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INVESTIGATIONS ON BIOCHEMICAL CHANGES DURING ACCELERATED AGEING OF GROUNDNUT (*Arachis hypogaea*, L.)

By

Kadam Balkrishna Sitaram

B. Sc. (Agri.) First Class

A Thesis submitted to the

MAHATMA PHULE AGRICULTURAL UNIVERSITY

RAHURI. DIST–Ahmednagar

(Maharashtra State, India)

In Partial Fulfilment of the requirements for the degree

of

Master of Science (Agriculture)

in

Biochemistry

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1990

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place : Rahuri

Dated : Feb 21, 1990

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CERTIFICATE

This is to certify that the thesis entitled "Investigations on Biochemical Changes During Accelerated Ageing of Groundnut (Arachis hypogaea L.)" submitted to the Mahatma Phule Agricultural University, Rahuri for the award of the degree of MASTER OF SCIENCE (Agriculture) in the discipline of Biochemistry, embodies the results of bonafide research carried out by Shri. B.S. Kadam under my guidance and supervision and that no part of the thesis has been submitted for any degree or diploma.

The assistance and help received during the course of these investigations have been duly acknowledged.

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CERTIFICATE

This is to certify that the thesis entitled "Investigations on Biochemical Changes During Accelerated Ageing of Groundnut (Arachis hypogaea, L.)" submitted to the Faculty of Agriculture, Mahatma Phule Agricultural University, Rahuri, District : Ahmednagar (Maharashtra) in the partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (Agriculture) in the discipline of Biochemistry embodies the results of a piece of bonafide research work carried out by Shri. B.S. Kadam, under the guidance and supervision of Dr. J.K. Chavan, Associate Professor of Biochemistry, Mahatma Phule Agricultural University, Rahuri. It is of sufficiently high standard to warrant it's submission to the University for the award of the said degree. No part of the thesis has been submitted for any other degree or diploma.

Rahuri:

Dated : 6.3.90

(N. K. Umrani)

ACKNOWLEDGEMENTS

I take this opportunity to express my profound debt of gratitude to Dr. J, K. Chavan, Research Guide, Chairman of Advisory Committee and Associate Professor of Biochemistry, Mahatma Phule Agricultural University, Rahuri, for suggesting this research problem, valuable guidance, constructive criticism, generous treatment, and keen interest shown by him throughout the period of this research and for his help in the preparation of the manuscript.

I wish to express my deep sense of gratitude to Dr. B.B. Desai, Head, Department of Biochemistry; Dr.S.S.Kadam, Professor and Head, Department of Food Science and Technology; Dr. R.N. Adsule, Associate Professor of Biochemistry and Dr. B.A. Chougule, Junior Wheat Analyst who are members of my advisory committee for their help and guidance.

My sincere thanks to my friends, Shri. S.B. Gangai, G.M. Dhumal, D.S. Nawadkar, H.B. Basate, P.M. Mangave, R.S. Jagtap for their help and cooperation during this study.

Words cannot express my gratitude to my parents who contributed to the completion of this study in their own way.

During the completion of this investigation, many hands pushed me forth and lit my knowledge and experience. I ever rest thankfully in debt to them all.

Place : Rahuri

Date : Feb 21, 1990

BSKadam

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ABSTRACT

INVESTIGATIONS ON BIOCHEMICAL CHANGES DURING ACCELERATED AGEING OF GROUNDNUT (*Arachis hypogaea*, L.)

By

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Rahuri

1990

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The present investigations were undertaken to study the influence of accelerated ageing of groundnut kernels on the changes in proteins, sugars and oil of groundnut kernels and to explore the possibility of using these data to predict the shelf-life of groundnut during long-term storage at ambient temperature. The groundnut kernels (Cv.JL-24 and SB-XI) of freshly harvested produce were dried at 45°C for 8 hours to about 5 percent moisture and stored in cloth bags at 25°, 35° and 45°C up to 60 days, in separate incubators. The samples were analysed after every 10 days for different chemical parameters. The results are compared with the data obtained in earlier experiment on long-term storage of groundnut at ambient temperature using same varieties grown at same locations.

The storage of groundnut kernels at 25°C up to 60 days did not cause any significant changes in various chemical parameters. However, storage of kernels at 35°C and 45°C caused a significant decrease in moisture content with an

Contd...

apparent increase in protein and oil contents. There was significant increase in free amino acids, acid value, peroxide value, saponification value, reducing sugars due to storage of kernels at higher temperatures. The results indicated that both hydrolytic and oxidative changes in storage proteins, oil and carbohydrates can be induced to occure at faster rate by holding the kernels at higher temperatures.

The values obtained for moisture, protein, oil free amino acids and acid value during accelerated ageing at 45^{o}C for 30 to 40 days are very close to the values obtained for these parameters from the kernels stored for 12 months at ambient temperature (Appendix I). These findings indicate that the shelf-life of groundnut kernels can be predicted by conducting a short duration experiment of accelerated ageing. Additional experiments are essential to standardise precise ageing conditions for each groundnut variety to predict its shelf-life during long-term storage.

Pages : 1 to 80

Introduction

1. INTRODUCTION

Groundnut (Arachis hypogaea L.) ranks first amongst the oilseed crops of India. It contributes to about 70 percent of the total oil seeds production of the nation. Other leading groundnut producers of the world include China and United States. The oil seed production of the world during 1986-87 was 160.2 million metric tonnes, out of which groundnut contributed 20.4 million metric tonnes (FAO, 1988). Among all the oil seed crops grown in India, groundnut alone claims the largest share in oilseed area (46%), and in edible oil production (59%). The area under groundnut crop in India during 1986-87 was 171.50 lakh hactarea with a production of 60.59 lakh tonnes (Anonymous, 1987). The area under groundnut in Maharashtra is 0.654 million hactares, with a production of 0.435 million tonnes (Anonymous, 1988).

Groundnut kernels contain 35.8-54.2 percent edible oil. (Hoffpauir, 1953; Cherry, 1977; Narashimhachar et al., 1985). Twenty three triacylglycerols have been identified and characterised in groundnut oil. The groundnut oil is an excellent source of unsaturated fatty acids (80%) of which 20-30 percent, are polyunsaturated (St.Angelo et al., 1979). The saturated fatty acids account for about 17-20 percent (Shitole, 1987).

Groundnut Kernels contain about 21-36 percent protein (Freeman et al., 1974). The nutritional composition and protein quality of groundnut have been studied by several workers. Groundnut seed proteins are high in acidic amino acids but are low in sulphur containing amino acids and lysine (Basha and Cherry, 1976).

The defatted edible groundnut flour contains about 38 percent total carbohydrate (Tharanathan et al., 1975). Ayres et al., (1984) reported that raffinose and stachyose are low in groundnut kernels. Sucrose is a dominant sugar in groundnut (kuo et al., 1988). The groundnut kernels are fairly good source of certain minerals. The total mineral content of groundnut ranges from 1.8 to 3.1 percent (Freeman et al., 1954). Derise et al. (1974) have reported contents of nine minerals viz. Ca, Mg, P, K, Na, Fe, Cu, Zn, Mn, in the groundnut kernels. Groundnut is a good source of some vitamines like vitamin B, vitamin E, and vitamin K (Hoffpauir, 1953). However, nicotinic acid is predominantly concentrated in groundnut varieties (Rao and Rao, 1981).

Groundnut shows a good potential as a source of food grade protein for human diet (Altschul, 1965; Woodroof, 1983). Numerous processes have been devised for the preparation of protein concentrates and protein isolates from raw groundnut kernels (Rhee et al., 1972; Rhee et al., 1973; Cater et al., 1974). Crisp and Snack type of foods are prepared from defatted groundnut flour itself or by blending with wheat flour and degermed corn meal (Ayres et al., 1974 . McWatters and Cherry, 1980). Various exatraction methods are tested for recovery of oil from deskined groundnut kernels which, after roasting are used in snack type foods (Belani and Pai, 1983). In India, toned and double tonned milk is being prepared by mixing reconstituted skim milk solids with buffalo and cow milk (Chandrashekhara et al., 1971). Fungal fermentation of groundnut flour increase it's nutritive value which can be used in different type of foods (Quinn et al., 1975). Beuchat and Nail (1978) prepared fermented groundnut milk which can be substituted for butter milk. It can be blended, flavoured and consumed as a beverage. Low fat groundnut kernels are being prepared by moistening removing of oil (20-60%) and roasting until it develop colour, flavour and uniform moisture (Wilkins and Gannis, 1984). Direct interesterification of groundnut oil using sodium alkoxide as a catalyst is idealy suited as salad oil for heart patients (Shitole, 1987). Thus, groundnut kernels, oil and defatted meal have enormous applications in food processing industry. To obtain a product of good nutritional and sensory properties, the quality of kernels need to be high. The kernels stored for considerable time before processing may undergo undersirable changes.

The chemical composition of the seeds affect the quality of oil and processed food products. During storage, the composition with respect to nature of oil, proteins and carbohydrates may undergo remarkable changes depending upon the moisture content, conditions and length of storage. The high amount of oil in groundnut kernels, moisture content and storage temperatures are responsible for changes occuring in groundnuts during storage . Raw shelled groundnuts require more careful and controlled storage than the inshell groundnut to retain their edible quality (Woodroof, 1983). Shelled, roasted and salted groundnuts require adequate package protection as the storage life of such groundnuts is about 3 weeks at room temperature (Sheufelt and Young,1977). Groundnut kernels containing about 8-9 percent moisture during storage allowed hydrolysis of complex compounds like protein and starch and increases in free sugars and free amino acids. There was also increase in total lipid content but iodine values were not changed (Pattee et al., 1982a). Free amino acids were found to be increased except phenylalanine in all seed sizes during cold storage of 6-7 months (Pattee et al., 1981b). Storage of groundnut pods and kernels in different containers under ambient conditions resulted in decrease in protein and oil content and increase in fatty acid content with storage time (Ramamoorthy and Karivarathraj, 1986).

In India, groundnuts are usually stored as inshell or shelled nuts over a period of 12 to 24 months in variety of containers and storage atmosphere. The storage conditions include container, temperature, relative humidity and access to pests, molds and rodents. A complex deteriorative chemical changes ih proteins, oil and carbohydrates of kernels occur under favourable conditions of high kernel moisture, storage temperature and relative humidity. In India, the temperature ranges between 10°C in winter to 45°C in summer in different parts of the country. Thus, large temperature variations can favour the varying detrimental changes in stored nuts. Secondly, to assess storage effect at ambient temperature, one has to store the material for 12 to 24 months. The information on the chemical changes in groundnut kernels during accelerated ageing is limited. The studies on accelarated ageing of kernels may help to know the possible undersirable changes that the stored kernels may undergo during long-term storage.

The possible deterioration that might occur after 12 months at 25°C, could be known within few days by holding the kernels at 35 or 45°C, artificially. Hence, the present investigations were planned to store the groundnut kernels at elevated temperatures up to 60 days and monitor the changes in storage proteins, oil and sugars.

Objectives

1. To investigate the changes in moisture, oil, proteins and sugar in groundnut kernels during accelerated ageing.
2. To predict the shelf-life of groundnut kernels.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Chemical composition

Groundnut is one of the most concentrated foods because of it's high oil and protein contents (Hoffpauir, 1953). Whole groundnut pod consists of about 20 to 26 percent shells and 74 to 80 percent kernels which include cotyledons (about 72%), skin (4%) and embryo (3%). The groundnut seed contains approximately 6 percent moisture, 50 percent oil, 25 percent protein, 15 percent carbohydrates, 2 percent crude fibre and 2 percent ash. It also contains an appreciable amount of B-group vitamins. However, vitamins A, C and D are present in small quantities (Cobb and Johnson 1973; Adsule et al., 1989).

2.1.1 Proteins and Amino Acids

The orotein content in groundnut kernels has been reported to range from 21 to 36 percent (Freeman et al., 1954; Cherry et al., 1973; Cobb and Johnson, 1973; Khalil and Chughtai, 1983). The protein content in 300 test lines grown at ICRISAT, India was found to range from 16.2 to 28.2 percent (ICRISAT, 1985). The protein content in groundnut is influenced by genetic make up of the cultivars (Basha and Cherry, 1976; Nagaraj et al., 1984a). The average protein content for three years in 105 genotypes varied from 22.7 to 29.3 percent (Young and Hammons, 1973). Amaya et al. (1977) reported that 31 commercial varieties of groundnut contained 22.2 to 30.4 percent protein. Other favours that influence the protein content include fertilizer application (Basha and Pancholy, 1981a), agricultural chemicals (Wagle et al., 1985) and location (Miller, 1980; Nagaraj et al., 1984a).

The groundnut protein are relatively rich in acidic amino acids and low in sulphur containing amino acids (Basha and Pacholy, 1981a). The lysine, methionine, threonine and tryptophan have been reported as deficient amino acids in groundnut proteins (Aykroyd and Doughty, 1964; Miller and Young, 1977). The genetic make up significantly influence the amino acid composition in groundnut (Singh and Nath, 1981; Hovis et al., 1982). Amaya et al. (1977) reported that tryptophan content in the protein of 37 commercial varieties of groundnut ranged from 1.1 to 1.4 percent.

The proteins of raw groundnut exhibited, true digestibility of 97 percent and biological value of 57.9 (Mitchell and Beedles, 1937). In general, the nutritive value of groundnut protein is low when compared with egg protein (FAO, 1970). This has been attributed to the deficiency of several essential amino acids in groundnut proteins. Groundnut flour supplemented with 0.3 percent methionine and 0.2 percent methionine, 0.2 percent lysine have been reported to increase protein efficiency ratio by 39 and 41 percent respectively (Alid et al., 1981).

2.1.2 Carbohydrates

The carbohydrates content of groundnut kernels range from 10 to 20 percent. The defatted groundnut contains about 39 percent total carbohydrates. The sucrose (14.2%) and starch (12.5%) constitute major carbohydrates in the kernels. The other carbohydrate fractions like hemicellulose (4%) verbascose (4%) stachyose (1.6%) and monosaccharides (1.2%) were present in minor quantities (Tharanathan et al., 1975). Gupta et al. (1982) observed that there was no appreciable difference in the reducing sugars content of 25 varieties of groundnut (1.18 to 1.78%), although there was considerable variation in the total sugars content which ranged from 7.44 to 14.24 content. Pattee et al. (1981a) determined carbohydrate content of groundnut kernels and observed significant, influence of seed size on the content of different type of sugars. These reports indicate that groundnut is rich source of dietary sugars. Crude fibre content of groundnut ranges from 1.2 to 4.3 percent. Amount of crude fibre is more in skin than in the kernels (Hoffpauir, 1953).

2.1.3 Lipids

Groundnut contains 36 to 54 percent total lipids. (Freeman et al., 1954, Sanders et al., 1982). Gupta et al. (1982) observed little variation in oil content (44.52 to 48.68%) of 325 varieties of groundnut. However, Nagaraj et al. (1984b) observed considerable variation in the oil content of 10 cultivars of groundnut. Similarly, Narashimhachar et al. (1985) reported 47 to 55 oil in 14 new cultivars of groundnut.

Most of the seed lipid are stored in cotyledons (Yatsu and Jacks, 1972). Groundnut oil contains 82 percent oleic and linoleic acids (Cobb and Johnson, 1973) which are considered as essential fatty acids. Hence groundnut oil has high nutritive value. Considerable genetic variation in the fatty acid composition between Spanish, Runner and Virginia types have been reported (Fore et al., 1953). Because of the high proportion of unsaturated fatty acids in the oil, it has high iodine value and low stability or keeping quality (Holley and Hammons, 1968). Narashimhachar et al. (1985) reported that the quality parameters such as free fatty acids (0.2 to 1%), saponification value (184 to 198) and iodine value (87 to 99) show considerable genetic variation. Groundnuts grown at different locations exhibited significant variation in iodine value (82.04 to 101.52). Saponification value (100 to 207) and percent free fatty acids (0.3 to 2.08%) (Nagaraj, 1984). Pattee et al. (1982a and b) reported that iodine value is significantly higher in smaller seeds. Thus, the oil quality in groundnut kernels is influenced by several parameters such as genetic background, location and seedsize.

2.1.4 Minerals

Total minerals content in groundnut ranged from 1.8 to 3.1 percent (Freeman et al., 1954 ; N.A.S., 1981; Gopalan, et al., 1982). It is a good source of phosphorus (0.25 to 0.66%). Potassium (0.5 to 1.13%), calcium (0.2 to 0.8%), magnesium (0.9 to 0.34%) and iron (18 to 1000 ug/g). The other minerals like zinc, sulphur, manganese, copper and boron are also present in appreciable amounts (Derise et al., 1974; Hullock, 1980; Gaines and Hammons, 1981; Branch and Gaines, 1983).

2.1.5 Vitamins

Groundnut is an excellent source of several B-complex vitamins such as riboflavin (1.05 to 1.57 ug/g), thiamine (8.5 to 14.00 ug/g) and niacin (88 to 200 ug/g) (Freeman et al., 1954). It also contains considerable amount of vitamin E but practically no vitamins A, C or D.

2.2 Effects of storage on nutritional quality of groundnut

The carbohydrate and protein components of groundnut are more preferable for mold and insect infestation especially under favourable conditions. The oil content of kernels is also prone to oxidative and hydrolytic deterioration. However, moisture content and storage temperatures of groundnut kernels are also critical in any situation. Storage stability of groundnuts and groundnut based foods is significantly influenced by the practices employed in harvesting, curing and shelling the peanuts.

The high amount of oil in groundnut kernel, moisture content, and storage temperatures are responsible for changes occuring in groundnuts during storage. Raw and shelled groundnuts require more careful and controlled storage than the inshell groundnut to retain their edible quality. (Woodroof, 1983). Mechanically dried groundnuts lost 1.8 percent grade points during 90 days storage as compared to only 0.3 percent grade point in field dried groundnut (Person, 1974). Sanders et al. (1981) carried out studies on groundnuts stored in five different warehouses. They found that excess moisture, high temperature, mechanical damage, and length of storage were related to storage losses in groundnut. But storage conditions had little effect on sensory characteristics (Branch et al., 1987; Branch et al., 1988).

The nitrogen introduction into porous interstices of partially defatted groundnut kernel provide a longer shelf-life than containing atmospheric air (Pominski Pearce et al., 1975). The stability of groundnut oil during storage was found to be dependent on its linoleic acid content (Fore et al., 1953). Hexane was found to be the best solvent to extract maximum oil from groundnut, but such hexane extracted oil was more susceptible to increase in the acidity (Heikal et al., 1972). Raw shelled groundnuts can be safely stored at 4^{o}C for at least 5-6 months before physiological changes occur in the seed and slow but constant oxidation of oil begins (St.Angelo and Ory, 1975). Increase in the free sugars and amino acid fractions was observed, due to hydrolysis of starch and proteins in groundnut kernels containing about 9 percent moisture during storage of 5 and 7 months. There was also a decrease in phospholipid content, increase in total lipid content but iodine values were not changed (Pattee et al., 1982a). During storage of groundnut butter, penta and hexa-hexanals were produced by autoxidation of linoleates and developed rancidity in the samples (Fore et al., 1976). Temperature near about 0^{o}C, RH 65 percent, initial moisture about 7 percent odourless and well ventilated storage environment is necessary for keeping initial quality of groundnut (Woodroof, 1983).

2.2.1 Effects of storage temperature and storage structure on nutritional quality of groundnut

Tango et al. (1974) concluded that 50 to 70 percent relative humidity offered best conditions for storage of groundnuts upto 9 months at ambient temperature. Free amino acids were found to be increased in all seeds during cold storage (4^{o}C and RH 65%) except phenylalanine for first 6 to 7 months, then again found to be reduced upto nine month of storage (Pattee et al., 1981b). The stability of oil was much greater within groundnut kernel during storage at 50^{o}C than corresponding extracted oil. (Adnan et al., 1981). Storage of unshelled groundnut at 10^{o}C and 70 percent relative humidity or at 5^{o}C and 60 percent relative humidity resulted in essentially unchanged acid value and peroxide number for 12 months. (Uematsu and Ishil, 1981). The percent free fatty acids were increased in innoculated groundnut kernels with Aspergillus and Rhizopus spp. within 5 days at 20^{o}C (Abdel-Rahman, 1982).

Groundnuts packaged in cloth bags and stored at 4^{o}C and 65 percent RH, were found to decrease in oxidative stability during storage (Pattee et al., 1982b). Storage of groundnut pods and kernels in different containers under ambient conditions (32.4 $\pm$ 5^{o}C and 52.1 $\pm$ 6.1% RH) results decrease in protein and oil content and increase in fatty acid content with storage time (Ramamoorthy and Karivarathraj, 1986). The defatted groundnut kernels, with 2.5 percent moisture and 25.9 percent fat packaged in flexible pouches had storage life of 120 days at 27^{o}C and 62 percent relative humidity (Mahadeviah et al., 1982). Shelled remoistened (16.6%) groundnuts were stored at 27^{o}C in atmosphere containing oxygen (1.5%) accumulated high levels of aflatoxins (Wilson and Jay, 1976). Groundnuts stored at 26^{o}C for one year in an atmosphere containing 60 percent CO_2, when CO_2 was not recirculated in the bin, the humidity and moisture were increased and altimately molding was occured (Wilson et al., 1985). Storage of refined groundnut oil in tin containers increased peroxide value and carbonyls in hot humid and cold climate but it was remained acceptable for a period of one year (Arya et al., 1975). However, Alikha-Yousuf et al. (1979) reported that raw groundnut oil was stable hydrolytically and oxidatively for 660 days in mild steel and tin containers. Arya et al. (1972) stored fresh refined and fortified groundnut oil with vit. A in tin containers, they found, free fatty acid content and peroxide value were increased and loss of vitamin A during storage of 6 and 12 months.

2.2.2 Effects of storage period on nutritional quality of groundnut

Storage of groundnuts, in shell or shelled in dark at 33^{o}F for three year did not show considerable changes in soluble proteins but stored at 75 $\pm$ 10^{o}F in dark or light dropped in soluble proteins content in both shelled and in shell form (Picket, 1948). During storage, peroxidation increased with time and recovery of salt soluble fraction increased and salt insoluble fraction decreased (St. Angelo and Ory, 1975). Losses in protein concentrates and isolates prepared from 36 and 18 month stored groundnut seeds have been reported by Ahmed and Schmidt (1979). Free amino acids were increased in all seed size except phenylalanine which was decreased with an increase in seed size during cold (4^{o}C, 65% RH), storage (Pattee et al., 1981b). The levels of carbohydrate content in groundnut kernel were significantly affected during cold (4^{o}C and 65% RH) storage with time, except raffinose and ribose (Pattee et al., 1981a). Stachyose and peptide (unidentified N containing compound) showed constant decrease with storage time (Pattee et al., 1982a).

Cold storage of groundnut (0-2^{o}C and 4-6^{o}C for two years) was found to exhibit 0.3 percent acid value and 85 percent germination power and quality of these seeds remained unaffected for about 2 months after removing from cold storage (Lam and Delbose, 1978). Groundnut kernels inoculated with *Aspergillus flavus* and non-inoculated samples contained 11.5 percent to 14.5 percent free fatty acids after six weeks of storage (Beuchat and Koehler, 1979). Groundnut kernels immersed in hot water (79^{o}C) for 90 seconds showed low peroxide value, acid value and lipoxygenage activity than non-immersed kernels during storage under non-refrigerated conditions of 2, 5 and 8 months (Branch et al., 1987 and Branch et al., 1988). Reconstituted partially defatted groundnut kernels lost 38 percent tocopherol in the first 8 days (Adnan et al., 1981). A peroxide value of 10 was attained in the ghani groundnut oil within a week and that of expeller oil attended in 7 weeks at 91 percent relative humidity (Gopalkrishnan and

Prabhakar, 1984). The aflatoxin in groundnut oil degraded in sunlight was not regenerated when stored in dark for 6 months (Shantha and Sreenivas, 1980).

2.2.3 Effects of storage on nutritional quality of groundnut products

Shelled, roasted and salted groundnut requires adequate package protection as the storage life of such groundnuts is about three weeks at room temperature (Shewfelt and Young, 1977). Roasted, salted-in-the-shell groundnut normally had a shelf-life of 4 to 6 weeks. The antioxidant, tertiary butyl hydroquinone (TBHQ) has been reported to double the shelf-life roasted peanuts (Hoover and Nathan, 1980). A shelf-life of crispness of roasted and salted spieced groundnuts under standard Indian condition (65% RH and 27^{o}C) was 180 days in vacuum packaged polyster and polyaminant pouches. The ultra violet rays and temperature increased the rancidity rates and peroxide value and made product unacceptable within short period (20-60 days) depending on storage conditions Balasubramanyam et al., 1983). Storage of groundnut butter is markedly influenced by temperature. Candies contain high amount of fat and this limits It's shelf-life (Woodroof, 1974). Storage of in about 50 percent loss of oil, increase in free fatty acids and unsaponifiable matter and poor hydrogenation characteristic was 15 percent in 48 hrs and 80 percent after 4 days of storage at 26^{o}C (Godwin, 1987).

Materials and methods

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Seeds

The freshly harvested pods of two popular groundnut varieties JL-24 and SB-XI, were obtained from the Post-Graduate Institute Farm, Mahatma Phule Agricultural University, Rahuri. The pods were shelled manually, the kernels cleaned, dried at 45^{o}C and healthy and bold kernels were stored at three different temperatures in cloth bags until used for further analysis.

3.1.2 Chemicals

Most of the chemicals used in this study were analytical grade (BDH, Sarabhai M. Chemicals or E. Merck.) DL-Methionine and Folin-Ciocalteul reagents were obtained from Sisco Research Laboratory, Bombay. Ninhydrin was obtained from Fluka AG, Chem-Bovine Serum albumin was obtained from Romali American Preparate while L. tryptophan was obtained from Loba Chemicals, Bombay.

3.2 Methods

An experiment in factorial completly randomised block design (F-CRD) was planned to investigate the effect of accelerated ageing on the groundnut kernels.

(a) Storage period - 6 (Dec. 1988 to Feb. 1989)
10, 20, 30, 40, 50 and 60 days
(Considering 0 days as control)

(b) Storage temperatures - 3

i) 25 ± 2^{o}C

ii) 35 ± 2^{o}C

iii) 45 ± 2^{o}C

(c) Varieties - 2

i) JL-24

ii) SB-XI

For each temperature treatment, a sample of 500 g kernels was stored in 6 separate cloth bags. Temperatures were adjusted to 25, 35 and 45 ^{o}C in three separate incubators. After appropriate storage period, one bag from each incubator was removed for analysis. The kernels were milled to 40 mesh to determine moisture, oil and ash. The defatted meal was further ground to 100 mesh to estimate proteins, sugars, amino acid and soluble proteins. The oil extracted in Soxhlet apparatus was used for analysis of fat constants. Each analyses was carried out in triplicate and the results are statistically analysed.

3.2.1 Moisture, crude protein and crude oil

These were estimated by the standard procedures of A.O.A.C. (1980).

3.2.2 Free amino acids

One gram of defatted groundnut meal was extracted with 25 ml of 80 percent (w/v) boiling ethanol. The extraction was repeated four times by centrifugation at 10,000 x g for 15 min. The combined ethanolic extract was then evaporated to about 8 to 10 ml and contents were diluted to 100 ml with distilled water. The aqueous extract was used for the determination of free amino acids and reducing sugars. The free amino acids content in the aqueous solutions were determined by the colorimetric method of Rosen (1957) using L-leucine as reference standard.

Reagents :

Cyanide-acetate buffer : Sodium cyanide (0.01 m) was prepared by dissolving 0.49 g of sodium cyanide in 1000 ml of distilled water. Acetate buffer (0.01 m, pH 5.4) was prepared by dissolving 360 g of sodium acetate in 266 ml of distilled water containing 66 ml of glacial acetic acid. The pH was adjusted to 5.4 and the volume made to 1000 ml with distilled water. The cyanide-acetate buffer was then prepared by mixing 20 ml sodium cyanide with 980 ml of acetate buffer.

Ninhydrin : It was prepared by dissolving 3 g ninhydrin in 100 ml of 2 methoxy-ethanol.

Isopropanol : (diluted) Isopropanol was diluted with water in 1:1 (v/v) proportion.

Standard L-leucine : It was prepared by dissolving 10 mg L-Leucine in 100 ml distilled water. This solution contained 100 Mg of L-leucine/ml.

Procedure :

The appropriately diluted extract (0.2 ml) was heated for 15 min, in a boiling water bath after addition of 0.5 ml of cyanide acetate buffer (pH 5.4) and 0.5 ml of 3 percent ninhydrin. Immediately after removing the sample tubes from water bath, 5 ml of iso propyl alcohol (1:1 water, diluent) was added. The tubes were then shaken vigorously on cyclomix for about one minute and allowed to cool at room temperature. The intensity of colour was read at 570 nm. The concentration of aminoacids was calculated from a standard curve prepared from L-leucine by the same procedure using 0, 10, 20, 40 and 60 ug of L-leucine.

3.2.3 Reducing sugars

The aqueous extract prepared for the determination of free amino acids was used for determining the reducing sugars by the arsenomolybdate reagent method of Nelson (1944).

Reagents :

Copper sulphate reagent : It was a mixture of 25 parts of reagent A and one part of reagent B (v/v).

Reagent A : This solution contained 25 g Na_2CO_3(anhydrous), 25 g Rochelle salt, 20 g $NaHCO_3$ and 200 g Na_2SO_4 (anhydrous) in 1000 ml.

Reagent B : 15 percent $CuSO_4$ containing 1 to 2 drops of concentrated H_2SO_4/ 100 ml.

Arsenomolybdate reagent : It was prepared by dissolving 25 g ammonium molybdate in 450 ml water. To this 21 ml of concentrated H_2SO_4 acid were added followed by 3 g sodium arsenate dissolved separately in 25 ml water. The contents were mixed and incubated of 37^o for 48 hrs,

Standard D-glucose : D-glucose (100 mg) were dissolved in 100 ml of distilled water. This solution contained 1 mg D-glucose per ml. From this stock solution (10 ml) were diluted to 100 ml with water. This solution contained 100 ug D-glucose/ml.

Procedure :

To 1 ml of the appropriately diluted sample extract, 1 ml of copper sulphate reagent was added, mixed and heated for 20 min. in a boiling water bath. The tubes were cooled and 1 ml of arsenomolybdate reagent was added to it. The mixture was diluted to 8 ml and absorbance was measured at 520 nm. The total reducing sugars were calculated from a standard curve prepared by the same procedure using D-glucose at 0, 10, 40, 80 and 100 ug/ml.

3.2.4 Acid value

Reagents : 1. Fat solvent (alcohol + pertrolium ether, 50:50 v/v)

2. Potassium hydroxide 0.01 N

3. Phenolphthalein indicator.

Procedure :

Oil samples (2 g) was accurately weighed and dissolved in 25 ml of fat solvent in a conical flask. Phenolphthalein indicator (3 to 4 drops) was added to flask and mixed thoroughly. The contents were titrated with 0.01 N potassium hydroxide until faint pink colour persisted for 20 to 30 seconds. The amount of alkali required for neutralization was used for the calculation of acid values.

$$\text{Acid value} = \frac{(\text{Vol. of KOH required for sample} - \text{Vol. of KOH required for blank})}{\text{Weight of sample}} \times 0.5611$$

3.2.5 Iodine value

1. Fat solvent ($CHCl_3$)
2. Hanus reagent : 13.2 g pure iodine dissolved in 1 litre acetic acid by heating, 3 ml of b romine solution was added to this after cooling.
3. Potassium iodide, 15 percent.
4. Sodium thiosulphate ($Na_2S_2O_3$) 0.1 N
5. Starch indicator 1 percent

Procedure : Oil (0.25 g) was weighed accurately in 250 ml glass stoppered flask and dissolved in 10 ml of fat solvent ($CHCl_3$). 25 ml of Hanus iodine solution was added to it by draining pipette in definite time and allowed to stand for 30 min in dark. The contents of the flask was shaken occasionally. Subsequently, 10 ml of 15 percent KI solution was added and mixed thouroughly and 100 ml freshly boiled and cooled water was added, washing down any free iodine on stopper. Titrated 'I' with standard 0.1 N $Na_2S_2O_3$, adding it gradually, with constant shaking until yellow turns almost colourless. The few drops of starch indicator were added and continued titration until blue entirely disappeared. Conducted two blank determinations along with samples.

$$\text{Iodine Value} = \frac{(B - S) \times N \text{ of } Na_2S_2O_3}{\text{Weight of sample (g)}} \times 12.69$$

Where

B = Vol. of $Na_2S_2O_3$ required for blank

S = Vol. of $Na_2S_2O_3$ required for sample

3.2.6 Saponification value

Reagents

1. Alcoholic KOH : 40 g KOH and 45 g CaO (granulated) were crushed to powder and dissolved in 1 lit. alcohol. Next day morning fitered in clean dry 9.5 bottle.
2. 0.5 N HCl
3. 0.2 percent phenolpthalein indicator solution in 50 percent ethanol

Procedure

About 2.5 g of oil sample was taken into 250 ml erlenmeyer flask, to this 25 ml of alcoholic KOH solution was added by draining pipet in definite time. The flask connected with air condenser and boiled until the fat was saponified (about 30 min.). The flask cooled, and titrated with 0.5 N HCl, using phenolpthalein as an indicator. Blank was carried out along with sample, used same pipet for measuring KOH solution and drained in same time.

Calculations

$$S.V. = \frac{(B - S)}{\text{Weight of sample (g)}} \times 28.05$$

Where

B = ml of 0.5 N HCl required for blank

S = ml of 0.5 N HCl required for sample

3.2.7 Peroxide value

Reagents

1. Acetic acid-chloroform solution
   - Mixed three volumes of acetic acid with two volumes of chloroform
2. Saturated potassium iodide solution
   - Dissolved excess KI in freshly bioled H_2O
3. Standard sodium thiosulphate solution : prepared 0.1 N $Na_2S_2O_3$ solution and diluted to 0.01 N with freshly boiled and cooled H_2O. Standardized by using $K_2Cr_2O_7$.
4. 1 percent starch solution

Procedure

Accurately weighed 2.5 g oil in erlenmeyer flask. To this 30 ml of acetic acid-chloroform solution was added and this was followed by addition of 0.5 ml of standard KI solution and swirrled to dissolve KI solution and kept for 2 minutes with occasional shaking. After 2 minutes shaking, 30 ml of water was added. The contents were slowly titrated with 0.01 N $Na_2S_2O_3$ with vigrous shaking until yellow colour was almost disappeared. Starch indicator was added and titration was continued with vigrous shaking, until blue colour just disappeared. A blank determination without oil sample was also conducted in the same manner.

Calculations

$$P.V.\ (Meq/kg) = \frac{(B - S) \times N}{\text{Weight of sample (g)}} \times 1000$$

Where B = ml of $Na_2S_2O_3$ required for blank
S = ml of $Na_2S_2O_3$ required for sample
N = normality of $Na_2S_2O_3$ solution

3.3 Statistical analysis

The data obtained in the experiment were analysed for statistical significance by the methods of Panse and Sukhatme (1967).

Results and Discussion

4. RESULTS AND DISCUSSION

The results obtained on the chemical changes in groundnut during accelerated ageing for 60 days are presented and briefly discussed in this section.

4.1 Moisture

The changes in moisture content during accelerated ageing are presented in Table 1. The test of significance for effects of storage period, temperature and variety are given in Table 2 while their interaction effects are summarised in Table 3. The moisture content was found to decrease during storage. There was a greater reduction in moisture content in the kernels stored at 45°C followed by the kernels held at 35°C. The trend was similar in both the varieties. There was a significant reduction in moisture content with the length of storage and storage temperatures (Table 2). Results indicated that the rate of moisture loss was greater in the groundnut kernels which were held at higher temperatures.

The reduction in moisture content during storage is desirable for the increased shelf-life of the groundnuts (Woodroof, 1945; Woodroof, 1983). The moisture content of the kernels is one of the most critical factors which determine the storage stability of the groundnuts (Sanders et al., 1981). The moisture content in the groundnuts stored in cloth bags at 25°C was found to decrease from 4.2 to 3.8 percent

Table 1 : Effects of accelerated storage condition on moisture (%) of groundnut kernels.

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	5.11	5.08	5.07	4.88	4.82	4.75	4.43	4.84
JL-24	35		4.78	4.40	3.90	3.41	3.13	3.0	3.77
	45		4.41	4.10	3.32	2.87	2.73	2.4	3.30
	25	4.8	5.60	5.01	4.35	4.35	4.35	3.97	4.60
SB-XI	35		4.33	3.46	3.31	3.14	3.08	2.90	3.37
	45		3.27	2.41	2.32	2.24	2.04	2.00	2.38
Mean			4.57	4.07	3.68	3.47	3.34	3.11	3.70

Table 2 : Test of significance for changes in moisture (%) in groundnut kernel

Factors	Moisture (%)
Storage period (days)	
10	4.57
20	4.07
30	3.68
40	3.47
50	3.34
60	3.11
CD at 5%	0.07
Storage temperature ± 2°C	
25	4.72
35	3.57
45	2.84
CD at 5%	0.06
Variety	
JL-24	3.97
SB-XI	3.45
CD at 5%	0.04

Table 3(a): Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	4.757	4.218	4.487
20	4.523	3.629	4.076
30	4.031	3.328	3.679
40	3.699	3.244	3.472
50	4.072	3.158	3.615
60	3.274	2.932	3.103
Mean	4.059	3.418	3.739
CD at 5%	0.104		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (^{o}C)			Mean
	25	35	45	
10	5.070	4.550	3.842	4.487
20	5.037	3.933	3.258	4.076
30	4.613	3.607	2.818	3.679
40	4.585	3.277	2.553	3.472
50	4.553	3.103	3.188	3.615
60	4.202	2.950	2.158	3.103
Mean	4.677	3.570	2.970	3.739
CD at 5%	0.127			

(c) Interaction between variety x temperature

Variety	Temperature (^{o}C)			Mean
	25	35	45	
JL-24	4.839	3.768	3.571	4.059
SB-XI	4.514	3.372	2.369	3.418
Mean	4.677	3.570	2.970	3.730
CD at 5%	0.073			

after 6 months and to 3.4 percent after 12 months (Gangai, 1989). In the present study, the value of 3.4 percent for moisture content was observed when the kernels were stored for 40 days at 35°C or 30 days at 45°C indicating that the changes in moisture after 12 months storage at 25°C may be similar to the changes in moisture content after only 30 days of storage at 45°C.

4.2 Crude protein

The changes in crude protein in defatted meal of groundnut during accelerated ageing conditions are shown in Table 4. The test of significance for factors of ageing (Table 5) and their interaction effects (Table 6) are also calculated. The protein content in the defatted meal of groundnut was about 50 percent in both the varieties. The protein content was found to increase significantly after 40 days of storage at storage temperature of 35°C and 45°C. The cumulative effects of period and temperature of storage were also significant. The protein content was found to increase from 50.3 percent (25°C and 10 days) to 51.6 percent (45°C and 60 days) in JL-24. The trend was also similar in the other variety.

An increase in the protein content during storage can be attributed to the loss in moisture from the kernels. The greater losses in the moisture occured at higher temperatures after 50 and 60 days of storage. Hence significant increase in the protein content was noticed at higher storage temperature for 50 and 60 days. The changes

Table 4 : Effects of accelerated storage condition on crude protein (%) content of the groundnut kernels.

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	49.9	50.3	50.3	50.08	50.2	50.3	50.4	50.26
JL-24	35		50.4	50.4	50.4	50.8	51.2	51.7	50.81
	45		50.7	50.8	51.4	51.4	51.9	53.3	51.58
	25	48.7	48.7	48.7	49.1	49.1	49.2	49.1	48.98
SB-XI	35		48.7	48.7	50.1	50.6	50.8	51.7	50.11
	45		49.1	49.1	51.2	51.3	53.7	52.3	51.11
Mean			49.65	49.66	50.38	50.56	51.18	51.41	50.47

Table 5. Test of significance for changes in crude protein content (%) in groundnut kernel

Factors	Crude protein (%)
Storage period (days)	
10	49.65
20	49.66
30	50.38
40	50.56
50	51.18
60	51.41
CD at 5%	0.44
Storage temperature ± 2°C	
25	49.62
35	50.46
45	51.34
CD at 5%	0.31
Variety	
JL-24	50.88
SB-XI	50.06
CD at 5%	0.25

Table 6 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	50.464	48.869	49.667
20	50.511	48.847	49.679
30	50.603	50.157	50.380
40	50.818	50.344	50.581
50	51.143	51.221	51.182
60	51.791	51.002	51.407
Mean	50.889	50.077	50.483
CD at 5%	0.62		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (^{o}C)			Mean
	25	35	45	
10	48.550	49.550	49.900	69.667
20	49.517	49.550	49.970	49.679
30	49.555	50.250	51.335	50.380
40	49.567	50.707	51.370	50.581
50	49.767	51.002	52.758	51.182
60	49.760	51.653	52.807	51.407
Mean	49.636	50.455	51.357	50.483
CD at 5%	0.76			

(c) Interaction between variety x temperature

Variety	Temperature (^{o}C)			Mean
	25	35	45	
JL-24	50.262	50.812	51.592	50.889
SB-XI	49.010	50.099	51.121	50.077
Mean	49.636	50.455	51.357	50.483
CD at 5%	0.44			

in the protein content are therefore, only apparant. The results obtained on protein content in groundnut kernels are in agreement with the literature values (Damame, 1988). The kernels of cultivar JL-24 when stored for 6 and 12 months at 25°C in cloth bags exhibited the protein content of 50.7 percent and 51.8 percent, respectively (Gangai, 1989). The similar protein content was observed during the accelerated ageing after 50 days of storage at 35°C or after 40 days at 45°C, respectively. Although the changes in total protein content during ageing are not very marked, the nature of changes are indicative to show the correlation of accelerated ageing to the long-term storage at ambient temperature. However, storage of groundnut pods and kernels in different containers under ambient conditions (32.4 ± 5°C and 52.1 ± 6.1 RH) caused a decrease in protein and oil content and, increase in fatty acid content with storage time (Ramamoorthy and Karivarthraj, 1986).

4.3 Free amino acids

The changes in free amino acids during accelerated ageing of groundnuts are presented in Table 7. The test of significance for the effects of storage period, storage temperature and variety are shown in Table 8, while their interaction effects are given in Table 9. The free amino acid content did not change markedly during 60 days of storage at 25°C. However, there was a significant increase in free amino acids when the groundnuts were held at 35°C

Table 7 : Effects of accelerated storage condition on free amino acids (%) of groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	0.40	0.41	0.41	0.41	0.42	0.42	0.43	0.416
JL-24	35		0.40	0.42	0.43	0.45	0.58	0.59	0.479
	45		0.41	0.42	0.46	0.60	0.62	0.62	0.521
	25	0.41	0.42	0.42	0.42	0.43	0.43	0.44	0.426
SB-XI	35		0.42	0.47	0.47	0.44	0.49	0.54	0.471
	45		0.42	0.49	0.51	0.49	0.50	0.59	0.50
Mean			0.414	0.438	0.451	0.471	0.506	0.535	0.469

Table 8 : Test of significance for changes in free amino acids (%) in groundnut kernel

Factors	Free amino acids (%)
<u>Storage period (days)</u>	
10	0.414
20	0.438
30	0.451
40	0.471
50	0.506
60	0.535
CD at 5%	0.003
<u>Storage temperature ± 2°C</u>	
25	0.42
35	0.47
45	0.51
CD at 5%	0.003
<u>Variety</u>	
JL-24	0.472
SB-XI	0.466
CD at 5%	0.001

Table 9 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	0.407	0.419	0.413
20	0.432	0.464	0.448
30	0.436	0.485	0.461
40	0.576	0.486	0.531
50	0.588	0.500	0.544
60	0.612	0.555	0.584
Mean	0.508	0.485	0.487
CD at 5%	0.0056		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (°C)			Mean
	25	35	45	
10	0.413	0.411	0.416	0.413
20	0.426	0.453	0.465	0.448
30	0.441	0.456	0.485	0.461
40	0.500	0.544	0.550	0.531
50	0.524	0.550	0.558	0.544
60	0.562	0.563	0.626	0.584
Mean	0.478	0.496	0.516	0.497
CD at 5%	0.0068			

(c) Interaction between variety x temperature

Variety	Temperature (°C)			Mean
	25	35	45	
JL-24	0.479	0.515	0.532	0.508
SB-XI	0.476	0.478	0.501	0.485
Mean	0.478	0.496	0.516	0.497
CD at 5%	0.0039			

and 45^{o}C. The tread in changes of free amino acids was similar in both the cultivars. Both storage period and storage temperature significantly increased the free amino acid content of the groundnut kernels. The effects were more pronounced in kernels stored at 45^{o}C than those stored at 35^{o}C.

The changes in free amino acids are related to metabolic degradation of storage proteins during storage. Pattee et al. (1981b) observed an increase in free amino acids during storage of groundnut for first 6 to 7 months. The faster rate of accumulation of free amino acids in the groundnuts stored at higher temperatures indicate an increased proteolysis in these seeds. The free amino acids were found to increase from 0.35 to 0.44 percent during storage at 25^{o}C for 12 months (Gangai, 1989). The similar values for free amino acids for the same variety were observed in the seeds stored at 35^{o}C after 40 days and in seeds stored at 45^{o}C after 30 days. The observations in the present study indicate the relationship between the normal long-term storage and accelerated ageing.

4.4 Reducing sugars

The changes in reducing sugars in the groundnuts during accelerated ageing are presented in Table 10. The test of significance for the effects of storage period, storage temperature and variety are summarised in Table 11, while their interaction effects are shown in Table 12. The storage period of 60 days did not change the sugar content markedly,

Table 10 : Effects of accelerated storage condition on reducing sugars (%) in groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	0.178	0.178	0.180	0.185	0.184	0.185	0.185	0.183
JL-24	35		0.219	0.219	0.232	0.211	0.214	0.217	0.219
	45		0.219	0.214	0.229	0.204	0.240	0.224	0.222
	25	0.214	0.219	0.210	0.235	0.210	0.217	0.189	0.213
SB-XI	35		0.216	0.214	0.219	0.227	0.235	0.305	0.236
	45		0.214	0.221	0.214	0.224	0.224	0.233	0.222
Mean			0.211	0.210	0.219	0.200	0.219	0.226	0.215

Table 11: Test of significance for changes in reducing sugars (%) of groundnut kernels

Factors	Reducing sugars (%)
<u>Storage period (days)</u>	
10	0.211
20	0.210
30	0.219
40	0.200
50	0.219
60	0.226
CD at 5%	0.004
<u>Storage temperature ± 2°C</u>	
25	0.198
35	0.227
45	0.222
CD at 5%	0.003
<u>Variety</u>	
JL-24	0.21
SB-XI	0.22
CD at 5%	0.002

Table 12 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	0.205	0.216	0.211
20	0.204	0.215	0.210
30	0.215	0.223	0.219
40	0.202	0.220	0.211
50	0.213	0.225	0.219
60	0.209	0.242	0.226
Mean	0.208	0.224	0.216
CD at 5%	0.0059		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (^{o}C)			Mean
	25	35	45	
10	0.199	0.218	0.216	0.211
20	0.195	0.216	0.218	0.210
30	0.210	0.226	0.222	0.219
40	0.197	0.219	0.217	0.211
50	0.201	0.224	0.232	0.219
60	0.187	0.261	0.228	0.226
Mean	0.198	0.227	0.222	0.216
CD at 5%	0.0073			

(c) Interaction between variety x temperature

Variety	Temperature (^{o}C)			Mean
	25	35	45	
JL-24	0.183	0.219	0.223	0.208
SB-XI	0.213	0.236	0.222	0.224
Mean	0.198	0.227	0.222	0.216
CD at 5%	0.042			

while the storage temperature had a significant effect on the sugar content. The sugar content was significantly increased in the groundnut kernels stored at higher temperatures. The trend was common in both the cultivars studied.

Storage of groundnut is known to significantly affect the carbohydrate content (Pattee et al., 1981a). A significant increase in the free sugars during the storage of groundnut has been reported (Pattee et al., 1982a, Pushpamma et al., 1981). This has been attributed to the degradation of the starch during storage. The higher values observed for reducing sugars in the groundnut stored at 35°C indicate that, the rate of the starch degradation is accelerated due to high temperature storage. Thus, storage temperature has a significant influence on the sugar content in stored groundnuts. The levels of reducing sugars observed for different samples are in agreement with the earlier report (Suryawanshi, 1989).

4.5 Oil

The changes in oil content during accelerated ageing of groundnuts are presented in Table 13. The test of significance for different storage factors is presented in Table 14. There was no significant change in the oil content in the groundnuts stored at 25°C for 60 days. However, the content of oil was found to increase slightly in the nuts stored at 35°C and 45°C. The influence of storage period and storage temperature on the oil content was, however, found to be nonsignificant.

Table 13 : Effects of accelerated storage condition on oil (%) content of groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	46.4	46.3	46.4	46.2	46.5	46.6	46.6	46.43
JL-24	35		46.7	46.9	46.8	46.8	47.0	47.3	46.91
	45		46.7	47.1	31.4	47.1	47.3	48.7	44.71
	25	46.8	46.6	46.9	47.3	47.4	47.2	47.5	47.15
SB-XI	35		47.2	47.8	47.9	48.0	48.7	49.4	48.21
	45		47.3	46.8	48.4	48.9	49.6	50.1	48.51
Mean			46.81	46.98	44.66	47.45	47.73	48.26	46.98

Table 14 : Test of significance for changes in oil content (%) of groundnut kernel

Factors	Oil (%)
Storage period (days)	
10	46.81
20	46.98
30	44.66
40	47.45
50	47.73
60	48.26
CD at 5%	NS
Storage temperature ± 2°C	
25	46.79
35	47.56
45	46.61
CD at 5%	NS
Variety	
JL-24	46.0
SB-XI	47.9
CD a5 5%	NS

The values observed for the content of oil in groundnuts are in agreement with the earlier report (Pattee et al., 1982a). The slight increase in oil content in the groundnuts stored at 35°C and 45°C can be attributed to the loss in moisture content from these seeds. Hence, these changes can be regarded as only apparant.

4.6 Acid value

The changes in acid value of kernel oil during accelerated ageing of groundnut are presented in Table 15. The test of significance for effects of different storage factors and their interaction effects are shown in Table 16 and 17, respectively. The acid value was found to increase in the groundnut kernels stored at 35°C or 45°C as compared to those stored at 25°C. The acid value of the kernel oil of cultivar JL-24 was significantly low (0.18) as compared to oil from cultivar SB-XI (0.6). However, the nature of changes in the acid value in both the cultivar were similar. Both storage period and storage temperature as well as variety significantly affected the acid value of kernels oil, particularly those held at higher temperatures. All the interactions were found to be significant.

The oil in stored nuts is known to undergo oxidative and hydrolytic changes. The hydrolytic changes catalysed by the enzyme lipase are responsible for increased accumulation of free fatty acids. The activity of the hydrolytic enzymes is known to be temperature dependent. The groundnuts stored at cold temperature (0 to 6°C) are reported to exhibit lower

Table 15 : Effects of accelerated storage condition on the acid value of the groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	0.18	0.18	0.18	0.17	0.18	0.18	0.20	0.182
JL-24	35		0.20	0.19	0.20	0.20	0.22	0.28	0.215
	45		0.20	0.21	0.25	0.28	0.31	0.38	0.272
SB-XI	25	0.60	0.61	0.61	0.62	0.62	0.63	0.64	0.622
	35		0.66	0.76	0.79	0.79	0.81	0.84	0.775
	45		0.69	0.84	0.87	0.86	0.87	0.88	0.835
Mean			0.423	0.465	0.483	0.488	0.503	0.537	0.483

Table 16 : Test of significance for changes in acid value of groundnut kernel

Factors	Acid value
Storage period (days)	
10	0.42
20	0.46
30	0.48
40	0.48
50	0.50
60	0.53
CD at 5%	0.0065
Storage temperatures ± 2°C	
25	0.40
35	0.49
45	0.55
CD at 5%	0.0046
Variety	
JL-24	0.22
SB-XI	0.74
CD at 5%	0.0038

Table 17 (a) Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	0.195	0.657	0.426
20	0.196	0.740	0.468
30	0.211	0.762	0.486
40	0.236	0.760	0.498
50	0.229	0.772	0.501
60	0.290	0.793	0.541
Mean	0.226	0.747	0.487
CD at 5%	0.0092		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (°C)			Mean
	25	35	45	
10	0.398	0.434	0.446	0.426
20	0.398	0.477	0.529	0.468
30	0.400	0.497	0.561	0.486
40	0.404	0.500	0.589	0.498
50	0.409	0.510	0.575	0.501
60	0.423	0.565	0.636	0.541
Mean	0.405	0.499	0.556	0.487
CD at 5%	0.011			

(c) Interaction between variety x temperature

Variety	Temperature (°C)			Mean
	25	35	45	
JL-24	0.186	0.218	0.275	0.226
SB-XI	0.625	0.779	0.837	0.247
Mean	0.405	0.499	0.556	0.487
CD at 5%	0.0064			

acid values as compared to those stored at ambient temperature (Lam and Delbose, 1978, Damame 1988 and Gangai, 1989). The higher values observed for acid values in the kernels oil stored at 35°C or 45°C can therefore be attributed to higher activity of hydrolytic enzymes, such as lipase at this temperatures. The results therefore, indicate that, the ageing of groundnuts kernel with respect to oil degradation can be accelerated by storing the nuts at higher temperatures. The storage of groundnut kernels in cloth bags at 25°C caused an increase in acid value from 0.65 to 0.80 in variety JL-24 and from 0.63 to 0.83 in variety SB-XI (Gangai, 1989).

Many investigators have studied the changes in free fatty acids during storage of groundnut kernels (Beuchat and Koehler 1979, Branch et al., 1987, Damame, 1988, Sabale et al., 1972). If the results obtained in the present study of accelerated ageing are extrapolated on the above findings, it can be seen that, the increased values for acid value in the kernels held at 35°C for 50 days or at 45°C for 30 days in both the cultivars, are very close to the values obtained for acid value of kernel oil stored for 12 months at 25°C.

4.7 Peroxide Value

The changes in peroxide value during accelerated ageing of groundnut are presented in Table 18. The test of significance for storage period, storage temperature and varieties are presented in Table 19, while their interaction effects are given in Table 20. The peroxide value of the kernel oil was found to increase significantly during 60 day

Table 18 : Effects of accelerated storage condition on peroxide value of groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	4.34	4.53	4.81	5.30	5.40	5.93	5.67	5.27
JL-24	35		4.74	5.38	5.47	7.26	7.89	8.40	6.52
	45		5.01	5.51	5.73	7.46	8.74	8.90	6.89
	25	5.6	5.93	6.15	6.41	6.52	6.69	6.84	6.42
SB-XI	35		5.63	6.95	7.32	7.59	9.30	9.40	7.69
	45		5.95	7.36	7.72	8.87	11.56	11.72	8.86
Mean			5.29	6.026	6.325	7.18	8.35	8.48	6.94

Table 19 : Test of significance for changes in peroxide value of groundnut kernel

Factors	Peroxide value
<u>Storage period (days)</u>	
10	5.29
20	6.02
30	6.32
40	7.18
50	8.35
60	8.48
CD at 5%	0.16
<u>Storage temperature ± 2°C</u>	
25	5.84
35	7.12
45	7.87
CD at 5%	0.112
<u>Variety</u>	
JL-24	6.23
SB-XI	7.65
CD at 5%	0.092

Table 20 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	4.761	5.836	5.298
20	5.232	6.819	6.026
30	5.501	7.148	6.326
40	6.708	7.660	7.186
50	7.522	9.181	8.332
60	7.654	9.318	8.487
Mean	6.230	7.660	6.945
CD at 5%	0.23		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (°C)			Mean
	25	35	45	
10	5.227	5.185	5.483	5.298
20	5.480	6.163	6.433	6.026
30	5.855	6.395	6.723	6.324
40	5.958	7.425	8.168	7.184
50	6.312	8.595	10.148	8.352
60	6.252	8.900	10.308	8.487
Mean	5.842	3.111	7.878	6.945
CD at 5%	0.27			

(c) Interaction between variety x temperature

Variety	Temperature (°C)			Mean
	25	35	45	
JL-24	5.273	6.525	6.892	6.230
SB-XI	6.422	7.696	8.963	7.660
Mean	5.857	7.111	7.878	6.965
CD at 5%	0.15			

storage particularly in the kernels held at 35°C or 45°C. The trade was similar in both the cultivars. The results indicate that, both storage periods and storage temperature significantly influenced the peroxidation of kernel oil. The kernels held at higher temperature showed significantly higher peroxide values. The peroxide values of kernel oil of the cultivar SB-XI was significantly higher than the values for cultivar JL-24. All the interactions were found to be significant.

The peroxide value of any oil indicates the autoxidation during storage. The peroxidation is carried out by oxidative enzyme such as lipoxygenase (St. Angelo et al., 1979). The activity of the enzyme is temperature dependent (St. Angelo and Ory., 1975). The groundnuts stored in cold conditions exhibit low peroxidation as compared to those stored at ambient or higher temperatures. Similarly, the extent of peroxidation increases during storage (Branch, et al., 1987, Branch et al., 1988 and Pattee et al., 1982b). The faster increase in peroxide value in the groundnut kernels held at 35°C or 45°C can therefore be attributed to higher storage temperatures. Branch et al., 1987 have reported that groundnuts stored at 27°C showed an increase in peroxide value from 2.5 after 2 months to 5.5 after 8 months. The groundnuts stored at 35°C for similar length of periods had a peroxide value of 3.0 and 6.0 for cultivar NC7. Similar storage treatments to cultivar Fla. Early exhibited the peroxide values of 2.5 and 6.0 at 27^{o}C and 4.0 and 7.5 at 35^{o}C, respectively. These reports indicated that peroxidation of kernels oil increases during storage at 25^{o}C and storing the groundnuts at higher temperature increase the rate of peroxidation. The peroxide value of 6.0 to 7.0 was found after a storage of 30 to 40 days at 45^{o}C or 35^{o}C in variety JL-24. Although considerable varietal variations exists for the peroxide value in the kernels oil, the results indicate that, peroxide value can be used as an indicater to assess or evaluate the shelf life of groundnut through accelerated ageing at higher temperature for short periods.

4.8 Saponification value

The changes in saponification value of kernel oil during accelerated ageing are presented in Table 21. The test of significance for different factors of ageing is given in Table 22, and their interaction effects are calculated in Table 23. Both storage period and storage temperature significantly influenced the saponification value of kernel oil. However, the varietal variations were nonsignificant. The interaction between the storage period and storage temperature was found to be significant, while other interactions were nonsignificant.

The results obtained for the saponification value of kernel oil are in agreement with the literature values (Nagaraj 1984, Narashimhachar et al., 1985). The saponification value has been found to increase significantly

Table 21 : Effects of accelerated storage condition on saponification value of the groundnut kernels

Variety	Storage temperature °C	Storage period (days) 0	10	20	30	40	50	60	Mean
	25	187	187	189	192	192	193	195	191
JL-24	35		184	192	198	201	203	211	198
	45		187	192	197	203	204	209	198
	25	187	187	188	190	192	194	195	191
SB-XI	35		185	192	196	199	201	211	197
	45		190	190	198	199	205	216	199
Mean			186	190	195	197	200	206	196

Table 22 : Test of significance for changes in saponification value of groundnut kernel

Factors	Saponification value
Storage period (days)	
10	186
20	190
30	195
40	197
50	200
60	206
CD at 5%	1.4
Storage temperature ± 2°C	
25	191
35	197
45	199
CD at 5%	1.0
Variety	
JL-24	196
SB-XI	196
CD at 5%	NS

NS = Non significant

Table 23 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	186	187	186
20	190	190	190
30	195	194	195
40	198	196	197
50	200	199	199
60	204	207	206
Mean	196	196	196
CD at 5%	1.97		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (°C)			Mean
	25	35	45	
10	187	194	188	186
20	188	191	191	190
30	191	196	197	195
40	191	199	201	197
50	193	201	204	199
60	195	210	212	206
Mean	191	197	199	196
CD at 5%	2.42			

(c) Interaction between variety x temperature

Variety	Temperature (°C)			Mean
	25	35	45	
JL-24				
SB-XI		N.S.		
Mean				
CD at 5%				

N.S. = Non significant

during storage of 5 months at 27°C (Damame, 1988). The saponification value of any oil is related to molecular weight of constituent fatty acids. An increase in the saponification value indicate the formation of low molecular weight compounds. The increase in saponification value during storage indicates that the kernel oil undergoes hydrolytic and oxidative changes resulting in complex breakdown of fatty acids. An increase in saponification value during storage of kernels at 35°C and 45°C over kernels stored at 25°C for respective storage period, clearly indicates the influence of storage temperature. Results therefore indicate that, the breakdown of fatty acids to low molecular weight acids which occurs at normal rate during storage at ambient conditions can be accelerated by holding the kernels at higher temperatures for short periods.

4.9 Iodine value

The changes in iodine value of kernel oil during accelerated ageing of groundnut are presented in Table 24. The test of significance for different storage factors are summarised in Table 25, while their interaction effects are shown in Table 26. The iodine value did not change significantly during storage of 60 days at 25°C in both the varieties. Similarly, the iodine value showed no significant changes during accelerated ageing for 50 days at 35 or 45°C. However, iodine value was found to decrease

Table 24 : Effects of accelerated storage condition on iodine value of the groundnut kernels

Variety	Storage temperature °C	Storage period (days)							Mean
		0	10	20	30	40	50	60	
	25	98.9	97.7	97.9	98.5	98.0	97.9	97.2	97.86
JL-24	35		98.5	98.4	97.9	97.2	98.3	95.7	97.66
	45		97.8	98.7	97.2	97.2	97.4	93.9	97.03
	25	97.1	96.5	96.6	98.6	97.1	97.2	95.5	96.91
SB-XI	35		96.7	96.4	97.9	96.1	96.9	92.5	96.08
	45		97.8	95.2	96.6	97.2	97.1	91.8	95.95
Mean			97.50	97.20	97.78	97.13	97.46	94.43	96.91

Table 25 : Test of significance for changes in iodine value of groundnut kernel

Factors	Iodine value
Storage period (days)	
10	97.5
20	97.2
30	97.7
40	97.1
50	97.4
60	94.4
CD at 5%	0.31
Storage temperature ± 2°C	
25	97.4
35	96.9
45	96.5
CD at 5%	0.22
Variety	
JL-24	97.5
SB-XI	96.3
CD at 5%	0.18

significantly in the kernels stored at 45°C for 60 days. All the interactions were found to be significant.

The iodine value of any oil reflects on the degree of unsaturation in the constituent fatty acids. The groundnut oil is made up of about 80 percent unsaturated fatty acids. Hence, it is more susceptible to oxidative spoilage. The oxidative spoilage of groundnut oil during storage catalysed by the enzymes like lipoxygenase produce various low molecular weight saturated and unsaturated products. Since, the iodine value is related to degree of unsaturation, the oxidative changes in oil may or may not reflect on the changes in iodine value. Damame (1988) did not find significant changes in groundnut oil during storage at 27°C for 150 days. However, a significant decrease in iodine value has been reported after severe heat treatments to groundnut kernels (Damame, 1988) and groundnut oil (Rai and Narayanan, 1984). The decrease in iodine value due to servere heat treatments such as frying and roasting has been attributed to losses in polyunsaturated fatty acids. The temperatures used in the present study (35 and 45°C) are much below the reasting (160°C) or frying (148°C) temperatures. The results therefore, indicates that ageing of groundnut kernels at 35 or 45°C for a period of 30 to 50 days is not enough to induce significant changes in iodine value of kernel oil.

Table 26 (a) : Interaction between storage period x variety

Storage period (days)	Variety		Mean
	JL-24	SB-XI	
10	98.00	96.99	97.50
20	98.34	96.07	97.21
30	97.85	97.70	97.78
40	97.47	96.79	97.13
50	97.88	97.08	97.48
60	95.59	93.28	94.43
Mean	97.52	96.32	96.92
CD at 5%	0.49		

(b) Interaction between storage period x temperature

Storage period (days)	Temperature (^{o}C)			Mean
	25	35	45	
10	97.08	97.63	97.78	97.50
20	97.25	97.43	96.94	97.21
30	98.54	97.86	96.94	97.78
40	97.55	96.68	97.17	97.13
50	97.54	97.62	97.27	97.48
60	96.35	94.12	92.83	94.43
Mean	97.38	96.89	96.49	96.92
CD at 5%	0.53			

(c) Interaction between variety x temperature

Variety	Temperature (^{o}C)			Mean
	25	35	45	
JL-24	97.86	92.68	97.03	97.52
SB-XI	96.91	96.10	95.94	96.32
Mean	97.38	96.89	96.49	96.92
CD at 5%	0.30			

The results obtained on iodine value of different samples are similar to those reported earlier (Damame, 1988, Pattee et al., 1982a and b; Nagaraj, 1984).

The groundnuts are stored either in the form of pods or kernels, mostly in traditional storage structures at ambient temperature for a period of 6 to 12 months and sometimes longer in India. The product containing about 7-10 percent moisture is usually stored. The ambient temperature in Indian climate ranges from as low as 10°C to as high as 45°C in different parts of the country. Both storage period and storage temperatures are known to greatly influence the metabolic changes in the groundnut kernels, which often lead to quantitative and qualitative losses in the groundnuts. Hence, the produce stored under warmer climatic conditions will exhibit different chemical composition than that stored under relatively low temperature climatic conditions. Consequently, the products prepared from such produce which has been stored for similar length of period but at varying temperatures will exhibit different nutritional and sensory qualities. Although it is advantageous to process the fresh produce to obtain products of good quality, it is not always practically feasible. Besides, the varietal variations for different chemical constituents in the freshly harvested produce as well as the produce stored under varying conditions differ significantly. Hence, it is important to know the nature of deteriorative chemical changes that have taken place in the produce stored for a longer time such as 12 months before it is processed into particular product. Such information is useful to modify the processing method to eliminate undesirable constituents during processing. For example, the oil extracted from the freshly harvested groundnuts will exhibit lower acid value and peroxide value. Hence it can directly be used for edible purposes. However, the oil extracted from the groudnnut kernels that have been stored for longer period at varying temperature will exhibit higher acid or peroxide values indicating the extend of spoilage or loss. The free fatty acids and low molecular weight oxidative products from such oil will have to be removed through oil refining process before such oil is used for edible purposes.

The groundnuts are used in variety of food products such as extraction of oil, roasted and salted peanuts, groundnut butter, dairy based products, number of confectionary items, fermented products, preparation of protein isolates and concentrates. It may not be always possible to analyse the raw material for various chemical constituents before the preparation of above mentioned products on commercial scale. It is therefore, important to develop a technique to predict such deteriorative changes in long-term stored groundnuts at ambient temperatures. An attempt has been made to develop a method to predict such changes by carrying out an experiment of accelerated ageing of groundnut kernels at 35 and 45°C up to 60 days. The results obtained on the nature of chemical changes during accelerated ageing are compared with the results obtained in earlier experiments in the same laboratory (Gangai, 1989) on the similar parameters in groundnut kernels stored at ambient temperature for 6 and 12 months, using same cultivars of groundnuts grown under similar agronomical conditions.

Ageing of groundnut kernels at higher temperatures induced the rate of chemical changes. The values for changes in moisture, protein, oil, free amino acids, acid value, peroxide value, and saponification value were significantly different in the kernels stored at higher temperatures as compared to those stored at ambient temperature. Secondly, it was tentatively possible to extrapolate the results obtained in changes in moisture, protein, oil, free amino acids and acid value during accelerated ageing for 30 to 40 days on changes occured in these parameters in the kernels stored for 12 months at ambient temperature. Although this is only a preliminary experiment and, the extrapolation is only approximate, the results indicate that such an approach is quiet **feasible** to predict the shelf-life of groundnut during long-term storage at ambient temperature. More experimentation is however, required to confirm these results and usefulness of accelerated ageing to predict the self-life of groundnut kernels.

Summary and Conclusions

5. SUMMARY AND CONCLUSIONS

The kernels of freshly harvested varieties SB-XI and JL-24 were stored at 25°, 35° and 45°C in cloth bags up to 60 days. The kernels were analysed for moisture, protein, free amino acids, oil, acids value, peroxide value, saponification value and iodine value after every 10 days of storage. The results obtained during such accelerated ageing are compared with the results obtained in earlier experiments conducted in the same laboratory during 1987-88 (Gangai, 1989), in which the same varieties were stored at ambient temperature for 6 and 12 months. The results obtained are briefly summarised in this section.

1. The storage of groundnut kernels at 25°C for 60 days did not cause any significant changes in various chemical parameters studied. However, storage of groundnut kernels at 35°C and 45°C resulted in significant changes in various chemical parameters. There was significant reduction in moisture content which caused an apparent increase in both protein and oil contents. The levels of free amino acids, acid value, peroxide value and saponification value significantly increased due to storage at higher temperature. Such effects were more pronounced in the kernels stored at 45°C than those stored at 35°C. Thus, an increase in the storage temperature to

$45^{o}C$ for a short period was found to be effective in inducing the faster rate of hydrolytic and oxidative changes in groundnut kernels.

2. The storage temperature appears to play an important role in the rate of ageing of groundnut kernels. Since the ambient temperatures in Indian conditions vary greatly from region to region and from season to season, the kernels of same variety stored in such conditions for longer time will exhibit different composition and degree of spoilage during long term storage.

3. An attempt has been made to compare the results obtained in present experiments of accelerated ageing at 35 and $45^{o}C$ with the results obtained for the same parameters of earlier experiments where same varieties were stored for 6 and 12 months at ambient temperatures in this laboratory. The values obtained for various chemical parameters during accelerated ageing at $45^{o}C$ for 30 to 40 days are approximately very close to the values obtained for the similar parameters of groundnut kernels stored for 12 months at ambient temperature. The results, therefore, indicate that it should be possible to predict the shelf-life of groundnut for long-term storage through the short duration accelerated ageing. However, additional experimentation is essential to confirm these results and standardise more precise ageing conditions to predict the shelf-life of groundnut.

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6. LITERATURE CITED

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APPENDIX I

Parameter	Control		Cultivar		
	JL-24	SB-XI	JL-24		SB-XI
			Storage period (Months)		
			6	12	12
Moisture	4.2	4.86	3.95	3.83	3.00
Crude Protein	47.6	45.43	50.66	54.99	46.59
Crude oil	46.90	49.00	48.40	48.97	55.00
Free amino acids	0.31	0.35	-	0.44	-
Reducing sugars	0.17	0.12	0.20	0.197	-
Acid value	0.63	0.66	0.79	0.82	0.83
Iodine value	94.1	92.3	-	94.2	92.61

Gangai S.B. (1989)

Vita

7. VITA

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of

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