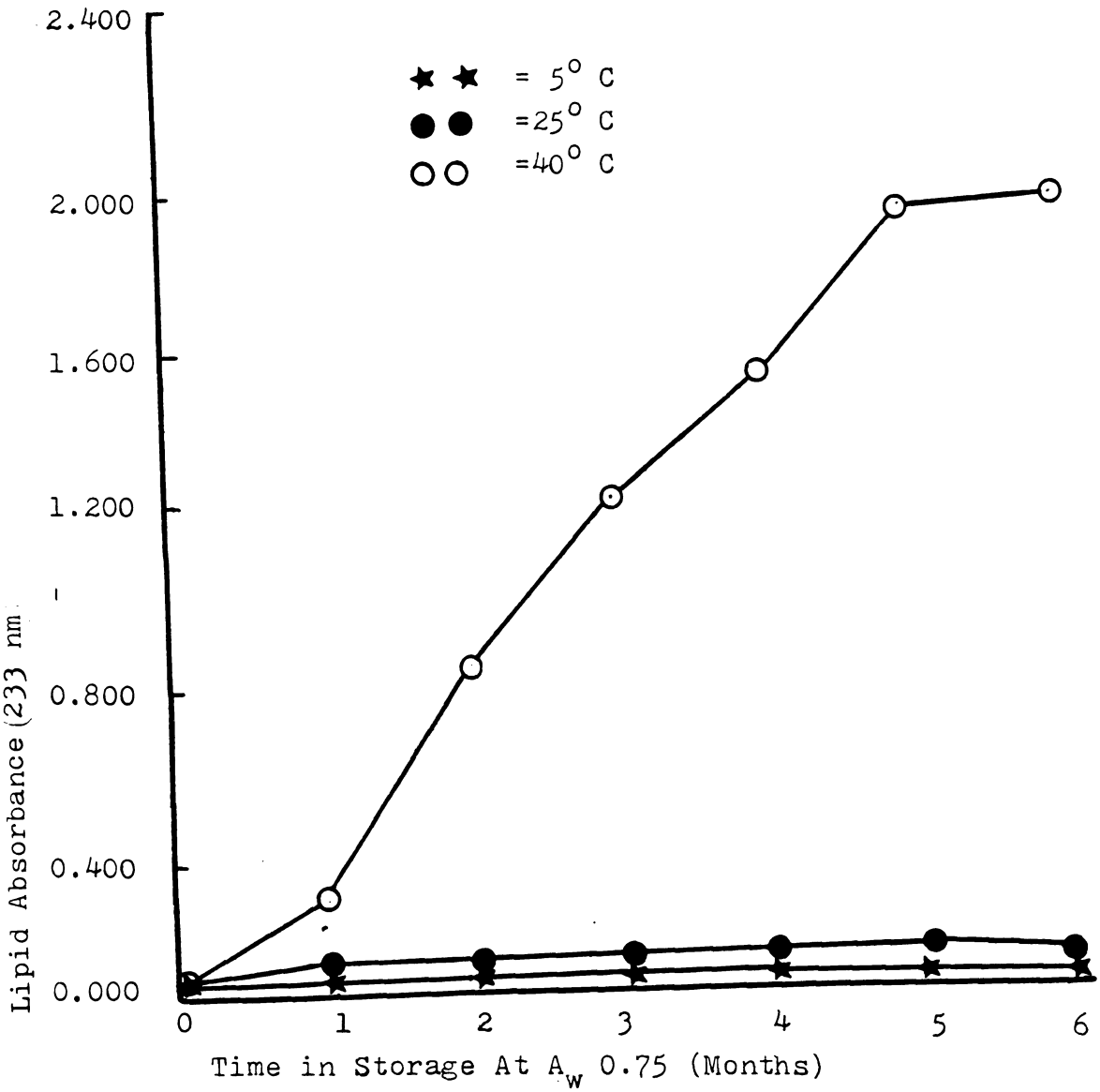


Figure 2. Effect of Temperature on Lipid Absorbance at 233 nm. In Drum-Dried Cowpea Powders During Storage

TABLE 2 EFFECT OF WATER ACTIVITY AND TEMPERATURE ON THE ABSORBANCE AT 233 NM* OF LIPID DERIVED FROM RAW COWPEA POWDER

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
0.030	0.030	0.030	0.030	0.030	0.030
FIRST MONTH'S READINGS					
0.038	0.030	0.039	0.032	0.033	0.700
SECOND MONTH'S READINGS					
0.041	0.036	0.042	0.031	0.036	1.007
THIRD MONTH'S READINGS					
0.073	0.038	0.074	0.033	0.075	1.409
FOURTH MONTH'S READINGS					
0.138	0.078	0.141	0.032	0.143	1.804
FIFTH MONTH'S READINGS					
0.165	0.115	0.148	0.034	0.146	1.843
SIXTH MONTH'S READINGS					
0.173	0.137	0.150	0.035	0.152	1.862

*Values are the means of duplicate readings

A_w was raised to 0.75 from 0.33, an increase in lipid oxidation was recorded. This means that there was a multistage trend in the relationship of lipid oxidation to changes in the A_w of the system. This trend is in agreement with many others described in the literature (Ayerst, 1965; Labuza, 1968; Labuza, 1970).

In attempting to explain the lowered lipid oxidation at higher water activities, Labuza (1971) stated that water at these high water activities may be facilitating the destruction of free radicals, reducing the rate of hydroperoxide conversion to other products by hydrogen bonding to them and by hydrating or reacting with prooxidant metal catalysts so that they are rendered ineffective. The recorded increase in lipid oxidation when the A_w was increased from 0.33 to 0.75 may also be explained according to Labuza (1971) who speculated that such high A_w water may be promoting catalyst mobility and or new catalyst surface exposure.

The effect of storage was to increase lipid oxidation as the length of storage increased. It was estimated that for the raw cowpea powders at 0.11 A_w the induction period for lipid oxidation was about two months. At 0.33 A_w, the induction period increased to 3½ months; but at A_w 0.75, the induction period decreased to 2 months again. This is another way of looking at the protective effect of water against lipid oxidation in the stored samples, since shorter induction periods indicate a greater tendency to oxidize.

It was not possible to determine precisely the induction periods for the drum-dried samples because of the small monthly changes in absorbance values as depicted in Figures 1 and 2.

In both the raw and drum-dried cowpea powders, increases in temperature from 5° C to 25° C to 40° C led to increases in lipid oxidation. Chemical reactions generally are accelerated when temperatures are increased. This is even more evident for the initiation process of lipid autoxidation. The energy requirement for radical production by rupture of a CH bond is about 80 Kcal which is regarded as excessive. Accordingly, any input of thermal energy is likely to increase oxidation as is demonstrated graphically in the Figures and Table. It is also obvious that a temperature of 5° C was quite effective in protecting the cowpea powders against any measurable increase in oxidation over the time span of this study. At this temperature, there was no discernible end of what might be called an induction period for the raw samples. At 25° C it was about 2 months and at 40° C it was less than 1 month. The drum-dried cowpea powder had no discernible end of an induction period. At 40° C, it was again less than 1 month. These values are shown in Table 3. The potency of temperature as high as 40° C in inducing cowpea lipid oxidation is apparent. Apart from supplying greater energy for the oxidation processes, the high storage temperature of 40° C may also have acted

TABLE 3 ESTIMATED INDUCTION PERIODS (MONTHS) OF STORED COWPEA POWDERS

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powder	-	-	-
Raw Powder	2	3.5	2

	A_w 0.75		
	5° C	25°C	40°C
Drum Dried Powder	±	±	1
Raw Powder	±	3	1

- = No clear cut induction period

± = No discernable end of induction period because of high stability

to increase the rate of oxygen diffusion into the system to promote faster lipid oxidation. This would have been particularly important in view of the milling and drum-drying operation which reduced the cowpeas to powders with more surface area and porosity for oxygen diffusion and oxidation reactions. It also means that, in the warm areas of this country and in all of Nigeria, low temperature storage (5^{o} C) is advised for these products if lipid oxidation and the consequent flavor problems are to be avoided.

Initially, the raw samples had lower oxidation status than the drum-dried samples but, at the end of the six month storage period, the raw samples had oxidized more than the drum-dried samples. The thermal treatment associated with the drum-drying process may have caused some initial oxidation of the drum-dried cowpea powder lipids to account for its higher oxidation status. The higher oxidation status of the raw powders at the end of storage may have been due to the fact that both autoxidation and lipoxygenase catalyzed oxidation were occuring in these samples. Lipoxygenase, which is principally a plant enzyme, is known to be distributed widely in legumes. For example, Erickson (1967) and Erickson and Suensson (1970) demonstrated lipoxygenase activity in peas, and recommended blanching to inactivate these enzymes. These would have been inactivated in the drum-dried samples and not in the raw powders in this study.

Table 4 shows the rate of oxidation (increase in lipid absorbance per month) for the cowpea powders which was calculated from the following devised formula:

$$R = \frac{\Delta X}{T}$$

Where

ΔX = Total change in value under consideration (lipid oxidation value as measured by diene conjugation absorbance at 233 nm), over a six month period.

T = length of storage

R = rate of change in oxidation, over the storage period. (increase in lipid conjugated diene absorbance per month).

The table reveals that the raw samples had a higher monthly rate of oxidation than the drum-dried samples at all the A_w's and at 5^o C and 25^o C. It is interesting that at 40^o C, the raw samples had a slightly lower value than the drum dried samples probably because exposure to that temperature for as long as six months was sufficient to cause lipoxygenase denaturation and or inactivation. The values tabulated in Table 4 also show the multistage phenomenon in lipid oxidation rate with changing A_w earlier on referred to.

Fatty-Acid Content of Cowpea Powders

The fatty-acid composition of the cowpea powders used in this study was analyzed soon after preparation and the results are shown in Table 5.

TABLE 4 CALCULATED* RATES OF LIPID OXIDATION IN STORED COWPEA POWDERS

	25°C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.007	0.006	0.007
Raw Powders	0.024	0.018	0.020

	A_w 0.75		
	5° C	25°C	40°C
Drum Dried Powders	0.001	0.008	0.312
Raw Powders	0.001	0.020	0.305

*Increase in lipid absorbance per month

TABLE 5 FATTY ACID ANALYSES OF LIPIDS DERIVED FROM ORIGINAL RAW AND DRUM-DRIED COWPEA POWDERS

	% FATTY ACID	
FATTY ACID	RAW	DRUM-DRIED
Oleic Acid	13.20	12.90
Linoleic Acid	27.81	27.41
Linolenic Acid	13.63	13.03
Palmitic Acid	35.14	37.16
Stearic Acid	7.44	7.52
Arachidic Acid	2.78	2.47

Palmitic acid was the predominant fatty-acid in both the raw and drum-dried samples followed by linoleic acid, oleic acid, stearic acid and arachidic acid in the order stated. Takayema et al. (1965) did report the predominance of palmitic acid in the cowpea variety (Vignia sinensis) which he studied but found no arachidic acid as a component fatty-acid of the bean lipids. Varietal differences apparently account for this. Although Oyenuga (1968) reported arachidic acid as a component fatty-acid of Vigna unguiculata lipid, he also reported traces of both behenic and lignoceric acids. The last two fatty-acids were not detected in the samples used in this research. It has to be borne in mind, however, that Oyenuga worked with samples obtained in Nigeria where climatic and agronomic practices are not exactly the same as in the United States of America.

The calculated ratios of saturated to unsaturated fatty acid (S/U ratios) at various stages of this experiment are shown in Tables 6 and 7. The initial S/U ratio represent results obtained by calculation from the fatty-acid profiles of both the raw and drum-dried cowpea powders immediately after preparation. In effect, they represent base values with which subsequent values in the course of storage were compared. The values indicate that the S/U ratios for both samples were slightly below unity indicating that the unsaturated fatty-acids were present at slightly higher levels than the saturated acids. The

TABLE 6 CHANGES IN THE *S/U RATIOS OF COWPEA POWDER LIPIDS STORED AT 25°C

	** INITIAL READINGS		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.875	0.875	0.875
Raw Powders	0.830	0.830	0.830
	FIRST MONTH READINGS		
Drum Dried Powders	0.925	0.882	0.918
Raw Powders	0.933	0.860	0.933
	THIRD MONTH READINGS		
Drum Dried Powders	1.117	0.971	1.120
Raw Powders	1.171	1.021	1.187
	SIXTH MONTH READINGS		
Drum Dried Powders	1.438	1.060	1.431
Raw Powders	1.620	1.204	1.620

*Saturated/Unsaturated fatty-acid ratio

**Readings obtained immediately after drum-drying and milling

TABLE 7 CHANGES IN THE *S/U RATIOS OF COWPEA POWDER LIPIDS STORED AT A_w 0.75

	** INITIAL READINGS A_w 0.75		
	5°C	25°C	40°C
Drum Dried Powders	0.875	0.875	0.875
Raw Powders	0.830	0.830	0.830
	FIRST MONTH READINGS		
Drum Dried Powders	0.875	0.918	1.339
Raw Powders	0.830	0.933	1.452
	THIRD MONTH READINGS		
Drum Dried Powders	0.875	1.112	1.798
Raw Powders	0.845	1.189	2.149
	SIXTH MONTH READINGS		
Drum Dried Powders	0.890	1.431	2.394
Raw Powders	0.865	1.622	3.149

**Readings obtained immediately after drum-drying and milling

*Saturated/Unsaturated fatty-acids ratio

slightly higher value for the drum-dried sample is to be expected from their somewhat higher oxidation status which would have caused a greater loss of the unsaturated fatty-acids vis-a-vis the saturated ones.

The Tables under consideration reveal also that at all temperatures except 5° C, and at all A_w's, the ratio of saturated to unsaturated fatty-acids increased with increasing length of storage. This probably was due to oxidation. That the samples oxidized at all A_w's and at all temperatures with the exception of 5° C has already been referred to and demonstrated by changes in the lipid diene conjugation.

If the unsaturated fatty-acids oxidized more than the saturated fatty-acids, the S/U ratio would be expected to increase in the course of storage. Moreover, in agreement with oxidation measurement obtained by lipid diene conjugation, the raw samples at all the conditions of storage had a somewhat lower S/U ratio at the beginning of storage. At the end of storage the raw samples attained slightly higher values than the drum-dried powders. Again, a combination of lipoxygenase catalyzed autoxidation and autoxidation is inferred. The stability of the S/U ratio at 5° C, is a reflection of the ability of low temperature to retard oxidation of the lipid unsaturated fatty-acids.

Using the same formula stated earlier, a calculation was made of the rate of loss, (decrease in percentage

fatty acid per month) of the unsaturated fatty-acids in the stored powders. The results are given in Table 8. There is a clear indication that the rate of loss for each fatty-acid tended to be related to its level of unsaturation with the more unsaturated acids showing losses greater than the less unsaturated ones, obviously, due to the greater oxidation rates of the former. The higher values obtained for the raw cowpea powders harmonize with the concept that the raw samples may have experienced both autoxidation and lipoxygenase catalyzed oxidation.

The effect of increasing the A_w from 0.11 to 0.33 was to decrease the S/U ratio indicating a protection of unsaturated fatty-acids against oxidation by the higher A_w of 0.33. However, the ratio tended to increase when the A_w was increased from 0.33 to 0.75. This is consistent with the multistage trend observed in oxidation assessment by lipid diene conjugation. It would appear that an A_w of 0.33 is the critical value for lipid stability in the samples studied here. An A_w of about 0.33 has been found to be the most protective against lipid oxidation for most dehydrated intermediate moisture foods (Labuza, 1971).

Increases in temperature from 5° C to 25° C to 40° C led to corresponding increases in the S/U ratio. The results are consistent with the expectation that oxidation would proceed faster at higher temperatures

TABLE 8 CALCULATED* RATES OF LOSS OF PERCENTAGE UNSATURATED FATTY ACIDS OF STORED COWPEA POWDER LIPIDS OVER A SIX MONTH PERIOD

	RAW POWDERS		
	25°C		
	A_w 0.11	A_w 0.33	A_w 0.75
Oleic Acid	0.597	0.367	0.60
Linoleic Acid	0.882	0.498	0.885
Linolenic Acid	1.267	0.680	1.260
	DRUM-DRIED POWDERS		
Oleic Acid	0.428	0.227	0.437
Linoleic Acid	0.622	0.238	0.600
Linolenic Acid	1.005	0.335	0.998

	RAW POWDERS		
	A_w 0.75		
	5°C	25°C	40°C
Oleic Acid	0.027	0.603	1.197
Linoleic Acid	0.040	0.882	1.622
Linolenic Acid	0.101	1.265	2.272
	DRUM-DRIED POWDERS		
Oleic Acid	0.008	0.433	0.932
Linoleic Acid	0.002	0.598	1.285
Linolenic Acid	0.065	1.002	1.763

*Decrease in percentage unsaturated fatty acid per month

in such a way that the unsaturated fatty-acids would be oxidized in preference to the saturated acids.

Finally, palmitic acid was the main saturated fatty-acid which tended to increase relatively in storage as the percentage unsaturated fatty-acid decreased. Stearic and arachidic acids showed smaller increases.

Lipid Free-Amino Groups

The effects of changing both the temperature and the A_w on the lipid free-amino groups of the stored cowpea powders are discernable in Figures 3 and 4 and Table 9.

Increases in temperature and A_w lowered the lipid free-amino group concentrations in both the raw and drum-dried cowpea powders. The reason probably lies in the fact that increasing A_w and increasing temperature promoted increased carbonyl-amine reactions. Indeed, the literature indicates that phospholipids which contain amino groups such as phosphatidyl ethanolamine and phosphatidyl serine are capable of contributing to the browning observed in foods. For example, Lea (1956) noted that glucose in dried egg yolk was able to enter into Maillard type browning reactions with the amino-phospholipids of the dried eggs. Similarly Folch (1948) reported that phosphatidyl ethanolamine isolated from brain changed color from white to a tan brown color only after two weeks of

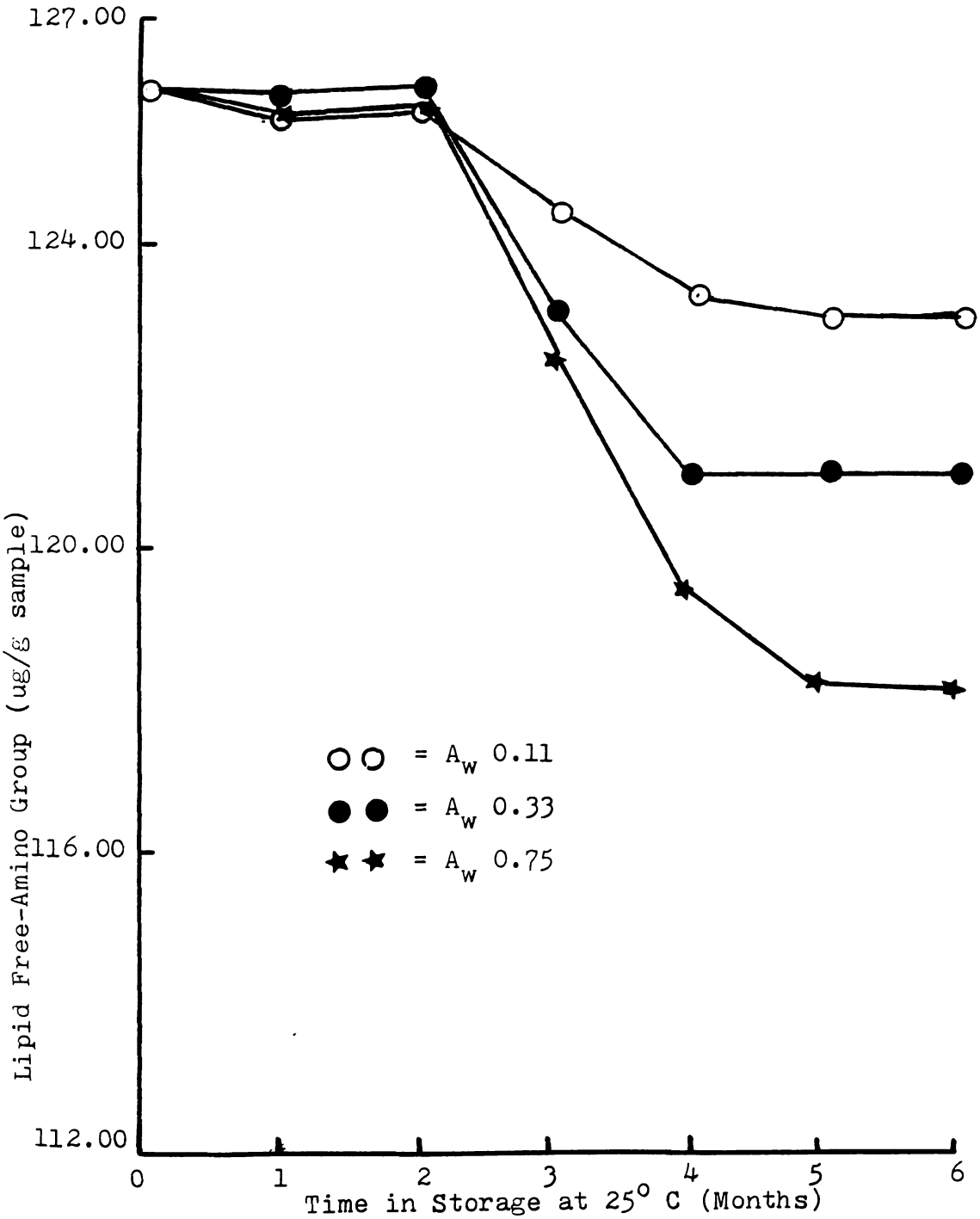


FIGURE 3 Effect of A_w on the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powders During Storage

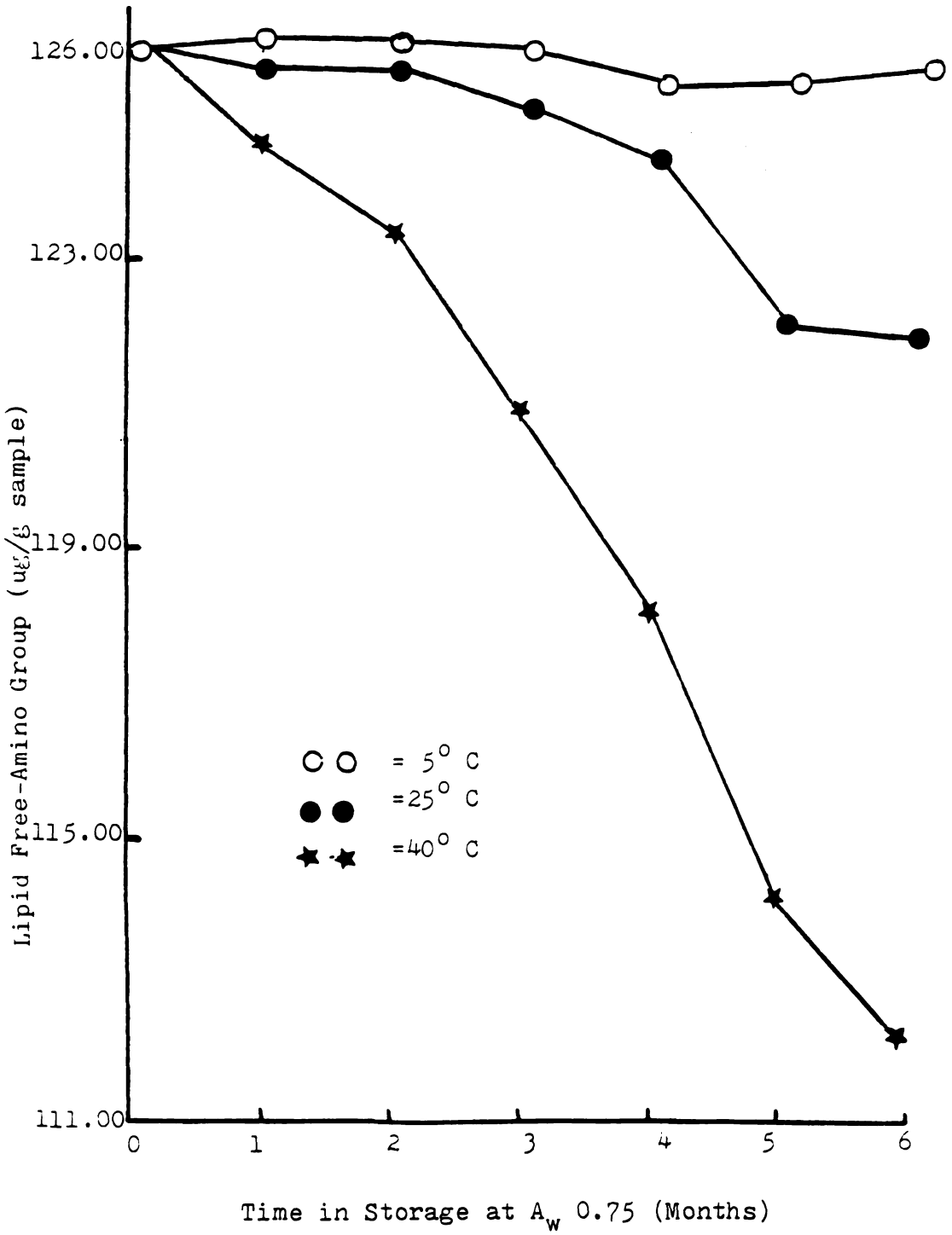


Figure 4: Effect of Temperature on the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powders During Storage.

TABLE 9 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE LIPID FREE-AMINO GROUP CONTENT (ug/g sample)* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
126.72	126.72	126.72	126.72	126.72	126.72
FIRST MONTH'S READINGS					
126.23	126.12	126.36	126.67	126.33	125.19
SECOND MONTH'S READINGS					
126.30	126.05	126.01	126.55	125.84	124.03
THIRD MONTH'S READINGS					
124.07	123.08	122.16	126.02	124.05	122.14
FOURTH MONTH'S READINGS					
122.04	120.04	118.00	125.00	122.14	116.04
FIFTH MONTH'S READINGS					
122.05	120.00	116.13	124.66	118.27	110.53
SIXTH MONTH'S READINGS					
122.01	120.02	116.04	123.44	117.42	111.00

*Values are the means of duplicate readings

storage in a vacuum desiccator implicating Maillard reactions as the occurring phenomenon in the browning process.

The calculated rate of losses (Decrease in lipid free-amino group (ug/g sample) per month) for the two types of cowpea powders are shown in Table 10. The raw samples again had higher rates of losses. This was probably because the enzyme system was left intact in the raw cowpea powders so that more oxidation products capable of reacting with the amino groups were formed. Even the so-called non-enzymatic Maillard browning may also have been accelerated by these intact enzyme systems such as by glycosyl hydrolase enzymes capable of releasing glucose from complex polymers (Hodge, 1967).

Increases in temperature and water activity had the effect of increasing this rate of phospholipid amino loss. This would be predictable from increasing browning in both cases. That increasing the A_w from 0.11 to 0.33 did not lead to reduced rate of lipid amino loss would seem to indicate that carbonyl-amine reactions are more important than oxidation in predicting losses in lipid amino groups. The multistage trend in rate which would be linked to lipid oxidation as was discussed earlier was not observed in the trend of lipid amino losses with changing A_w.

Finally, the efficacy of the refrigeration temperature of 5^{o} C in slowing the rate of lipid amino losses

TABLE 10 CALCULATED* RATES OF LOSS OF LIPID FREE-AMINO GROUPS

	A_w 0.75		
	5°C	25°C	40°C
Drum Dried Powders	0.038	0.800	2.060
Raw Powders	0.538	1.495	2.365

	25°C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.452	0.700	0.800
Raw Powders	0.703	1.017	1.501

*Decrease in lipid free-amino group (ug/g powder) per month

and that of 40° C in accelerating it is quite obvious from Table 8. This again would be predictable from the observed ability of 5° C to slow browning rate and/or oxidation and of 40° C to do just the opposite.

Color Changes and Browning

The level of whiteness in both the drum-dried and the raw cowpea powders were assessed by recording the "L" values in a standardized Hunter Colorimeter as has already been described. The trend in color changes for both samples are shown graphically in Figures 5 and 6 and Table 11.

The figures and table show loss of whiteness with increasing A_w and with increasing temperature and storage time. The effectiveness of the refrigeration temperature of 5° C in maintaining whiteness or in inhibiting browning is also apparent. In contrast, a storage temperature of 40° C was quite effective in inducing browning in both types of powders. Changing the A_w from 0.11 to 0.33 and 0.75 did not appear to alter seriously the intensities of browning of the powders, yet the small differences in the levels of coloration were persistent and consistent throughout the period of storage. Johnson (1966) assessed loss of whiteness in flour by taking reflectance values on a disc reflectance equipment and was able to relate loss of whiteness to decreased reflectance values.

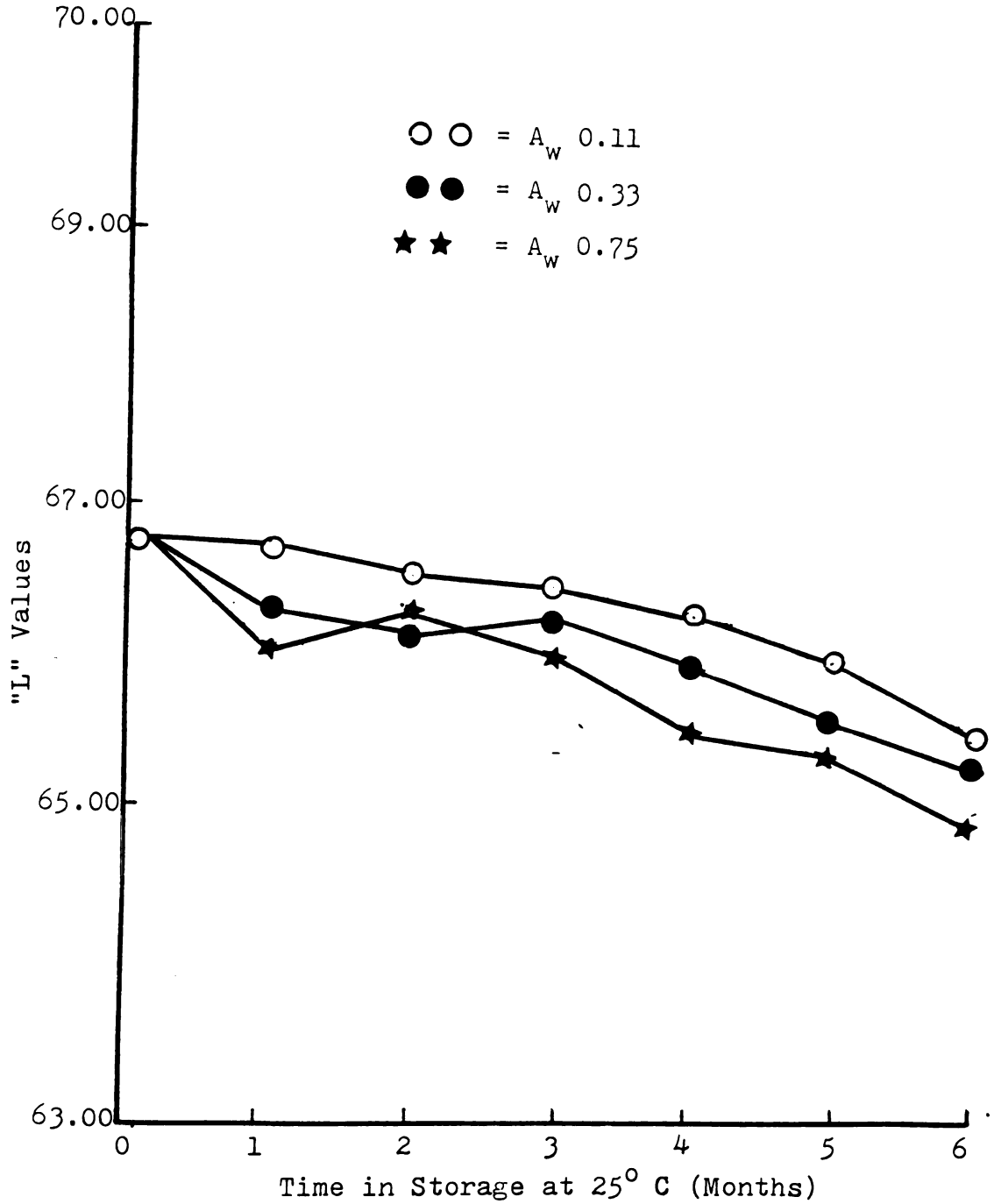


FIGURE 5 Water Activity Effect on the "L" Values of Drum-Dried Cowpea Powders During Storage

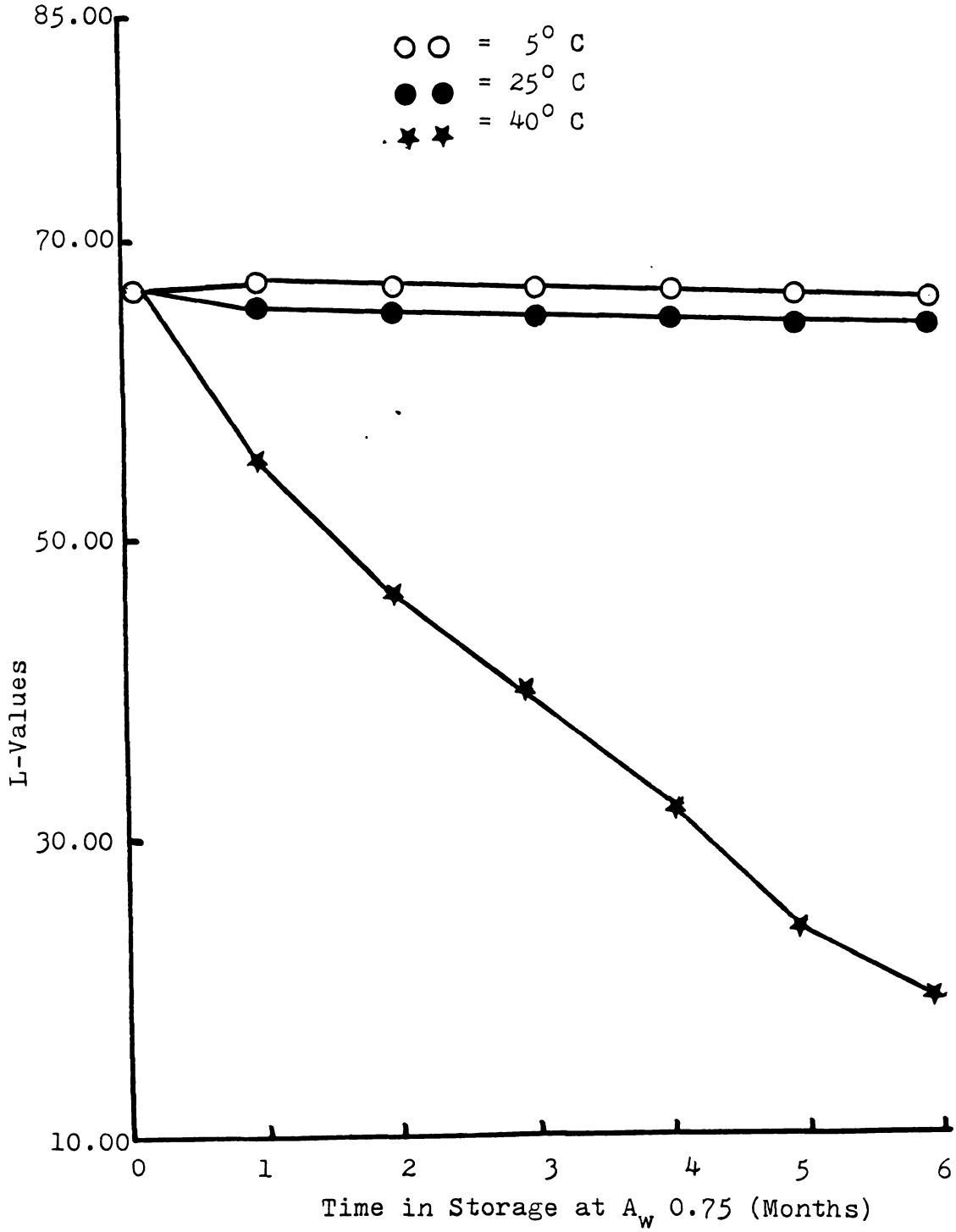


FIGURE 6 Temperature Effect on the "L" Values of Drum-Dried Cowpea Powders During Storage

TABLE 11 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE BROWNING INTENSITY ("L" VALUE)* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5^{o} C	25^{o} C	40^{o} C
INITIAL READINGS					
81.94	81.94	81.94	81.94	81.94	81.94
FIRST MONTH'S READINGS					
81.45	81.45	81.20	81.70	81.60	44.60
SECOND MONTH'S READINGS					
81.25	81.20	80.60	81.50	81.00	38.70
THIRD MONTH'S READINGS					
81.30	81.00	80.20	81.25	80.15	31.10
FOURTH MONTH'S READINGS					
81.00	80.90	80.20	81.00	80.10	27.70
FIFTH MONTH'S READINGS					
81.00	80.55	80.05	80.60	80.05	23.40
SIXTH MONTH'S READINGS					
80.65	80.15	79.60	80.20	79.55	15.00

*Values are the means of duplicate readings

Counter (1969) did not observe consistent changes in color by using the Hunter Colorimeter, on stored navy bean powders. In anticipation of the type of problem hinted by Counter, a second method of color measurement described by Choi et al. (1949) and modified by Karel and Labuza (1968) was used in this work. There was good agreement with the data obtained from the Hunter Colorimeter measurements in this study.

Loss of whiteness was obviously due to browning and browning itself may have been caused by the Maillard type reactions between the epsilon-amino group of lysine and other such reactive amino groups in various kinds of compounds such as phospholipids and the carbonyl groups of such compounds as reducing sugars and lipid oxidation products such as malonaldehyde, aldehydes and ketones. The literature cites many such instances (Henry and Kon, 1948; Ben-Gera and Zimmerman, 1964; Hodson and Miller, 1957; Ponting et al. 1964; Carpenter, et al. 1962; Braddock and Dugan, 1973 and Schwenke, 1975). Browning in stored food may also be due to ascorbic acid browning. However, neither the raw nor the drum-dried cowpea powders contained any measurable ascorbic acid; and as such it cannot be assigned a role in the loss of sample whiteness here.

Table 12 shows the rate of browning (decrease in "L" values per month) of the raw cowpea powders to be generally greater than that for the drum-dried samples.

TABLE 12 CALCULATED* RATES OF LOSS OF WHITENESS (INCREASE IN BROWNING) IN STORED COWPEA POWDERS

	A_w 0.75		
	5°C	25°C	40°C
Drum Dried Powders	0.158	0.208	6.142
Raw Powders	0.280	0.342	4.933

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.208	0.167	0.206
Raw Powders	0.133	0.217	0.343

* Decrease in "L" values per month

Simply, this may have been due to the activity of polyphenolase enzymes in the raw bean powder which caused the so-called enzymatic browning. This would be in addition to the so-called non-enzymatic or Maillard browning. In addition, it is speculated here that amylase hydrolytic enzymes in the raw sample may also have acted to release reactive glucose and or fructose molecules which were subsequently mobilized in Maillard browning reactions. Thirdly, the lipoxygenase enzymes which may have been present in the raw cowpea powders may have caused some oxidation of the cowpea lipid to yield reactive carbonyl groups to further increase their potential for Maillard browning. Because of the possible thermal inactivation of these enzymes in the drum-dried cooked samples, they would not have contributed to browning.

If the initial values of browning are examined in the figures and table in reference which represent the level of whitenss of the powders immediately after drum-drying and milling respectively, it would be seen that the drum-drying process greatly increased browning of the drum-dried powders. The high temperatures of the drum-dryer are enough to induce caramelization reactions described by Hodge and Osman (1976) in addition to some Maillard type reactions between free and reactive amino groups and reactive carbonyl groups. The drum-dehydration

process is known to cause pre-storage browning as has been indicated by many workers such as Bakker-Arkema et al. (1967), Onayani and Potter (1976), Morris (1961) and Kon et al. (1974).

The loss of whiteness, if it is not accompanied by serious nutritional losses, should not be a problem in these products. The changes in nutritional parameters brought about by the drum-dehydration applied in this work are discussed later. Loss of whiteness could only be a marketing problem if people consciously or unconsciously expect these products to be white. Since these are new products, only marketing research can tell what consumers expect their color to be.

Changes in Reducing Sugar

The percentage changes of the reducing sugar in the stored cowpea powders are shown in Figures 7 and 8 and Table 13.

Increasing the A_w from 0.11 to 0.33 and to 0.75 led to decreases in the percentage amounts of reducing sugar in the cowpea powders. The effect of increasing the temperature from 5° C to 25° C to 40° C similarly caused decreases in the amount of reducing sugar.

An explanation for this loss would appear to be that higher A_w and temperature promoted greater browning reaction involving the carbonyl groups of the reducing sugars and reactive amino groups in the powders. Higher

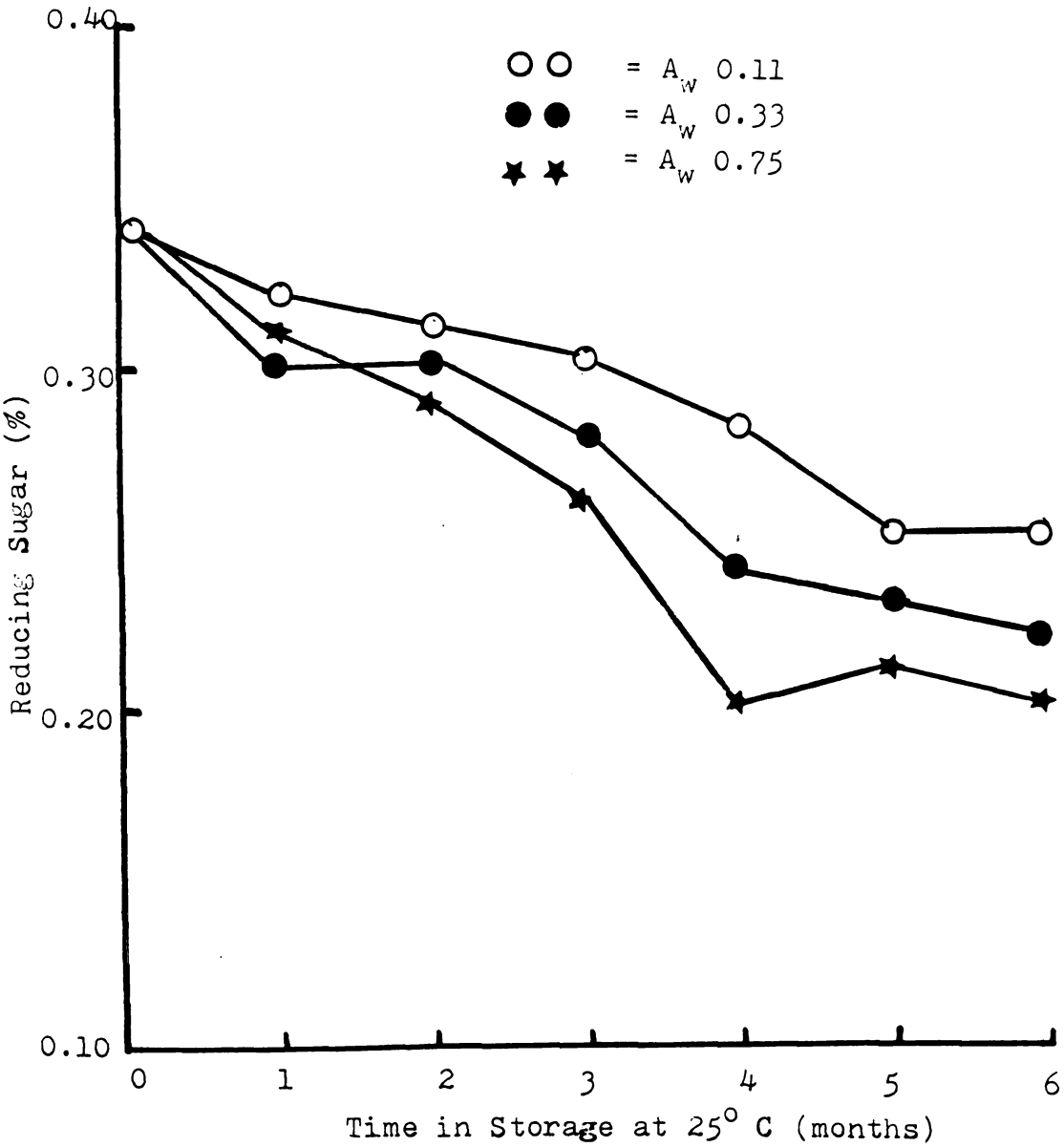


FIGURE 7 Effect of A_w on the Reducing Sugar Content of Drum Dried Cowpea Powders During Storage

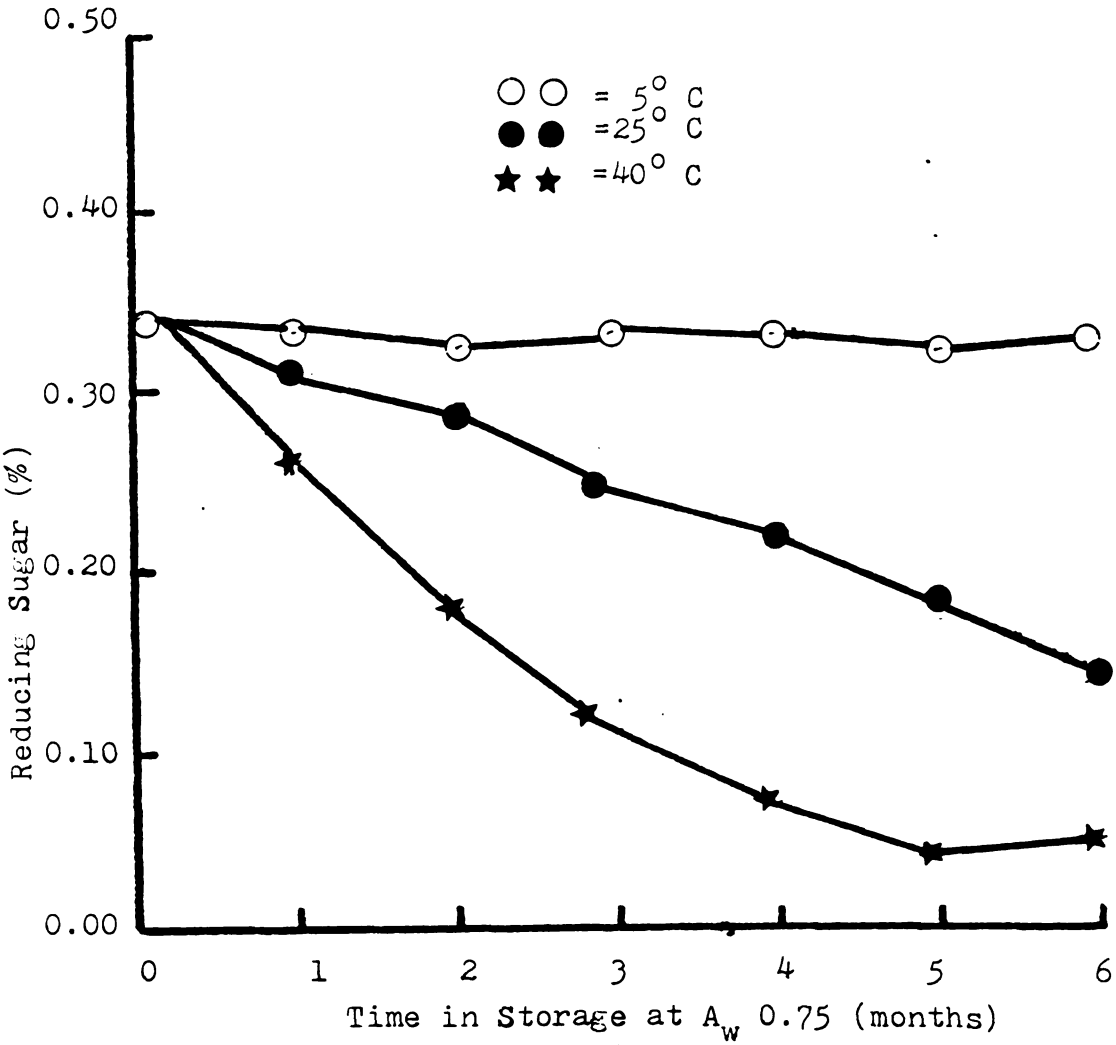


FIGURE 8 Effect of Temperature on the Reducing Sugar Content of Drum-Dried Cowpea Powders During Storage.

TABLE 13 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE PERCENTAGE REDUCING SUGAR CONTENT* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
0.40	0.40	0.40	0.40	0.40	0.40
FIRST MONTH'S READINGS					
0.39	0.37	0.36	0.39	0.36	0.29
SECOND MONTH'S READINGS					
0.36	0.35	0.34	0.39	0.33	0.20
THIRD MONTH'S READINGS					
0.33	0.31	0.28	0.37	0.30	0.13
FOURTH MONTH'S READINGS					
0.31	0.27	0.23	0.35	0.27	0.09
FIFTH MONTH'S READINGS					
0.28	0.24	0.19	0.36	0.22	0.07
SIXTH MONTH'S READINGS					
0.26	0.24	0.18	0.36	0.20	0.04

*Values are the means of five readings

A_w and temperature have been reported to cause greater browning. The speculation here that Maillard browning may account for the observed trend is consistent with the reports of many workers in the field. Hodge (1953) indicated the involvement of reducing sugar carbonyl groups in such browning reactions. Overend et al.(1961) made mention of the role of the reducing sugars in these browning reactions when he indicated that the initial rate of browning of a reducing sugar with an amino compound is dependent on the rate at which the sugar's ring opens to the reducible form. Fragne and Adrian (1971) listed some reducing sugars such as D-galactose, D-mannose, D-glucose, D-fructose and even sucrose which are capable of reacting with amino acids and N-terminal amino acids of proteins in the Maillard browning reactions.

The amount of reducing sugar at 0.11 was higher than that at 0.33 probably because, as Fennema (1976) has hinted, the browning intensity is lower at 0.11 because the degree of water binding at the A_w of 0.11 is greater and thus reactions depending on solution such as Maillard browning would be slower at A_w 0.11 than at A_w 0.33 or at A_w 0.75. Non-enzymatic browning may have been accelerated at the higher A_w's because the reactants were water soluble. Love (1975) reported a similar loss of reducing sugars as the A_w was increased from 0.11 to 0.33.

Increasing storage time led to losses of reducing sugars in the samples.

The calculated rates of loss (decrease in percentage reducing sugar per month) of reducing sugars are given in Table 14. The rate of loss was higher in the raw samples than in the drum-dried samples. In the raw cowpea powders, browning phenomenon may have been coupled with other phenomena to account for the higher rate of reducing sugar loss. With the enzyme system intact, mitochondrial linked respiration starting with the glycolytic pathway of sugar metabolism is possible. It might be expected that, since the hydrolytic enzymes responsible for the release of reducing sugars from their complex carbohydrate polymers would be expected to be functional in the raw powders, their rate of reducing sugars loss should be lower. That would be true if such released sugars were allowed to accumulate. If they were mobilized in browning and in respiration as fast as they were released this speculation would not hold. In fact, it is known that, in contrast to what operates in potatoes, some commodities notably seeds (peas, corn, beans, among others), synthesis of starch rather than degradation may predominate after harvest. (Amir et al. 1971). Then Levitt (1972) has noted that most stress conditions such as mechanical damage (milling in the case of this study) and exposure to extreme temperatures may result in a burst of respiratory activity and, as stated by Haard (1976), "the relatively rapid rate of respiration

TABLE 14 CALCULATED* RATES OF LOSS OF REDUCING SUGAR IN STORED COWPEA POWDERS

	A_w 0.75		
	5° C	25°C	40°C
Drum Dried Powders	0.002	0.033	0.048
Raw Powders	0.007	0.034	0.060

	25°C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.015	0.020	0.033
Raw Powders	0.023	0.028	0.037

*Decrease in percentage reducing sugar per month

in leguminous seeds and certain vegetables, such as sweet corn and asparagus, appears to relate to starch synthesis."

The refrigeration temperature of 5^{o} C gave a comparatively minimal rate of loss of reducing sugar indicating again that one of the ways to guarantee the wholesomeness of this product would be refrigeration.

As can be seen from the Figures and Table the drum-dried samples initially, had slightly lower reducing sugar content than the raw powders. The difference does not appear to be considerable. The preparation process of the drum-dried samples used in this work was carefully done to minimize leaching losses during the cooking stage. All the water present in the beans after cooking was used to mash them before drying on the drum-drier. The very small loss of reducing sugar indicated in the results may have occurred by some limited reactions with other components of the beans or by similar losses during the drum-drying operation itself, such as by caramelization.

Changes In The Available Lysine Content

Figures 9 and 10 and Table 15 show the changes in the available lysine content of the cowpea powders and measured spectrophotometrically according to the procedure described by Kakade and Liener (1969).

Increasing A_w from 0.11 to 0.33 and to 0.75 led to decreases in the available lysine content of both the raw

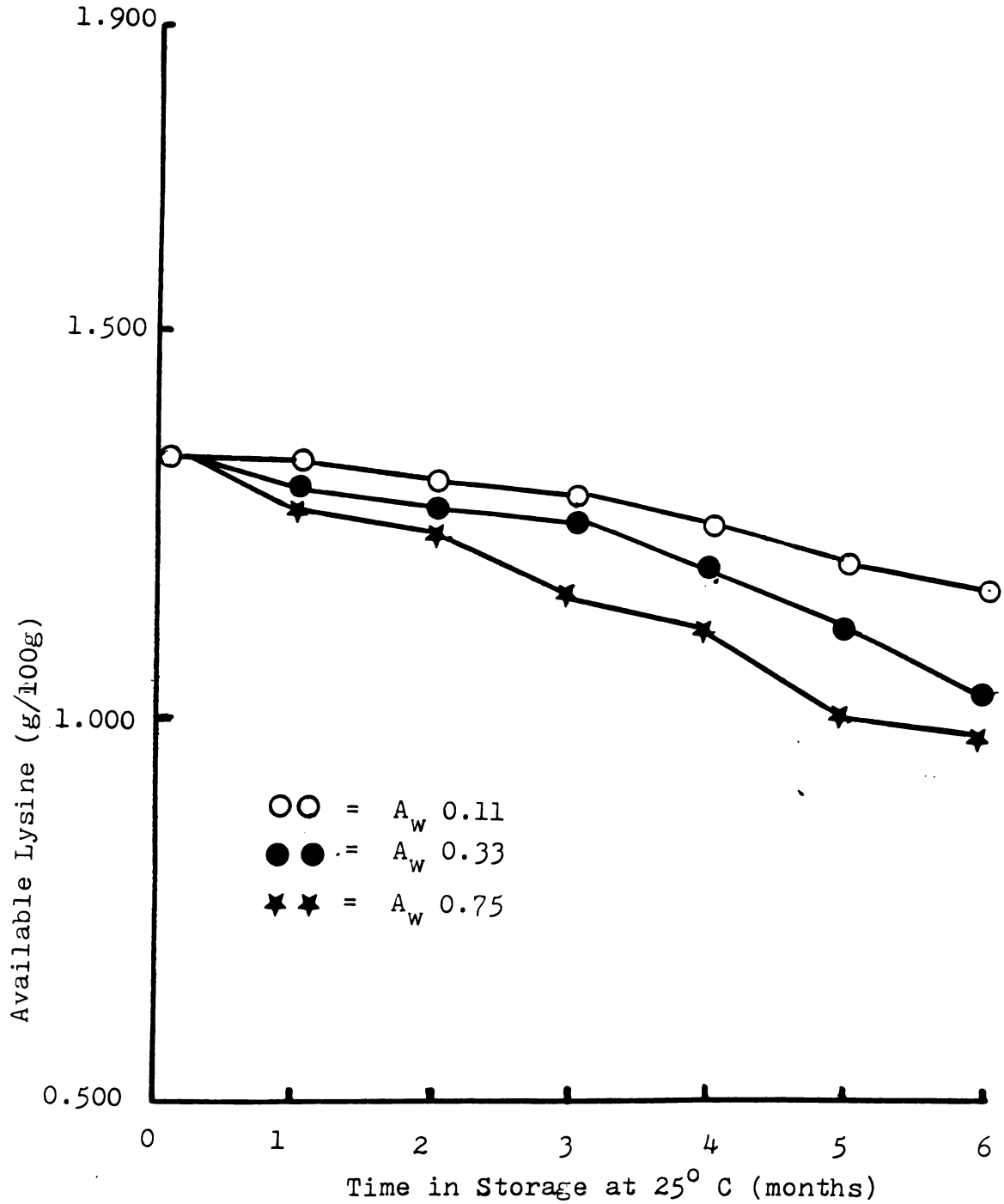


FIGURE 9 Water Activity Effect on the Available Lysine Content of Drum-Dried Cowpea Powders During Storage

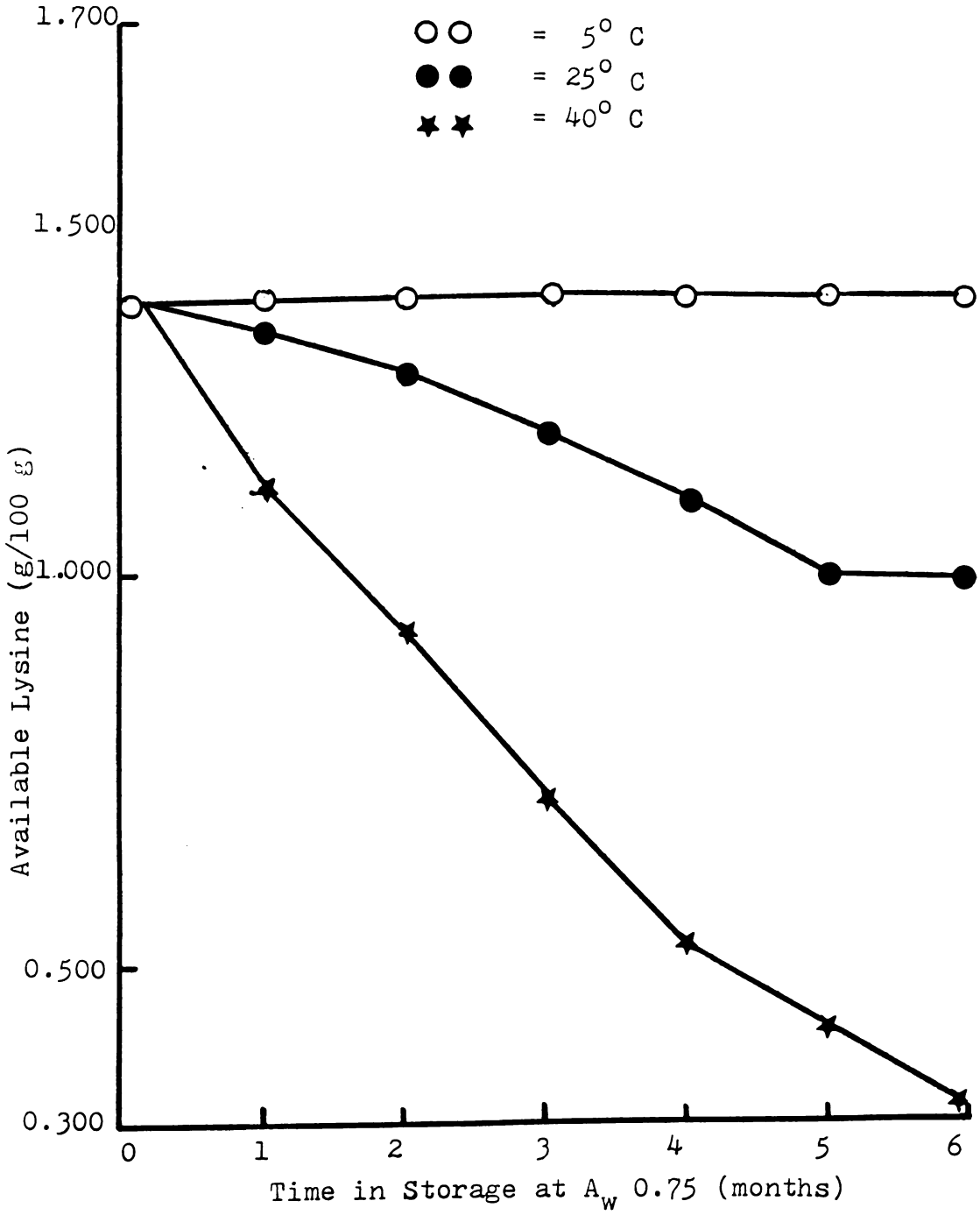


FIGURE 10 Temperature Effect on the Available Lysine Content of Drum-Dried Cowpea Powders During Storage

TABLE 15 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE AVAILABLE LYSINE CONTENT OF RAW COWPEA POWDER DURING STORAGE (g/100g powder)**

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS*					
1.762	1.762	1.762	1.762	1.762	1.762
FIRST MONTH'S READINGS					
1.701	1.692	1.688	1.760	1.684	1.276
SECOND MONTH'S READINGS					
1.640	1.613	1.584	1.761	1.585	1.027
THIRD MONTH'S READINGS					
1.600	1.562	1.403	1.758	1.407	0.978
FOURTH MONTH'S READINGS					
1.541	1.484	1.306	1.757	1.301	0.768
FIFTH MONTH'S READINGS					
1.472	1.395	1.208	1.759	1.204	0.607
SIXTH MONTH'S READINGS					
1.393	1.304	1.200	1.756	1.202	0.402

*Readings obtained immediately after milling
**Values are the means of duplicate readings

and drum-dried cowpea powders. The inference here is that because Maillard browning also increased with increasing A_w as has already been demonstrated here in agreement with the description of Labuza (1970), the available lysine content also decreased in correspondence. This would have been because of the well documented ability of the epsilon-amino group of lysine to enter into Maillard browning reactions with the carbonyl groups of such compounds as reducing sugars and carbonyl-amine reactions with oxidation products such as malonaldehyde and similar products. According to Carpenter and Booth (1973), the lysine portion of a protein is able to enter into such reactions because it is the only essential amino acid that still has a free amino group in its condensed form as it exists in the protein molecule. Similarly, Bjarnason and Carpenter (1969) have speculated on the possibility of amide linkage formation between the epsilon-NH_2 groups of lysine and carbonyl containing molecules. So also have Eldred and Rodney (1946), Henry et al. (1948) among many others.

Increase of temperature from 5° C to 25° C and to 40° C also caused decreases in the available lysine content of the powders. The results here agree with those of many others. Onayemi and Potter (1976) reported that the available lysine content of freshly dried cowpea powders was of the order 97% of total lysine and that this value decreased to 94% at 37° C in 24 weeks. In their

work with lysine availability in rice meal, Tsao et al. (1978) indicated that elevated temperatures led to decreased lysine availability as similarly reported for the cowpea powder used in the study.

Again, it is obvious that Maillard browning explains the increased loss of lysine availability with increasing temperature. It would be recalled that these temperature increases also led to increased browning. In agreement with this, Baldwin et al. (1951), working with several protein sources, concluded that, Maillard browning reactions between the free amino groups of the protein lysine and the reducing sugars present were responsible for the reduced protein quality when they were subjected to adverse temperatures.

The effect of length of storage time was to decrease the available lysine content of both powders as the storage time increased. The samples held at 5^{o} C recorded only very small losses when compared to those held at room temperature of 25^{o} C and at 40^{o} C in particular. Of interest too is the way these decreases were recorded. When Wolf et al. (1979) subjected soy protein isolates to thermal treatment, they reported that the loss of available lysine was initially very rapid followed by a transition phase which itself was followed by an equilibrium and final phase in which there was no measurable loss of available lysine. The results of this study at all A_w's and at 5^{o} C and 25^{o} C do not appear to follow

the sequence observed by Wolf *et al*. The only one that approaches that type of description to any remarkable degree are the samples stored at 40° C. This is not surprising in view of the fact that only the 40° C storage temperature could be considered thermal stress on the cowpea powders when compared to 0° C and 25° C. It would even become a more important stress condition the longer the powders were held in storage.

Using the formula designated previously, the rates of loss (decrease in available lysine (g/100 g sample) per month) of available lysine at all A_w's and at all temperatures were calculated and the results are shown in Table 16. The rates increased with increased temperatures and with increased water activities with very negligible rates incurred at 5° C and comparatively remarkable rates recorded at 40° C. The trend agrees with what was seen in the case of browning and with other measures of Maillard browning such as by the rate of loss of reducing sugar.

The table also reveals that the rate of loss of available lysine was definitely higher in the raw powders than in the drum-dried material. This is a plus. It means that once the drum-dehydration process has been optimized to minimize adverse effects on the quality of the finished product, one can expect smaller losses of available lysine during storage of the drum-dried powders than the raw or undehydrated powders. A look at the base values illustrated in Table 27 demonstrates this point. The

TABLE 16 CALCULATED* RATES OF LOSS OF AVAILABLE LYSINE (g/100 of sample) IN STORED COWPEA POWDERS

	A_w 0.75		
	5° C	25° C	40°C
Drum Dried Powders	0.0005	0.058	0.172
Raw Powders	0.001	0.093	0.227

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.030	0.051	0.058
Raw Powders	0.062	0.076	0.094

*Decrease in available lysine (g/100 of sample) per month

available lysine content of the drum-dried samples was somewhat higher apparently because of processing effects, but once in storage, the rate of loss of available lysine of the raw samples was higher than that of the drum-dried. It is likely that over a very long period of storage, the higher rate of loss would have acted to compensate for the initial lower available lysine content of the drum-dried samples. Eventually, they would probably have higher available lysine values than the raw samples.

Comparison of HPLC and Spectrophotometric Methods in the Determination of Available Lysine

The determination of available lysine of a protein food stuff is a very important parameter for evaluating its protein quality. Block and Mitchell, (1946) and Harris and Mattil (1940) noted that the discrepancy sometimes observed between amino acid analyses and feeding trials in assessing protein quality was associated with the amino-acid lysine. Then Finley and Friedman (1973) asserted that, among other things, the availability of essential amino-acids such as lysine affected the nutritive value of a protein food. Moreover, according to Carpenter and Booth (1973), lysine is of great nutritional importance because it is the only essential amino-acid in a protein molecule which still has a reactive amino group in the molecule to engage in various types of reactions of which the most important is probably Maillard browning. Because

of this, many workers have attempted to establish precise procedures to evaluate the lysine availability of protein foods. This is of particular interest in a protein food like cowpea where the high content of lysine is one reason it is seen as a potential for meeting the world's protein needs. A correct estimation of its available lysine content, rather than total lysine, will give a more correct picture of its usefulness as a source of protein.

With that in mind, two methods were employed in evaluating the available lysine content of the cowpea powders used in this research: A spectrophotometric method described by Kakade and Liener (1969) and that by Peterson and Warthesen (1979) which uses high performance liquid chromatography. The aim was to learn whether they showed agreement at critical points in the course of this research: immediately after milling and drum-dehydration, mid-way (3 months) in storage and at the end of storage. The results are tabulated in Table 17. The results appear to agree reasonably well, with minor differences here and there. Such differences may be accounted for by the different reagents used in this study. The spectrophotometric method described by Kakade and Liener (1969) uses the reagent 2,4,6-trinitrobenzene sulfonic acid which specifically reacts with primary amino groups coupled with the ability of the method to differentiate between free epsilon amino and N-terminal amino groups of proteins. The method is therefore likely to be very precise.

TABLE 17 COMPARISON OF SPECTROPHOTOMETRIC AND HPLC METHODS IN THE DETERMINATION OF AVAILABLE LYSINE (g/100g powder)

	5° C	25°C	40° C
		INITIAL READINGS	
		A_w 0.75	
Drum Dried Powders	1.346, 1.207*	1.346, 1.207*	1.346, 1.207*
Raw Powders	1.762, 1.313*	1.762, 1.313*	1.762, 1.313*
		THIRD MONTH READINGS	
Drum Dried Powders	1.344, 1.208*	1.176, 1.003*	0.714, 0.531*
Raw Powders	1.758, 1.309*	1.407, 1.159*	0.978, 0.726*
		SIXTH MONTH READINGS	
Drum Dried Powders	1.343, 1.204*	1.00 , 0.864*	0.314, 0.175*
Raw Powders	1.756, 1.307*	1.202, 0.951*	0.402, 0.322*
	A_w 0.11	A_w 0.33	A_w 0.75
		INITIAL READINGS	
		25° C	
Drum Dried Powders	1.346, 1.207*	1.346, 1.207*	1.346, 1.207*
Raw Powders	1.762, 1.313*	1.762, 1.313*	1.762, 1.313*
		THIRD MONTH READINGS	
Drum Dried Powders	1.290, 1.153*	1.264, 1.123*	1.175, 1.034*
Raw Powders	1.600, 1.184*	1.562, 1.160*	1.403, 1.162*
		SIXTH MONTH READINGS	
Drum Dried Powders	1.164, 1.029*	1.042, 0.807*	1.001, 0.765*
Raw Powders	1.393, 1.106*	1.304, 0.853*	1.200, 0.795*

* Readings obtained by HPLC

The HPLC method uses the reagent 1-fluoro 2,4-dinitrobenzene. It is said to have the advantage of speed of resolution and the avoidance of interference from some products formed during sample hydrolysis. Peterson and Warthesen (1979) however explained the differences between their HPLC method and the spectrophotometric method described by Carpenter (1960) and modified by Booth (1971) as being due to carbohydrates in the latter method causing over-estimation in the spectrophotometric method. Precisely for this reason, the method of Kakade and Leiner (1969) which uses a reagent that is not only specific for primary amino group but also differentiates epsilon-amino group was used in these studies.

Changes In Soluble Protein

Figures 11 and 12 and Table 18 depict the changes in the water soluble protein content of the drum-dried cowpea powders when the A_w was varied from 0.11 to 0.33 and to 0.75 at a constant temperature of 25° C and when the temperature was varied from 5° C to 25° C and to 40° C at a constant A_w of 0.75. The effects of length of storage time under these conditions are also discernible from the same figures.

The water soluble content of the drum-dehydrated powders tended to decrease as the A_w was increased. The

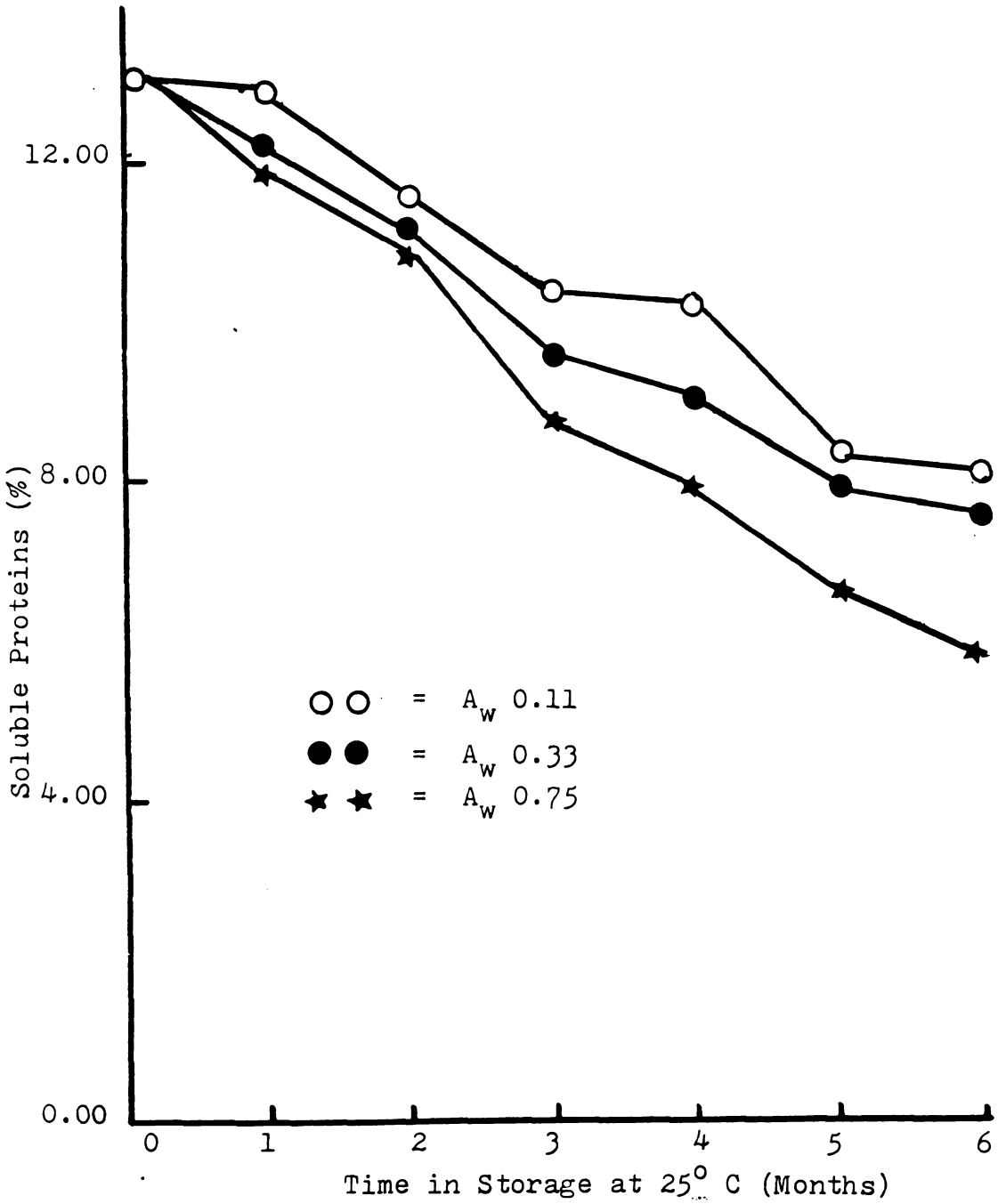


FIGURE 11 Effect of Water Activity on the Soluble Protein Content of Drum-Dried Cowpea Powders During Storage.

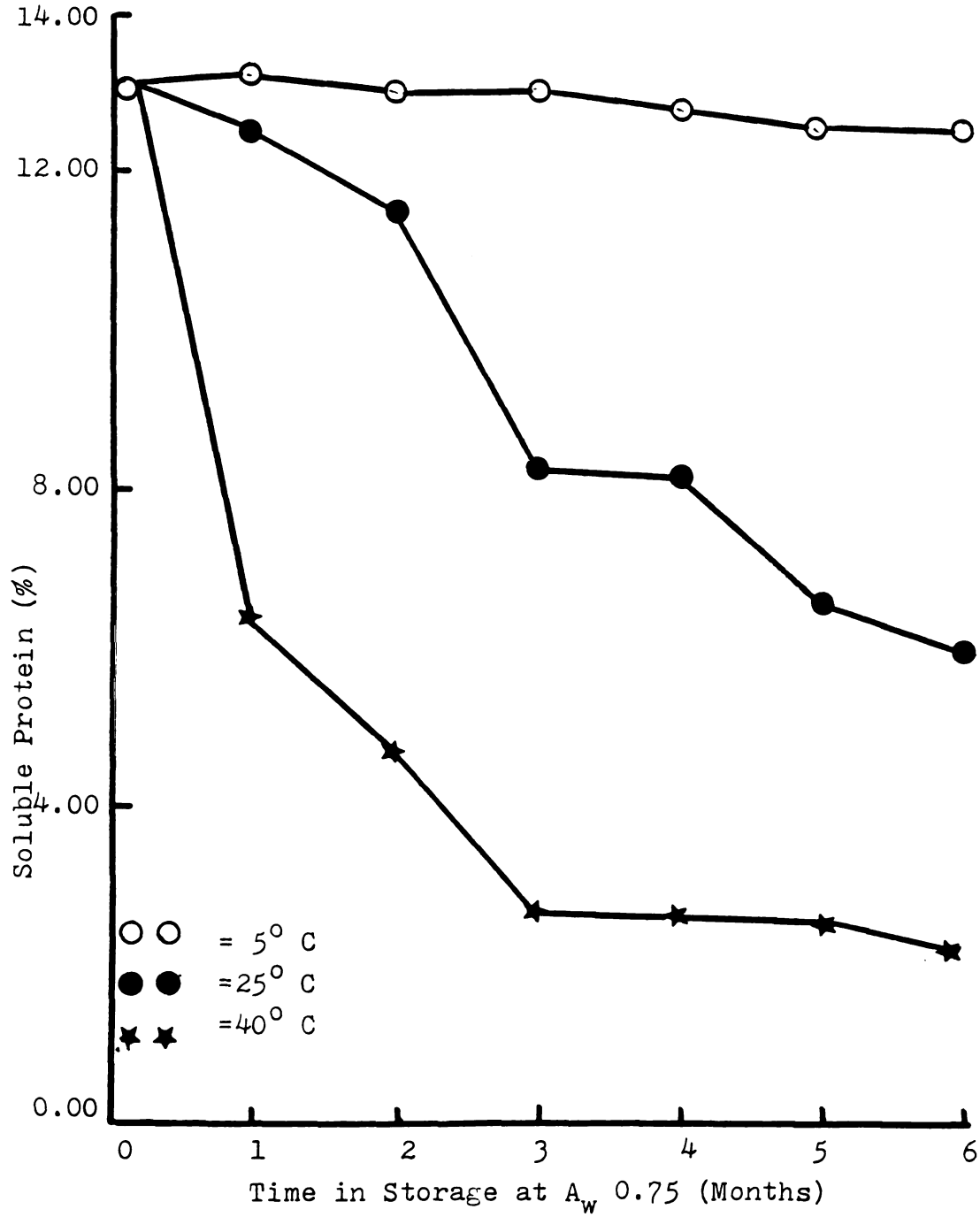


FIGURE 12 Effect of Temperature on the Soluble Protein Content of Drum-Dried Cowpea Powders During Storage

TABLE 18 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE SOLUBLE PROTEIN CONTENT (%)* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
10.83	10.83	10.83	10.83	10.83	10.83
FIRST MONTH'S READINGS					
6.60	7.60	7.70	10.60	7.30	4.00
SECOND MONTH'S READINGS					
5.30	7.20	7.40	10.40	7.20	2.40
THIRD MONTH'S READINGS					
4.77	4.80	4.80	10.25	4.80	2.40
FOURTH MONTH'S READINGS					
4.01	3.91	3.76	10.01	3.53	1.79
FIFTH MONTH'S READINGS					
4.03	3.64	3.49	9.82	3.60	1.69
SIXTH MONTH'S READINGS					
4.00	3.48	3.23	9.50	3.41	1.64

*Values are the means of duplicate readings

same tendency was exhibited when the temperature was increased.

The explanation for the above is more likely to be because of increased Maillard browning as both the A_w and temperature were increased. It has been established here that browning and, with justification, Maillard browning, increased with increasing A_w and temperature. The trend reported for the soluble protein is one more indication that one of the deteriorative reactions to be expected from these products when stored under appropriate conditions is browning—Maillard browning inclusive.

The trend reported here, and the explanation offered for the trend, is supported by the findings of other workers in the field. Working with unsaturated fats which had been oxidized and with proteins, Tappel (1955) was able to state that these unsaturated fatty-acids reacted with protein to yield stable complexes with decreased solubility. Schwenke (1975) noted that some of the protein characteristics affected by Maillard browning reactions were the iso-electric point, electrophoretic behavior, solubility and precipitation characteristics.

The differences between the water soluble protein content of the drum-dried cowpea powders at the beginning and at the end of storage appear to be marked. This is important in view of the associated implications of lowered protein solubility. This would be expected if it were true that browning of the Maillard type involving the

epsilon-amino group of lysine and the carbonyl group of reducing sugars or of lipid oxidation products occurred. Decreased protein solubility is also associated with decreased digestibility which of course lowers the biological value of the powder. Melnick and Oser (1949) were able to establish a relationship between protein quality and digestibility which was itself determined among other things by its solubility. More specifically, McInroy et al.(1945) reported that increased browning led to decreased solubility and protein digestibility.

Since the result of this study shows that a temperature of 5^{o} C was quite effective in maintaining the soluble protein content of the drum-dried powders, it is recommended that low temperature storage be used to store this rather new product.

The picture for the raw samples is somewhat different. Increases in A_w from 0.11 to 0.33 to 0.75 for the first two months led to some increases in their soluble protein content. After this, the trend was the same as for the drum-dried powders. The result is difficult to explain. The speculation here is that, possibly, during the first two months, proteolytic enzymes were able to cause some fragmentation of some high molecular weight proteins into small molecular weight proteins or peptides. This may have been accompanied by configurational changes which exposed more polar groups. For the first three months, this enzymatic proteolysis and/or configurational changes may

have been the dominant process in the raw powders. After three months, probably because of end product accumulation and feedback inhibition (a process common with enzymatically controlled reactions), the browning phenomenon may have become the dominant process affecting protein solubility.

It is worth noting that Wolf and Briggs (1958) have reported association-dissociation reactions for soybean proteins which affect their solubilities.

A contrast with work reported by other workers is observed here: Smith *et al.* (1958) reported the absence of water soluble proteins in kidney beans which were coagulable by heat. The effect of temperature increases up to 40° C in this study was to decrease the soluble protein content indicating the presence of heat sensitive water soluble proteins in both the raw and drum-dried cowpea powders. Wolf and Tamura (1969) observed that heating disrupted the quaternary structure of the 11S protein fraction of soybeans, separating the subunits into, first, a soluble and then, later, an insoluble fraction.

Table 19 shows the calculated rate of loss (decrease in percentage soluble protein per month) of H_2O soluble proteins over the six month storage period. The results agree with the trend previously discussed. The slightly lower value for the raw than the drum-dried sample at 40° C which contrasts with what has always been observed so far

TABLE 19 CALCULATED* RATES OF LOSS OF SOLUBLE PROTEIN IN STORED COWPEA POWDERS

	A_w 0.75		
	5° C	25°C	40° C
Drum Dried Powders	0.08	1.163	1.783
Raw Powders	0.222	1.237	1.532

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	0.847	0.88	1.197
Raw Powders	1.138	1.225	1.267

*Decrease in % soluble protein per month

may be due to long term systematic inactivation of enzymes in the raw samples.

The base values for the water soluble protein obtained immediately after the drum-drying and milling operations show that the drum-dried powders had a higher water-soluble protein content than the raw samples. One would have expected it to be the other way around in view of the well known heat denaturation phenomenon which invariably lowers protein solubility. The reason for the figures reported here probably lies in hydrolysis of cowpea protein by the moist heat to smaller and therefore more water soluble peptides.

Changes in pH

Changes in pH were followed in the course of this research because of the knowledge that the phenomenon of Maillard browning is frequently accompanied by changes in pH. However, pH influences other characteristics of foods such as protein solubility which has already been referred to.

Examination of Figures 13 and 14 and Table 20 reveals that increases in A_w and temperature led to lowering of pH and that increasing storage time also led to pH lowering. Browning, which includes Maillard, was also shown to give the same trend. Some form of link between browning and pH change is being suggested here. This link is predictable from the fact that in Maillard browning the

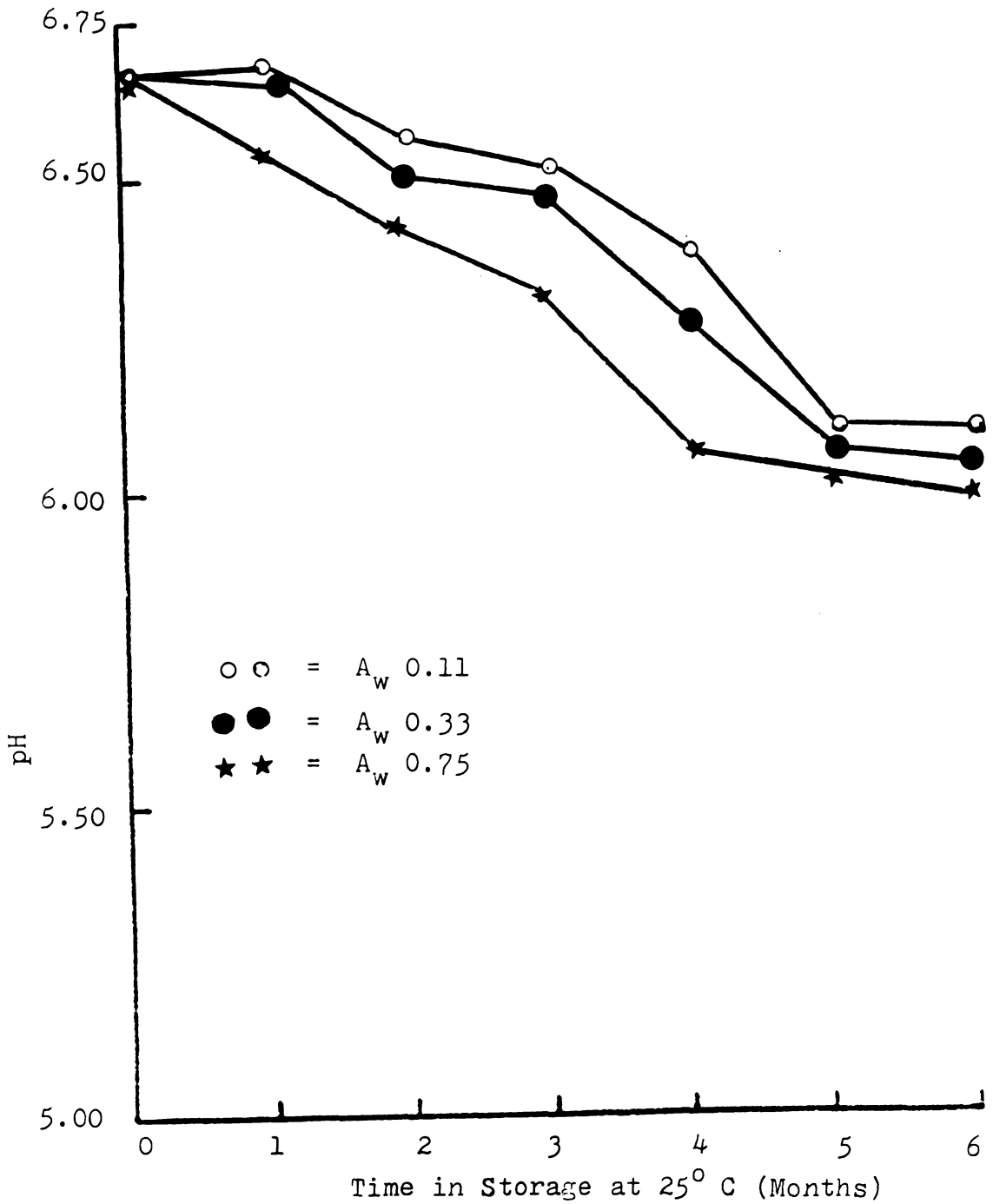


FIGURE 13 Effect of Water Activity on the pH of Drum-Dried Cowpea Powders During Storage

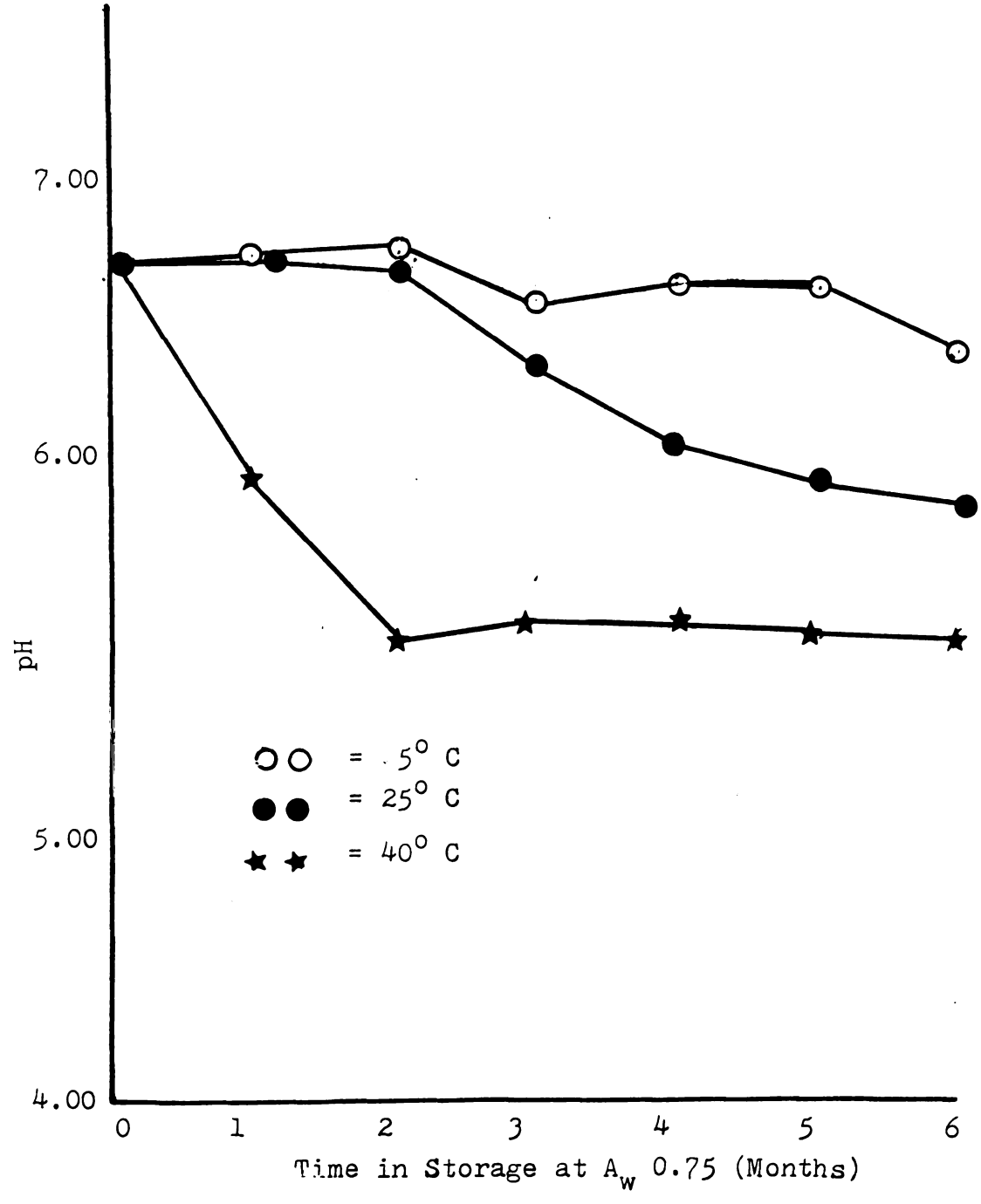


FIGURE 14 Effect of Temperature on the pH of Drum-Dried Cowpea Powders During Storage

TABLE 20 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE PH* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
6.56	6.56	6.56	6.56	6.56	6.56
FIRST MONTH'S READINGS					
6.62	6.53	6.51	6.50	6.48	5.59
SECOND MONTH'S READINGS					
6.40	6.33	6.27	6.36	6.33	5.04
THIRD MONTH'S READINGS					
6.35	6.35	6.26	6.31	6.28	4.98
FOURTH MONTH'S READINGS					
6.30	6.31	6.04	6.31	6.00	5.20
FIFTH MONTH'S READINGS					
5.89	5.88	5.60	6.27	5.90	4.81
SIXTH MONTH'S READINGS					
5.81	5.59	5.01	5.94	5.26	4.65

*Values are the means of duplicate readings

amino group of lysine, for example, condenses as a first step of the process, with carbonyl-containing compounds. This translates to a loss of amino groups which would then decrease the pH. The loss of the lipid free amino groups in carbonylamine reaction may also have led to some decrease in pH values.

The Figures and Table in reference, however, also indicate that pH changes especially at all A_ws and at 5° C and 25° C, were minimal. This simply points to the buffering capacity of the system. Since most foods are complex materials of biological origin, they contain many substances and buffering systems that can participate in pH control. Included are proteins, organic acids, and weak inorganic acid phosphate salts. Lactic acid and phosphate salts along with proteins are important for pH control in animal tissue while polycarboxylic acids, phosphate salts and proteins are important in plant tissues (Lindsay,1976).

The consistency of the changes in pH with the recorded intensity of browning in this work suggests that pH readings may be used to follow the trend of browning.

Another reason which may account for the observed changes in pH relates to the phenomenon of Strecker degradation referred to by Hodge (1967). At elevated temperatures, α-dicarbonyl compounds such as 3-deoxyglucosone, pyruvaldehyde, glyoxal, or dehydroascorbic acid will cause the degradation of an α-amino acid to an aldehyde with one

carbon less than that of the amino acid. It will be recalled that α-dicarbonyl compounds are some of the intermediates of Maillard browning. If α-amino groups were utilized in the system, the pH might be expected to be lowered to reflect the lowered basicity and increased acidity of the system. This may have been particularly important in the case of samples stored at 40° C over the six month period. Moreover, Strecker degradation is also accompanied by the production of CO_2.

The raw samples consistently and persistently had lower pH readings than the drum-dried samples. This agrees with previous observations made in this work. In addition, it may be that as part of the general metabolism of a living system like the raw cowpea powders, deamination phenomena were taking place throughout the storage period. Deamination would help to lower the pH even more. In addition to that, lipolysis catalyzed by lipolytic enzymes in the raw powders would be expected to release free fatty-acids which in themselves might lower the pH slightly or engage in reactions with the amino groups to further lower the pH.

Ascorbic Acid

Ascorbic acid was measured in the samples to learn if there was a relationship between the intensity of browning in the samples and the amount of ascorbic acid. In the

oxidative browning contributed by ascorbic acid, ascorbate oxidase may act on the enediol to form reactive carbonyl compounds and the reactions that follow this enzymic oxidation are non-enzymic. Furthermore, the initial oxidations occur, although much more slowly, in the absence of oxidases. According to Ranganna and Setty (1974), ascorbic acid can be oxidized enzymically and non-enzymically to dehydroascorbic acid which is a vicinal tricarbonyl compound that reacts non-enzymically with amino-acids to product red and brown polymers.

However, measurements made in this work did not indicate the presence of measurable ascorbic acid in both the raw and drum-dried cowpea powders either at the beginning or at the end of the storage period. This was probably because of the stage of maturity of the batch of cowpea seeds used to prepare both the raw and drum-dried powders. Oyenuga (1968) reported no measurable ascorbic acid in Nigerian varieties of cowpeas. Hoover (1955) showed that cowpeas were relatively high in ascorbic acid, but he noted that this was true only if the seeds were harvested at an early stage in their development and used as such immediately. Holding the seeds at temperatures no higher than 40° F was also indicated to help maintain their ascorbic acid level. According to his findings, if the cowpeas are allowed to mature such that the green color begins to disappear from the pod, the cowpeas cease to become a valuable source of this vitamin. Storage

temperatures of 75° F and 100° F respectively led to decreases of 42% and 54% in the ascorbic acid content.

So, it is likely that the samples used in this work may have been harvested late as far as maintaining the ascorbic acid content is concerned.

Changes In Riboflavin Content

Figures 15 and 16 and Table 21 give the trend in the riboflavin content of stored cowpea powders. Losses of riboflavin occurred in both the drum-dried and raw cowpea powders at 25° C and 40° C and at all A_w's as the length of storage time increased. The refrigeration temperature of 5° C was very effective in minimizing such storage losses.

As a food nutrient, every vitamin is important as part of a balanced diet that will ensure normal growth and development of consumers; but we are interested in the riboflavin stability of these products because, compared to other vitamins, the B-vitamin complex of which riboflavin is an integral part is high in the cowpea.

The loss of riboflavin here agrees with the generally reported losses of vitamins in stored foods. Schroeder (1972) reported losses of vitamins resulting from processing and preservation of a variety of foods and suggested that vitamins such as pyridoxine and panthothenic acid may have

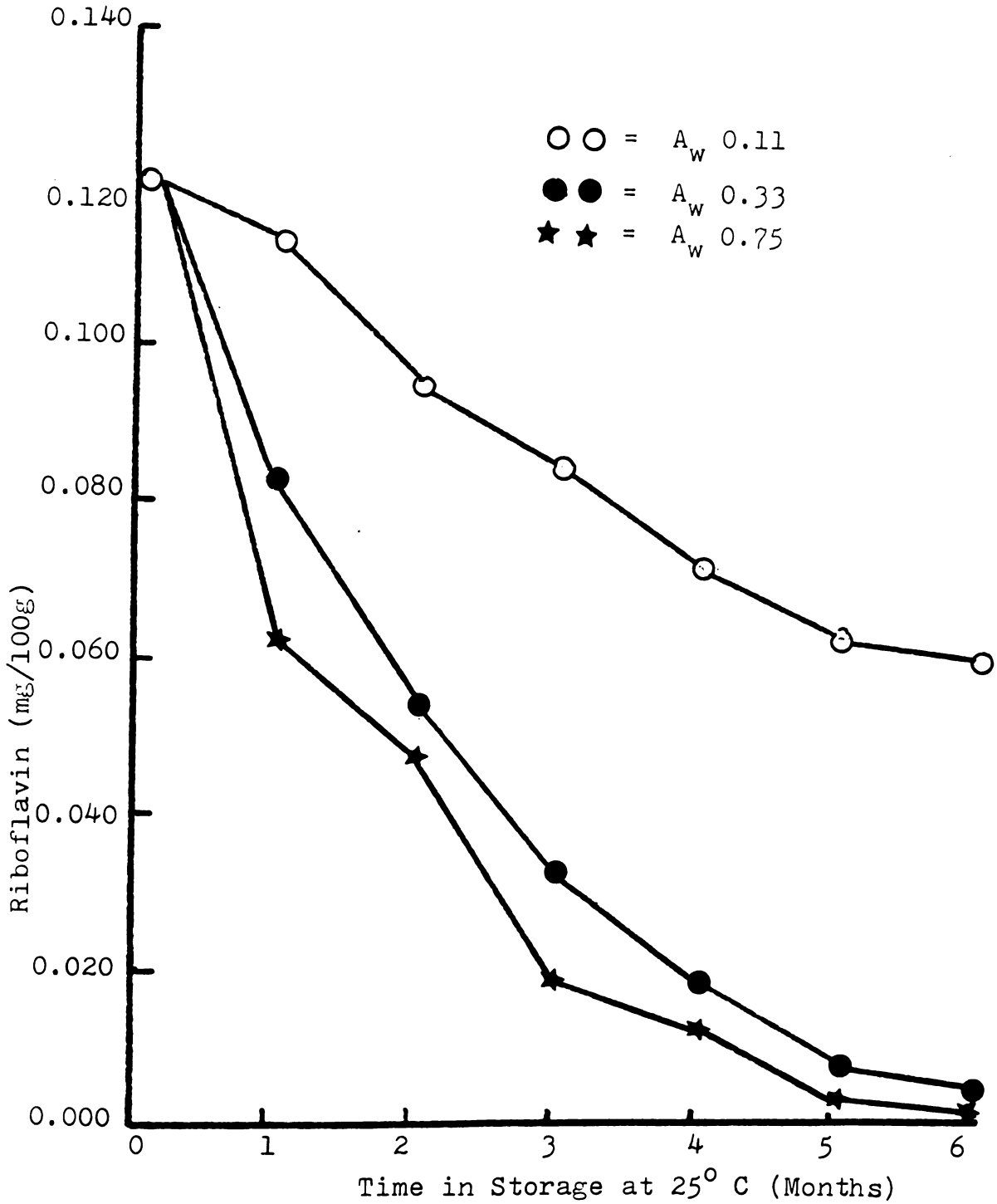


FIGURE 15 Effects of Water Activity on The Riboflavin Content of Drum-Dried Cowpea Powders During Storage

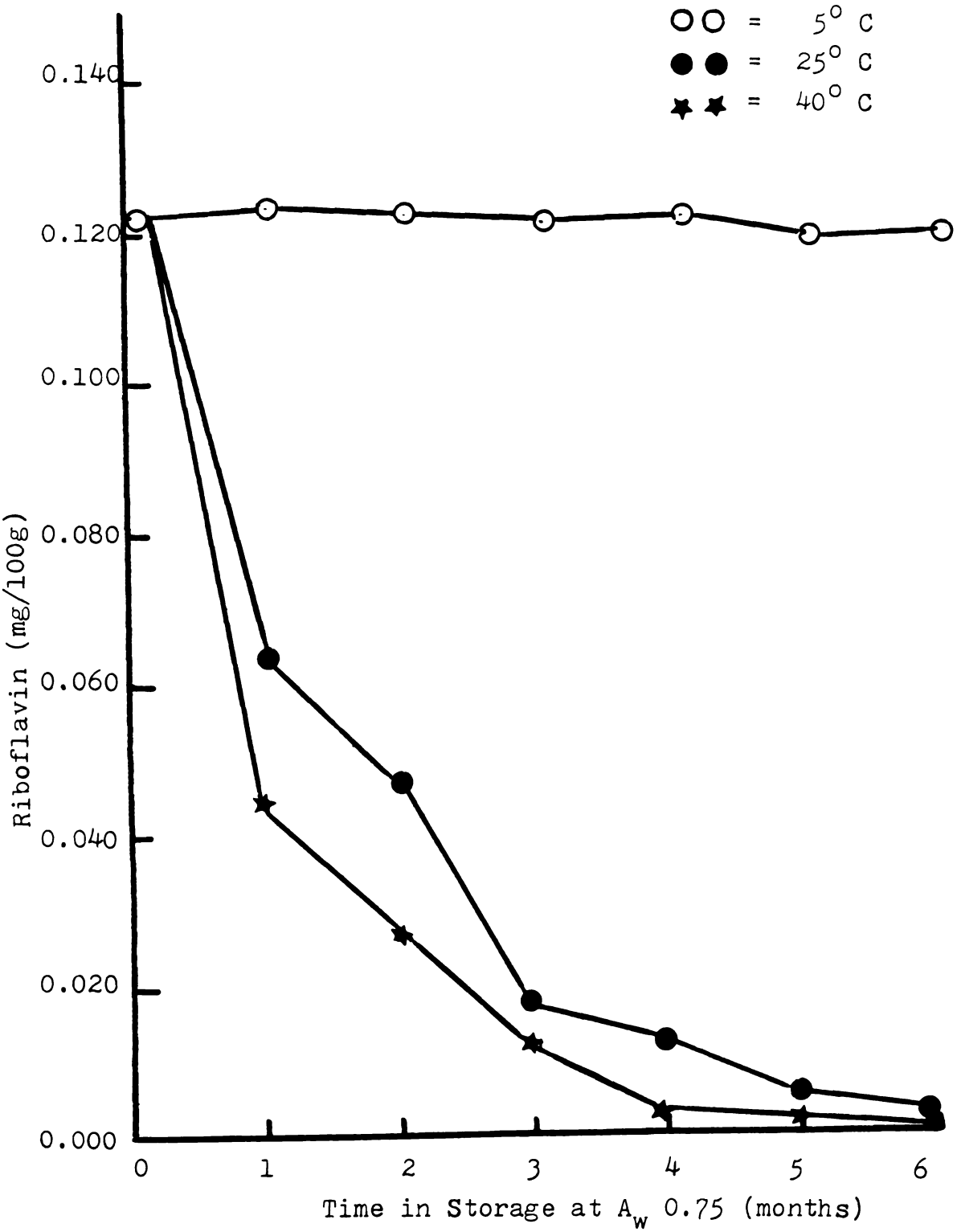


FIGURE 16 Effect of Temperature on the Riboflavin Content of Drum-Dried Cowpea Powders During Storage

TABLE 21 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE RIBOFLAVIN CONTENT (MG/100G SAMPLE)* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
0.163	0.163	0.163	0.163	0.163	0.163
FIRST MONTH'S READINGS					
0.092	0.114	0.134	0.162	0.133	0.105
SECOND MONTH'S READINGS					
0.043	0.085	0.115	0.163	0.117	0.098
THIRD MONTH'S READINGS					
0.006	0.054	0.096	0.161	0.095	0.064
FOURTH MONTH'S READINGS					
0.003	0.023	0.075	0.162	0.076	0.032
FIFTH MONTH'S READINGS					
--	0.021	0.074	0.160	0.076	--
SIXTH MONTH'S READINGS					
--	0.018	0.072	0.159	0.071	--

-- = no measurable amount of riboflavin by the fluorometric procedure used.

* = values are means of duplicate readings

to be supplemented in the diets of peoples eating such foods.

Examination of Table 22 shows that losses of riboflavin ranged from about 2% to 100%. The same phenomenon has been observed in other foods. Gleim et al. (1944) working with spinach and asparagus reported losses of 5 and 22% respectively during storage for twenty-four hours at 18.8 to 25.5° C but that the losses on freezing were 40 and 42% respectively.

What is perhaps more interesting in these results is the percentage loss of potency as the A_w's and temperature are varied in both the raw and drum-dried cowpea powders. Increasing the temperature from 5° C to 25° C and 40° C had the effect of decreasing the potency of the vitamin as measured by the percentage loss of the vitamin at the end of the six-month storage period. Storage at 5° C greatly reduced the loss of riboflavin potency while storage at 40° C increased the loss of vitamin potency. In fact, at 25° C, the drum-dried powders had no measurable riboflavin activity, while at 40° C, both the drum-dried and raw cowpea powders had no measurable vitamin activity at the end of the storage period. The percentage losses reported here do not testify to the generally accepted and reported heat stability of riboflavin by Levine and Remington (1937). Schweigert et al. (1943) noted that storage either by quick-freezing or sterilization led to relatively small losses of riboflavin. The difference in

TABLE 22 *PERCENTAGE LOSSES OF RIBOFLAVIN IN STORED COWPEA POWDERS

	A_w 0.75		
	5° C	25° C	40° C
Drum Dried Powders	8.20	100	100
Raw Powders	8.50	56.40	100

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	50.80	96.70	100
Raw Powders	100	89.00	55.80

*Over a six month storage period

this work may lie in the condition of storage. In elucidating the chemistry of riboflavin destruction by light, Theodor Wagner-Jauregg (1967) stated that in acid or neutral solution riboflavin is converted by light to lumichrome (6,7-dimethyl alloxazine) but that in alkaline solution, it is converted to lumiflavin (6,7,9-Trimethylisoalloxazine). Under neutral conditions and in the absence of oxygen, riboflavin is converted to "Deuteroflavin" (6,7-Dimethyl-9-formylomethylisoalloxazine). Data for the pH readings in this work correspond to acid-neutral condition.

Based on the above, it is here postulated that the large losses incurred in this project may have been due to the fact that deliberate steps were not taken to insulate the cowpea powders from light during storage. For example, the loss in milk which is allowed to stand in glass containers on consumer's doorstep may be as high as 85% after 2 hours' exposure to bright sunlight. (Ziegler, 1944). This may be compared with practically no loss of riboflavin during pasteurization procedures, where light effects are minimal (Holmes, 1944). If the loss of riboflavin was by light, then riboflavin was converted to lumichrome in the powders used in this study because of the prevailing pH conditions.

The Figures and Tables already referred to show that in the drum-dried samples higher losses were incurred with increasing A_w. In contrast, higher A_w led to lower

losses in the raw cowpea powders. The trend is interesting but difficult to explain. It may be that in the drum-dried samples, increasing solubility at the higher A_w led to increasing losses. It is frequently observed that where reactants in a chemical process are water soluble, increasing the amount of available water generally increases the rate or extent of reaction until a dilution effect begins to take effect. The contrast which is observed with the raw samples may be a reflection of a different mechanism for riboflavin destruction and loss in these powders. The speculation here is that in the raw powders, oxidation-linked destruction of riboflavin may be the prevailing mechanism in riboflavin loss. It is true that moisture may protect a food substance from oxidative destruction by several mechanisms already referred to in the discussion of lipid oxidation. High A_w may present a physical barrier to the diffusion of oxygen into the system. In addition, if free radicals produced in lipid oxidation played a part in riboflavin loss, then the observed trend for the raw samples would be predictable.

The drum-drying process led to a 25% loss of riboflavin activity. The figure is not too far from the 20% most frequently quoted but the source of loss in this work has to be different. Mayfield and Hedrick (1949) and McIntire et al. (1943) among many others have given figures close to 20% as representing processing losses but most of

the losses were attributed to the extraction of the vitamin by the water used in the cooking or blanching operations. In preparing the drum-dried powders used in this work, all cooking water was incorporated into the making of the puree before the drum-drying operation itself. So, leaching losses must have been non-existent or minimal. Steam blanching rather than water blanching was also used. Steam blanching reduces leaching losses common with water-blanching. It is therefore reasonable to suggest that the 25% loss of riboflavin here may have been due to thermal effects of the entire process of steam blanching, cooking and drum dehydration. Also, losses of riboflavin due to exposure to light during cooking may also have been important. Cheldelin et al. (1943) have shown that large losses, up to 48%, occurred in the cooking of eggs, milk and pork chops in uncovered dishes under conditions where there was no loss of riboflavin when cooking dishes were covered.

Changes in Thiamine Content

As Figures 17 and 18 and Tables 23 and 24 show, the changes in the thiamine content of the cowpea powders at the different conditions of storage were basically the same as observed for riboflavin; and the same explanation for these trends probably hold: (a) losses of thiamine at 25° C and 40° C and at all A_w's as the length of storage increased (b) effectiveness of 5° C in minimizing such

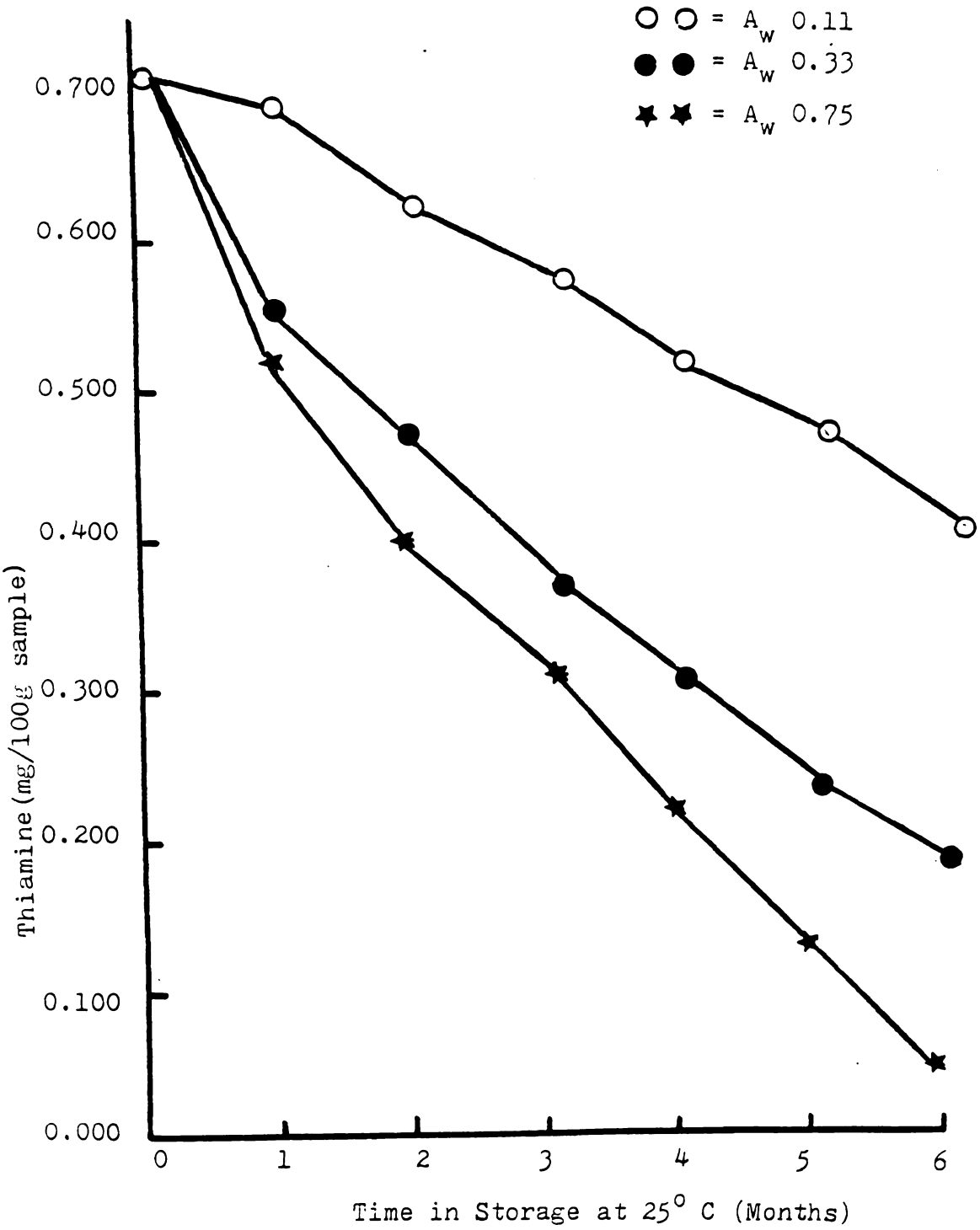


FIGURE 17 Effect of Water Activity on the Thiamine Content of Drum-Dried Cowpea Powders During Storage

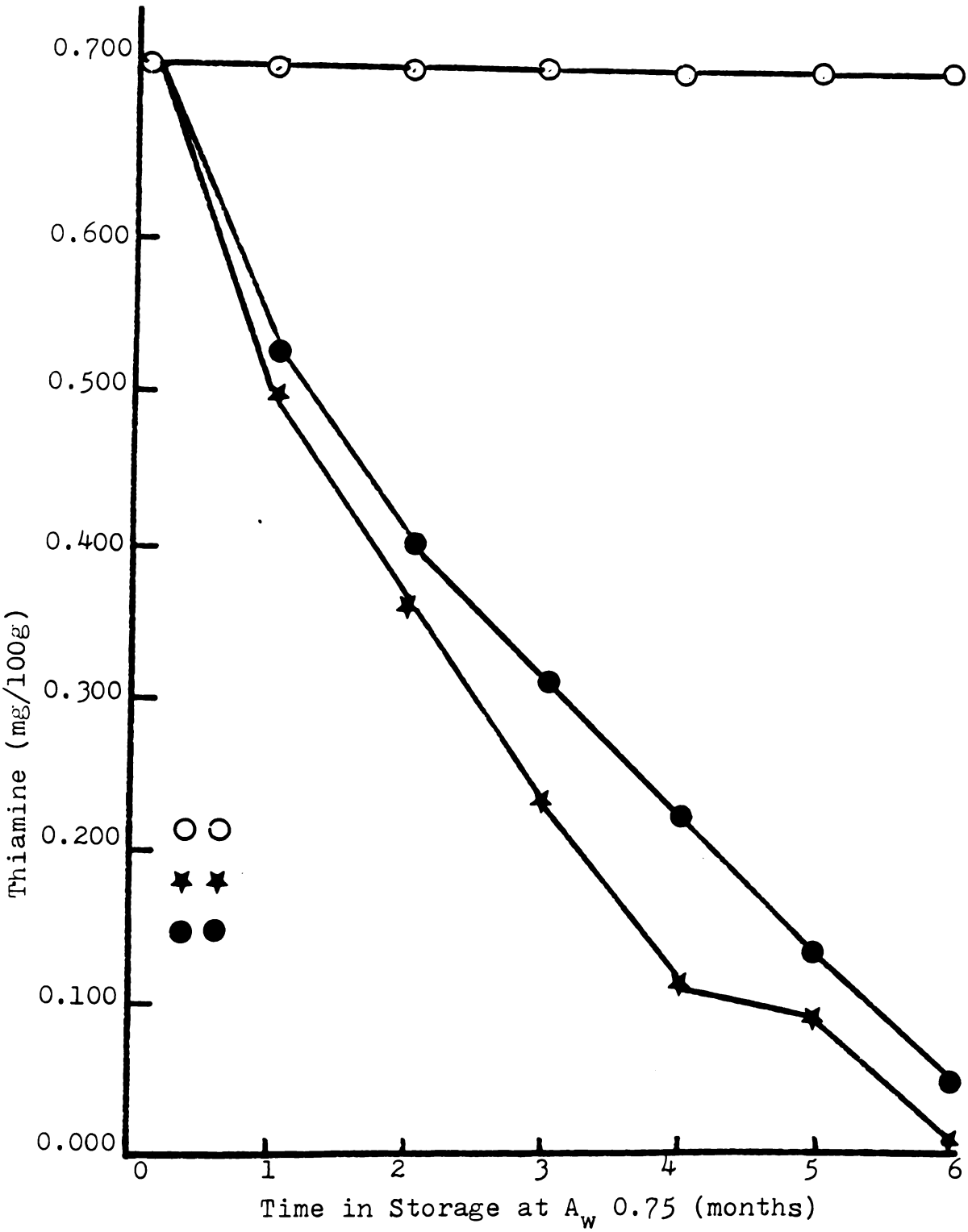


FIGURE 18 Effect of Temperature on the Thiamine Content of Drum-Dried Cowpea Powders During Storage

TABLE 23 EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON THE THIAMINE CONTENT (mg/100g sample)* OF RAW COWPEA POWDER DURING STORAGE

A_w			Temperature		
0.11	0.33	0.75	5° C	25° C	40° C
INITIAL READINGS					
0.863	0.863	0.863	0.863	0.863	0.863
FIRST MONTH'S READINGS					
0.722	0.782	0.843	0.861	0.841	0.544
SECOND MONTH'S READINGS					
0.611	0.726	0.813	0.862	0.814	0.353
THIRD MONTH'S READINGS					
0.530	0.664	0.772	0.865	0.771	0.204
FOURTH MONTH'S READINGS					
0.461	0.612	0.743	0.863	0.741	0.065
FIFTH MONTH'S READINGS					
0.392	0.567	0.710	0.861	0.712	--
SIXTH MONTH'S READINGS					
0.322	0.511	0.686	0.858	0.688	--

-- = no measurable amount of thiamine by the fluorometric procedure used

* = values are the means of duplicate readings

TABLE 24 *PERCENTAGE LOSSES OF THIAMINE IN STORED COWPEA POWDERS

	A_w 0.75		
	5° C	25° C	40° C
Drum Dried Powders	2.80	93.20	100
Raw Powders	5.80	20.30	100

	25° C		
	A_w 0.11	A_w 0.33	A_w 0.75
Drum Dried Powders	44.00	74.40	93.40
Raw Powders	62.70	40.80	20.50

*Over a six month storage period

losses (c) for the drum-dried powders, increasing losses of thiamine with increasing A_w probably because of greater solubility of the vitamin; the raw samples had higher vitamin values with higher A_w probably because of lower oxidation linked destruction with increasing A_w.

Some of the above findings are supported by literature reports. Temperature is an important factor in thiamine stability. Freed et al. (1949) reported that peas stored at 38° C had a 68% vitamin retention after 12 months storage. At 1.5° C retention was 100%. Green beans had 8% retention at 38° C but retention increased sharply to 76% when storage was at 1.5° C. The values for lima beans were 48 and 92% at 38° C and 1.5° C respectively.

The information on thiamine stability in dehydrated foods is limited. In studies with dehydrated cornsoy milk (CSM), Bookwalter et al. (1968) indicated that thiamine degradation was influenced strongly by moisture content. They noted that storage at 100° F for 182 days caused no loss when the system was maintained below 10% moisture content, but that extensive loss occurred at 13% moisture. This clearly agrees with the rather well marked changes in vitamin loss at the three A_w's of 0.11, 0.33, and 0.75 which were 44.00, 74.40 and 93.40% respectively for the drum-dried samples.

The drum-drying process led to about 17% loss of thiamine. This does not appear to amount to much in view of the fact that thiamine is one of the least stable vitamins. The literature summarized by Farrer (1955) point to extensive losses of the vitamins in cereals as a result of cooking or baking; in meats, vegetables and fruits, as a result of various processing operations; and during storage. Roasting of beef and pork was reported to incur losses as high as 36-53% while boiling of vegetables has been indicated to reduce their thiamine content by as much as 40%. The loss of only 17% in thiamine potency in the drum-dried powders means that the drum-dehydration process employed in this research was such as to cause minimal damage to the thiamine content of the cowpea powder.

Table 24 does not show any consistent pattern in the degree of loss when the raw cowpea powders are compared with the drum-dried powders. In some instances, for example, at 5° C and at 0.11 A_w, the raw samples recorded higher percentage loss. At the other conditions of storage, the drum-dried samples had higher losses. The inconsistency may have arisen from the possible differences in the mechanism of thiamine losses in the powders under the different conditions of storage.

Comparison of Processing and Storage Stabilities of Thiamine and Riboflavin

The sum of the processing steps involved in drum-drying led to 17 and 25% losses of thiamine and riboflavin respectively. After a six-month storage period, losses of riboflavin were higher than those for thiamine.

This means that thiamine was more stable than the riboflain in the cowpea powders used in this work. Most literature indicates the reverse. It must be realized, however, that differences can occur in the reports of different workers depending on the types of methods used in analyses, the form of the vitamin released and measured by the method employed and the type of storage conditions used. The photo-lability of riboflavin is well documented. It is not known what would have happened if the cowpea powders used in this research were completely protected from light.

Some Physical Problems Observed In The Cowpea Powders

Some of the problems associated with the processing and storage of the powders have already been discussed. Most of them were chemical and not very obvious or very serious. From the marketing standpoint, the physical problems of color, cakiness, and mold growth are more serious because they are easily discernible by consumers.

In the raw cowpea powders used in this study, mold growth was observed only in powders stored at A_w 0.75 and

25° C. All other samples were free of this problem, indicating the role of a combination of high A_w and ambient temperature in promoting mold spoilage in the products. It should be noted that mold growth occurred at the sixth month of storage. In a country like Nigeria where refrigeration facilities are not as widespread as they are in the United States, this could be a serious problem in the use of these products. It is recommended to store and use them for not more than five months in a country such as Nigeria where temperature/A_w combinations are similar to the 25° C/A_w 0.75 which permitted mold growth in the raw powders, if chemical additives have not been added to prevent mold growth.

Similar incidences of mold growth have been observed in other legumes by other workers, when the temperature and relative humidity of storage were high enough (Swanson, et al., 1977; Weston and Morris, 1954; Christensen and Kaufman, 1969).

A way to get around the problem of mold growth in these powders may involve the use of antimicrobial agents such as sulfites, SO_2 and sorbic acid (Chichester and Tanner, 1972).

Another physical problem observed in the products was that of cakiness. This occurred in powders stored at A_w 0.75 at 5° C, 25° C, and 40° C temperatures. This indicates that cakiness in these products is a function of A_w and not of temperature. Cakiness affects adversely the

free flowing characteristics of granular and powdered forms of foods, such as those prepared in this study, that are hygroscopic in nature.

Several anticaking agents are available that could be tried in these powders. They include calcium silicate ($CaSiO_3 \times H_2O$), calcium stearate, sodium silico-aluminate, tricalcium phosphate, magnesium silicate, and magnesium carbonate as recommended by the Committee on Food Protection (1972).

The decrease in protein solubility and the browning of the powders have already been discussed. Browning may be minimized in these products by low temperature storage, and use of sulfites.

Statistical Analyses of Data

Statistical evaluation involved the calculation of t-values to see if the differences in the various quality parameters of the cowpea powders with changes in water activity and temperature were statistically significant at the α levels of 0.01 and 0.05.

Figures and Tables referred to previously clearly indicate that variations in water activity and temperature led to obvious and definite changes in the values of the various quality parameters of the cowpea powder. The statistical analyses indicated in Table 25, however, reveal that changes in A_w did not cause statistically significant differences in the values of most of these parameters. Temperature variations from 5° C to 25° C and to 40° C gave more statistically significant differences.

It is possible however that over a longer period of storage the differences in the values of these quality parameters with changing A_w would have been statistically significant.

TABLE 25 STATISTICAL ANALYSES OF DATA

Parameter	Drum Dried Powders (t values*)		
	5°C vs 25°C	25°C vs 40°C	5°C vs 40°C
Lipid oxidation	-3.095[c]	-3.578[c]	-3.649[c]
Lipid Free-Amino Groups	0.027	2.111[c]	0.119
Color by "L" Values	1.145[b]	3.908[c]	3.957[c]
Reducing Sugar	0.222[b]	0.013[b]	0.013[c]
Available Lysine	3.248[c]	2.814[b]	4.046[c]
Soluble Protein	3.072[c]	2.511[b]	5.354[c]
pH	1.941[c]	2.770[b]	4.934[c]
Riboflavin	5.026[c]	0.397	5.496[c]
Thiamine	4.313[c]	0.366	4.426[c]
	Raw Powder		
Lipid Oxidation	-2.555[c]	-4.351[c]	-4.576[c]
Lipid Free-Amino Groups	1.713	1.217[c]	2.377[c]
Color by "L" Values	1.321[b]	5.215[c]	5.283[c]
Reducing Sugar	2.781[c]	2.169[b]	1.250
Available lysine	3.568[c]	2.484[b]	4.606[c]
Soluble Protein	4.130[c]	1.391[b]	5.265[c]
pH	1.113[c]	2.879[b]	4.148[c]
Riboflavin	4.341[c]	1.46,[c]	4.185[c]
Thiamine	3.439[c]	3.907[c]	4.696[c]
	Drum Dried Powder (t values*)		
	A_w		
	0.11 vs 0.33	0.11 vs 0.75	0.33 vs 0.75
Lipid Oxidation	0.756	0.064	-0.670
Lipid Free-Amino Groups	1.097	1.549	0.679
Color by "L" Values	1.004	1.490	0.569
Reducing Sugar	0.943	1.362	0.524
Available Lysine	0.921	1.680	0.804
Soluble Protein	0.443	0.985	0.765
pH	0.186	0.854[b]	0.674
Riboflavin	2.145	2.557[b]	0.325
Thiamine	1.979	2.378[b]	0.602
	Raw Powder		
Lipid Oxidation	0.952	0.154	-5.968[c]
Lipid Free-Amino Groups	0.728	1.273	0.692
Color by "L" Values	0.741	2.038	1.306
Reducing Sugar	0.679	1.300	0.702
Available Lysine	0.542	1.376	0.889
Soluble Protein	-0.197	-0.168	0.024
pH	0.303	0.997[b]	0.745
Riboflavin	-0.774	-2.226[b]	-1.450
Thiamine	-1.375	-2.871[b]	-1.887

*12 degree of freedom. [b]significant at the α level of 0.05

[c]significance at both 0.01 and 0.05

Relationship Between the Various Quality Parameters of Stored Cowpea Powders

To gain more insight into the various mechanisms that occurred in the stored cowpea powders and to attempt to explain the observed changes in the parameters studied in this work, plots of one parameter against another were made. Simple correlation coefficients were also calculated and are shown in Table 26.

1. Riboflavin Content of Stored Powders as Affected by Lipid Oxidation

Figures 19-22 show this relationship. At the water activities of 0.11, 0.33 and 0.75, a negative correlation existed between the riboflavin content and the degree of lipid oxidation as evinced by diene conjugation in lipids of the drum dried and raw cowpea powders (Table 26). Lipid oxidation may, at least, partially account for the loss of riboflavin in these powders in the course of storage.

In the raw powder, the first few months were marked by large losses of the vitamin which were not marked by similar increases in the lipid conjugated diene absorbance at 233 nm. For example, at A_w 0.11, decreases in riboflavin were matched by corresponding increases in lipid conjugated diene absorbance at 233 nm only after about 2 months in storage. At A_w 0.33, it was about 3 months and at A_w 0.75, it was again about 2 months. The fact that

TABLE 26 CORRELATION COEFFICIENTS BETWEEN THE VARIOUS QUALITY PARAMETERS

1. Soluble Protein vs. Available Lysine	A_w		
	0.11	0.33	0.75
Drum Dried Powders	0.96^{c}	0.93^{c}	0.99^{c}
Raw Powder	0.82^{b}	0.91^{c}	0.96^{c}
	Temperature		
	5°C	25° C	40°C
Drum Dried Powders	0.31_{b}	0.98^{c}	0.89^{c}
Raw Powder	0.86^{b}	0.94^{c}	0.86^{c}
2. Reducing Sugar vs. Available Lysine	A_w		
	0.11	0.33	0.75
Drum Dried Powder	0.98^{c}	0.93^{c}	0.96^{c}
Raw Powder	0.99^{c}	$0,98^{c}$	0.83^{b}
	Temperature		
	5°C	25°C	40°C
Drum Dried Powder	-0.09	0.98^{c}	0.98^{c}
Raw Powder	0.90^{c}	0.98^{c}	0.98^{c}
3. Riboflavin vs. Lipid Oxidation	A_w		
	0.11	0.33	0.75
Drum Dried Powder	-0.98^{c}	-0.93^{c}	-0.89^{c}
Raw Powder	-0.76^{b}	-0.42	-0.24
	Temperature		
	5°C	25°C	40°C
Drum Dried Powder	-0.48_{c}	-0.89^{c}_{b}	-0.85^{b}
Raw Powder	-0.98^{c}	-0.86^{b}	-0.98^{c}

TABLE 26 (Cont)

4. Lipid Oxidation vs. Lipid Free-Amino Groups	A_w 0.11	0.33	0.75
Drum Dried Powder	-0.96^{c}	-0.96^{c}	-0.98^{c}
Raw Powder	-0.97^{c}	-0.88^{c}	-0.99^{c}
	Temperature 5°C	25°C	40°C
Drum Dried Powder	-0.75$_{b}$	-0.92^{c}	-0.96^{c}
Raw Powder	-0.86^{b}	-0.94^{c}	-0.88^{c}

5. Color (L) vs. Lipid Free-Amino Group	A_w 0.11	0.33	0.75
Drum Dried Powder	0.86^{b}	0.83^{b}	0.92^{c}
Raw Powder	0.84^{b}	0.87^{c}	0.84^{c}
	Temperature 5°C	25°C	40°C
Drum Dried Powder	0.82^{b}	0.92^{c}	0.97^{c}
Raw Powder	0.09	0.87^{b}	0.79^{b}

6. Color "L value" vs. Lipid Oxidation	A_w 0.11	0.33	0.75
Drum Dried Powder	-0.89^{c}	-0.92^{c}	-0.95^{c}
Raw Powder	-0.86^{b}	-0.89^{c}	-0.83^{b}
	Temperature 5°C	25°C	40°C
Drum Dried Powder	-0.73$_{c}$	0.99^{c}	-0.99^{c}
Raw Powder	-0.92^{c}	0.93^{c}	-0.95^{c}

7. Lipid Free-Amino Groups vs pH	A_w 0.11	0.33	0.75
Drum Dried Powder	0.90^{c}	0.97^{c}	0.94^{c}
Raw Powder	0.84^{b}	0.77^{b}	0.87^{b}
	Temperature 5°C	25°C	40°C
Drum Dried Powder	0.48	0.94^{c}	0.68
Raw Powder	0.92^{c}	0.93^{c}	0.72

TABLE 26 (Cont)

8. Available lysine vs pH	A_w 0.11	0.33	0.75
Drum Dried Powder	0.98^{c}	0.97^{c}	0.97^{c}
Raw Powder	0.95^{c}	0.94^{c}	0.86^{c}
	Temperature 5°C	25°C	40°C
Drum Dried Powder	0.58_{b}	0.98^{c}	0.83^{b}
Raw Powder	0.83^{b}	0.86^{b}	0.94^{c}

N/B

b means that the calculated t value is significant at α level 0.05 with 5 degrees of freedom.

c means that the calculated t value is significant at α level 0.01 with 5 degrees of freedom.

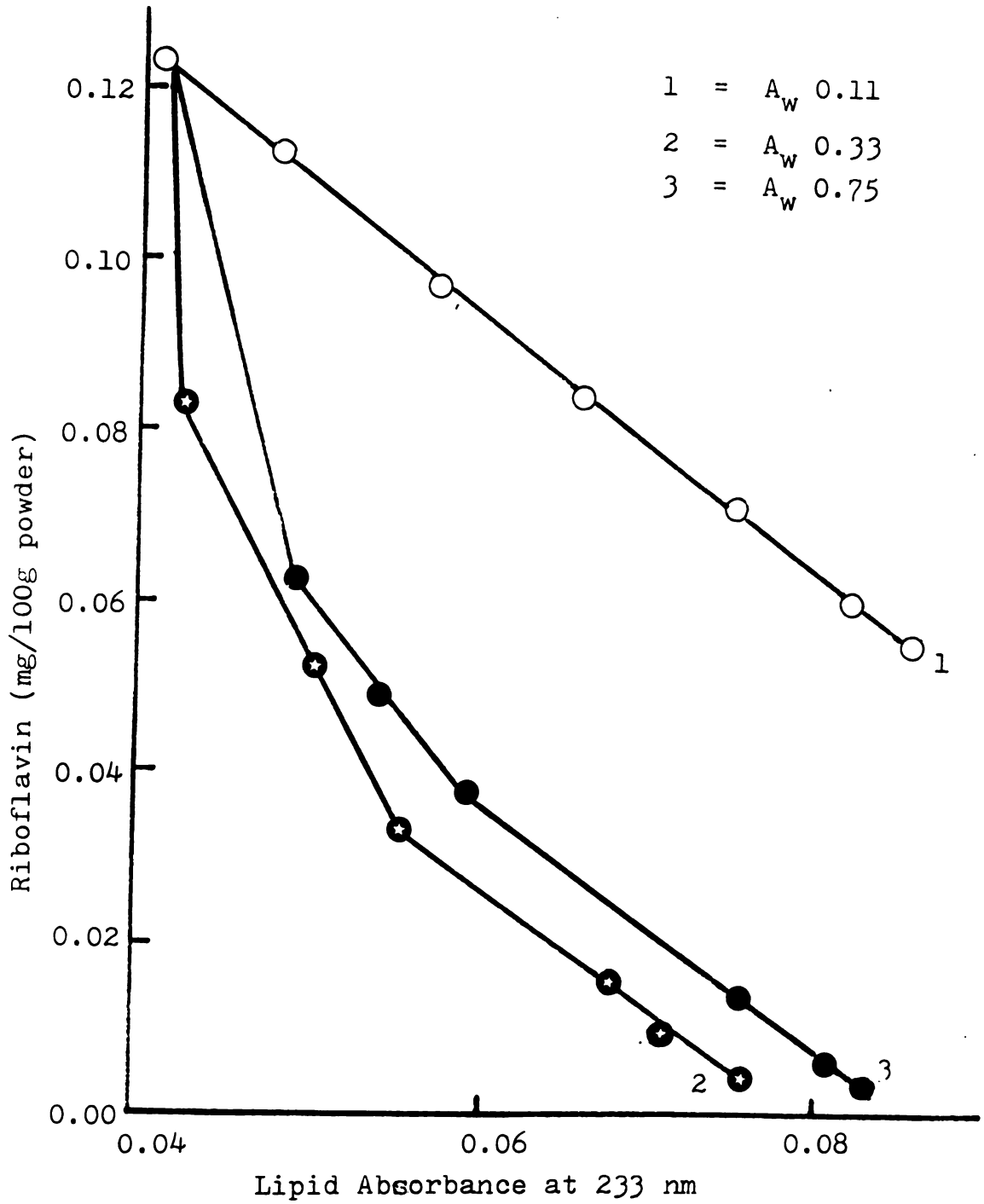


FIGURE 19 Effect of Lipid Oxidation on the Riboflavin Content of Drum Dried Cowpea Powder Stored for Six Months at 25° C

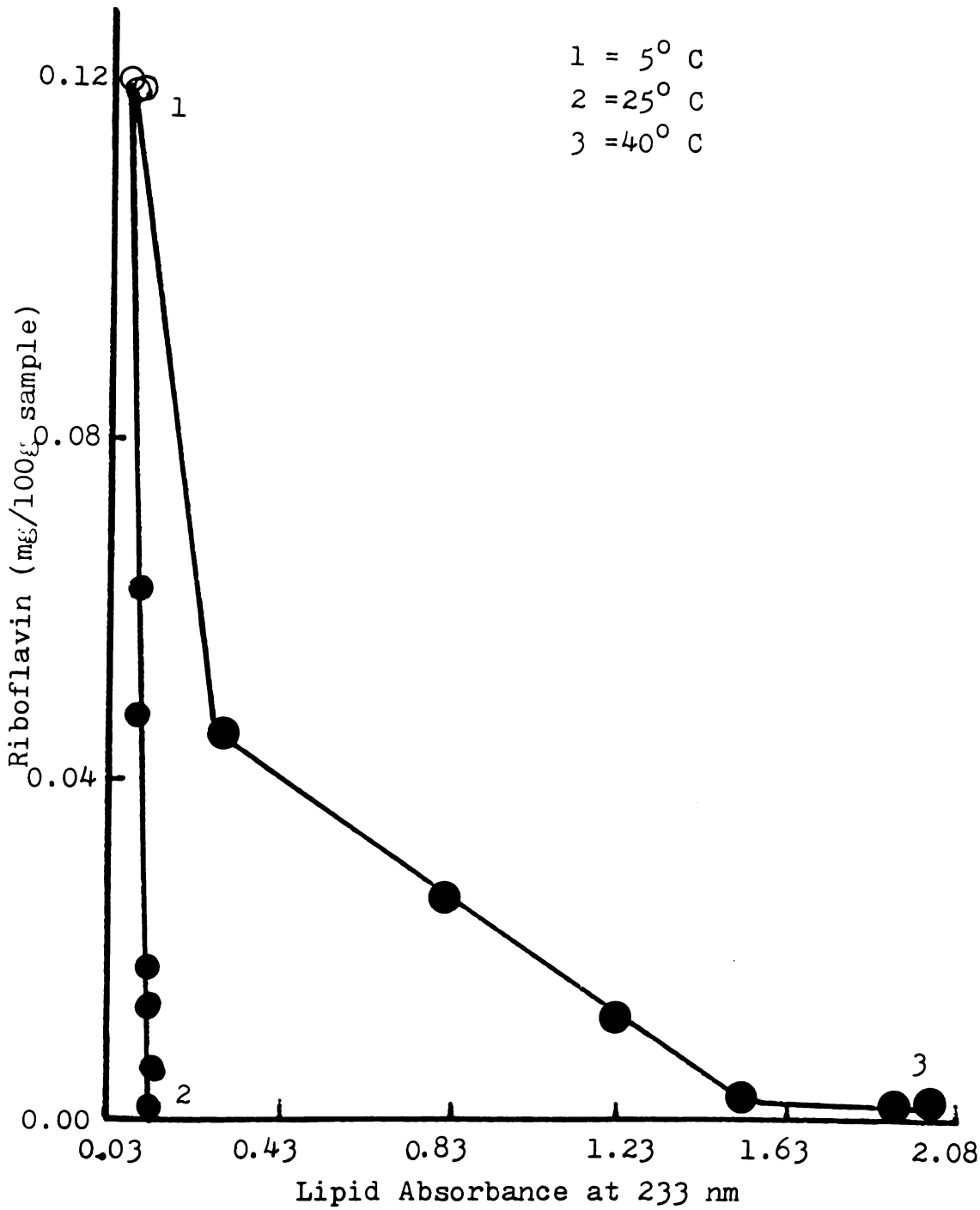


FIGURE 20 Effect of Lipid Oxidation on the Riboflavin Content of Drum Dried Cowpea Powder Stored for Six Months at A_w 0.75

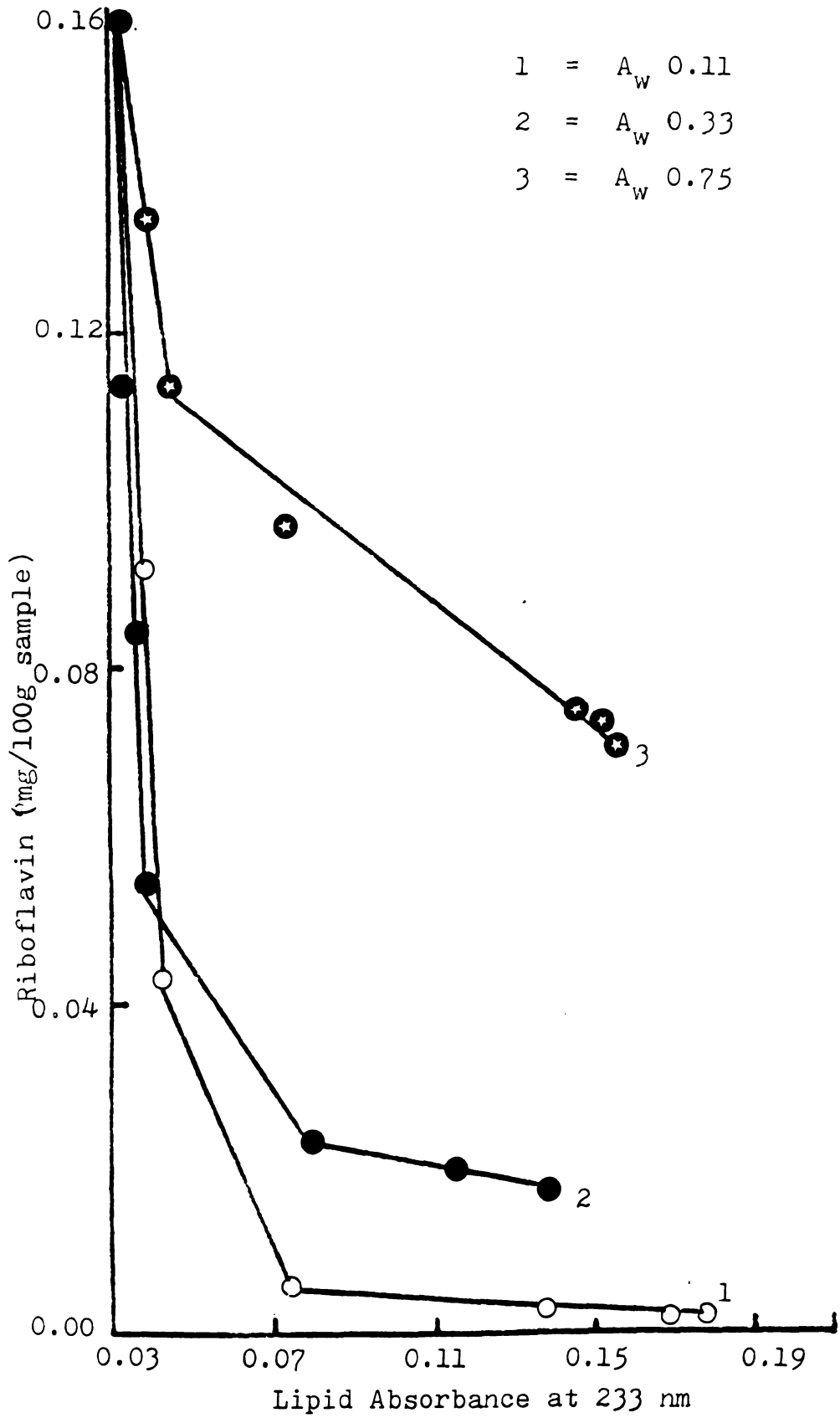


FIGURE 21 Effect of lipid Oxidation on the Riboflavin Content of Raw Cowpea Powder Stored for Six months at 25 °C.

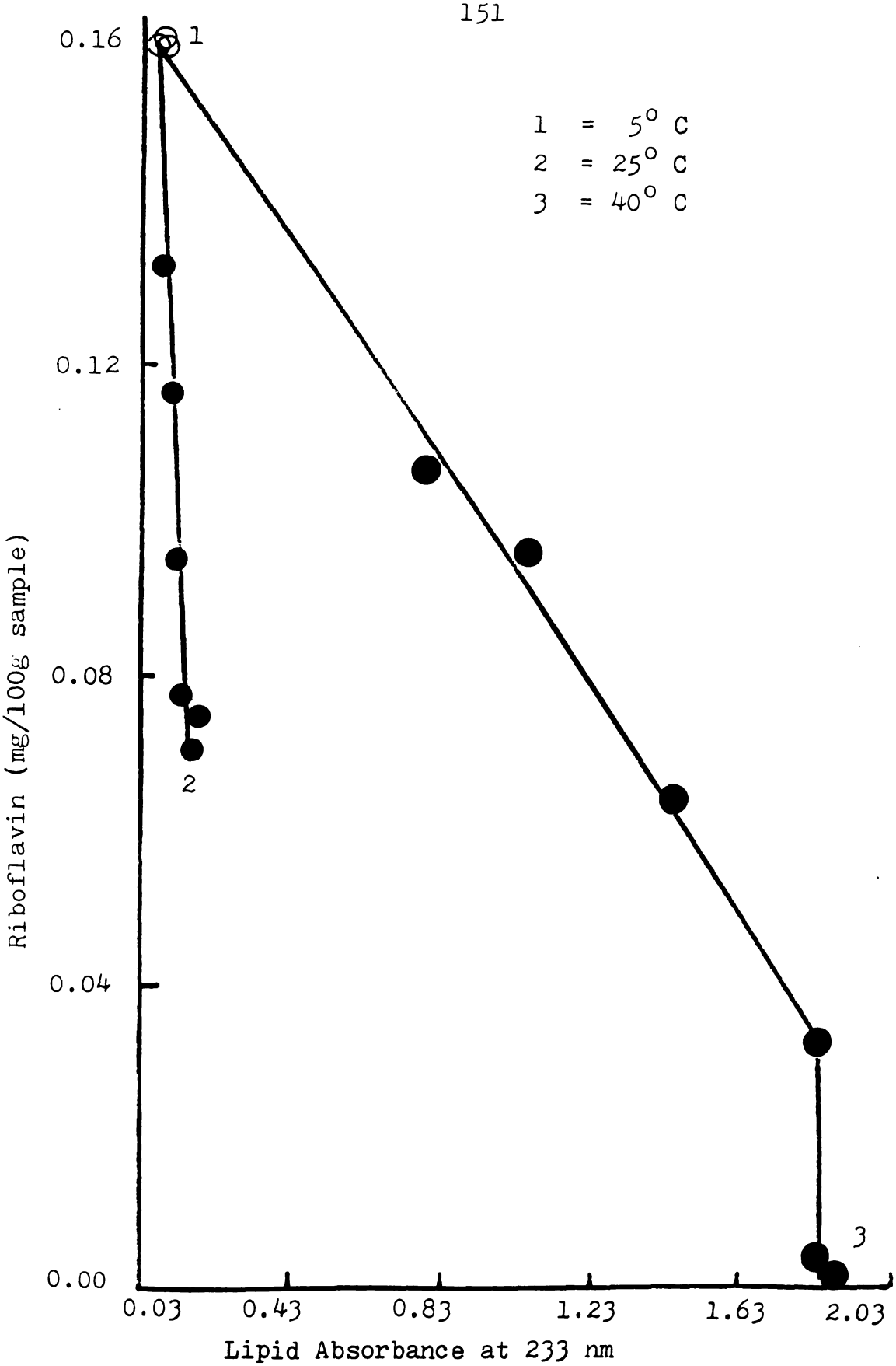


FIGURE 22 Effect of Lipid Oxidation on the Riboflavin Content of Raw Cowpea Powder Stored for Six Months at A_w 0.75

these time periods correspond to the estimated induction periods for the cowpea lipid seem to indicate that oxidation-linked destruction of riboflavin accounts partially for the loss of riboflavin as the A_w was increased from 0.11 to 0.33 and to 0.75.

Before the end of the induction period, the photodestruction of riboflavin may have been the prevailing phenomenon, since the samples were not shielded from light during storage.

Figure 21 also reveals the multistage phenomenon in the oxidation of the raw cowpea powder lipid with increases in A_w. In accordance with the concept that oxidation-linked destruction is one mechanism for the loss of riboflavin in these powders, the higher riboflavin content of samples with an A_w of 0.33 than of those with an A_w of 0.11 is interesting. However, samples stored at an A_w of 0.75 had the highest riboflavin content at the end of storage. This does not fit the multistage phenomenon in lipid oxidation depicted in Figure 21. Clearly, other mechanisms are called into play here given the complexity of the system. Could it be that by some mechanism that the high A_w of 0.75 acted to mitigate the destructive effect of light? Increased browning with increased A_w has already been noted in this work. Could it be that the reductones, α-dicarbonyl compounds and similar antioxidant compounds produced in the course of browning operated to protect the riboflavin against oxidative destruction? In addition, there may have been a

dilution effect of other reactants capable of causing loss of riboflavin in these powders at the high A_w of 0.75.

Figure 19 also reveals the multistage phenomenon in the oxidation of the drum-dried cowpea lipid. The effect of A_w on the relationship between lipid oxidation and the riboflavin content of the cowpea powder is different in some respect from that observed with the raw powder. First, it has been established that the negative correlation that exists between lipid oxidation and the riboflavin contents of these powders suggest a role for lipid oxidation in the loss of riboflavin. However, the lack of correspondence between the multistage phenomenon in lipid oxidation depicted in Figure 19 and the riboflavin content of the drum dried powder suggest that other mechanisms are involved. It was suggested earlier that increases in A_w in these powders may have acted to accelerate the reactions involved in the destruction of riboflavin by increasing the solubility of the vitamins.

Riboflavin loss at low A_w is more directly related to oxidation changes than at higher A_w where more rapid losses of riboflavin occur prior to extensive evidence of lipid oxidation. Temperature also has a strong role since, even at high A_w, there is extensive loss of riboflavin prior to much evidence of lipid oxidation—this is apparent in both drum-dried and raw powders.

The nature of the curves with increases of temperature from 5° to 25° and to 40° is equally interesting. In

the drum-dried powder stored at 5° C, the riboflavin content was maintained and the degree of lipid oxidation was less than in the raw powders. This is predictable from the ability of low temperature to greatly reduce the rate of lipid oxidation as was earlier discussed and from the ability of peroxidase enzymes in raw powders to accelerate riboflavin destruction through their oxidation products.

At 25° C, the relationship between both parameters was essentially linear. However, during the first two or three months of storage, large losses of riboflavin did not relate very strongly with the diene conjugation of lipids. This was apparently because lipid oxidation was still in the induction phase, and that the photo-destruction of riboflavin was the dominant process. At 40° C, losses of riboflavin followed diene conjugation of lipids much earlier (within a month in storage) because of the effect of the high temperature in greatly accelerating oxidation.

2. Effect of Lipid Oxidation On The Lipid Free-Amino Group

As seen in Figures 23 and 24, the relationship between the lipid free-amino group and the degree of lipid oxidation in the drum-dried powder at all A_w was essentially linear and the calculated correlation coefficients were negative indicating that one way by which the lipid free-amino groups were lost in the powder during storage was through lipid oxidation, which provided

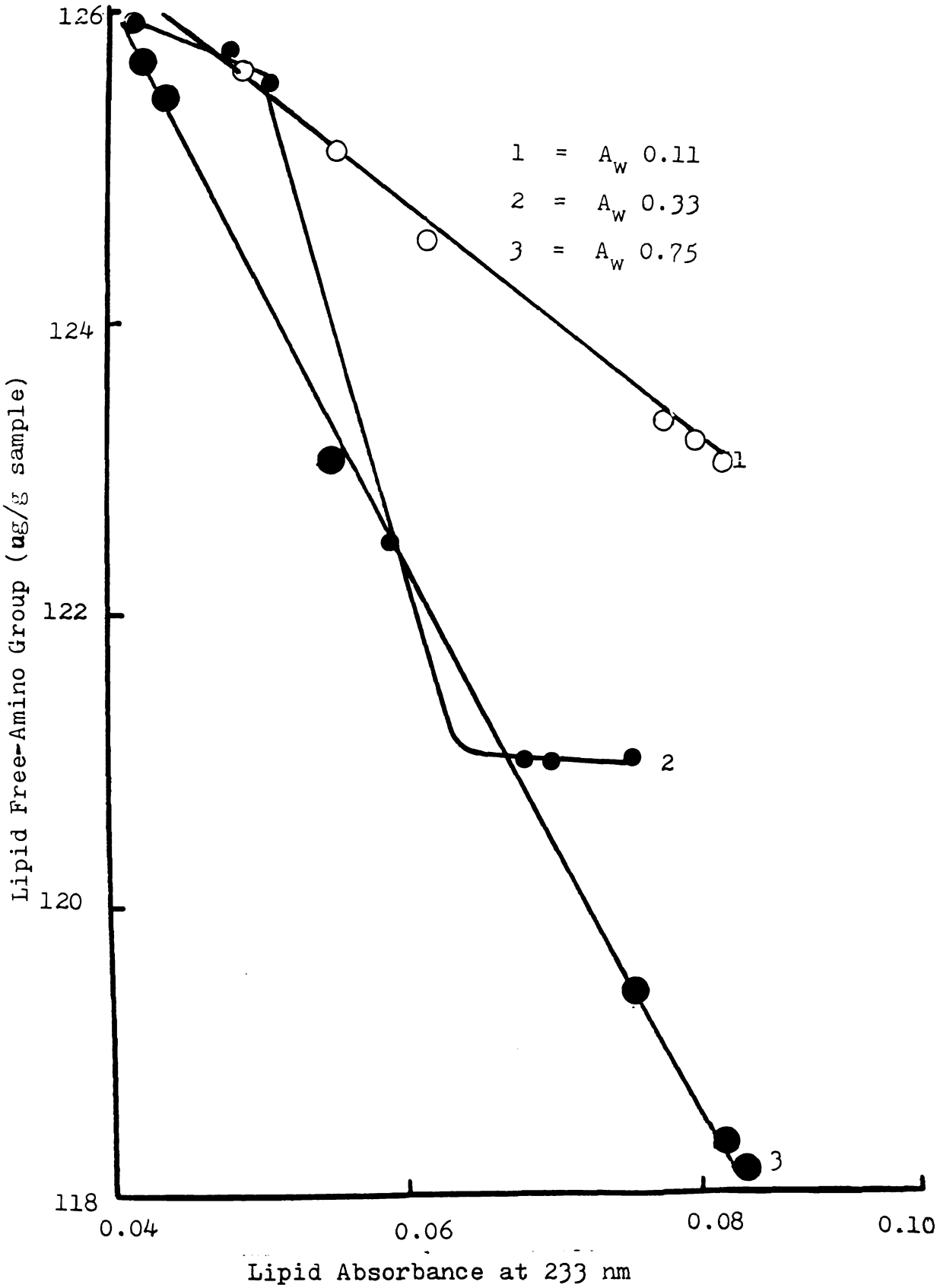


FIGURE 23 Effect of Lipid Oxidation on the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powder Stored for Six Months at 25° C

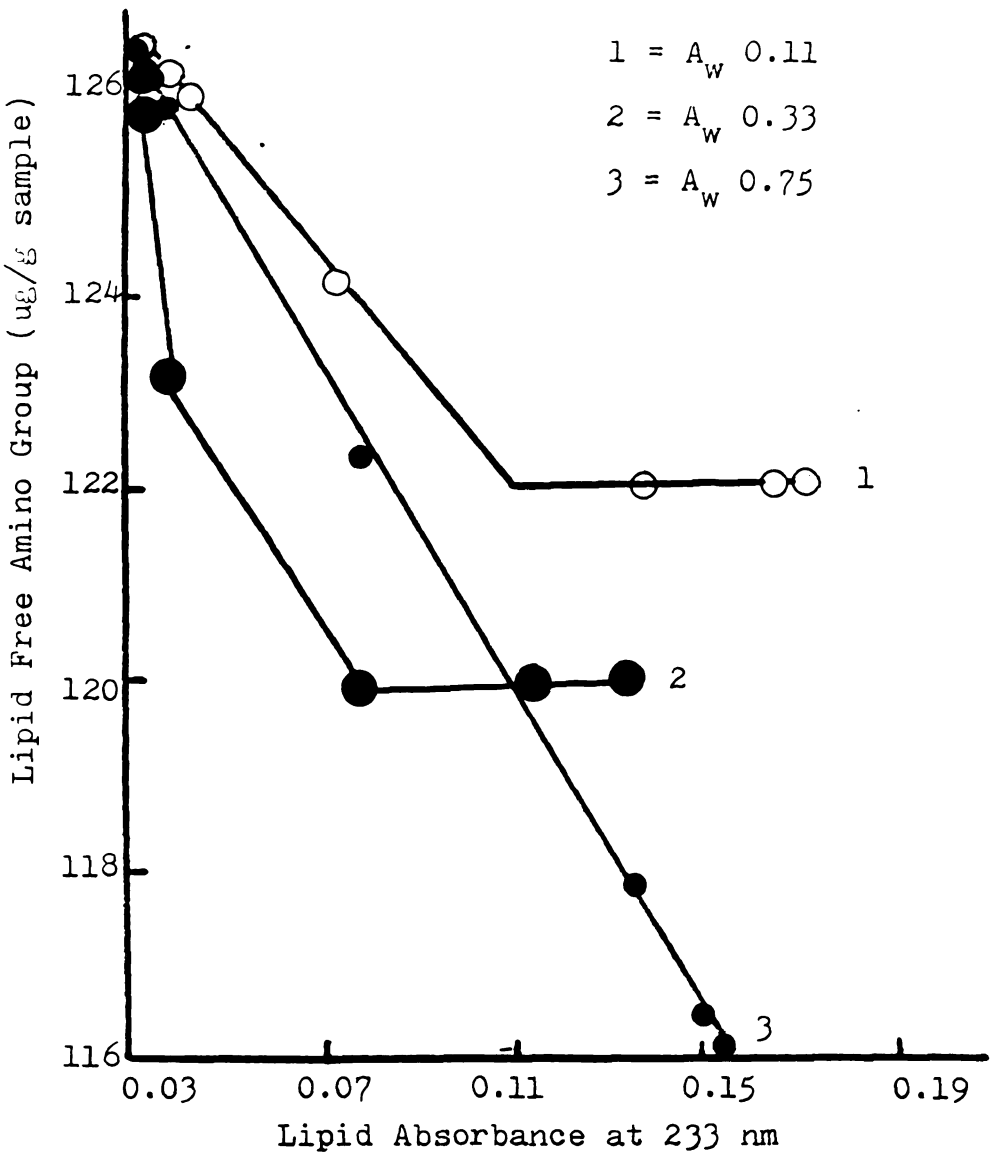


FIGURE 24 Effect of Lipid Oxidation on the Lipid Free Amino-Group Content of Raw Cowpea Powder Stored For Six Months at 25° C

carbonyl-containing compounds. The carbonyl groups may then have engaged in carbonyl-amine reactions with the lipid free-amino groups.

In the raw powders, the relationship was also essentially linear and the calculated correlation coefficients negative. However at A_w 0.11 and 0.33, the last three months were marked by a weak relationship between both parameters. It means that late in storage under the conditions indicated above, loss of lipid free-amino groups may occur by other mechanisms.

A mechanism may have been reactions or interactions between the amino group of the phospholipids and the carbonyl group of reducing sugar.

Another indication that other mechanisms are involved in the loss of lipid free-amino groups in these powders during storage is that their lipid free-amino group content, at the end of storage, did not correspond to that predictable from the multistage phenomenon in lipid oxidation depicted clearly in Figures 23 and 24. For example, one would have expected the cowpea powder with an A_w of 0.11 to have the lowest lipid free-amino group content if lipid oxidation was solely responsible for the loss of these groups during storage. The fact that increasing browning with increasing A_w was previously noted in this work suggests that the higher lipid free-amino group content observed in Figures 23 and 24 with increasing A_w was due to browning losses involving reactions between the free-amino groups of the cowpea phospholipid and the

carbonyl groups of carbonyl-containing compounds such as reducing sugars and lipid oxidation products.

Indeed, Figures 25 and 27 reveal that the higher the A_w the lower was the lipid free-amino group content of the cowpea powders at the end of storage. This was matched by decreasing "L" values (increasing browning) as the A_w was increased from 0.11 to 0.33 and to 0.75.

3. Relationship Between Lipid Free-Amino Group and Browning

Browning as estimated by "L" values were plotted against the lipid free-amino groups of the cowpea powders as shown in Figures 25-28. Decreases in "L" value (increased browning) correspond to decreases in the lipid free-amino group. It appears, therefore, that the loss of lipid free-amino group relates in part to carbonyl-amine reactions which lead to browning.

The extent of this relationship depended on the condition of storage. At 25°C/A_w 0.75, for example, there was virtually no observable relationship between the loss of lipid free-amino group and changes in the browning intensity in the raw powder throughout the six months of storage. While the lipid free-amino group was decreasing, the browning intensity remained virtually constant. However, it was shown earlier that after about three months in storage, lipid oxidation was negatively related to the lipid free-amino group content of the raw powder under the same conditions of storage. It is suggested, therefore, that the loss

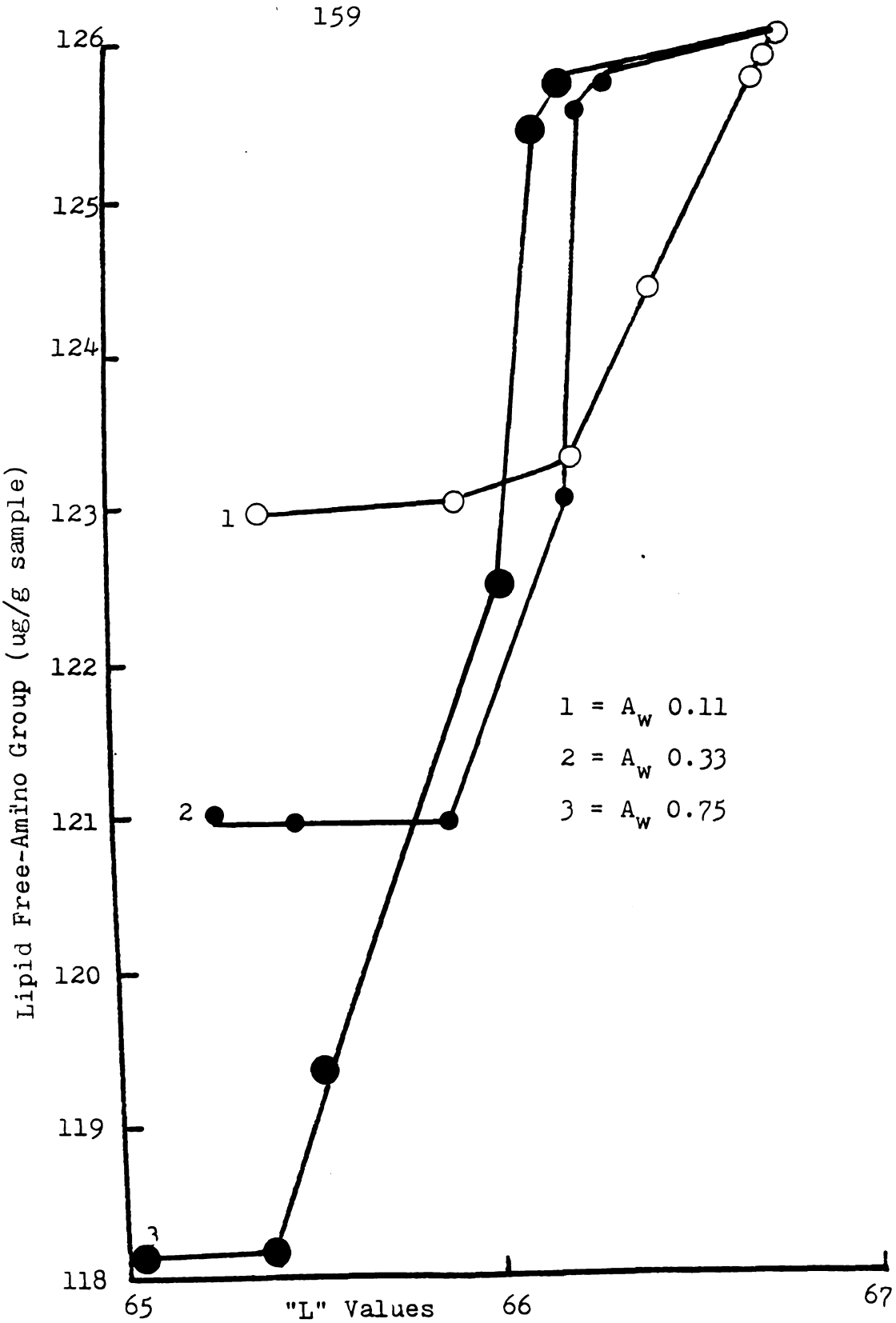


FIGURE 25 Effect of Browning on the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powder Stored For Six Months at 25° C

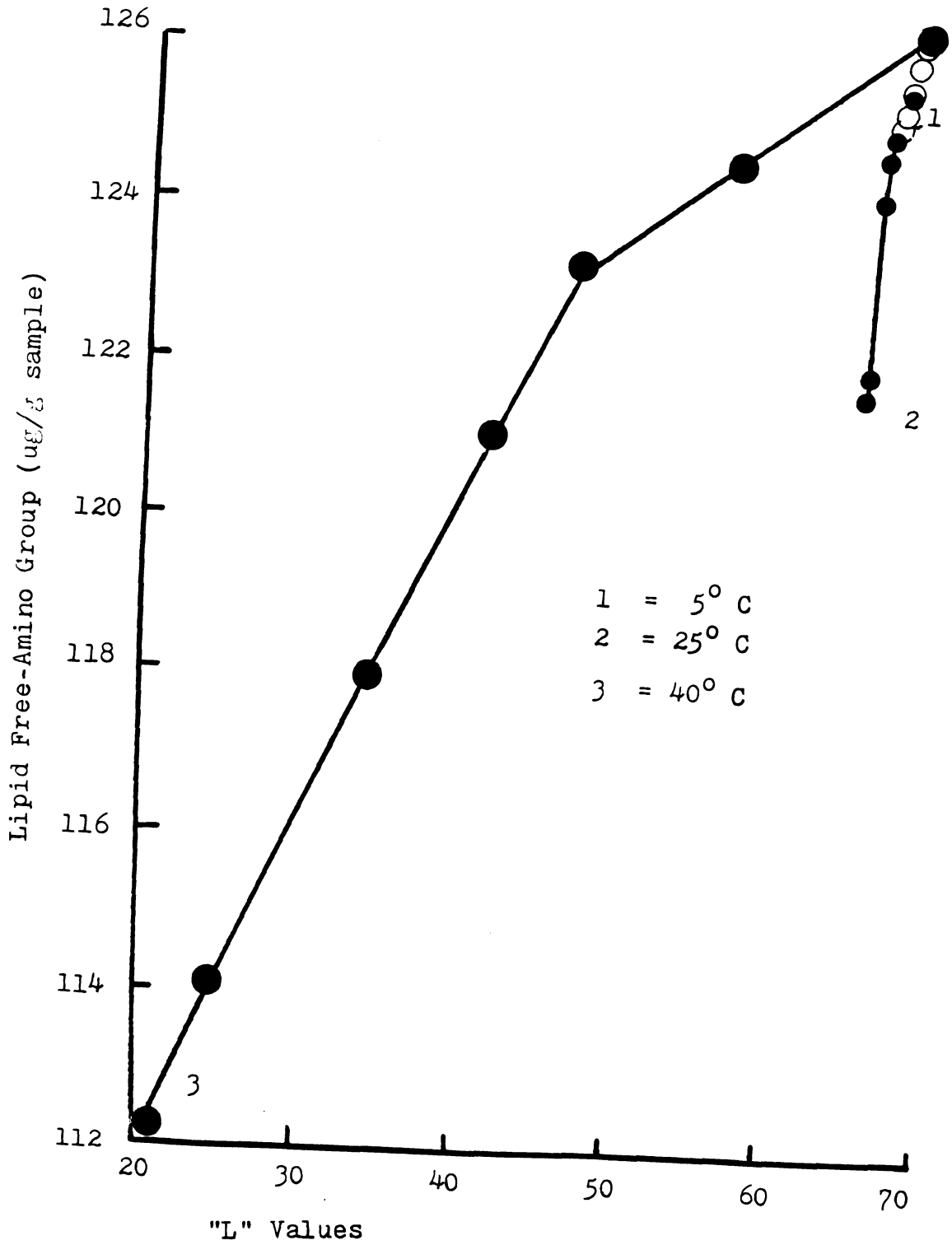


FIGURE 26 Effect of Browning on the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powder Stored For Six Months at A_w 0.75

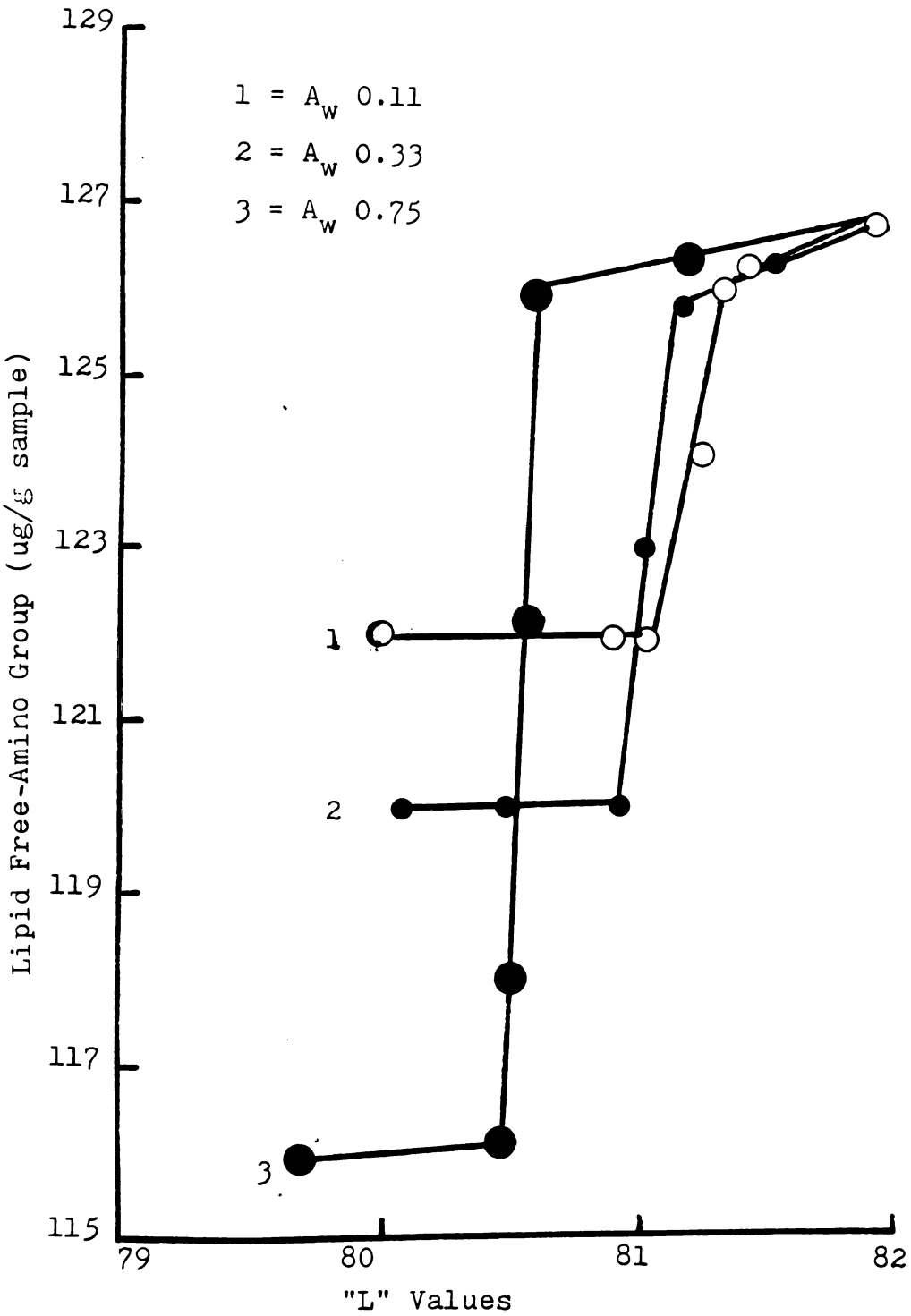


FIGURE 27 Effect of Browning on the Lipid Free-Amino Group Content of Raw Cowpea Powder Stored For Six Months at 25° C

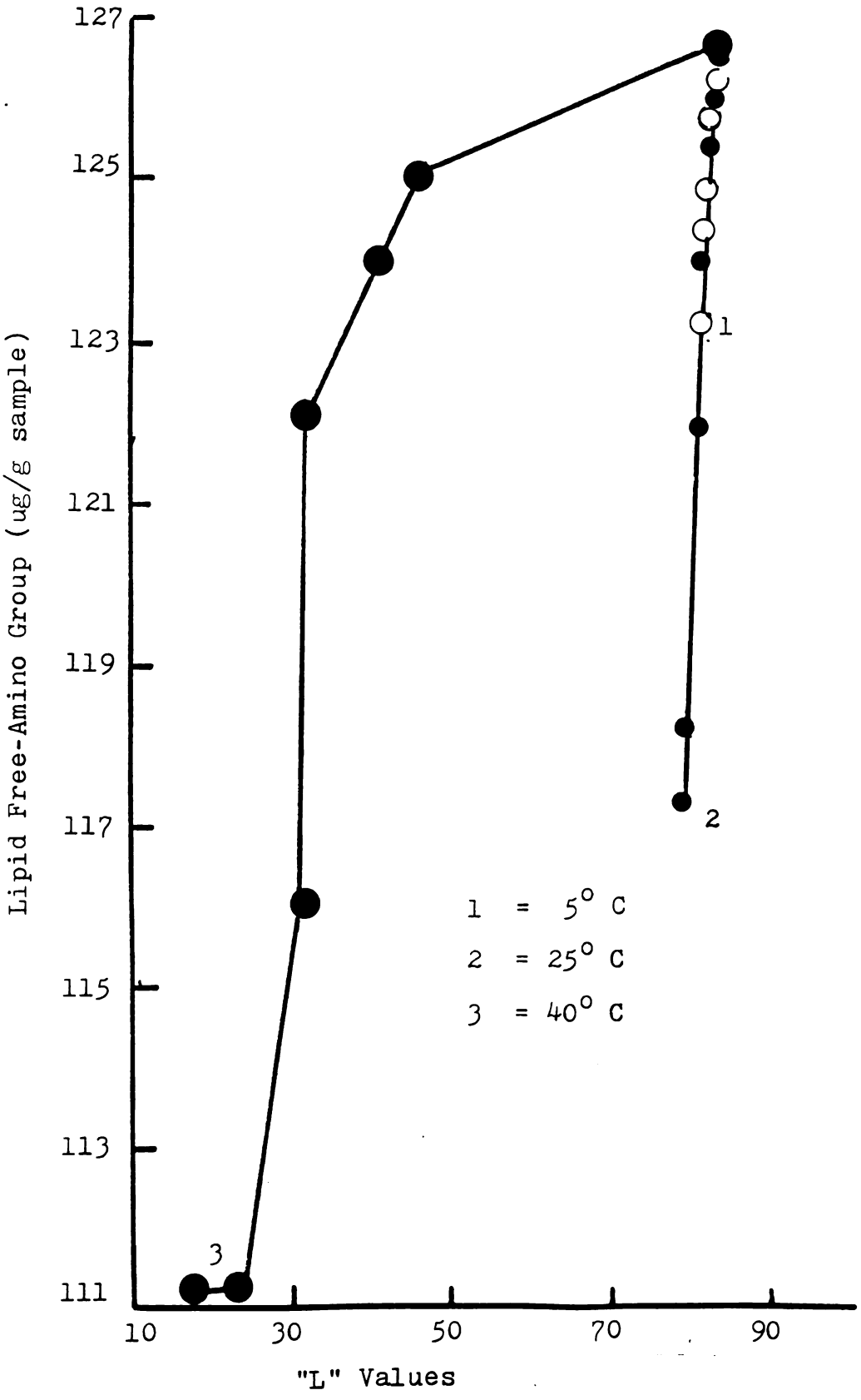


FIGURE 28 Effect of Browning on the Lipid Free-Amino Group Content of Raw Cowpea Powder Stored for Six Months At A_w 0.75

of the lipid free-amino group in the raw powders stored at 25° C and at A_w 0.75 from about the third to about the sixth month of storage was a function of lipid oxidation rather than of browning.

At 40° C, the losses of lipid free-amino groups followed decreases in "L" values (increased browning) during the first three months. This implies that browning may also lead to losses of lipid free-amino groups, although, after this period, the relation between both parameters was weaker. Data previously referred to reveal that during the first three months of storage at 40° C, lipid oxidation was also contributing to the losses of the lipid free-amino groups.

The same picture emerges when other figures relating browning to lipid free-amino groups and those relating oxidation to lipid free-amino groups, in the drum-dried powders are examined.

It is quite obvious, therefore, that a combination of the browning phenomenon and lipid oxidation account for the loss of the lipid free-amino groups in these powders during storage.

In fact, Acosta et al. (1966), Roubal (1967) and Mattsson and Swartling (1963) have all reported on the great susceptibility of phosphatidyl ethanolamine and phosphatidyl serine to oxidation. Malonaldehyde and other substances which can react with 2-thiobarbituric acid, produced during the oxidation of polyunsaturated fatty-acids can react with

amino-acids and proteins (Kwon et al., 1965). More specifically, according to Lea (1956) oxidation products such as aldehydes, epoxides and hydroperoxides could contribute to the loss of the free-amino group of phosphatidyl ethanolamine. Lea (1956) similarly noted specifically that the decrease in the free-amino group of phosophatidyl ethanolamine might result from its reaction with carbonyl-containing lipid oxidation products.

4. Relationship Between Lipid Free-Amino Groups and pH

As depicted graphically in Figures 29 and 30, decreases in lipid free-amino groups corresponded to decreases in the pH of the powders during storage. This is another indication that the free-amino groups of the cowpea lipids were involved in carbonyl-amine reactions as in browning, since losses of basic amino groups should lead to the lowering of pH. The slopes of the curves reflect the buffering capacity of the system.

Earlier, it was shown that losses of lipid free-amino groups related in part to browning and that increases in A_w led to increases in browning of the cowpea powders. Figures 29 and 30 show that higher A_w correspond to lower pH and lower lipid free-amino group contents of both the raw and drum-dried cowpea powders. The indication here is clear, therefore, that carbonyl-amine reactions between lipid free-amino groups and carbonyl groups of compounds such as those of lipid oxidation products and reducing sugars

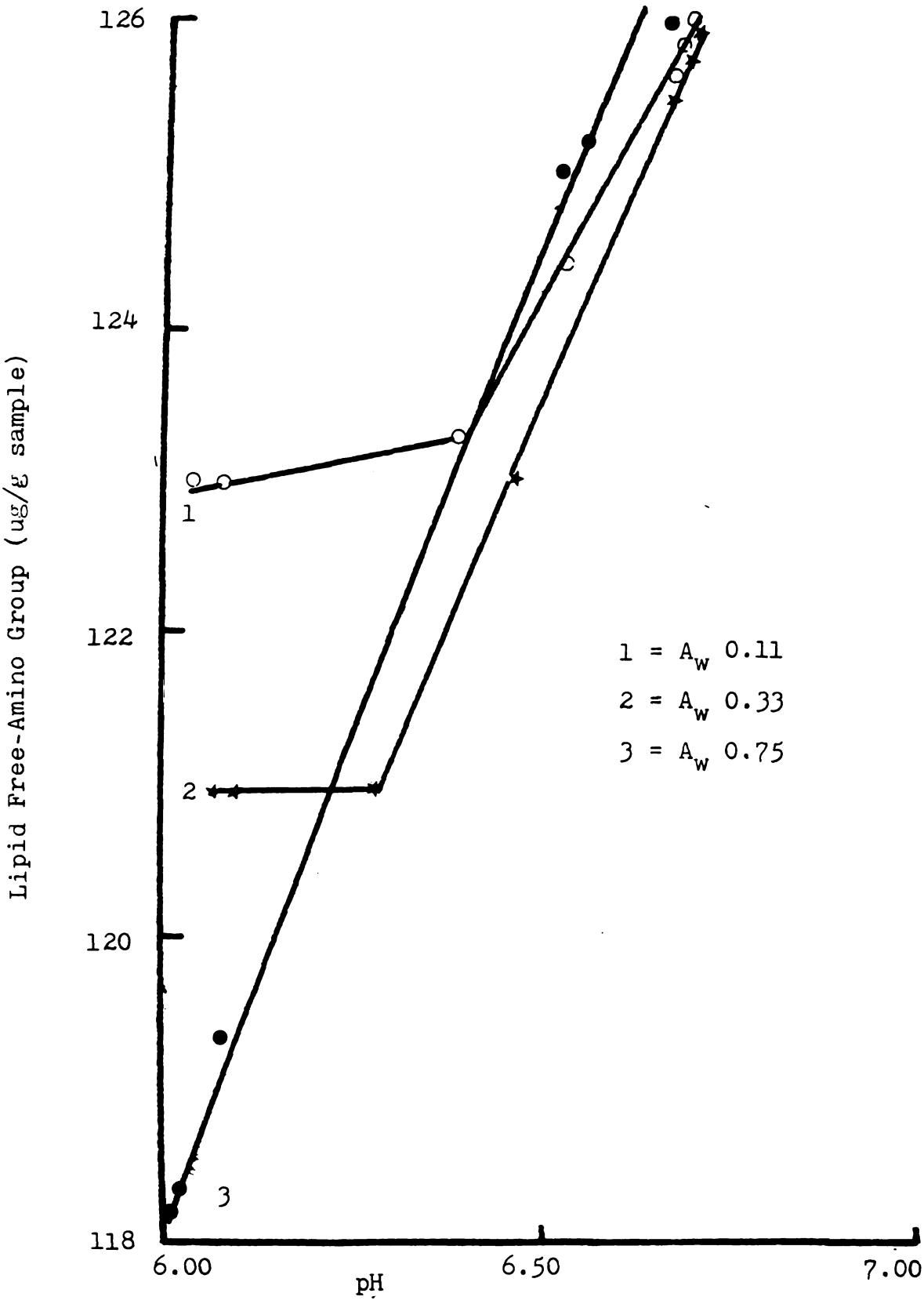


FIGURE 29 Relationship Between the pH and the Lipid Free-Amino Group Content of Drum-Dried Cowpea Powder Stored for Six Months at 25° C

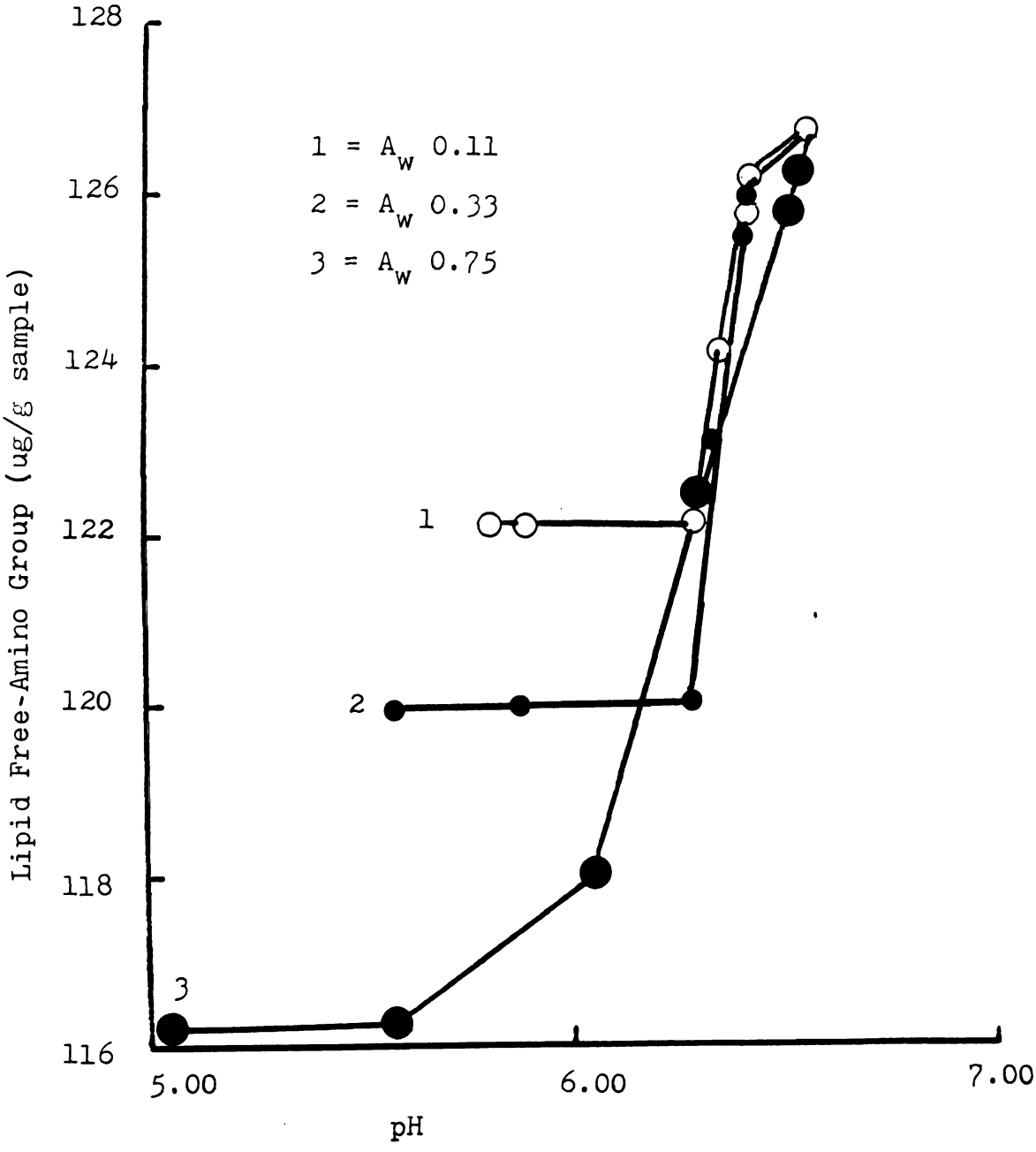


FIGURE 30 Relationship Between the pH and the Lipid Free-Amino Group Content of Raw Cowpea Powders Stored for Six Months at 25° C

referred to earlier, and which lead to browning, is one way by which lipid free-amino groups are lost. This loss then translates, in part, to lower pH values for these powders because of the decreased basicity and corresponding increased acidity.

Apparently, pH values were affected not only by the loss of the basic amino groups of lysine and lipid but by other interactions or reactions. The exact nature of these interactions or reactions is not clear from these studies. The N-terminal amino groups of proteins, peptides, glyco-proteins, lipo-proteins, organic acids produced in the course of lipid oxidation and cell metabolism could be involved in various types of reactions or interactions to influence the pH of these powders. Phenomena such as lipolysis and Strecker degradation have already been referred to as possible mechanisms leading to the lowering of pH.

5. Relationship Between the Available Lysine Content and the pH of Stored Powder.

Figure 31-34 depict the relationship between the available lysine content and the pH values of the stored cowpea powder. In the drum-dried powders, the low correlation coefficient between both parameters at 5° C is due to the already discussed inhibition of loss of available lysine at this temperature. In the drum-dried powder stored at 40° C/A_w 0.75, loss of available lysine did not lead to observable changes in the pH of these powders between the

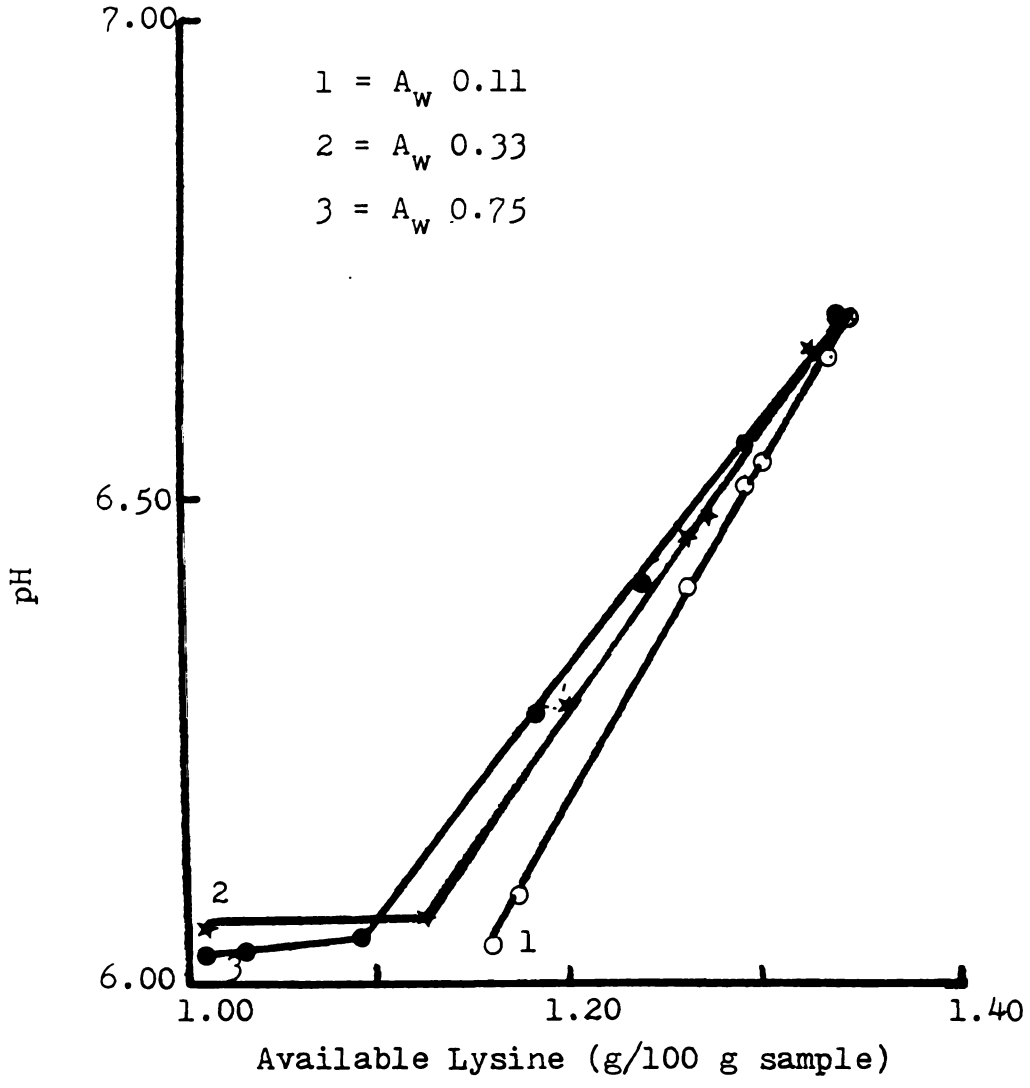


FIGURE 31 Relationship Between Available Lysine Content and pH of Drum-Dried Cowpea Powders Stored For Six Months at 25° C

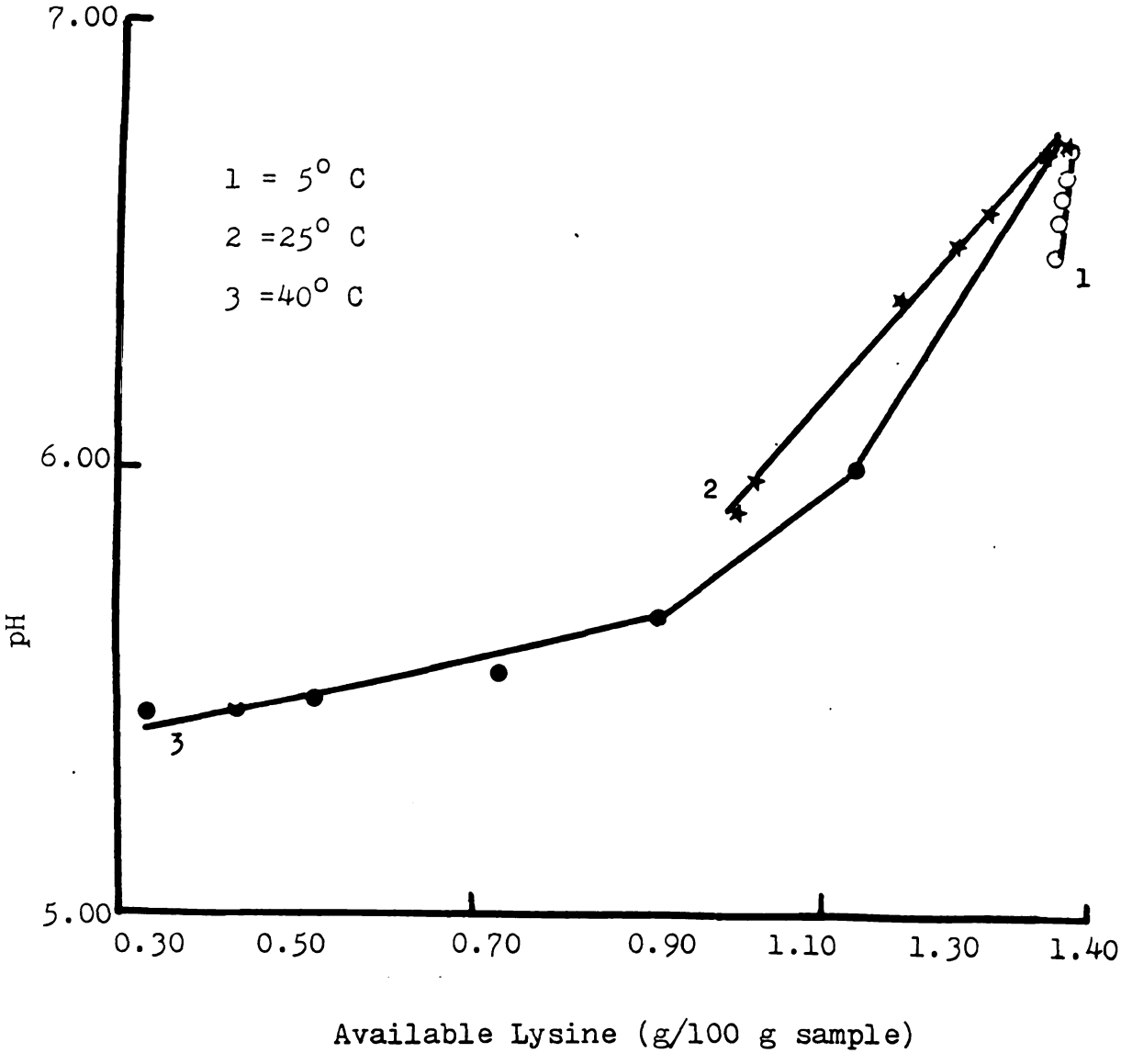


FIGURE 32 Relationship Between Available Lysine Content And pH of Drum-Dried Powder Stored for Six Months At A_w 0.75

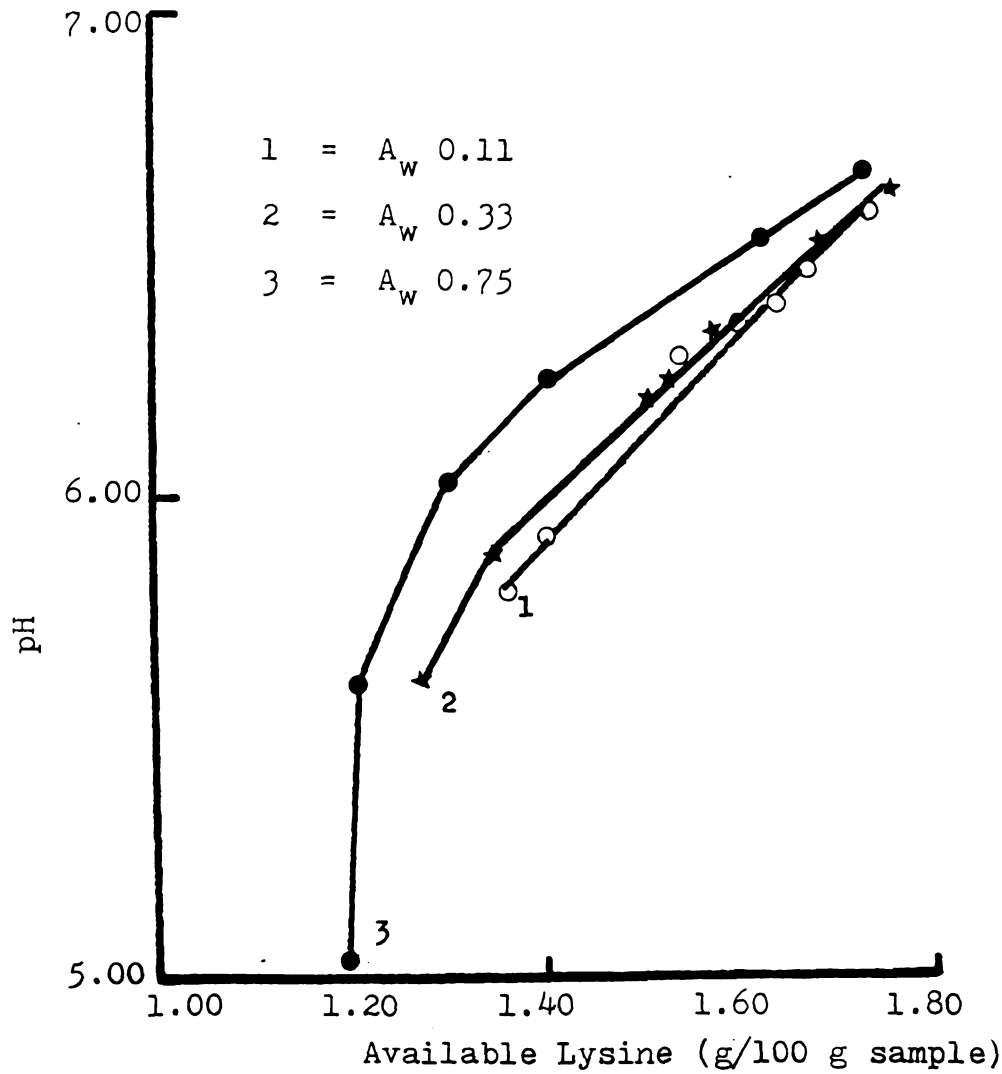


FIGURE 33 Relationship Between Available Lysine Content and pH of Raw Cowpea Powders Stored for Six Months at 25° C

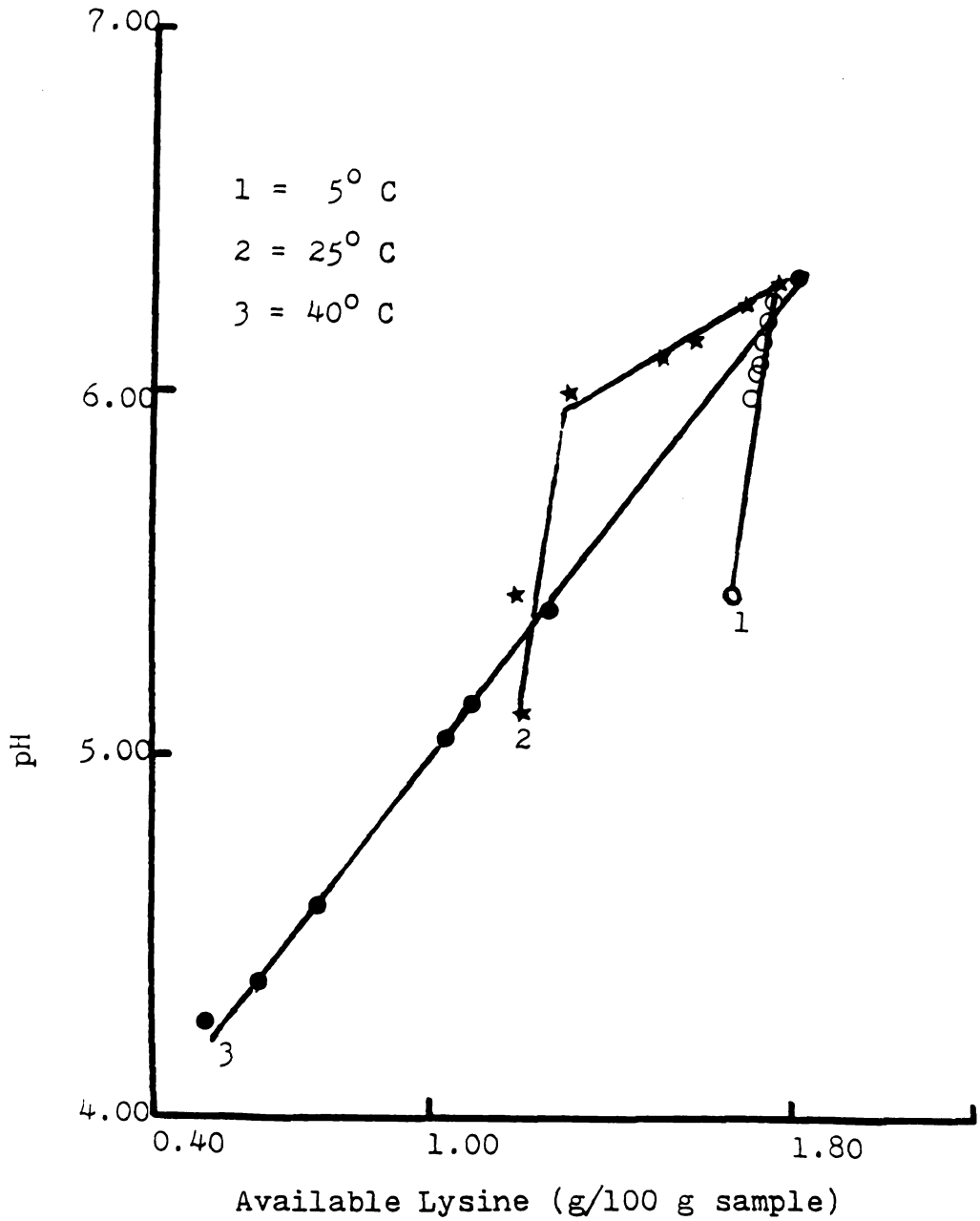


FIGURE 34 Relationship Between Available Lysine and pH of Raw Cowpea Powder Stored for Six Months at A_w 0.75

2nd and 6th month of storage. This relationship is difficult to explain. Apart from this apparent discrepancy, the relationship between the available lysine content and the pH of the cowpea powders in storage was as would be expected from the fact that losses of the basic amino groups of lysine in carbonyl-amine reactions would lead to the lowering of pH.

The nature of some of the curves with changing A_w's and temperature is also interesting. As shown in Figure 32, at 5° C a vertical curve signifying a weak relationship between the pH and the available lysine content of the drum-dried powder was obtained. At both 25° C and 40° C, however, the relationship was stronger as is obvious from the correlation coefficients shown in Table 26.

As depicted in Figure 33, an essentially linear relationship exists between both parameters in the raw powder stored at A_w 0.11 and at A_w 0.33. At an A_w of 0.75, however, while the first four months of storage gave an essentially linear relationship between both parameters, the last two months were characterized by a weak relationship between the pH and the available lysine content. This indicates that at this time in storage, decrease in pH occurred by other mechanisms such as by reaction between lipid free-amino group and reducing sugar and/or the products of lipid oxidation. Examination of Figures 29 and 30 indicates a role for the reactions involving lipid free-amino groups in causing pH decreases. In particular, Figure 29 shows that

for the drum-dried cowpea powder stored at an A_w of 0.75, the weak relation between available lysine content and pH during the last two months in storage could be explained by the loss of lipid free-amino groups in browning reactions as shown in Figures relating browning to lipid free-amino group content of the powder.

Figures 31 and 33 reveal that higher A_w corresponded to lower pH values. This in combination with the observation that high A_w corresponded to higher browning and that strong correlation exists between reducing sugar and available lysine (Table 26) and between lipid oxidation and browning leads to the conclusion that browning reactions account, at least partially, for the decreases in pH and available lysine content in these powders during storage.

Figure 34 shows the nature of the curves as a function of temperature. The weak relationship at 5° C has already been discussed here. At 25° C, the first four months were marked by an essentially linear relationship between the pH and the available lysine content of the raw powder. The last two months were characterized by a weak relationship probably because of the same reason already advanced. At 40° C, the relationship between both parameters is linear for the whole six months of storage.

6. Relationship Between the Reducing Sugar and the Available Lysine Content of the Cowpea Powder in Storage.

Figures 35 and 36 clearly show that essentially linear relation existed between the reducing sugar and the

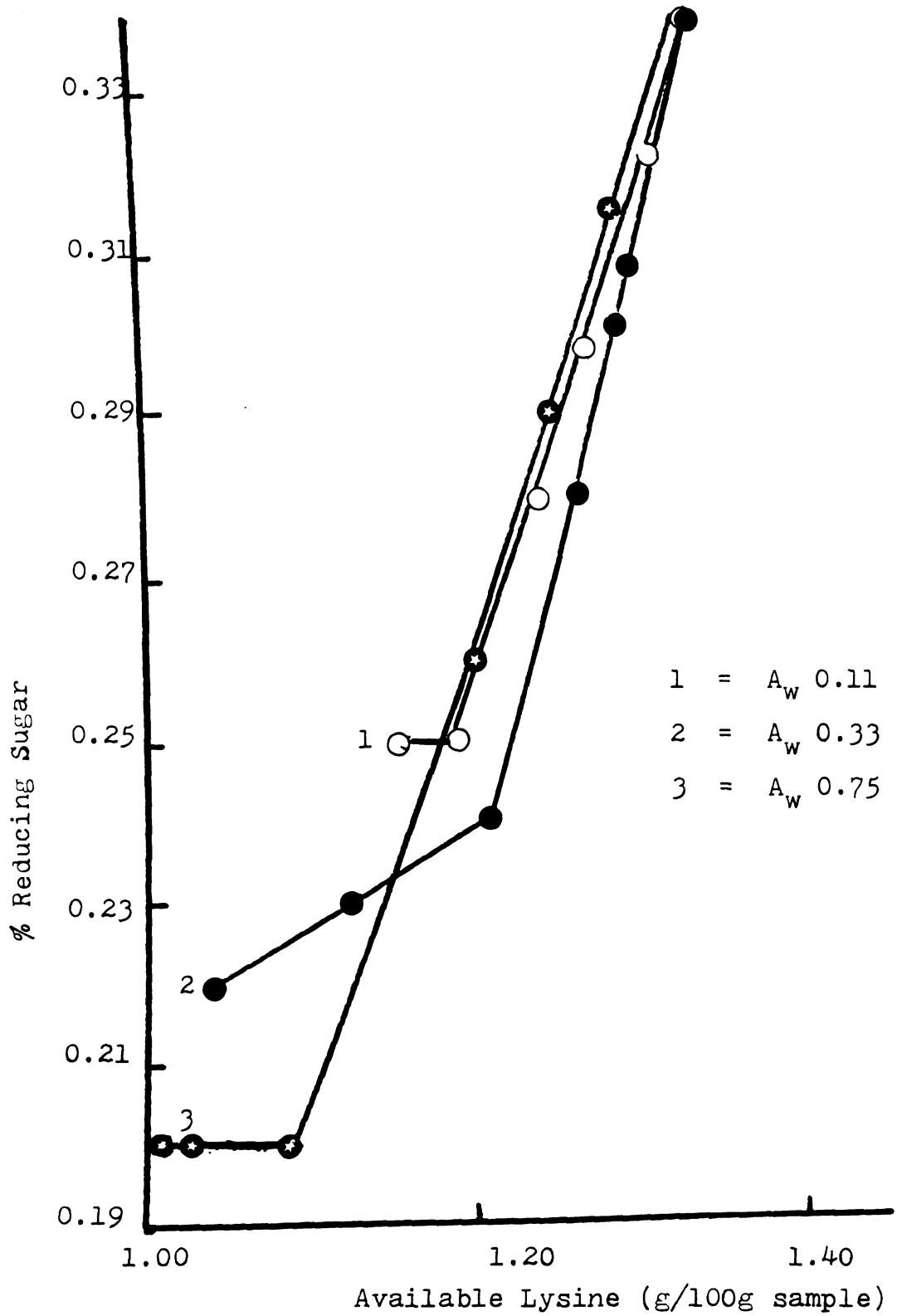


FIGURE 35 Relationship Between Available Lysine and Reducing Sugar Contents of Drum-Dried Cowpea Powder Stored for Six Months at 25° C

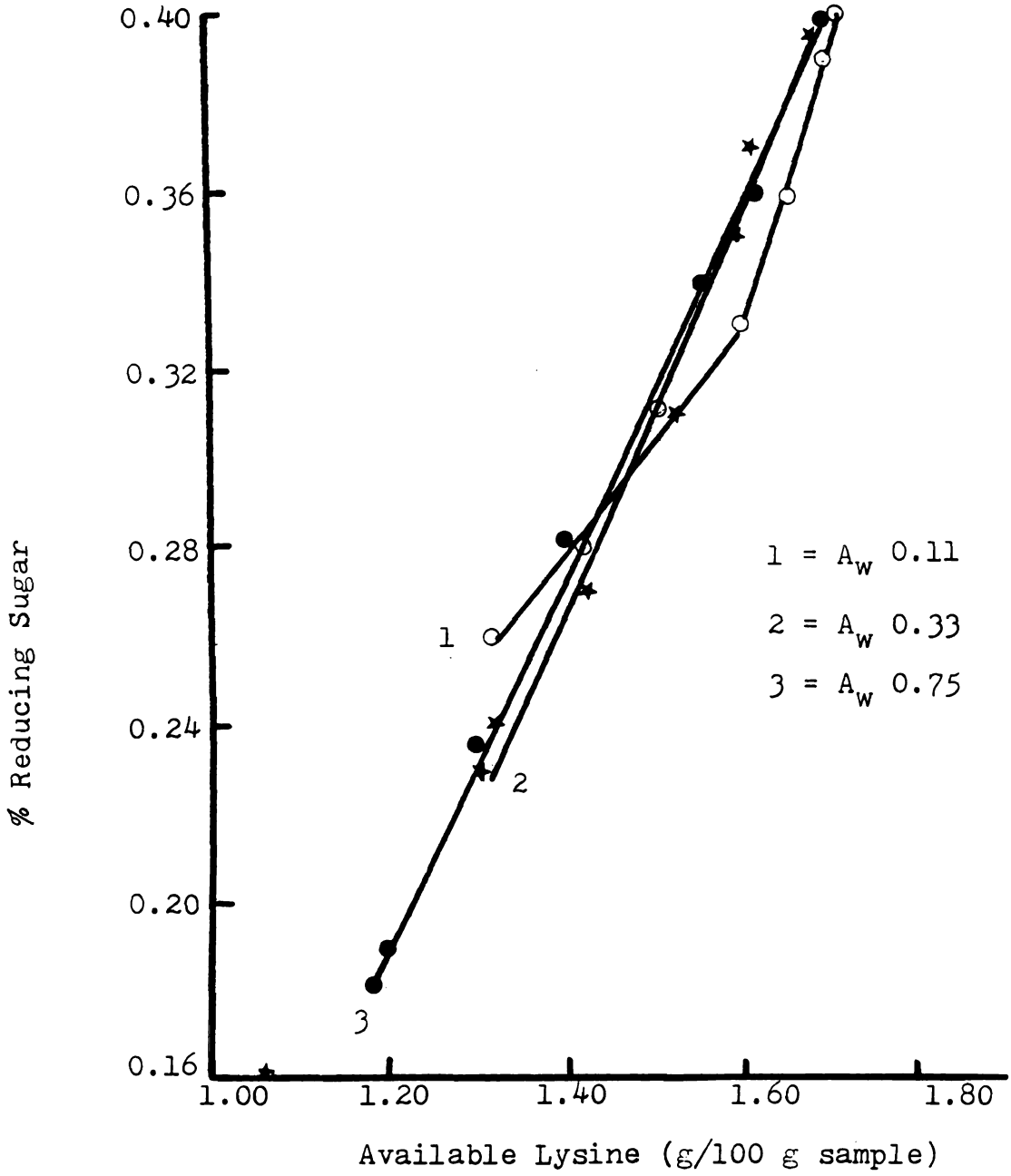


FIGURE 36 Relationship Between Available Lysine and Reducing Sugar Contents of Raw Cowpea Powder Stored for Six Months at 25° C

available lysine contents of the cowpea powders in storage. A positive correlation exists between both parameters as shown in Table 26 and implicates both in Maillard browning reactions. The literature in support of this has been referred to earlier in the discussion.

However, at A_w 0.75/25° C, and between the 4th and 6th month of storage, decreases in the available lysine content of the drum-dried powder was not matched by corresponding decreases in the reducing sugar content. Similarly, at 25° C/A_w 0.75 and between the 5th and 6th month of storage, decrease in the reducing sugar content of the drum-dried powder did not lead to any measurable change in the available lysine content. In the first case, reaction between the epsilon amino group of lysine and the carbonyl group of lipid oxidation product may have occurred. This is borne out by later observation that lipid oxidation showed a strong relationship with browning. In the second case, the positive correlation between browning and lipid free amino group implies that the loss of reducing sugar was by reaction with lipid free-amino group.

Another aspect of Figures 35 and 36 which shows that reducing sugar reaction with lysine is a dominant cause of lysine loss is the amounts of both constituents at the end of storage as influenced by the A_w. The higher the A_w the lower were both the available lysine and the reducing sugar contents of the powders. This observation is predictable from the fact that in the Maillard reaction

the epsilon amino group of lysine reacts with the carbonyl group of reducing sugar to produce a Schiff's base compound from which other products of the browning reaction such as the amadori compound and melanoidin pigments are produced successively.

7. Relationship Between the Available Lysine and Soluble Protein Contents

Except at 5^{o} C/A_w 0.75 the generally recorded positive correlation between the available lysine and the soluble protein contents of the cowpea powders during storage is predictable from the documented effect of Maillard browning and other carbonyl-amine reactions between proteins, peptides, amino acids and carbonyl containing compounds in lowering protein solubility and digestibility because of the formation of new and resistant linkages.

However, in the raw powder, the nature of the slopes of the curves in Figures 37-40 as the A_w was increased from 0.11 to 0.33 and to 0.75 indicate a weaker relationship between the available lysine and the soluble protein during the first three months in storage. Large decreases in the soluble protein content were accompanied by much smaller decreases in the available lysine content. This agrees with earlier suggestion that during the first three months, other processes other than non-enzymatic browning reaction between the epsilon amino group of lysine and carbonyl group may account for the loss of protein solubility in the raw

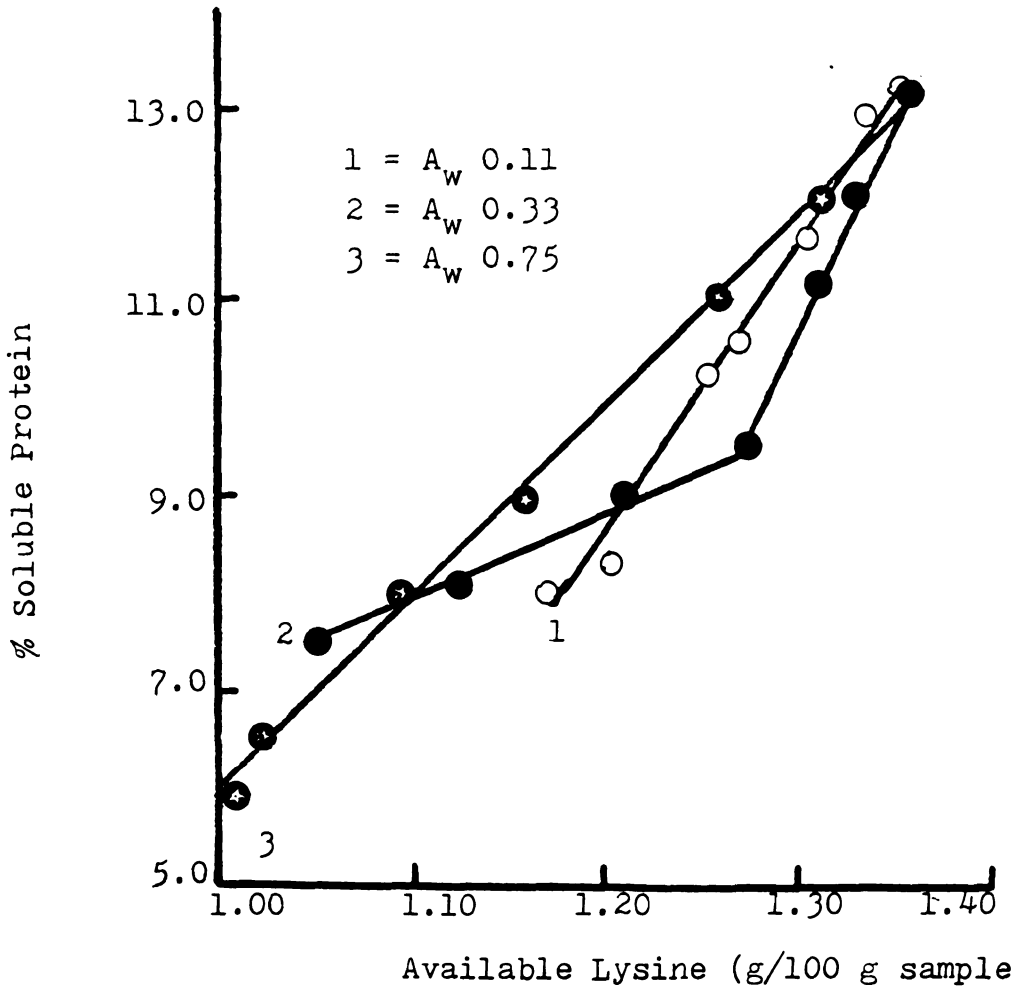


FIGURE 37 Relationship Between the Available Lysine and Soluble Protein Contents of Drum-Dried Cowpea Powders Stored for Six Months at 25° C

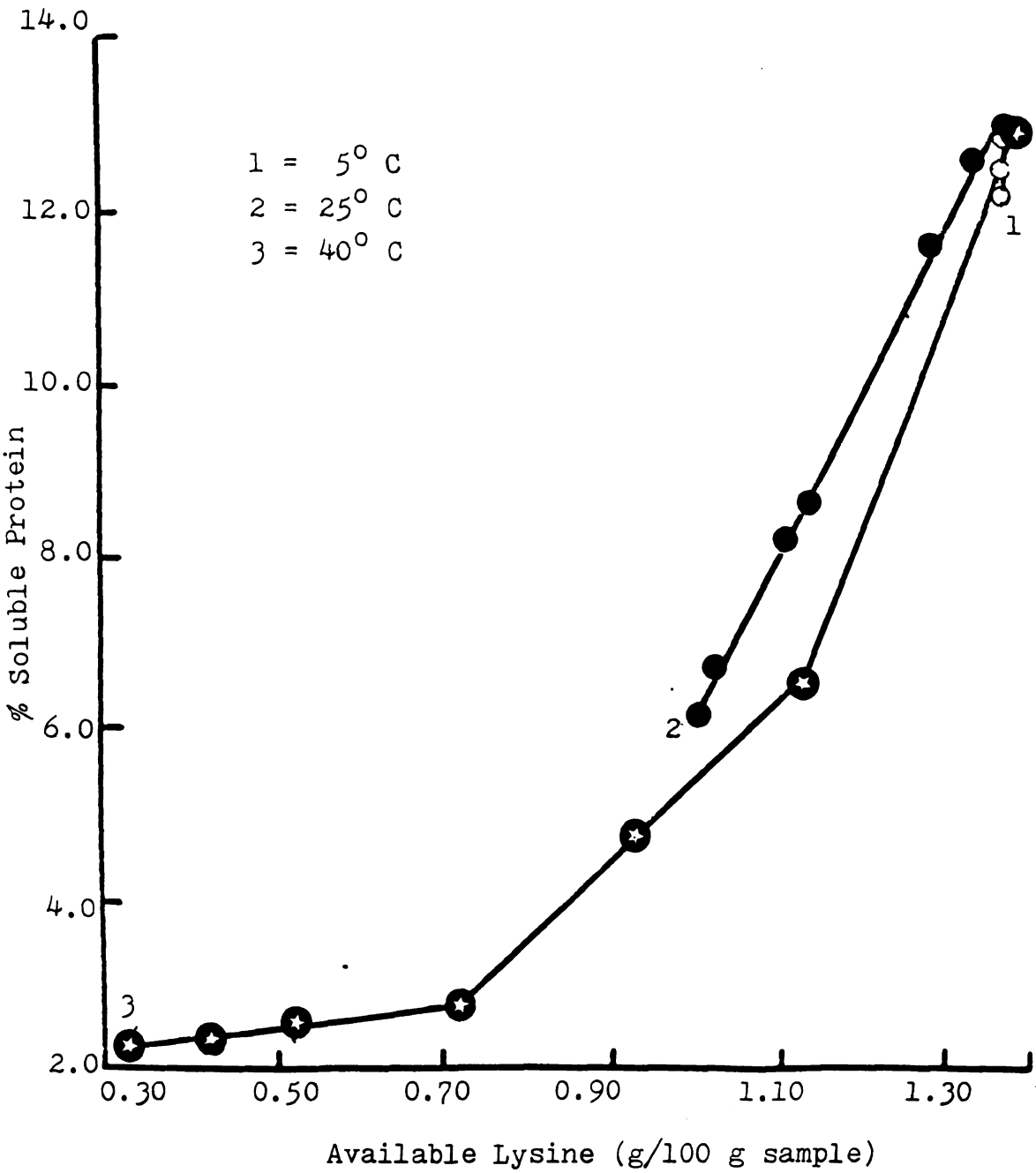


FIGURE 38 Relationship Between the Available Lysine and Soluble Protein Contents of Drum-Dried Cowpea Powders Stored for Six Months At A_w 0.75

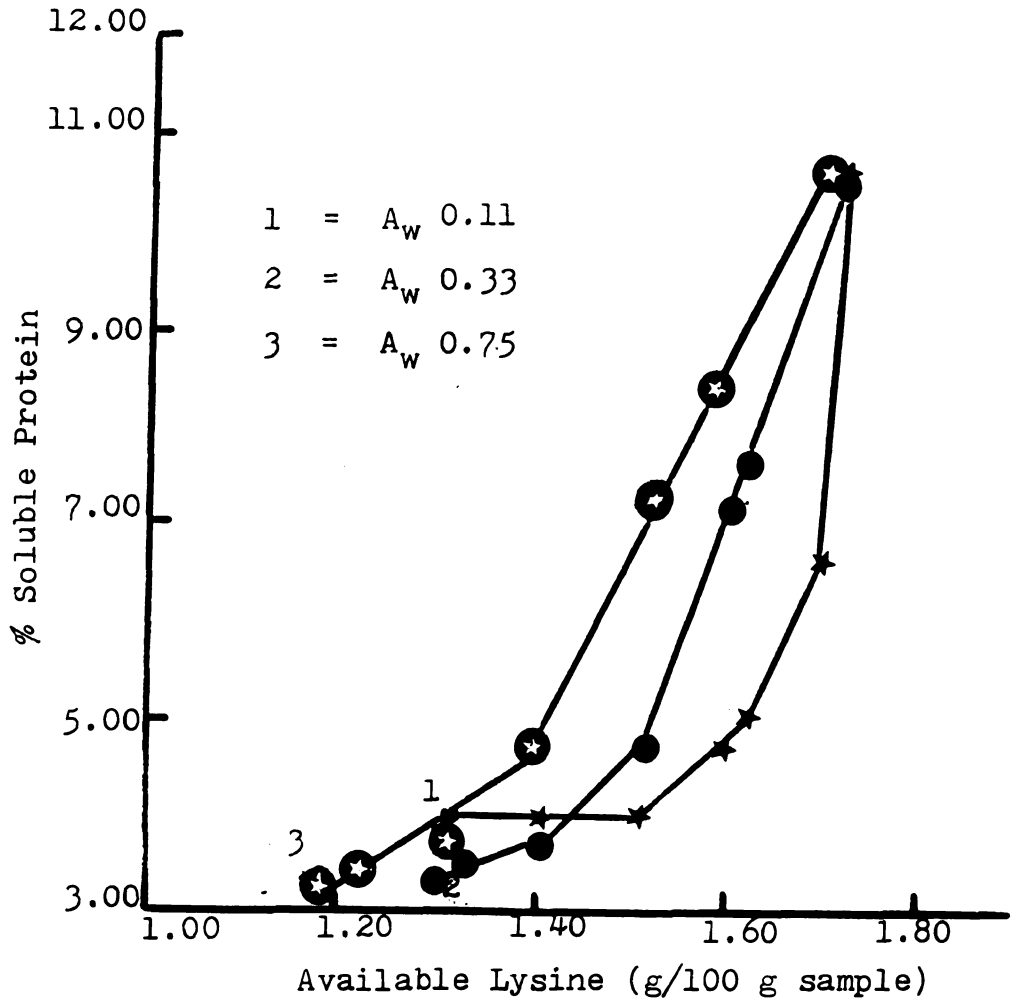


FIGURE 39 Relationship Between the Available Lysine and Soluble Protein Contents of Raw Cowpea Powders Stored for Six Months at 25° C

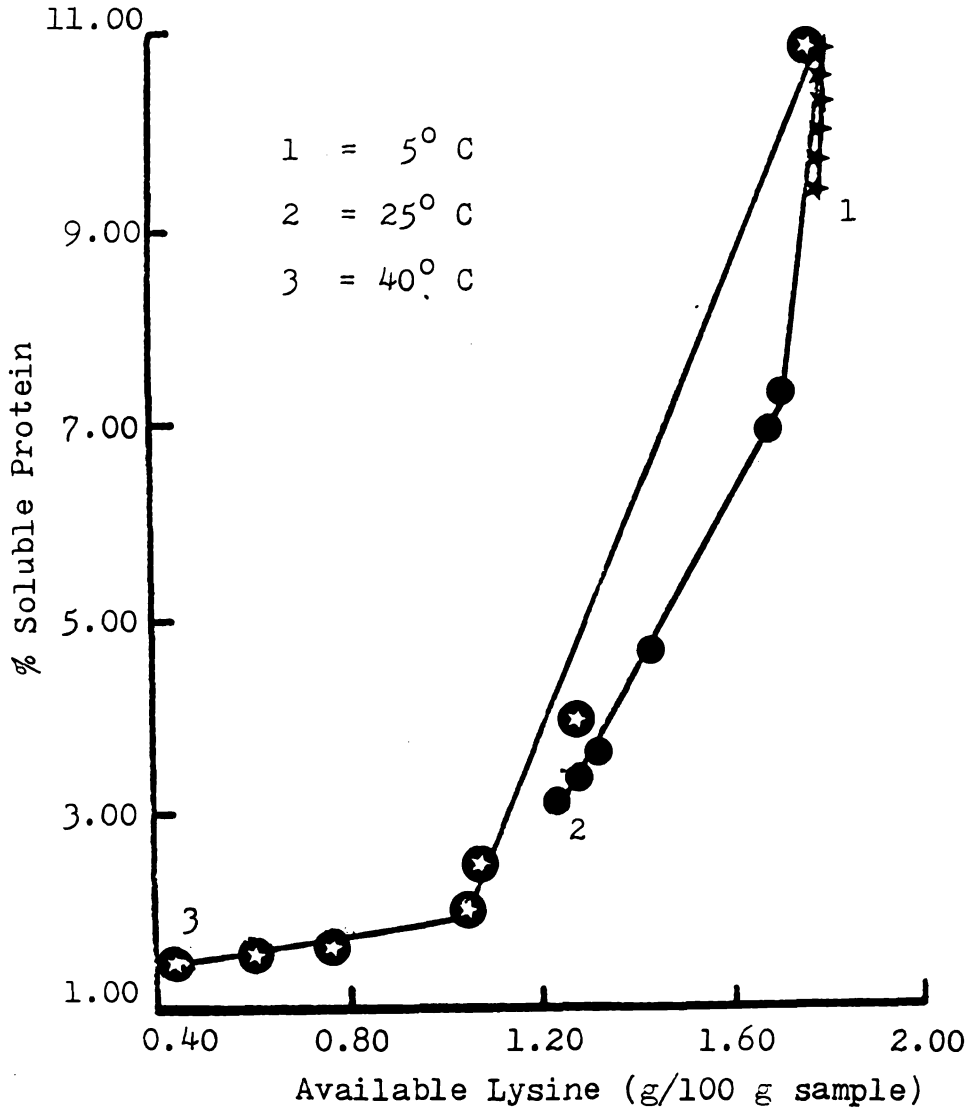


FIGURE 40 Relationship Between the Available Lysine and Soluble Protein Contents of Raw Cowpea Powders Stored for Six Months at A_w 0.75

powder. Proteolysis by enzymes and/or configurational changes which exposed more polar groups was suggested as the dominant mechanism.

The effect of increases in temperature in shifting the curves to the left as is apparent in Figures 38 and 40 is also noteworthy. This agrees with the stronger correlation between the available lysine and the soluble protein contents of the powders, as depicted in Table 26.

At 40° C, however, especially at the last months of storage, great changes in the lysine contents of the powders occurred without corresponding large losses of protein solubility. Obviously protein solubility in these powders is affected by other reactions apart from those involving lysine.

8. Relationship Between Lipid Oxidation and Browning

The nature of the relationship between lipid oxidation and browning of the cowpea powders as depicted in Figures 41-44 clearly indicates that as speculated earlier in the discussion, lipid oxidation products apparently can take part in the browning reactions. At certain periods of storage, the figures also show that lipid oxidation may not account for browning trends. In these cases, it is interesting to note the strong relationship that exists between reducing sugar and available lysine; whic provides indirect evidence for the involvement of reducing sugar,

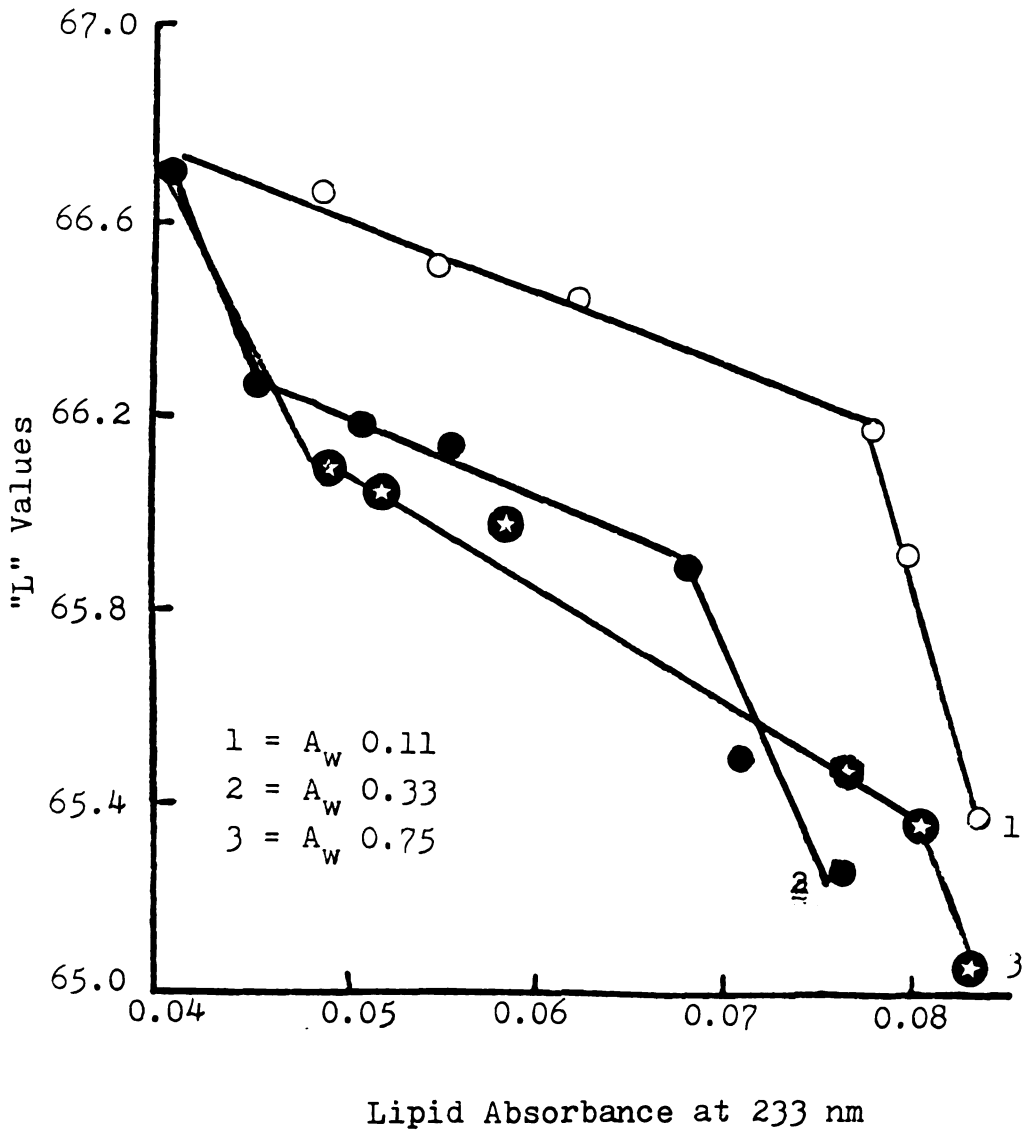


FIGURE 41 Relationship Between Diene Conjugation of Lipid and the Browning Intensity of Drum-Dried Cowpea Powders Stored for Six Months at 25° C

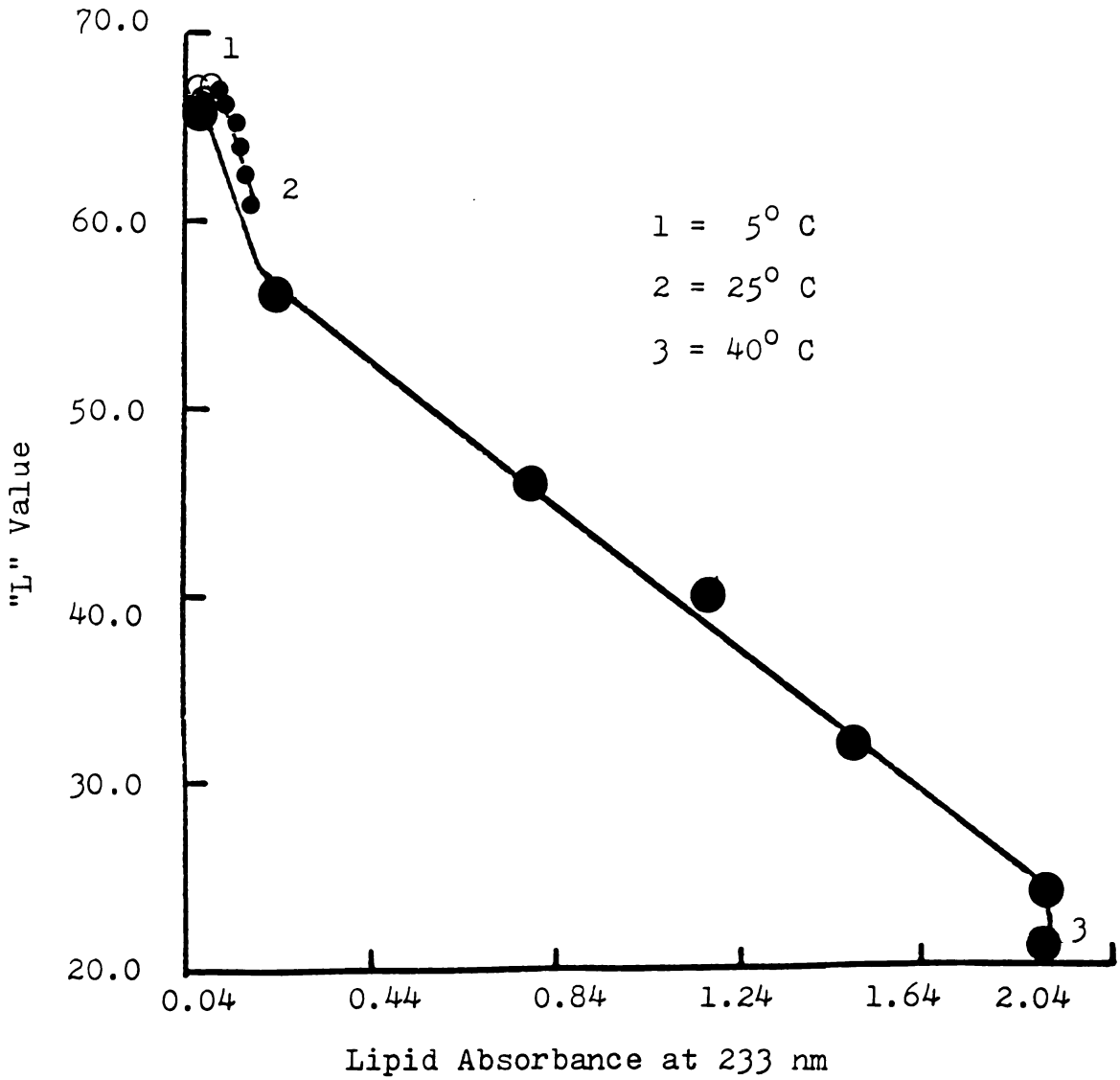


FIGURE 42 Relationship Between Diene Conjugation of Lipid and the Browning Intensity of Drum-Dried Cowpea Powder Stored for Six Months at A_w 0.75

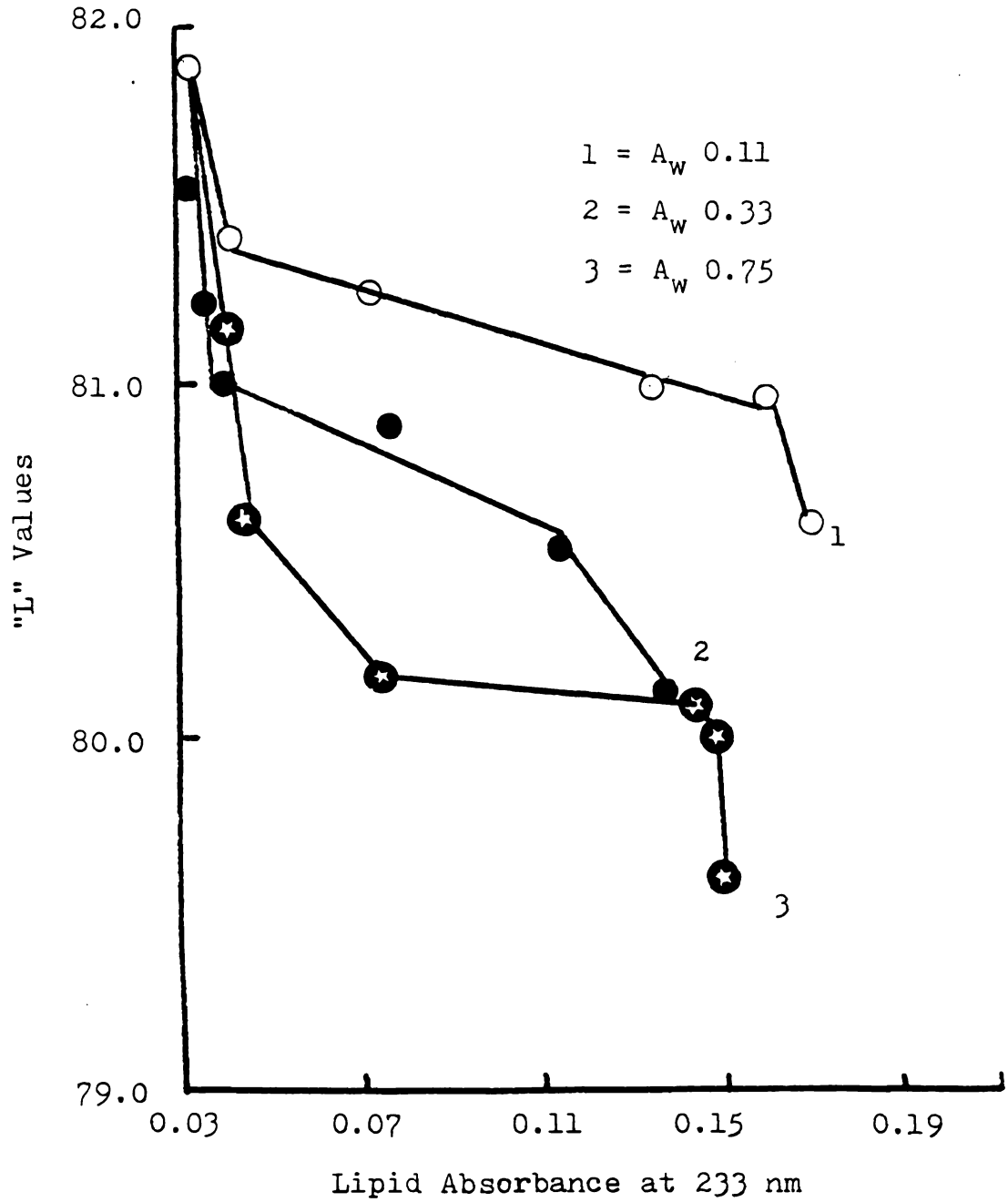


FIGURE 43 Relationship Between Diene Conjugation of Lipid and the Browning Intensity of Raw Cowpea Powder Stored for Six Months at 25° C

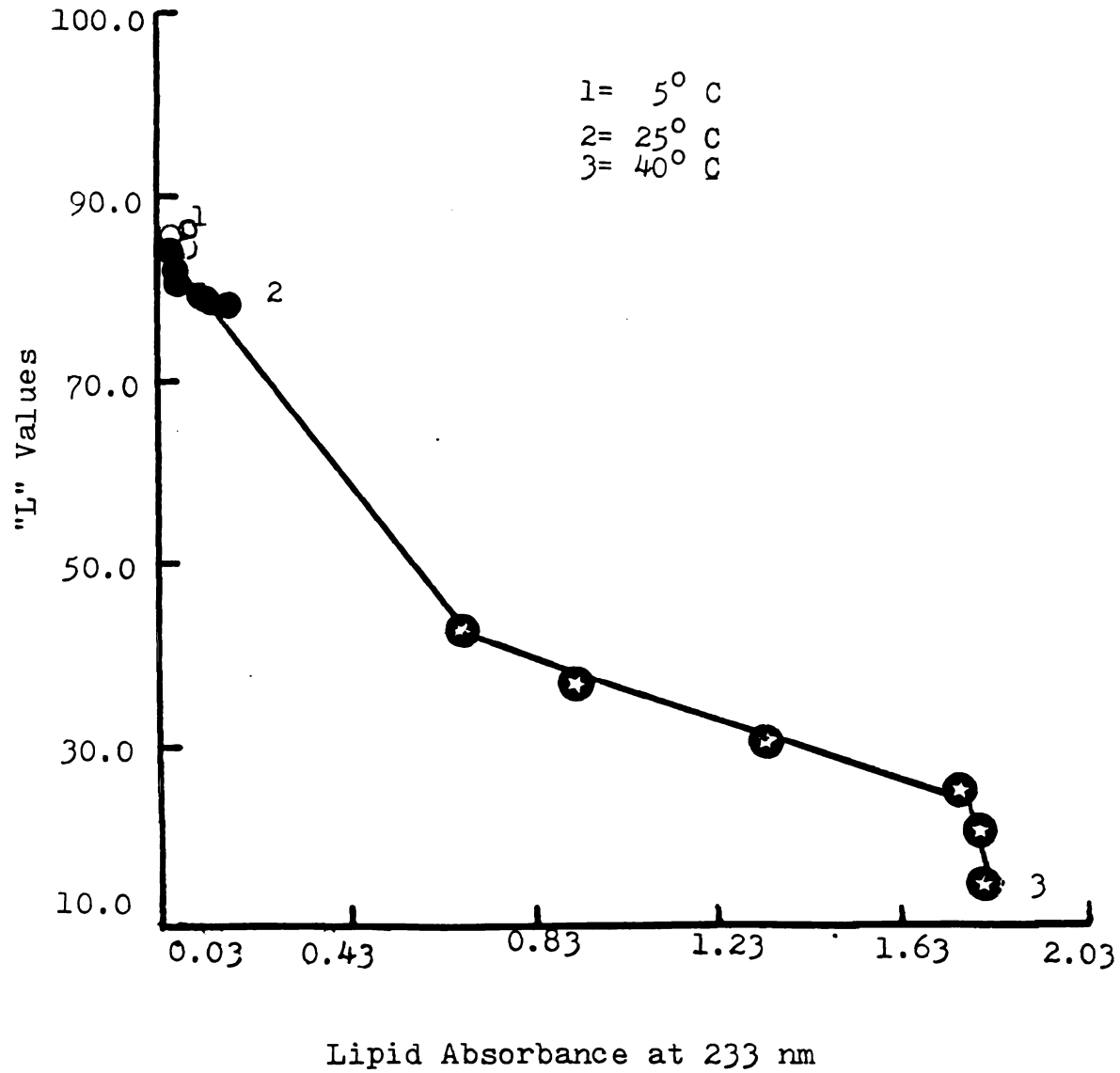


FIGURE 44 Relationship Between Diene Conjugation of Lipid and the Browning Intensity of Raw Cowpea Powder Stored for Six Months at A_w 0.75

rather than lipid oxidation products, in the browning reaction.

It is inferred, therefore, that both lipid oxidation products and reducing sugars may constitute the sources of the carbonyl groups needed for the browning phenomenon responsible for effects of pH, soluble protein, lipid free-amino groups and available lysine.

The effect of A_w on the nature of the curves in the drum-dried powder deserves mention. At A_ws of 0.11 and 0.75, the last three months are marked by virtually no lipid oxidation with large increases in browning intensities, in contrast to the observation that during the first three or four months, increased lipid oxidation led to increased browning. This means that A_ws of 0.11 and 0.75 promoted or allowed for lipid oxidation at the early months of storage and that the products of lipid oxidation took part in carbonyl-amine reactions which led to browning. Interestingly, at 0.33 A_w, the first few months in storage showed large losses in the whiteness of the powders which were not accompanied by similar increases in lipid oxidation. This indicates the ability of an A_w of 0.33 to inhibit lipid oxidation in these powders.

The similarity in this regard between the A_ws of 0.11 and 0.75 fits the multistage nature of lipid oxidation noted earlier in the discussion.

A similar protective effect of an A_w of 0.33 in minimizing lipid oxidation in the raw cowpea powder is seen in Figure 43.

As shown in Table 26, the effect of increasing temperature from 5° C to 25° C and to 40° C was to increase the correlation between browning and lipid oxidation in the raw and drum-dried cowpea powders during storage. This apparently is a reflection of increasing lipid oxidation and browning with increasing temperature.

Temperature is a greater factor in the relationship of lipid oxidation to browning than is A_w which is quite variable.

SUMMARY AND CONCLUSION

The objective in this project was to prepare cowpea powders by drum-drying and simple milling operations and study physico-chemical changes in the powders during six months' storage at water activities (A_w's) of 0.11, 0.33, and 0.75 with temperatures held constant at 25° C and at different temperatures of 5° C, 25° C and 40° C with A_w held constant at 0.75. The effects of the type of processing on their physico-chemical characteristics were also examined by analyses of the freshly prepared products the values of which also represented base values against which storage changes were evaluated.

Physico-chemical characteristics examined and the observed changes were:

1. Lipid Oxidation: This was assessed by absorbance at 233 nm due to conjugated diene in the system. A multistage trend in lipid oxidation was obtained with lipid oxidation decreasing when A_w was increased from 0.11 to 0.33 and increasing when A_w was increased from 0.33 to 0.75. The classical explanation of Labuza (1971) accounts for this trend.

With the raw samples showing the greater rate of oxidation, increases of temperature from 5° C to 25° C and to

40° C led to increases in lipid oxidation. The storage temperature of 5° C proved quite effective in mitigating oxidation in both powders.

2. Fatty-Acids: Gas liquid chromatography of the total lipid from the powders revealed palmitic acid to be the dominant fatty-acid in these products. The ratio of saturated to unsaturated fatty-acid (S/U ratio) was slightly below unity. The unsaturated fatty acids were oleic, linoleic and linolenic.

At all A_w's and at all temperatures except 5° C, the S/U ratio increased as the length of storage increased due to preferential loss of unsaturated fatty acids via oxidation.

The rate of loss for each fatty acid tended to be related to its level of unsaturation, with the more unsaturated acids having the greater losses.

Increase of A_w from 0.11 to 0.33 led to a decrease in the S/U ratio indicating a reduced loss of unsaturated fatty-acids by oxidation at A_w 0.33; but the rate tended to increase slightly when the A_w was increased from 0.33 to 0.75. This was in good agreement with the results obtained from oxidation measurements of diene conjugation absorbance at 233 nm.

Palmitic acid was the saturated fatty-acid which tended to increase more in a relative sense in storage as the unsaturated fatty-acids decreased. Other saturated

fatty acids—stearic and arachidic—showed much smaller increases.

3. Lipid Free-Amino Group: Measurement of lipid free-amino groups by the rapid spectrophotometric method of Siakotos (1967) showed that increases both in temperature and A_w led to lowering of lipid free-amino groups and that the rate of loss was higher for the raw samples than those which had been drum-dried.

4. Color Changes and Browning: Color changes were followed by measuring the "L" values ("whiteness values") on a Hunter Colorimeter and it was observed that loss of whiteness followed increases in A_w and temperature although at 5^{o} C there was virtually no loss of whiteness.

With the rate of browning and loss of whiteness higher again in the raw samples than in the drum-dried powders, it is apparent that while browning in the former may have included both enzymic and non-enzymic, browning in the latter probably was non-enzymic.

5. Reducing Sugar: Increases both in A_w and temperature meant higher losses of reducing sugars and as the length of storage time increased the loss of reducing sugars in the powders increased.

Probably because of respiratory losses and sugar-starch interconversions, in combination with Maillard browning losses, the raw powders registered higher reducing sugar losses than the drum-dried powders.

6. Available Lysine: The loss of available lysine, as estimated by the spectrophotometric method of Kakade and Liener (1969) and by the high performance liquid chromotography method of Peterson and Warthesen (1979), was as would be expected from carbonyl-amine reactions: higher losses with increasing A_w and temperature and with increasing length of storage. The effectiveness of 5° C in minimizing losses was again demonstrated.

7. Soluble Protein: Soluble protein was assessed by the Biuret procedure.

The soluble protein in the drum-dried powders decreased as the A_w and temperature were increased and thereby implicated the browning process.

In the raw samples, the effect of increasing A_w for the first three months was to increase the water soluble protein content probably because of hydrolytic actions of proteolytic enzymes and/or configurational changes which expose more polar groups. After this three month period, increases in A_w led to decreases in water soluble protein probably because Maillard browning became the dominant process.

Protein solubility was virtually unchanged during storage at 5° C.

8. pH: Changes in pH followed browning and the small changes in pH after a six month storage period indicate buffering capacity of the system. At 40° C,

however, there were more marked and noticeable decreases in pH than at other storage conditions.

The small decreases in pH observed in the powders may have been caused by a combination of loss of basic amino group in carbonyl-amine reactions, Strecker degradation phenomenon, especially, at 40° C, deamination phenomenon in the raw and lipolysis also in the raw.

9. Ascorbic Acid: There was no measurable ascorbic acid in either the raw or drum-dried cowpea powders.

10. Riboflavin: Losses of riboflavin occurred at 25° C and at 40° C at all A_w's as the storage time increased, probably by conversion to lumichrome, because of the prevailing pH condition in the powder. 5° C was, however, very effective in minimizing these losses.

Higher losses with increasing A_w were noted in the drum-dried powders probably because of greater solubility of the vitamin; but for the raw powders, higher vitamin values with higher A_w's were observed probably because of lower oxidation-linked destruction.

11. Thiamine: The trend was the same as described for riboflavin.

Relationship Between the Various Quality Parameters

Examination of the relationships between the various cowpea quality parameters indicated that:

a. lipid oxidation may account for some of the losses of riboflavin
b. the lipid free-amino groups may have been lost in storage as a result of both oxidation and carbonyl-amine reactions; as in browning
c. the decrease in the pH of the powders during storage may be partly accounted for by the loss of the basic amino groups of the lipid free-amino groups and the available lysine
d. the loss of reducing sugar and of the available lysine was at least partly due to Maillard browning reaction
e. the browning process was probably responsible for the loss of protein solubility.

Some Effects of Drum Drying

Some of the effects of the process of drum-drying, when compared to the milled powders (Table 27) were that there was slight oxidation, slight increase in pH, slight reduction in the percentage reducing sugar content, some increase in the soluble protein content, loss of whiteness, decreased available lysine content, about 25% loss of riboflavin, about 17% loss of thiamine, very slight loss of lipid free-amino group and some loss of unsaturated fatty-acid with corresponding increases in the percentage of saturated fatty-acids.

TABLE 27 BASE VALUES* FOR RAW AND DRUM-DRIED SAMPLES

PARAMETER	DRUM DRIED SAMPLES	RAW SAMPLE
Absorbance at 233 nm of lipid extract	0.041	0.030
pH	6.67	6.56
% Reducing Sugar	0.34	0.40
% Soluble Protein	13.08	10.83
Color by "L-value"	66.71	81.94
Available lysine g/100g sample	1.346 (1.207)*	1.762 (1.313)*
Riboflavin mg/100 g sample	0.122 (25.153% loss)	0.163
Thiamine mg/100 g sample	0.714 (17.265% loss)	0.863
Lipid free-amino group ug/g sample	126.03	126.72

*Obtained by HPLC procedure
*Values are the means of at least duplicate readings obtained immediately after processing.

Proximate Analyses: Results from the proximate analyses for both powders indicated that the products are high in protein content, low in total lipid, high in carbohydrate content and fair in crude fiber and ash.

The drum-drying process was able to reduce moisture content from about 10% to about 4%.

Some Physical Problems Observed:

Some of the physical problems observed in the course of storage of these powders included cakiness at A_w 0.75 at all temperatures. Cakiness appears to be determined more by A_w than by temperature.

Another was mold growth which occurred after five months of storage at A_w 0.75/25° C storage condition.

General:

These powders will keep well if they are stored under appropriate conditions. The drum-drying process does not appear to cause serious losses of nutrients immediately after processing. In storage, the process is even more beneficial. It will slow most of the deteriorative reactions in these powders which appear to center around browning and loss of vitamins, if they are stored under improper conditions.

The powder if stored at A_w, 0.11 and 5° C, will maintain most of its useful nutritional and physical

attributes. At an A_w of 0.11, the only problem might be lipid oxidation but this is only after two or three months in storage. The use of antioxidants should alleviate this problem.

Therefore, these powders represent one more potential useful convenience food item and should also be of importance in meeting the protein problem of the world.

Suggestions For Further Research

Every research undertaking raises new and interesting issues. This has been the case in this work.

Most of the explanations offered for the observations made in this work have been based on hard data; but some were largely speculative as is indeed true of science in its drive to uncover the truth.

It would be interesting to monitor changes in lipoxygenase activity in the raw powders to see if this can account for the higher rates of lipid oxidation observed in the raw powders when compared to the drum-dried powders.

It should also be of interest to isolate the phospholipids of cowpeas, characterize them and then make oxidation studies at the different A_w's and temperatures to see what actual role, if any, phospholipid oxidation plays in the loss of lipid free-amino groups which were reported in this work.

It was noted in this work also that for about the first three months increase of water-activity from 0.11 to 0.33 and to 0.75 resulted in higher water soluble protein contents of the powders. It was postulated that proteolytic enzymes and/or configurational changes which expose more polar sites may account for this. It would be useful to isolate or at least identify the enzyme involved if it is true that enzymes actually were involved. Once identified, experiments should be conducted to relate the activity of the enzyme to changes in A_w. Only then would it be possible to explain in precise terms what was responsible for the increased water soluble protein content in the first two or three months in the powders.

An interesting observation was made with regard to the thiamine and riboflavin contents of the powders as the A_w was increased. In the drum-dried powders, increases in A_w led to increases in loss of these vitamins, but the raw samples recorded lower losses with increasing A_w. Work should be conducted into the mechanisms of destruction of the vitamins in the cowpea powders. This would be a useful step in trying to prevent such losses.

Because of the recommendation that the cowpea powders be stabilized against lipid oxidation by using BHA, BHT and preferably ascorbic acid which is absent in these powders, research should be conducted into the effectiveness of these and other antioxidants in the cowpea powder systems. An antioxidant effective in one system might be

ineffective in another. Moreover, the optimum level of such antioxidants which protects these products without causing deleterious effects and which meet Federal standards needs to be worked out. The same would be true of the suggested antimycotic and anti-caking agents referred to in the discussion.

It might also be useful to investigate the effects of various types of packaging such as air, vacuum and storage conditions such as hypobaric on the shelf-life of these products. The effect of using light screening packaging materials would be particularly interesting research in terms of riboflavin content. The packaging method employed in this research (beaker exposed to light and atmosphere) is not the best for maintaining shelf-life of stored foods.

Finally, it should also be useful to prepare powders by other dehydration techniques such as spray drying, vacuum puff drying, freeze-drying, even sun drying and oven drying so as to be able to compare physico-chemical attributes which might influence their storage stability and marketing appeal.

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