The First Flower of my Life Dedicated to my Beloved Parents

KANTILAL

BIOCHEMICAL CHANGES IN ONION BULBS (Allium cepa L.) DURING STORAGE

By

Kantilal Ramlal Marmat

۰,

ACC NUL T-1955 Call No. 574.1921

E 159

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

Agricultural Biochemistry



DEPARTMENT OF BIOCHEMISTRY POST- GRADUATE INSTITUTE, MAHATMA PHULE AGRICULTURAL UNIVERSITY. RAHURI 413 722

BIOCHEMICAL CHANGES IN ONION BULBS (Allium cepa L.) DURING STORAGE

By

KANTILAL RAMLAL MARMAT

A Thesis submitted to the MAHATMA PHULE AGRICULTURAL UNIVERSITY, RAHURI-413722, DIST.AHMEDNAGAR Maharastra State, India

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (Agriculture)

in

AGRICULTURAL BIOCHEMISTRY

٩ų 2

-

Approved:

Professor or Mai 1c Adsule) (Dr. Chavan) Committee Member Committee Member

(Prof Z.V. Deshmukh) Committee Member

(Dr. R.N.

MAHATMA PHULE AGRICULTURAL UNIVERSITY POST-GRADUATE INSTITUTE, RAHURI-413 722, DIST. AHMEDNAGAR

CANDIDATE'S DECLARATION

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

AIL Ozur

Place : Rahuri-413 722. Dated : 10 . (., 876

(Kantilal R. Marmat)

ii

Dr. S.S. Kadam, M.Sc.(Agri.),Ph.D. Head, Department of Biochemistry, Mahatma Phule Agricultural University, Rahuri-413 722, District-Ahmednagar, Maharashtra State.

CERTIFICATE:

This is to certify that the thesis entitled, "BIOCHEMICAL CHANGES IN ONION BULBS (<u>Allium cepa</u> L.) DURING STORAGE", submitted to the Faculty of Agriculture, Mahatma Phule Agricultural University, Rahuri, District-Ahmednagar, in partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE (Agriculture) in AGRICULTURAL BIOCHEMISTRY, is a record of bonafied research carried out by SHRI. KANTILAL RAMLAL MARMAT, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or publication.

Rahuri-413 722, Dated :

(Dr. S.S. Kadam) Major Professor Dr. B.H. Mogal, Associate Dean, Post-Graduate Agricultural Institute, Mahatma Phule Agricultural University, Rahuri-413 722, District-Ahmednagar, Maharashtra State (INDIA).

:CERTIFICATE:

This is to certify that the thesis entitled, "BIOCHEMICAL CHANGES IN ONION BULBS (<u>Allium cepa L.</u>) DURING STORAGE", submitted to the Faculty of Agriculture, Mahatma Phule Agricultural University, Rahuri, District-Ahmednagar, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (Agriculture) in AGRICULTURAL BIOCHEMISTRY, embodies the results of a piece of bonafied research work carried out by SHRI. KANTILAL RAMLAL MARMAT, under the guidance of Dr. S.S. Kadam, Head and Professor, Department of Biochemistry, Rahuri, Dist.Ahmednagar, and that no part of the thesis has been submitted to any other degree or publication.

Place : Rahuri-413 722, Dated : $\Re(\delta)$ 86

B.H. Mogal

: ACKNOWLEDGEMENTS :

I wish to express my sincere gratitude to my Major Advisor, Dr. S.S. Kadam, Professor and Head, Department of Biochemistry, Mahatma Phule Agricultural University, Rahuri, District-Ahmednagar, for his valuable inspiring guidance, constant encouragement, keen interest and constructive criticism throughout the progress of these investigations and for his help in the preparation of the manuscript.

I express my deep sense of gratitude to my Committee Members, Dr. R.N. Adsule and Dr. J.K. Chavan, Associate Professors of Agricultural Biochemistry, and Prof. Z. V. Deshmukh, for their valuable suggestions and constructive criticism during the course of this investigations.

Heartful gratitude is extended to Prof. A.L. Pharande, S/Shri. K.M. Lawande, S.D. Bagal, G.D. Patil, V.A. Dhotre, for their valuable suggestions, helpful comments and constructive criticism.

I would like to express my sincere appreciation to S/Shri. Pardeshi, Patil, Kulkarni, Shah, Gawande, Girase and Bhavsar, for their help and continous encouragement.

It would be ungreatful, if I fail to mention the enormous help I received from Prof. V.V. Thomas, English Teacher, for checking this manuscript grammaticaly and Shri. P.P. Shete, for the meticulous effort he has taken to type this manuscript in a tidy manner.

Last but not the least, I express my profound gratitude to my beloved parents who built up my educational career.

Place : Rahuri-413 722; Dated : 10, 6. 86

(Kantilal R. Marmat)

(omathe

v

TABLE OF CONTENTS

Page

CAN	DIDATE'S DECLARATION	11
CER	TIFICATE - 1. MAJOR PROFESSOR	iii
	2. ASSOCIATE DEAN (PGI)	iv
ACK	NOWLEDGEMENTS	v
LIS	T OF TABLES	viii
lis	T OF FIGURES	ix
ABS	TRACT	x
1. INTRO	DUCTION	1
2. REVIE	W OF LITERATURE	5
2.1	Dry matter	5
2.2	Crude protein	6
2.3	Crude fat	7
2.4	Reducing and Non-reducing sugars	8
2.5	Total phenolic compound	11
2.6	Enzymes	12
3. MATER	IAL AND METHODS	14
3.1	Materiel	14
	3.1.1 Chemicals	14
3.2	Methods	14
	3.2.1 Dry matter content	15
	3.2.2 Crude protein	15
	3.2.3 Crude fat	17
	3.2.4 Reducing sugars	18
	3.2.5 Total sugars	21
	3.2.6 Total phenolics	22
3.3	Enzymes	23
	3.3.1 Amylases	23
	3.3.2 Proteases	27

contd.....

TABLE OF CONTENTS (Contd....)

Page

4.	RESULTS	S AND DISCUSSION	30
	4.1	Dry matter	30
	4.2	Crude protein	32
	4.3	Crude fat	34
	4.4	Sugars	34
		4.4.1 Reducing sugar	34
		4.4.2 Non-reducing sugar	37
		4.4.3 Total sugar	39
	4.5	Total phenolics	41
	4.6	Amylases	41
	4.7	Proteases	45
5.	SUMMAR	Y AND CONCLUSION	49
6.	LITERA	TURE CITED	52
7.	VITA .		58

LIST OF TABLES

Т	ab	<u>le</u>
_		

<u>Title</u>

Page

1	Changes in the content of dry matter (%) during storage	••	31
2	. Changes in the content of crude protein(%) during storage	••	33
3	• Changes in the content of crude fat(%) during storage	••	35
4	. Changes in the content of reducing sugar(%) during storage	••	36
5	. Changes in the content of non-reducing sugar (%) during storage	••	38
6	. Changes in the content of total sugar (%) during storage	••	40
7	. Changes in the content of total phenolic compound (%) during storage	••	42
8	. Changes in the activity of ≪ -amylase during storage	••	43
9	. Changes in the activity of β -amylase during storage	••	44
10	• Changes in the activity of protease at pH 5.5 during storage	••	46
11	. Changes in the activity of protease at pH 7.00 during storage	••	47

ix

LIST OF FIGURES

Figure

Title

Between page

1.	Changes in dry matter content of two onion cultivars during storage	••	31-32
2.	Changes in the protein content of two onion cultivars during storage	••	33-34
3.	Changes in the crude fat content of two onion cultivars during storage	••	35-36
4.	Changes in the content of reducing sugars of two onion cultivars during storage	••	36-37
5.	Changes in the content of total pheno- lic compounds of two onion cultivars during storage	••	42-43

ABSTRACT

BIOCHEMICAL CHANGES IN ONION BULBS (<u>Allium</u> <u>cepa</u> L.) DURING STORAGE

By

K.R. MARMAT

Agricultural Biochemistry Mahatma Phule Agricultural University

Research Guide	\$	Dr. S.S. Kadam
Department	:	Agricultural Biochemistry

Several biochemical changes take place during storage of onion. The biochemical changes in onion bulbs were studied during storage, in two varieties of onion. The varieties selected for investigation were N-2-4-1 and N-53. The variety N-53 has been shown to have poor keeping quality. whereas variety N-2-4-1 is known to have good keeping quality.

The changes in the content of dry matter, sugars, crude protein, crude fat, phenolic compounds and the enzyme amylase and protease activities were studied every fifteen days interval upto two months, starting from curing of the two onion cultivars viz., N-2-4-1 and N-53.

On dry weight basis, on an average onion contains about 12% dry matter, 7% crude protein, 0.55% crude fat, 34% total sugar and 1.50% phenolic compounds. During storage protein, reducing sugar and phenolic compounds increased steadily while dry matter, non-reducing sugar and crude fat decreased gradually during storage.

contd....2

M.Sc.(Agri.)	Abstract	(contd)	K.R.	Marmat
--------------	----------	---------	------	--------

The contents of dry matter, crude fat, sugars and phenolic compounds were positively correlated with better keeping quality while crude protein content was negatively correlated with good keeping quality.

The activities of both hydrolytic enzyme amylase and protease increased during storage.

Pages 1 to 58

x3

CHREAT ONE MENT



1. INTRODUCTION

Onion is an important vegetable crop of India. It is widely used in salads, pickles, <u>chutney</u>, stew for flavouring and as a cooked vegetable. Onions bulbs as well as top leaves are used as a vegetable. Onion powder is prepared from bulbs. Onion is consumed by the people almost every day in one form or other. On fresh weight basis, onion contains about 87% moisture, 1.2% proteins, 0.1% fat, 0.6% fiber and 11% carbohydrates. It has calorific value of 50 Cal/100g. Onion is an important source of minerals like calcium, phosphorus, iron and vitamins like thiamien, riboflavin, niacin, vitamin C and folic acid (Gopalan, <u>et al</u>., 1983). Most of the essential amino acids are found in significant amount in onion bulbs. The pungency in onion is due to sulphide compound namely allyl propyl disulphide.

The world production of dry onion was 22.4 million metric tonnes in 1981-82 and out of which, India produced 2.7 million metric tonnes. About 30% of the world onion production is derived from tropical countries like India, China and Japan (FAO, 1982). In India, onion is grown over an area of about 2.6 lakh hectares (FAO, 1982). Maharashtra State has cultivable area of 50,000 hectares under onion with an annual production of 10 lakh tonnes of onion (Hassanpour, 1983). Maharashtra State stands first in the production as well as cultivable area under onion in the country. Nasik and Pune districts are the major producers of onion in the State. A large part of area under onion cultivation in the State (about 84%) is under the jurisdiction of Mahatma Phule Agricultural University. The average yield of onion is 150 q/ha during <u>kharif</u> season and 225 to 250 q/ha during <u>rabi</u> season in the State of Maharashtra. The commonly cultivated varieties of onion are N-53, N-2-4-1, Pusa Red (all with red skin) and No.257-9-1 (white skin).

Onion is a seasonal crop and has comparatively low storage ability. Onion bulbs are usually stored until the harvest of next season is available, or for longer period due to seasonal gult in the market. Significant losses both in quality and quantity of onion occur during storage. Storage of onion bulbs has therefore, become a serious problem in the tropical countries like India. Maharashtra State Co-operative Marketing Federation Ltd., suffered a total loss over rupees five crores during 1979-80 and over rupees six crores during 1982-83 due to the postharvest losses of onions. This could be attributed to poor storage and marketing facilities.

In Maharashtra, generally traditional methods of storage are used for onion storage i.e. storage in gunny

bags, in huts and in wooden crates. Under such storage, the fungal spoilage is most frequently observed. The fungus <u>Botrytis allis</u> attacks first on the surface of the onion bulb and then moves towards the centre, causing blackening between the layers. Severe infection of this fungus makes the onions unfit for consumption.

£

Sprouting is another problem associated with onion storage. Several physico-chemical changes occur in onion bulbs during storage. The poor keeping quality of onion cultivars has been attributed to the low dry matter content, relatively high rate of water loss and a high total water loss. Other physical and chemical parameters such as neck thickness, dry matter content, total soluble solids, nonreducing sugars and proteins have been correlated with the keeping quality and other storage characteristics of onion.

Phenolic compounds are closely related with keeping quality of onion. Onion cultivars with high phenolic compounds were resistant to fungus spoilage.

Rutherford and Whittle (1984) observed that the alkaline invertase present in storage onion bulbs was directly related to their longevity. They observed possitive correlation between amount of fructose and alkaline invertase activity in the onion bulbs. Fructose amounts at any other time

during storage were not related to storage longevity. In view of the foregoing observations, experiments were conducted at the Post-Graduate Institute of the Mahatma Phule Agricultural University, Rahuri to study the relationship between the biochemical parameters and the keeping quality of onion. The study was, however, restricted to following specific objectives.

- To study the changes in biochemical constituents in onion bulbs during storage.
- To study the changes in levels of hydrolytic enzymes in onion bulbs during storage.

CHREAT ONE MENT



2. REVIEW OF LITERATURE

Several physico-chemical changes take place during storage of onions. The literature available on the biochemical changes during storage of onion and related matter is reviewed in this chapter.

2.1 Dry matter

Bajaj et al. (1981) reported that the average dry matter content of five white and seven red cultivars of onion on dry weight basis ranged from 10.66 per cent to 14.80 per cent. The average dry matter content of onion bulb has been reported as 13.4 per cent (Gopalan et al., 1983) and 11.3 per cent(Rutheford and Whittle, 1984). Malkki and Nikkila (1978) observed that the dry matter content of onion varieties ranged from 11.9 per cent to 13.5 per cent for seed raised onion and from 17.4 per cent to 18.6 per cent for set raised onion.

Kodic (1971) found that the onion varieties with higher dry matter content stored well and retained their aroma better than those onion varieties having low dry matter. Zeceva and Minkov (1965) studied a large number of onion varieties and concluded that varieties 957A and 957B having 12% dry matter content were found to have satisfactory storage capacity. Macollum (1971) observed that there was a negative correlation between bulb size and dry matter content. It has been reported earlier that higher dry matter of onion bulbs causes less sprouting during storage (Foskett and Peterson, 1950). Butarin (1958) also observed a strong positive correlation between dry matter and keeping quality. Cultivars having high dry matter content can be stored for longer periods than those having low dry matter content (Jones and Mann, 1963; Toul and Pospisilova, 1968).

Yamaguchi et al. (1975) noted that the content of dry matter was positively correlated with storage temperature of onion bulbs. Saxena et al. (1974) evaluated the percentage loss in white, yellow and red cultivars of dry onion stored for various periods and found that red cultivars had higher storage potential in comparision with yellow and white under 'Guyana' conditions.

2.2 Crude protein

The average protein content of onion bulbs is 1.2% on fresh weight basis (Gopalan et al.,1983). Toul and Pospisilova (1968) reported that good storage variety had low content of protein. Magdum (1981) found that the protein, in general, was positively correlated with the total sugar losses as well as the losses due to sprouting, rotting and shrinkage. Singh and Kumar (1969) observed gradual increases in protein content

and total N during storage. Similar, observations were recorded earlier (Karmarkar and Joshi, 1941).

In comparision with other fresh vegetables, onions are intermediate in the protein content (Jones and Mann, 1963, Gopalan et al., 1983). Bordia et al. (1973) reported that Bombay red variety had the highest protein content (1.2%) on fresh weight basis followed by joint red (1.19%), English red globe (0.99%) and country white (0.89%).

Ketiku (1976) studied the four onion varieties of Nigerian origin and given the detail contents of amino acid. The average content of amino acids was 305-735 mg/100gm. The concentration of individual essential amino acids was (in mg/100gm): valine 17-47, leucine 16-69, isolaucine 11-47, threonine 7-33, methionine 9-19, phenylalanine 17-49, lysine 15-53 and tyrosine10-34.

2.3 Crude fat

Gopalan et al. (1983) reported that the average fat content in the onion bulbs was 0.1 g/100gm on fresh weight basis. Ketiku (1976) studied the chemical composition of four varieties and reported the average crude fat content of onion in the range of 0.63-1.9 g/100gm. The crude fat content of onion bulbs varied from 0.51 to 0.68 per cent on dry weight basis (Khade, 1985).

The role of crude fat on the keeping quality of onion has not investigated fully. However, the higher crude fat content of onion bulbs has been correlated with their better keeping guality (Aksoy, 1983).

Aksoy (1983) reported that oil content of Imrali and Kantartopu onion cultivars increased on storage (3-9 months) from 0.016-0.019 and 0.031-0.047 ml/100 gm, respectively. Khade (1984), however, reported that crude fat content of onion decreased slightly during storage.

2.4 Reducing and non-reducing sugars

Ketiku (1976) studied the four Nigerian origin varieties of onion and observed the following carbohydrate composition : oligosaccharides containing ketose portion - 1.06-7.66%, sucrose 0.62-21+15%, fructose - 6.96-28.38%, gluclose - 4.57-25.88%, total sugars - 13.21-71.80%, total hemicellulose-8.46-13.20%, cellulose - 10.88-24.57% and total carbohydrates -50.98-83.70%. Bajaj et al. (1980) studied total water soluble sugars and reducing sugars of five white and seven red cultivars of onion. The total water soluble sugar content ranged from 42 to 74% and reducing sugar content ranged from 12 to 22% on dry weight basis.

Bennett (1941) studied chemical composition of onions harvested in different years. In the first year study, bulbs contained 60% of non-reducing sugars and 5% of reducing (sugars on dry weight basis. In the second year, the contents were 42% and 17%, respectively. Bhati and Asghar (1965) reported that among the onion cultivars studied, Viraval and Nasik red had highest total sugar contents while white skin and red skin cultivars from Quetta were found to have higher reducing sugar contents.

Khodzhaeva (1979) reported that the water soluble polysaccharide content of 15 onion species grown in Central Asia ranged from 41 to 76 per cent. All the polysaccharides were glucofructan and those isolated from <u>Allium</u> cepa were of the inulin type. Joslyn and Peterson (1958) identified sucrose, glucose and fructose in onions, but did not specify the amount present. Karmarkar and Joshi (1941) found that reducing sugar increased during low temperature storage.

Butarin (1958) found that the ratio of sucrose to monosaccharide in onion was positively correlated with storage properties. Similar observation was reported by Bajaj et al. (1980). Garvisheva and Orlava (1980) studied the chemical composition of onion bulbs and reported that the ratio of monosaccharide to disaccharide in the bulbs varied according to variety and found direct correlation between keeping quality and ratio of monosaccharide to disaccharide.

1

The low content of reducing sugar was associated with improved storage ability of onion (Chang, 1981). According to Rutherford and Whittle (1984), low fructose in onion at harvest indicates poor long term storage quality. Hadokova and Ito (1968) suggested that a high soluble sugar content at harvest would be a indicator of good keeping quality in the bubls. Gorin and Borcsok (1981) reported that freshness of onion bulbs was more closely related to the total sugar content than to any other parameters.

Yamaguchi et al. (1975) noted that the quality of reducing sugar was inversely proportional to the temperature of storage. Toul and Pospisilova (1968) observed that the reducing sugar content of onion increased during storage at low temperature (33 to 35° F). During storage for two years, the reducing sugar increased from 5.2 to 21 per cent in the first year and from 17 to 29 per cent during the second year.

Rutherford (1981) stated that storage of onion at 4° C and 70% relative humidity had very little effect on the amount of polysaccharide present in the bulbs. However, the two

reducing sugars, glucose and fructose, increased by about 33% during the storage. Although the amount of total soluble sugars showed no change, there was an increase in the amount of sucrose.

2.5 Total phenolic compounds

Numerous free phenolic compounds have been identified in onions. Herrmann (1976) observed that most vegetable crops including onion contained flavone and flavonol in addition to ester and glycosides of hydroxycinuaric acid and hydroxybenzonic acid. The total phenolic content of onion cultivars has been reported to vary from 1.75 to 2.95 per cent (Bagul, 1984).

Bajaj et al. (1981) analysed twenty onion cultivars, five white and fifteen red, for various chemical constituents, the level of lachrometry factor ranged from 12.50mg to 62.25 mg/100 gm on a fresh weight basis. The white cultivar, Punjab-48 had a optimum level (25 mg) lachrometry factor.

Link and Walkar (1933) noted the significance of dihydroxy-benzonic acid and dihydroxy benzene (catechol) in relation of disease resistance in the onion. The catechol was present only in pogmented onion and not in white onion. The catechol and protocatechuric acids are toxic to the fungus

collectotrichum (Berk), the organisms responsible for onion diseases known as 'smudge'. Bulb et al. (1979) investigated the phenolic constituents in the neck tissue of onion and found that phenolic concentration in neck was weakly and negatively correlated with the subsequent diseases in storage.

Bajaj et al. (1981) reported that the pigments are formed in onion by enzymatic and non-enzymatic oxidation of dihydroxy phenolic compounds such as quercetin and protocatechuric acid. The cultivars with high phenolic contents are rich in colour in the dehydrated state. The red cultivars were less succeptible to the fungal diseases because of their high phenolic content.

2.6 Enzymes

The metabolic activity of onion bulbs continues even after storage. Several enzymes, particularly the hydrolytic and oxidative types remain active during storage of onion. The activities of these enzymes depend upon the conditions of storage and period of storage. Although these generalizations are known for some fruits and vegetables, the information on the nature and activity of enzymes during storage of onions is limited.

Hadacova and Klozava (1981) screened onion seed cultivar 'Vsetatska' to detect prospective markers for taxonomic studies.

Of the 17 enzymes detected, NAD⁺ malate dehydrogenase and non-specific esterase had 8 and 9 isoenzymes, respectively and 7 other enzymes each had more thant 3 isoenzymes. Rutherford and Whittle (1984) observed that the alkaline invertase present in stored onion bulbs was directly related to their longevity. They observed positive correlation between amount of fructose and alkaline invertase activity in the onion bulbs. Fructose amounts at any other time during storage were not related to storage longevity, nor the alkaline invertase levels earlier in storage or at the time of harvest.

Polascsck-Recz and Pozsar-Hajnal (1976) reported the method of assay for pectolytic enzymes in fruits and vegetables including onion. They observed that the pectin-methyl estarase (PMR) activity decreased substantially, whereas polygalacturonase (PG) activity increased during storage.

Selby and Galpin (1979) reported that the system of fl=vour production in the onion consists of the enzyme allinase and the flavour precursors, the 5-alkyl-L-cystine sulphoxide peroxidase activity in three onion genotype differing in dormancy characteristics was investigated at two stages of bulbs development, the activity of all the three genotypes was the same in young bulbs (1.2-2 cm in diameter) but in mature bulbs it differed with the genotype. Bulbs with short dormancy had higher activity and a greater number of isoenzymes than bulbs with long dormancy.

CHREAT ONE MENT



3. MATERIAL AND METHODS

3.1 Material

Bulbs of two promising varieties of onion, viz. N-53 and N-2-4-1, were obtained from the Onion Scheme, Mahatma Phule Agricultural University, Rahuri. These two varieties were developed at the Agricultural Research Station, Pimpalgaon-Baswant (Maharashtra) of this University. The bulbs of these varieties were collected immediately after curing. The bulbs of the both the varieties were deep purple in colour and flat round in shape. N-53 has comparatively low keeping quality while N-2-4-1 has good keeping quality.

3.1.1 Chemicals

The chemicals used in the present investigation were from Sarabhai M., E. Merk and B.D.H. Cysteine hydrochloride was obtained from the Loba Chemical Industries, Bombay.

3.2 Methods

Bulbs of both onion varieties were kept for storage in three sets of wooden crates at room temperature for 60 days i.e. from 30th November, 1985 to 30th January, 1986. The bulbs were removed at fifteenth days interval (i.e. after 0, 15, 30, 45 and 60 days of curing) starting from curing and were analysed for different biochemical characteristics and enzyme activities.

About one kg bulbs were taken for analysis at each stage of interval randomly. The bulbs were cut into small pieces with stainless steel knife and the chips were dried in hot air oven (60°C) to constant weight. The dried chips were then ground in pestle and mortar to obtain fine powder passing through 100 mesh. This powder material was utilized for analysis of various chemical and biochemical constituents. For enzyme assay the fresh cured bulbs collected at different stages of storage were used.

3.2.1 Dry matter content

For the determination of dry matter content, the bulbs were cut into small pieces. Ten gram chips were weighed accurately and dried in hot air oven at 60° C to a constant weight. The dry matter content was calculated from the differences in weight.

3.2.2 Crude protein

Crude protein content was determined by Kjeldahl's nitrogen method (AOAC, 1980). The nitrogen content was multiplied by a factor of 6.25 to obtain crude protein content. 3.2.2.1 Reagents

<u>0.01 N hydrochloric acid</u>: 0.89 ml of concentrated
A.R. HCl was dissolved in distilled water and volume was made
to 100 ml.

ii) <u>30% sodium hydroxide</u>: 150 gm of sodium hydroxide pellets were dissolved in 500 ml of distilled water.

iii) <u>2% boric acid</u> : 10 g of boric acid (crystals) dissolved in 500 ml of boiling distilled water.

iv) <u>Mixed indicator</u>: Mixed indicator was prepared by dissolving 0.1 g of bromocresol green and 0.1 g of methyl red in 100 ml of 95% alcohol, separately. Ten parts of bromocresol green solution and 2 parts of methyl red solution were mixed together and transferred a bottle provided with a dropper.

3.2.2.2 Procedure

Exactly 2.0 q of onion powder was weighed and transferred to 250 ml Kjeldahl flask. To this, 25 ml of concentrated H_2SO_4 were added and flask was placed on heating unit in the digestion chamber. Initially, the flask was heated gently over a low flame until the initial frothing ceased. Heating was continued for about an hour or more until the colour of of the digest was clear and pale blue. The flask was cooled and contents were transferred to 100 ml volumetric flask. Blank digestion was carried out similarly without the sample. An aliquot (5 ml) of the digested material was distilled with 10 ml of 30% NaOH. The ammonia liberated was collected in 5 ml of 2% boric acid to which 4 drops of mixed indicator were previously added. It was titrated with standard 0.01 N HCl. The end point of titration was determined when the blue colour just disappeared.

The per cent nitrogen was calculated with following formula :

Nitrogen X =

	Sample Blank titer titer	x	Normality of HCl x 14 x	Volume made up of the digest	•••
22	Aliquot of the digest taken	x	Weight of the x sample taken	1000 X I	00

The content of crude protein was calculated as follows : Crude protein % = Nitrogen % x 6.25.

3.2.3 Crude fat

Fat content of onion was determined by Soxhlet extraction method (A.O.A.C., 1980).

Five grams of dried sample of onion was transferred to a thimble. The top of the thimble was pluged with a wad of
fat free cotton. Thimble was fixed into the fat extraction tube of a soxhlet appratus. The bottom of the extraction tube was attached to a Soxhlet flask.

Approximately 75 ml of anhydrous ether were poured through the sample in the tube into the flask. The top of the fat extraction tube was attached to the condenser. The water bath was regulated so that the ether drops continuously upon the sample without any appreciable loss.

At the end of the extraction period, the ether in the flask was evaporated on a steam bath at low heat, cooled and weighed.

The crude fat content of onion sample was calculated as follows :

% Crude fat = Wt. of the fat soluble material Wt. of sample x 100

3.2.4 Reducing sugars

Sugar content of onion samples was determined by Shaffer-Somogyi micro_method (A.O.A.C., 1980).

3.2.4.1 Reagents

i) <u>Copper sulphate solution</u> : 100 g of copper sulphate were dissolved in water and volume was made to 1000 ml.

<u>0.1 N potassium iodate solution</u>: 3.567 g of
potassium iodate (KIO₃) were dissolved in water and volume
was made to 100 ml.

iii) <u>Shaffer-Somoqyi carbonate reagent</u>: 25 g each of anhydrous sodium carbonate (Na_2CO_3) and Rochelle salt (Potassium-sodium tartarate) were dissolved in approximately 500 ml distilled water. To this, 75 ml of copper sulphate solution were added with stirring followed by addition of 20 gm of sodium bicarbonate. Then 5 g of potassium were added followed by addition of 250 ml of 0.1N KIO₃ solution and the volume was made upto 1 liter. The solution was filtered and stored overnight before use.

iv) <u>Iodide-potassium oxlate solution</u>: 2.5 gm potassium iodide (KI) and 2.5 g potassium oxalate $(K_2C_2O_4)$ were dissolved in water and diluted to 100 ml.

v) <u>0.005 N thiosulphate standard solution</u> : 0.124 mg of thiosulphate was dissolved in 100 ml distilled water.

vi) <u>2 N sulphuric acid solution</u> : 5.6 ml of concentrated H_2SO_4 were dissolved in distilled water and volume was made to 100 ml.

3.2.4.2 Preparation of sample

2.5 g onion sample was weighed and transferred to a 250 ml beaker. About 50 ml of water were added and the beaker was heated to boil. After cooling, the contents were transferred to a 250 ml volumetric falsk. To this flask, lead acetate solution was added, mixed well and allowed to stand for 10 min. The excess of lead was precipated using potassium oxalate solution. The extracts were filtered and the volume was made with distilled water to 250 ml.

3.2.4.3 Procedure

5 ml extract containing approximately 2 mg dextrose was pipetted into test tube. To this, 5 ml of Shaffer-Somogyi reagent were added and mixed well. The blank was prepared using 5 ml of water and 5 ml of Shaffer-Somogyi reagent. The tubes were capped with funnels were placed in a boiling water bath for 15 minutes. The tubes were then carefully removed without disturbing and were cooled in running water for 4 minutes To each tube, 2 ml of iodine oxalate solution were added carefully by the side of the tube followed by addition of 3 ml 2N H_2SO_4 . The contents were mixed thoroughly to ensure that all cuprous oxide was dissolved. The tubes were allowed to stand in cold water bath for 5 minute; The liberated iodine was

titrated with 0.005 N thiosulphate solution using starch as an indicator. The titer value of test solution was subtracted from that of blank and the amount of dextrose in 5 ml of solution was determined from table values of Shaffer-Somogyi equivalents (A.O.A.C., 1980).

3.2.5 Total sugar

25 ml filtrate obtained from 2.5 g of onion samples was taken in 50 ml volumetric flask. To this 5.0 ml hydrochloric acid was added and allowed to stand for 24 hrs at room temperature. The excess acid was neutralized with sodium hydroxide and the volume was made to 50 ml with distilled water. Suitable volume of aliquot was taken and the total invert sugars were determined in the same manner as reducing sugar. The amounts of reducing, non-reducing and total sugars were calculated follows :

 Reducing sugar % = mg of dextrose x volume made up x 100 5 x wt. of sample taken x 1000
Total = Calculated as in 1 above making the use of titer value obtained in the determination of total sugar after inversion.

3. Non-reducing = (% total sugar - % reducing sugar) x 0.95 sugar %

21

3.2.6 Total phenolics

The total phenols in onion were determined by the method of Swann and Hills (A.O.A.C., 1980).

3.2.6.1 Reagents

i) <u>Folin-Denin reagent</u>: 100 g of sodium tungstate (Na₂W0₄ 2H₂0) were dissolved in 750 ml of distilled water. To this 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid (H₃P0₄) were added and contents were refluxed for 2 hours. After cooling to 25° C, the volume was made to 1000 ml with water.

ii) Na₂CO₃ solution : 100 ml of hot water were added to 35 g of anhydrous sodium carbonate.

iii) <u>Standard tannic acid solution</u> : 100 mg of tannic acid per 100 ml distilled water.

3.2.6.2 Procedure

The sample was extracted as it was done for sugar estimation. 0.5 ml sample was mixed with 6.5 ml of distilled water in a test tube. To this, 0.5 ml Folin-Denin reagent was added, mixed well and exactly after three minutes, 1 ml of Na_2CO_3 solution (saturated) and 1.5 ml of distilled water were added. The tubes were centrifuged for fifteen minutes. Exactly after 60 minutes, the absorbance of the supernatant was measured at 630 nm. Total phenolic compounds were calculated from the standard curve prepared by using standard tannic acid.

Standard curve for total phenols

Standard tannic acid solution 0 to 10 ml was pipetted into 100 ml volumetric flasks containing 75 ml distilled water and 5 ml Folin-Denim reagent and 10 ml saturated NaCO₃ solution. The contents were mixed well and the absorbance was measured at 540 nm. A graph of concentration of tannic acid vs absorbance was prepared.

3.3 Enzymes

Assay of enzymes - For assay of enzyme activity, representative samples of onion bulbs were collected, chilled and cut into small pieces. These samples were used for the assay of amylases and proteases.

3.3.1 Amylases

The method given by Bernfed (1955) was followed for the assay of α - and β - amylases. The increase in reducing sugar was measured by the reaction with dinitrosalicylic acid reagent.

3.3.1.1 Reagents

i) 0.02 M phosphate buffer (pH 6.9) containing 0.0067 M NaCl

152.2 ml of 0.04 M Na_2HPO_4 and 97.5 ml of 0.04 M NaH_2PO_4 were mixed together and 1.96 g of NaCl was dissolved in it. The pH was adjusted to 6.9 and the volume was made upto 500 ml.

11) 0.016 M acetate buffer (pH 4.8)

20 ml of 0.032 M acetic acid and 30 ml of 0.032 M sodium acetate were mixed together, the pH was adjusted to 4.8 and the volume was made upto 100 ml.

iii) 0.05 M tris-HCl buffer (pH 7.5) containing 0.005 M cysteine hydrochloride

50 ml of 0.02 M tris-hydroxymethyl amino methane and 40ml of 0.2 M HCl were mixed. To this 0.1576 g of cysteine hydrochloride was added, the pH was adjusted to 7.5 and the volume was made upto 200 ml with distilled water.

iv) Soluble starch

One gram of soluble starch was dissolved in 100 ml of 0.02 M phosphate buffer (pH 6.9) containing 0.0067 M NaCl for C-amylase. For assay of β -amylase, 1 g of soluble starch was dissolved in 100 ml of 0.016 M acetate buffer (pH 4.8).

v) Dinitosalicylic acid :

One gram of 3, 5 dinitrosalicylic acid was dissolved in 20 ml of 2N NaOH and 50 ml of distilled water. Thereafter, 30 gm of sodium potassium tartarate were dissolved in it. The volume was made upto 100 ml with distilled water.

3.3.1.2 Procedure

Five grams of chilled onion cuts were ground in chilled pestle and morter with 25 ml of 0.05 M tris-HCl buffer (pH 7.5) containing 0.005 M cysteine hydrochloride. The homogenate was centrifuged at 12,000 g for 20 minutes and the supernatant was used as a source of enzyme.

3.3.1.3 🖍 -- amylase

A portion of the supernatant was heated at 70°C for 15 minutes as described by Bernfed (1955) and 1 ml of this was incubated with 1 ml of soluble starch at 37°C. The reaction was stopped exactly after 15 minutes by addition of 2 ml of dinitrosalicylic acid reagent. The mixture was heated in boiling water for 5 minutes and then cooled under running tap water. The red-brown reduction product was diluted by addition of 21 ml of distilled w MPKV LIBRARY clour was read at 540 nm.

A calibration curve established water standard maltose (0.2 to 2.0 mg in 2 ml of distilled water) was used to convert T-1955



the colorimeter reading into mg of maltose. The activity was expressed as mg of maltose. The activity was expressed as mg of maltose liberated per mg of protein.

3.3.1.4 B-amylase

One ml of the remaining portion of enzyme extract, which was brought to pH 4.8, was incubated with 1 ml of the soluble starch at 37° C and assayed in the same way as described under $\sqrt[6]{-amylase}$.

The activities of \propto -amylase and β -amylase were expressed as mg of maltose liberated per mg of protein.

<u>Standard curve for maltose</u> - Standard maltose solution was prepared by dissolving 1 g maltose in 100 ml. From this stock solution, 10 ml was diluted to 1000 ml. This solution contained 1 mg maltose per ml for standard curve, different concentrations of maltose (1, 2, 4 and 6) were taken.

3.3.1.5 Protein content of enzyme extract

The protein content of enzyme extract was determined by Folin-Ciocalteu method (Lowry, et al., 1951).

3.3.1.5.1 Reagents

 Alkaline sodium carbonate solution (20 g Na₂CO₃ in 0.1 ml NaOH).

- 2. Copper sulphate Sodium potassium tartarate solution (5 g CuSO₄ 5 H₂0 in 10 g Na, K tartarate).
- 3. Alkaline copper reagent Alkaline copper solution was prepared by mixing 50 ml of (1) and 1 ml of (2) just before use.
- Folin-Ciocalteau reagent Commercially available reagent was diluted with an equal volume of water.
- 5. Standard bovine serum albumin.

3.3.1.5.2 Procedure

5 ml of alkaline copper reagent were added to 1 ml of the test solution, mixed throughly and allowed to stand at room temperature for 10 minutes. To this, 0.5 ml of diluted Folin-Ciocalteau reagent was added. After 30 minutes, the colour was read against the appropriate blank at 750 nm.

The standard curve was prepared using different concentrations of bovine serum albumin (1 to 10 µg/ml). The protein content of enzyme extract was calculated from the above curve.

3.3.2 Proteases

The two proteases (active at pH 5.5 and pH 7.0) were assayed by the method of Beevers (1968) using casein as a substrate. The TCA-soluble hydrolysate was subjected to amino acid estimation by ninhydrin method (Rosen, 1957).

3.3.2.1 Reagents

i) 0.05 M tris-HCl buffer containing 0.005 M cysteine hydrochloride (pH 7.5)

50 ml of 0.2 M tris-(hydroxymethyl) amino methane and 40 ml of 0.2 M HCl were mixed thoroughly. To this 0.1576 g of cysteine hydrochloride was added, the pH was adjusted to 7.5 and the volume was made upto 200 ml with distilled water.

ii) 0.2 M citrate buffer (pH 5.5)

14.8 ml of 0.4 M citric acid and 35.2 ml of 0.4 M sodium citrate were mixed, the pH was adjusted to 5.5 and the volume was made upto 100 ml with distilled water.

iii) 0.2 M phosphate buffer (pH 7.0)

152.5 ml of 0.4 M Na_2HPO_4 and 97.5 ml of 0.4 M NaH_2PO_4 were mixed, pH adjusted to 7.0 and the volume made to 500 ml.

iv) 1% Casein

1 g of casein suspended in 50 ml of 0.01 N NaOH. The pH was adjusted to 7.0 with the help of 0.1 N HCl added drop by drop and the volume was made upto 100 ml with distilled water.

v) 20% trichloroacetic acid (TCA)

20 gm of trichloroacetic acid was dissolved in 100 ml of distilled water.

3.3.2.2 Procedure

Seven grams of onion cuts were homogenised in 35 ml of 0.05 M tris HCl buffer (pH 7.5) containing 0.005 M cysteine hydrochloride. The homogenate was filtered through muslin cloth and centrifuged at 12,000 g for 20 minutes. The whole operation was carried out in cold.

One ml of the supernatant was incubated with 1 ml of 1 per cent casein and 1 ml of 0.2 M buffer, pH 5.5 (citrate) or pH 7.0 (phosphate). The reaction mixture was incubated at $37^{\circ}C$ for 90 minutes. The reaction was terminated by addition of 1 ml of 20 per cent TCA. After 1 hour the precipitate was filtered through Whatman No.40 filter paper and the filtrate was subjected to the determination of amino nitrogen by ninhydrin procedure (Rosen, 1957) usually 0.1 ml of the filtrate was sufficient for determination of amino nitrogen.

The protein content of the enzyme extract used in the assay was estimated by Folin-Ciocalteu method of Lowry et al. (1951) by using bovine serum albumin as standard.

The activity of both the proteases was expressed as Au moles of amino acid (expressed in terms of alanine) formed per mg of protein and corrected to zero time control. CHREAT ONE MENT

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

An experiment was conducted at the central campus of Mahatma Phule Agricultural University, Rahuri, to study the biochemical changes in the two improved varieties of onion (N-2-4-1 and N-53) during storage upto two months at the ambient conditions. The biochemical changes were recorded at an interval of every fifteen days starting from the date of curing. The results obtained are presented and discussed in this section.

4.1 Dry matter

The average dry matter content of two cultivars of onion during two months storage at room temperature is presented in Table 1 and Fig.1. The dry matter contents in the freshly cured onion varieties viz. N-2-4-1 and N-53 were 14.12 and 12.90%, respectively. The dry matter content in both the varieties decreased gradually during storage for 60 days i.e. from 14.12 to 11.70% in case of cultivar N-2-4-1 and from 12.90 to 10.90% in N-53. The variety N-2-4-1 contained higher dry watter than in N-53 at all the stages during storage.

The values of dry matter content of onion as reported by Nilson (1980) varied from 12.32 to 13.26 per cent, Bajaj et al. (1980) reported that 20 onion cultivars differed remarkebly in their dry matter content, ranging from 7.66 to 14.46 per cent. In the present study, the cultivar N-2-4-1, which has better keeping quality, was found to contain higher level of dry matter than in N-53 throughout the storage period.

Table 1 Changes in the content of dry matter during storage (%) of onion

Cultivar	Storage (days)					
	0	15	30	45	60	
N-2-4-1	14.12	13,90	13.54	12.00	11.70	13.05
N-53	12.90	12.56	12.00	11.54	10.90	11.98
Mean	13.51	13.23	12.77	11.77	11.30	

S.E.	for cultivars <u>+</u>	0.654
C.D.	at 5%	N.S.
S.E.	for storage stages <u>+</u>	0. 860
C.D.	at 5%	N.S.



Fig.1. Changes in dry matter contents of two onion cultivars during storage.

4.2 Crude proteins

The protein content of two cultivars of onion is given in Table 2 and in Fig.2. Both varieties under study significantly differed in their protein content. The fresh cured bulbs of varieties N-53 contained more protein (8.75%) than in N-2-4-1 (5.93%). In both the varieties, there was a gradual increase in the protein content with increase in the duration of storage period. The cultivar N-53 had consistantly higher protein content than in N-2-4-1 throughout the storage period.

The values of the protein content of onion obtained in the present study compared well with those reported by Ketiku (1976) and Magdum (1981). The low protein content onion has been shown to be correlated with the better keeping quality (Toul and Pospisilova, 1966). The onion cultivars having good keeping quality (N-2-4-1) had comparatively lower average protein content than in N-53.

Singh and Kumar (1969) observed increase in the total 'N' during storage. Similar observations were recorded by Carlous (1963), Peterson et al. (1960) and Karmarkar and Joshi (1941).

Table 2 Changes in the content of crude protein (%) during storage (on dry weight basis) of onion.

Cultivar		Mean				
	0	15	30	45	60	
N-2-4-1	5.93	6.35	7.00	7.30	7.34	6.78
N-53	8.75	9.00	9.19	9.50	9.63	9,21
Mean	7.34	7.67	8.10	8.40	8.48	

S.E.	for cultivars <u>+</u>	0.320
C.D.	at 5%	0.74
S.E.	for storage stages <u>+</u>	0.277
C.D.	at 5%	N.S.



Fig. 2. Changes in the protein contents of two onion cultivars during storage.

4.3 Crude Fat

The changes in the crude fat contents of two cultivars on onion are given in Table 3 and Fig.3.

The crude fat contents of the fresh cured onion bulbs were 0.58 and 0.66 per cent, for N-53 and N-2-4-1, respectively. The cultivar N-2-4-1 recorded the significantly higher average crude fat content as compared to N-53 at all stages during storage. The fat content of onion bulbs gradually decreased during storage.

The higher crude fat content of onion bulbs has been correlated with their better keeping quality (Aksoy, 1983). The onion cultivar (N-53) having less content of crude fat has comparatively poor keeping quality. The values of the crude fat content of onion obtained in the present study compared well with those reported by Khade (1984).

4.4 Sugars

4.4.1 Reducing sugars

Changes in the reducing sugar contents of two cultivars of onion during two months storage are presented in Table 4 and Fig.4.

Table 3	Changes in the	content of curde	fat (%)
	during storage	(on dry weight ba	asis) of onion.

Cultivar	Storage (days)					
	0	15	30	45	60	
N-2-4-1	0.66	0.64	0 .59	0.58	0.56	0.61
N-53	0.58	0.55	0.55	0.53	0.50	0.54
Mean	0.62	0.59	0.57	0.55	0.53	

S.E.	for cultivars 🛨	0.0002
C.D.	at 5%	0.001
S.E.	for storage stages <u>+</u>	0.0001
C.D.	at 5%	0.003

Fig. 3. Changes in the crude fat contents of twoonion cultivars during storage.

Table 4 Changes in the content of reducing sugars (%) during storage of onion (on dry weight basis) of onion

,

Cultivar		Storage (days)					
	0	15	30	45	60		
N-2-4-1	18.20	21.53	26.35	22.22	16.35	20.93	
N-53	17.03	19.20	23.16	20.30	13.85	18.71	
Mean	17,61	15.36	24.75	21.26	15.10		

S.E.	for cultivars	±	2.212
C.D.	at 5%		N.S.

S.E.	for storage	stages	±	1.729
C.D.	at 5%			4.444

Fig. 4. Changes in the contents of reducing sugars of two onion cultivars during storage.

The average reducing sugar contents (on dry weight basis) in freshly cured bulbs of two cultivars of onion, N-2-4-1 and N-53, were 18.20 and 17.03 per cent, respectively.

The reducing sugar content of onion bulbs did not differ significantly within the cultivars during the entire period of storage. However, the cultivar N-2-4-1 had consistently higher reducing sugar content as compared to N-53 at all stages of storage.

In both the varieties of onion, the reducing sugar content increased upto 30 days of storage and decreased thereafter. Rutherford (1981) also observed increase in reducing sugar, glucose and fructose during storage of onion at 4° C and 70% relative humidity. The decrease in reducing sugar, during prolonged storage of onion may be attributed to their probable utilization during respiration at high temperature storage (Gorin, 1981).

4.4.2 Non-reducing sugars

The non-reducing sugar contents of freshly cured onion bulbs of N-2-4-1 and N-53 varieties were 16.10 and 16.00 per cent, respectively (Table 5). There was a gradual decrease in the non-reducing sugar contents of both the varieties during storage (i.e. from 16.05 to 13.76%) for two months. There was, however, no significant difference between

Cultivar		Storage (days)					
	0	15	30	45	60		
N-2-4-1	16.10	15.00	14.97	13.70	14.77	14.90	
N-53	16.00	15.39	14.97	13.82	13.82	14.80	
Mean	16.05	15.19	14.97	14.20	13.76		
	S.I	S. for cu	ltivars	±	().56 7	
C.D. at 5%				N.S.			
	S.1	. for st	orage sta	ages <u>+</u>	0.	104	

C.D. at 5%

Table 5 Changes in the content of non-reducing sugars(%) during storage (on dry weight basis) of onion

.

.

38

0.269

the two varieties in non-reducing sugar content at any of the stages during storage.

Karmarkar and Joshi (1941) and Yamaguchi et al. (1957) did not observe significant differences in the non-reducing sugar content of the onion cultivar during the entire period of six month storage at room temperature. These observations are in conformity with the results of the present study.

4.4.3 Total sugar

Changes in total sugar content of two cultivars of onion during two month storage are presented in Table 6. The average total sugar content on dry weight basis in freshly cured onion bulbs was 34.30 per cent for N-2-4-1 and 33.03 per cent for N-53. In both the two varieties of onion, the total sugar content increased upto 30 days of storage and decreased thereafter.

The total sugar content of onion has been reported to be positively correlated with the ratio of sucrose to monosaccharide and the keeping quality of the cultivars during storage (Butarin, 1958). In the present study the cultivar N-2-4-1, which has relatively better keeping quality, also had higher total sugar content than that of N-53.

Cultivar	Storage (days)					
	0	15	30	45	60	
N-2-4-1	34.30	36.53	41.14	36.32	30.05	35.67
N-53	33.03	34.59	38.13	34.60	27.67	33.60
Mean	33,66	35.56	39.63	35.46	28.86	

Table 6 Changes in the content of total sugars(%) during storage (on dry weight basis) of onion.

S.E. for cultivars	±	2.21
C.D. at 5%		N.S.
S.E.for storage stages	±	1.62
C.D. at 5%		1.85

4.5 Total phenolics

The freshly cured bulbs of onion varieties N-53 and N-2-4-1 contained 0.85% and 1.05% total phenolics, respectively (Table 7 and Fig.5). The two cultivars of onion N-2-4-1 and N-53, did not differ significantly with respect to the contents of phenolic compounds. However, the cultivar N-2-4-1 contained relatively higher amounts of phenolic compounds than in N-53 during different stages of storage.

The total phenolic content of both cultivars of onion increased gradually during the storage. Bajaj et al.(1981) reported that the total phenolics content of onion cultivars varied from 1.75 to 2.95 per cent.

4.6 Amylases

The activity of \mathcal{K} -and β -amylases of onion cultivars increased significantly during the storage. The activity of \mathcal{K} -amylase increased during storage from 0.26 to 0.47 and from 0.25 to 0.45 mg maltose/mg protein in N-2-4-1 and N-53 cultivars, respectively (Table 8). Similarly, the activity of β -amylase increased from 0.26 to 0.45 and 0.25 to 0.47 mg maltose/mg protein in N-2-4-1 and N-53 cultivars, respectively (Table 9). There was no significant difference between the varieties with respect to the activity of both \mathcal{K} -amylase and β -amylase at any stage during storage. Table 7 Changes in the content of total phenolic compounds (%) during storage (on dry weight basis) of onion

Cultivar		Storage (days)							
<u> </u>	0	15	30	45	60				
N-2-4-1	1.05	1.35	1.85	2.25	2.40	1.78			
N-53	0.85	1.00	1.37	1.70	2.00	1.38			
Mean	0.95	1.17	1.61	1.87	2.20				

S.E.	for cultivars	<u>+</u>	0.228
C.D.	at 5%		N.S.
S.E.	for storage stages	±	0.405
c.D.	at 5%		N.S.

Fig.5. Changes in the contents of total phenolic compounds of two onion cultivars during storage.

Cultivar		Storage (days)					
	0	15	30	45	60		
N-2-4-1	0.26	0,35	0.38	0.42	0.47	0.376	
N-53	0.25	0.33	0.37	0.43	0.45	0.366	
Mean	0.255	0.34	0.375	0.425	0.46		

Table 8 : Changes in the activity of ∞ -amylase during storage of onion

S.B.	for cultivars	±	0.109
C.D.	at 5%		N.S.
s.e.	for storage stages	±	0.001
C.D.	at 5%		0.003

Cultivar	Storage (days)						
	0	15	30	45	60		
N-2-4-1	0.26	0.34	0.38	0.40	0.45	0.365	
N-53	0.25	0.35	0.40	0.42	0.47	0.378	
Mean	0.255	0.345	0.39	0.41	0.46		

Table 9.	Changes in the activity of B-amylase
	during storage of onion

S.E.	for cultivars	±	0.049
C.D.	at 5%		N.S.
S.E.	for storage stages	±	0 .001
C.D.	at 5%		0.003

4.7 Proteases

The activity of two proteases (one active at pH 5.5 i.e. acid protease) and other active at pH 7.0 (neutral protease) of onion during storage did not differ significantly in both cultivars of onion (Tables 10 and 11).

The activity of both the proteases increased significantly during storage. The rate of increase was more with acid protease than neutral protease. The acid protease actively increased from 0.47 to 1.50 and 0.44 to 1.54 A moles of alanine equivalent per mg protein at the end of 60 days storage in N-2-4-1 and N-53 cultivars, respectively (Table 10). The corresponding values for neutral protease activity were 0.40 to 1.03 and 0.42 to 1.00 A moles of alanine equivalent per mg protein.

There was no significant differences between the varieties with respect to the activity of protease (acid as well as neutral) at any stage during storage. The interaction of the changes in protease activity during storage of onion is not available in the literature.

Table	10	Changes	in	the a	activity	of	protease
		at pH 5	.5	durinç	g storage	of	Onion

Cultivar		Mean				
	0	15	30	45	60	
N-2-4-1	0.47	0.75	0.98	1.16	1.60	0.972
N-53	0.44	0.70	1.00	1.22	1,58	0.988
Mean	0.455	0.725	0 .9 9	1.19	1,54	

S.E.	for cultivars ±	0.264					
c.D.	at 5%	N.S.					
S.E.	for storage stages <u>+</u>	0.004					
c.Đ.	at 5%	0.012					
Cultivar		Storage (days)					
----------	------	----------------	-------	-------	------	---------	--
	0	15	30	45	60	<u></u>	
N-2-4-1	0.40	0.48	0.71	0.86	1.03	0.969	
N-53	0.42	0.57	0.70	0.85	1.00	0.708	
Mean	0.41	0.575	0.705	0.855	1.15		

Table 11	Changes in	the activity of	protease
	at pH 7.00	during storage	of onion

•

S.E. for cultivars ±	0.155
C.D. at 5%	N.S.
S.E. for storage stages ±	0.001
C.D. at 5%	0.005

Among the various factors responsible for spoilage of onion during storage, sprouting has been shown to be an important factor. Several biochemical changes occur during sprouting. These include conversion of polysaccharide into simple sugars and hydrolysis of proteins into amino acids. During storage, the activities of both enzymes increases steadily. In the present investigation, the activities of both amylases and proteases were negatively correlated with good keeping quality of onion during storage.

CHREAT ONE MENT

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Ah experiment was conducted to study the biochemical changes in onion bulbs during storage at the Central Campus of Mahatma Phule Agricultural University, Rahuri. The main findings of the study are as follows.

a) The dry matter content of onion decreased steadily during storage of two months from curing. It decreased from 14.1 to 11.7% and 12.9 to 10.9% in N-2-4-1 and N-53, respectively.

b) The crude protein content of onion increased during storage for two months from curing i.e. from 5.93 to 7.34 % in case of cultivar N-2-4-1 and 8.75 to 9.63 % in N-53.

c) The crude fat content of onion steadily decreased during storage from 0.66 to 0.56% in case of cultivar N-2-4-1 and from 0.58 to 0.50% in N-53.

d) The reducing sugar content of onion increased upto thirty days of storage and decreased thereafter upto sixty days. In the cultivar N-2-4-1,it decreased from 18.20 to 16.35% while in N-53 it decreased from 17.03 to 13.85%. e) The non-reducing sugar content of onion decreased steadily during entire period of storage. It decreased from 16.10 to 13.70% in the cultivar N-2-4-1 and from 16.00 to 13.82% in N-53.

f) Like reducing sugars, the total sugar content increased upto thirty days of storage and then decreased. During entire period of storage it decreased from 34.30 to 30.5% and 33.03 to 27.67% in N-2-4-1 and in N-53, respectively.

g) The total content of phenolic compounds increased steadily during entire period of storage. It increased from 1.05 to 2.40% in the cultivar N-2-4-1 and from 0.85 to 2.00% in the cultivar N-53.

h) Activities of both the \mathcal{A} - and β -amylases increased steadily during the entire period of storage. The activity of -amylase increased from 0.26 to 0.47 in case of N-2-4-1 and from 0.25 to 0.33 mg maltose/mg protein in N-53. The activity of β -amylase increased from 0.26 to 0.45 and from 0.25 to 0.47 mg of maltose/mg of protein in the cultivars of N-2-4-1 and N-53, respectively.

i) The protease activity increased steadily during the entire period of storage. The acid protease activity increased from 0.47 to 1.50 and from 0.44 to 1.58 (/u moles of alanine/mg of protein) in cultivars N-2-4-1 and N-53, respectively. The neutral protease activity increased from 0.40 to 1.03 and from 0.42 to 1.00 (/u moles of alanine/mg of protein) in the cultivars N-2-4-1 and N-53, respectively.

CHREAT ONE MENT



6. LITERATURE CITED

- *Aksoy, H.A. 1983. Investigation of the essential oils of onion. Res. J. Technology. University Istambul, Turkey, 177(1):34-36.
 - A.O.A.C. 1980. Official Methods of Analysis. Association of Official Analytical Chemists. 11th Edn. Washington, DC.
 - Bagul, K.M. 1984. Studies on post-harvest behaviour of some onion (<u>Allium cepa</u>) cultivars. M.Sc.(Agri.) thesis, MPAU, Rahuri.
 - Bajaj, K.L., Kaur, G. and Singh, J. 1981. Lachrometry factor and other chemical constituents of some varieties of onion. J. Plant Food. 3(3):190-203.
 - Bajaj, K.L., Gurdeep, K., Singh, J. and Gill, S.P.S. 1980. Chemical evaluation of some important varieties of onion. Quat. Plant/Plant & Food Hum., Nutr. 30,117.
 - Beevers, L. 1968. Protein degradation and proteolytic activity in the cotyledon of germinating pea seed (<u>P. sativum</u>). Phytochemistry. 7(7):1837-44.
 - Bennett, 1941. The effect of the storage on the carbohydrates of the onion. American Soc. Hort.Sci. 39, 293.4.

- Bernfed, P. 1955. In "Methods in Enzymology" (S.P. Colowick and N.V. Kaplan, eds) Vol.1 Acad. Press, New York, P.149.
- Bhati, B.H. and Ashgar, N. 1965. Certain dehydration studies on Pakistan onion composition and changes in various constituents, Agric. Pakistan. 16(3):299-306.
- Bordia, P.C., Smilot, M.M. and Kaur, S. 1973. Growth and chemical composition of eleven varieties of onion. Hort. Madras Agril. Journal Abs. 43(1):37-321.
- Bulb, C.E., Richardson, D.G. and Manson, M.S. 1979. Preharvest foliar deciccation and onion storage quality, J. American Soc. of Hort. Sci. 104(6): 773-777.
- Butarin, B.V. 1958, Utilizing refractometer in the selection of farms onion suitable for keeping. Plant Breed. Abst. (1960), 30(1):185-193.
- Chang, W.N. 1981. Storability of onion and it's improvement. Plant Breed. Abstr. 51(3):235.
- F.A.O., FAO production year book Vol.34, Food & Agriculture Organization, Rome. (1982).
- Foskett, R.L. and Peterson, C.E. 1950. Relation of dry matter content to storage ability in some onion varieties and hybrids. Proc. of Am. Soc. of Hort. Sci. 55:314-318.

Garvisheva, I.F. and Orlava, K.B. 1980. Evaluation of initial material of onion for chemical composition during growth, ripening and storage. Plant Breed. Abstr. 50(9):70 (8205).

Gopalan, C., Rama Sastri, B.V. and Balasubramanium, S.C. 1983. "Nutritive Value of Indian Foods", National Institute of Nutrition (I.C.A.R.), Hyderabad.

Gorin, N., and Borcsok, S. 1981. Chemical composition of stored onion, cultivar hydro as a criterion of freshness, Hort. Abstr. 51(6):397.

- *Hadocova, M.A. and Klozova, L. 1981. The screening of the enzyme and isoenzyme pattern in seed of <u>Allium</u> ceps, Biologia Plantarum, 23(6):442-448.
- *Hadokova, N.L. and Ito, M.N. 1968. Some biological changes in vegetable during storage. Hort. Abstr. J.(11): 764 (8284).
- *Herrmann, K. 1976. Contents and localization of a phenolic constituents in vegetables, Qualita Plantarum Plant Foods Hum. Nutr. : 231-246.
 - Hassanpour, M. 1983. Influence of nitrogen fertilizers, irrigation and preharvest foliar application of malic hydrazide on postharvest behaviour of onion (<u>Allium cepa</u>, L.) bulbs under several storage conditions. Ph.D. Thesis, M.P.A.U., Rahuri.
 - Jones, H.A. and Mann, L.K. 1963. Onion and Their Allies, Leonard Hill (Books), London.

*Joslyn, U. and Peterson, A.N. 1958. Reddening of white onion bulbs. J. Agric. Food Chem. 6, 754-65.

- Karmarkar, D.V. and Joshi, B.M. 1941. Investigation on the storage of onions. Indian J. Agric. Sci. 11:82-84.
- Khade, U.M. 1985. Effect of different ratios and forms of N, P, K fertilization on the yield, qualitv and post-harvest behaviour of orion. M.Sc.(Agri.) Thesis, M.P.A.U., Rahuri.
- *Khodzhaeva, M.A. 1979. Carbohydrates of <u>Allium</u> species, isolation and characteristics of polysaccharides, Khima Priridyka Soedieni, USSR 2, 137-142.
- Ketiku, A.L. 1976. Chemical composition of Nigerian Onion, Food Chemistrv. 1(1):41-47.
 - Kodic, B. 1971. Comparative cold storage trial of onions exposed to the air and crates. Hort. Abstr.41(1): 173.
 - Link, K.P. and Walkar, J.C. 1933. The isolation of catechol
 from pigmented onion and it's significance in
 relation to diseases in onion. J. Biol. Chem.10(2):
 379.
- *Lowry, O.H., Rosebrough, N.I., Farr, A.C. and Randall, R.J. 1951. Protein measurements with Folin-phenol reagent. J. Biol. Chem. 193, 265-75.

- Macollum, G.D. 1971. Heritability and genetic correlation of soluble solids, bulb size and shape in white spanish onion, Canadian J. Genet. Cyt. 10:508-14.
- Magdum, S.B. 1981. Genetic diversity of onion (<u>Allium cepa</u> L.) germplasm with special reference to storage guality. M.Sc.(Agri.) Thesis, M.P.A.U., Rahuri.
- Malkki, Y. and Nikkila. 1978. The composition and aroma of onion and influencing factors. Vegetables J. Sci. Agric. Soc. Finland. (798):50(2):103-104.
- *Mihicci, K. 1983. Determination, localization and heat activation of peroxidase in some vegetables. Food Sci., Central Food Research Institute, Budapest, Hungery.
- *Nilson, T. 1980. The influence of the time of harvest on the chemical composition of onions. Swedish J. Agric. Res. 10(2):77-88.
 - Polascsck-Recz, M. Possar-Hajnal. 1976. Determination of pectinmethylesterase, polygalacturase and pectic substances in some fruit and vegetable J. Acta -Alimataric. (1976). 5(3):189-204.
- *Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67, 10-17.
 - Rutherford, P.P., 1981. Some biological changes in vegetables during storage, Hort. Abstr. (11):764,(8254).

- Rutherford, P.P. and Whittle, R. 1984. Methods of predicting the long term storage of onions. J. Hort. Sci. 1984. 59(4):537-543, Bath Univ. Bath BAZ, 7AY, UK.
- Saxena, G.K., Halsay, L.H., Gull, D.D. and Prasad, N. 1974. Evaluation of onion and carrot cultivars for commertial production in Guyana, Sci. Hort. 2,257.
- Singh, K. and Kumar, S. 1969. Morphological and biochemical changes in stored onion as influenced by nitrogen and phosphorus fertilization. Plant Sci. 1969:1:181-188.
- Selby, C. and Galpin, I.J. 1979. Comparison of onion plant and onion tissue culture, New Pathologist. 83(2): 351-359.
- Toul, V. and Pospisilova, P. 1968. The chemical composition of onion varieties. Plant Breed. Abstr. 38(3):679 (5456).
- Yamaguchi, M., Pratt, H.K. and Harris, L.L. 1975. Effect of storage temperature on keeping quality and composition of onion bulbs and on subsequent darkening of dehydrated flakes. Proc. Am. Soc. Hort. Sci. 69, 421-246.
 - Zaceva, Z. and Minkov, I. 1965. The results of intervarietal hybridization of onion (<u>Allium cepa</u> L.) Plovdiv.14 (2):105-14 (Pl.Breed. Abstr. 1966, 36(3):5131).

* Original not seen.



CHREAT ONE MENT



7. VITA

KANTILAL R. MARMAT A Candidate for the degree of MASTER OF SCIENCE (Agriculture)

Thesis	3	Biochemical Changes in Onion Bulbs (<u>Allium cepa</u> L.) During Storage".
Major field	1	Agricultural Biochemistry
Biographical information		
- Personal data	3	Born at Kasampura, District- Jalgaon, on 25th August,1962, unmarried, son of Late Shri. Ramlal Malharsing Marmat, of Kasampura, Dist. Jalgaon.
- Educational data	:	Attended S.S.C. in S. B. High- School, Aurangabad and H.S.C. in Deogiri College, Aurangabad, received the Bachelor of Science (Agriculture) degree from Mahatma Phule Agricultural University, Rahuri, in July, 1984.

<u>T-1955</u>

