EFFECT OF GERMINATION, COOKING, CANNING AND STORAGE ON THE NUTRIENT COMPOSITION OF FOUR VARIETIES OF LENTILS

(*Lens esculenta* MOENCH)

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ABSTRACT

Various parameters (physical and biochemical) of the dry seeds of four varieties of lentils (Lens esculenta Moench) : two Syrians, one Jordanian, and one American were studied as were those of seedlings grown for up to 5 days in the dark (25 C and 35 C) and in the light (25 C).

The nutrients studied were : carbohydrates, lipids, crude fibre, proteins, and the vitamins: vitamin C, thiamine, and riboflavin. On the whole, there were no wide differences in nutritional values between the four lentil varieties studied.

1. Dark-grown seedlings (25 C) were found to be nutritionally better in retaining their vitamins than those grown at 35 C, and also than those grown at 25 C in the light. On the other hand, light-grown seedlings were nutritionally better in terms of protein quantity.

2. The four varieties of lentil seeds were also home processed (soaked in water and cooked for 1 h at 100 C). Total available carbohydrates, individual soluble sugars, free amino acids, and the three vitamins decreased significantly, lipids and proteins decreased slightly, while crude fibre increased significantly after home processing. Two chemicals, ammonium carbonate and trisodium phosphate, added in the soaking solution significantly decreased cooking time.

3. The lentil seeds were also canned. Most nutrients studied decreased after canning.

4. Storage of the canned products for 1 year at 25 C caused further losses in total available carbohydrates, proteins, free amino acids, and the 3 vitamins, while crude fibre again increased. Storing the canned products at 35 C for one year did affect the various nutrients but the decreases were not all that

different from those stored at 25 C.

5. Parallel studies using scanning electron microscopy showed changes in the cellular structure of germinated, cooked, and canned lentil seeds. During germination, starch granules change their shape, become porous and easily breakable, and the gaps between starch granules become larger. In cooked lentils, the cells become more separate, and starch granules change their shapes. In canned seeds, starch granules become rough and show a loss of birefringence.

6. These studies have shown that compared to seeds germinated under the different conditions, dry unprocessed seeds are the more nutritious.

It has been shown that canned seeds too can offer certain advantages nutritionally. However, they are more costly to produce than are dry seeds. Therefore, the home processing of lentil seeds is very beneficial nutritionally in a diet sadly lacking in carbohydrates, in proteins, and in vitamin C, in thiamine, and in riboflavin.

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ABBREVIATIONS

Control	Con
Canning	Can
Food and Agriculture Organisation of the United Nations	FAO
Fructose	FRU
Glucose	GLU
International Centre for Agriculture Research in the Dry Areas	ICARDA
Retention	Ret
Raffinose	RAF
Stachyose	STA
Sucrose	SUC

Varieties of lentils used:

var. American	AMR
var. Jordanian	JOR
var. Large	LAG
var. Small	SML

Temperatures are written : 100 C for 100 °C, 25 C for 25 °C etc.

GENERAL INTRODUCTION

FAO (1974) has reported that 10-15% of the World are undernourished and up to half of them suffer hunger or malnutrition or both. It has been estimated that nearly two billion people do not have enough to eat and that perhaps as many as ten thousands die every day either from lack of food or from lack of enough protein and vitamins to prevent malnutrition (POTTER, 1978; BERRY, 1981). ADAMS et al. (1978) have reported that edible legume seeds have the highest protein content of all commercial crops and on a worldwide basis may provide one of the most important sources of protein for human food.

There is a necessity for protein-rich food to meet an increasing world population. Legume seeds form the major part of protein-rich food, and are less expensive than non-legumes, e.g. cereals. LITZEN-BERGER (1973) has reported that about two thirds of the World, the poor countries consume only half of the World's protein, and this is mostly cereal protein. The nutritional deficiencies are most serious within developing countries, diets are generally deficient in protein quantity and quality because of the prevailing food consumption pattern and preferences. Many of the legumes grown in tropical areas are potentially a good protein source. However, food supply is not keeping pace with population growth, which is increasing at an enormous rate especially in Third World countries. The consumption of food legume is to be encouraged and therefore there is a need for Scientific and Technological research to achieve that goal. ROCKLAND and RADKE (1981) have reported that legumes require less energy per unit protein than cereal grains, and although many of the legume proteins have lower nutritional qualities than beef muscle protein, yet, up to ten times as much energy is required to produce the same amount of animal protein.

The Protein Advisory Group (PAG) have issued the following statement "Upgrading Human nutrition through the Improvement of

Food Legumes" (MILNER, 1974). PAG recommends urgent research and attention to legumes. World production of grain legumes is falling when compared to cereals, mainly due to considerably greater yields obtained with the latter.

Governments and International organisations are therefore continuing in encouraging the production of legumes.

Legumes have been the subject of intense investigations for many years due to their increasing importance as a human food source, and after a long time of relative neglect, they are again receiving attention as dry legume products and as legume sprouts (BATES et al., 1977). HENRY et al. (1982) have reported that germination of seeds for 72 h improves the nutritive value of beans. Also, preliminary studies have shown that sprouts may be used as raw material for the preparation of some food ingredients (POMERANZ et al., 1977). At the 40th meeting of the Institute of Food Technologists, VANDERSTOEP (1981) stated that it was still not clear whether temperature or light affected the protein content of germinated legume seeds. Therefore, more research is needed to study the composition of different kinds of sprouts and the best way to prepare them. The intestinal discomfort of flatulence which follows the consumption of legumes, e.g. beans and peas, is associated with oligosaccharides especially stachyose and raffinose (RAO and BELAVADY, 1978; EAST et al., 1972; SUGIMOTO and VANBURN, 1970). Germination of legume seeds is believed to affect the oligosaccharide content.

KAY (1979) has reported that lentils are the oldest of the grain legumes and are thought to have originated in Asia Minor from where they spread to Eygpt, Central and South Europe. They are also now cultivated in the U.S.A.

CUBERO (1981) has reported that three groups of lentils are restricted to certain areas : PILOSAE to India, AETHIOPIAE to Ethiopia and Yemen, and SUBSPONATANEAE to the Afghan regions. The

three groups are characterized by very small and dark coloured seeds. The five lowest protein cultivars originated in Ethiopia and Yemen (SOLH and ERSKINE, 1981). There is however considerable variations in the response of cultivars to temperature. KAY (1979) has also reported that lentils constitute about 2.4% of the total World output of grain legumes, and World production averaged 1,094,400 t/a for the period 1970-74; the major producing countries being India, Ethiopia, Turkey, U.S.S.R. and Syria. They occupy a place second only to Bengal and black gram among the pulses commonly consumed in India (SAINT-CLAIR, 1972). Lentils are considered the most easily digestible of grain legumes (BRESSANI et al., 1973). DARLING (1981) has mentioned that although lentils are among the oldest cultivated plants in the World, yet they have not been studied to any large extent. Most of the work done has concentrated on their protein content. ABU-SHAKRA and TANNOUS (1981) have reported that very few studies have been carried out on the quality of lentil as it relates to human nutrition and food supply. Priorities in research should therefore be given to screening numerous lentil varieties for their amino acid contents, for decreasing cooking time, and also for processing the grains. Lentils are a cheap source of protein, therefore an increase in lentil consumption will lead to an increase in protein intake in a diet which is otherwise sadly lacking in it.

ADAMS et al. (1978) have also emphasized that further research should be directed toward the important physical, chemical and biological properties of legumes as follows :

1. physical properties,
   (a) influence of temperature on cooking rate of dry legumes,
   (b) changes in tissues and cell structures related to soaking and cooking,
   (c) gelatinization characteristics of legume starches,

2. chemical properties,
   (a) vitamins, proteins,
   (b) other chemical constituents of dry and cooked legumes.

If such research goals were attained, it would improve the acceptance of many legumes in general and lentils in particular.

CULL and GEORGE (1981) have reported that FAO has a seed Industry programme, which is taking into consideration factors such as seed products, processing and storage conditions. Although the more popular legumes, e.g. beans and peas have been processed into different forms, there are relatively fewer studies on the processing of lentil seeds (ABU-SHAKARA and TANNOUS, 1981). Most legumes contain substances that inhibit digestive enzymes. Destruction of these substances increases the nutritive value of the food, while heat teatment beyond that needed to destroy these substances causes a reduction in their nutritive value (BENDER, 1978).

Knowledge about the extent of damage of nutrients during processing and storage stability is important. The nutrients, e.g. vitamins, lost during storage depend upon the rate of oxidation of other nutrients e.g. fat, since they are attacked by peroxides and free radicals formed from fat. Destruction therefore depends upon many factors, e.g. temperature, and oxygen (BENDER, 1978). Because of the different chemical and biological properties of legumes, processing and utilization technologies must be adopted for specific legume varieties and cultivars (ROCKLAND and RADKE. 1981). Processing of foods can serve a number of functions, including minimizing post-harvest losses and introducing the food in different forms which might increase consumption.

On the whole, studies on lentils have been carried out on unknown varieties and therefore the behaviour of lentil genotypes under different kinds and conditions of processing is unknown. Furthermore, some studies have been based on split lentils and

others on whole seeds (MEINERS et al., 1976; MORCOS et al., 1976).

The present study which has investigated a number of physical, nutritional and technological aspects of four varieties of lentils (Lens esculenta MOENCH) from different locations, will be divided into two Sections.

Section 1 made up of 5 chapters will investigate factors associated with the germination of these seeds, while Section II (3 chapters) will be on the processing of lentil seeds.

In chapter 1 the quality of dry seeds and other physical attributes, e.g. moisture, germination will be discussed. As previously mentioned, in Ethiopia and in Yemen, the lentil seeds are very small and dark (CUBERO, 1981), and they are considered among those cultivars with low protein content (SOLH and ERSKINE, 1981). The present study will attempt to recommend to these countries better varieties in terms of nutritive values, seed weight and cooking properties. It would then be possible to obtain these from Specialized International organizations, e.g. The International Centre for Agricultural Research in the Dry Areas (ICARDA). Investigation of the following chemical substances in dry lentil seeds as well as the effect of germination in the dark and in the light at 25 C, and in the dark at 35 C will be the theme of the following chapters : chapter II, carbohydrates; chapter III, lipids; chapter IV, proteins; and chapter V, the 3 vitamins, vitamin C, thiamine (vit. B_1), and riboflavin (B_2). The present study will attempt also to answer VANDERSTOEP (1981)'s question as to whether temperature or light affects the protein content of germinated lentil seeds.

In Section II, the effect of home processing (soaking, and cooking) on the same chemical constituents, and improvement in the cookability of lentils by the aid of simple salts and other chemical compounds will be discussed in chapter VI. In chapter VII, the effect of canning and storage of canned lentils at 25 and 35 C on these same chemical constituents over a twelve month period will be discussed, while chapter VIII will include Scanning Electron Microscopy (SEM) studies of the cell contents of lentils subjected to the different treatments, e.g. germination, cooking, and canning.

SECTION I

EFFECT OF GERMINATION IN THE DARK AND IN THE LIGHT ON THE NUTRIENT COMPOSITION OF DRY LENTIL SEEDS

CHAPTER I

PHYSICAL ATTRIBUTES OF LENTIL SEEDS AND THE EFFECT OF PROCESSING ON THEM

INTRODUCTION

Legumes have maintained their position as an important source of supplementary protein. Leguminoseae comprise approximately 600 genera with around 13,000 species, but only about 20 are of economic importance as legume food, and are consumed by humans (BRESSANI and ELIAS, 1974). KAKADE and EVANS (1965) have found that certain navy bean varieties are higher in protein than others, and also they show differences in methionine content. ELIAS et al. (1964) have only shown a small variation in the gross chemical composition and in amino acids of eight varieties of cowpeas. With a few exceptions, the variability in specific amino acids between species is not great, but those showing a great deal of variation are lysine and phenylalanine.

Studying varietal differences are of some importance, because varieties which are richer in certain nutrients may then be selected. BRESSANI and ELIAS (1974) have reported that hardly any research has been carried out on the evaluation of differences among legume varieties, and in cases where this has been done, no real attempt has been made to relate these differences to nutritional quality. Their significance to processing has been studied only too briefly.

As well as nutritional attributes, there are also some physical attributes of the quality of the dry seeds: these include cooking time, uniformity in colour, weight and size of the seeds (ELIAS et al., 1973).

In this chapter, the following physical attributes of four varieties of lentils will be studied : dry weight, colour, moisture content, and germination.

Throughout this study, four different varieties of lentil seeds obtained from different locations were used. The varieties were as follows :

1. American (Chilean) abb. AMR were obtained from Athens, Georgia, U.S.A.,
2. Syrian small abb. SML, (ILL 4401),
3. Syrian large abb. LAG, (ILL 4400), and
4. Jordanian large abb. JOR (ILL 4354). The last three varieties were obtained from ICARDA, Aleppo, Syria. The dried seeds (1-year old) were kept in closed containers and stored in a cold room (10 C). Dry, unprocessed seeds were always used as controls.

1. Determination of dry weight of 100 seeds

Samples of lentil seeds were cleaned, and the broken ones removed. The seeds were weighed accurately (about 20 g), and the number of individual seeds were counted. The weight of 100 seeds for all four varieties were calculated using the following formula :

$$\text{weight of 100 seeds} = \frac{\text{weight of seeds (g)} \times 100}{\text{number of seeds}}$$

RESULTS AND DISCUSSION

Table 1 shows that the dry weight (g) of 100 seeds for AMR, LAG, JOR, and SML are 4.76, 7.05, 4.13, and 2.71 g respectively; LAG are the heaviest, while SML the lightest.

PYLOV (1978) has reported that the large grain Soviet lentil (Petrovskaya 4/103 var.) has a stable green colour and a weight

Table 1: The weight of 100 seeds and moisture contents of four varieties of lentil seeds.

Variety	Weight of 100 seeds/(g) ± SE	Percentage moisture (g/100g)
AMR	4.76 (0.13)	9.60 (0.52)
LAG	7.05 (0.15)	10.10 (0.45)
JOR	4.13 (0.20)	8.50 (0.34)
SML	2.71 (0.07)	8.14 (0.28)

( ) = SE

of 5.6g/100 seeds. SOLH and ERSKINE (1981) have reported that the weight of 100 seeds is still an important factor of quality, and ICARDA have chosen arbitrarily for practical purposes a seed weight of 4.5g/100 seeds to separate the microsperma from the macrosperma accessions. According to the above classification, AMR (4.76) and LAG (7.05) are macrospermae while JOR (4.13) and SML (2.71) are microspermae.

2. Colour of seeds (colour code for lentils)

The colours of the seeds were classified according to the HORTICULTURAL COLOUR CHART (WILSON, 1938-41).

AMR :	Mars orange	013/1
LAG :	Saturn red	013/3
	Carrot red	012/3
JOR :	Mars Orange	013/3
	Lavender green	000761/3
SML :	Delft Rose	020/2
	Burnt orange	014/2
	Mars orange	013/2
	Jasper red	018/2
	Azalea pink	618/2

As can be seen from the above, SML shows a wider range of colour while AMR is more uniformly coloured : with all four varieties showing predominantly red, orange or pink. Apart from some seeds of JOR which appear green, the others do not and therefore do not appear as a stable green colour as did those of the Soviet variety Petrovska (PYLOV, 1978).

3. Determination of moisture content in lentils

Several methods are available for the determination of moisture in foods. They include drying, and direct distillation methods (PEARSON, 1976). Drying methods are the most commonly used and have been used in the present study.

Procedure

Two g of seed powder or 5 g of germinated seeds were weighed accurately in a flat-bottomed aluminium dish with light-fitting slip-over cover. The sample was placed in an oven at 105 C for 5 h. After that, the dish was covered, transferred to a desiccator, and weighed soon after reaching room temperature. This was repeated until constant weight (AOAC, 1975).

Calculation

The moisture content of the samples was calculated by using the following formula :

$$\text{Moisture (\%)} = \frac{\text{Loss in weight of seeds} \times 100}{\text{Weight(g) of seeds.}}$$

RESULTS AND DISCUSSION

Table 1 shows that the moisture content in AMR, LAG, JOR, and SML were 9.6, 10.1, 8.5, and 8.1% respectively. The high moisture content of legume seeds is a disadvantage during the storage of legumes, for as moisture content increases, their quality deteriorates rapidly as micro-organisms can severely attack them.

4. Soaking time and germination

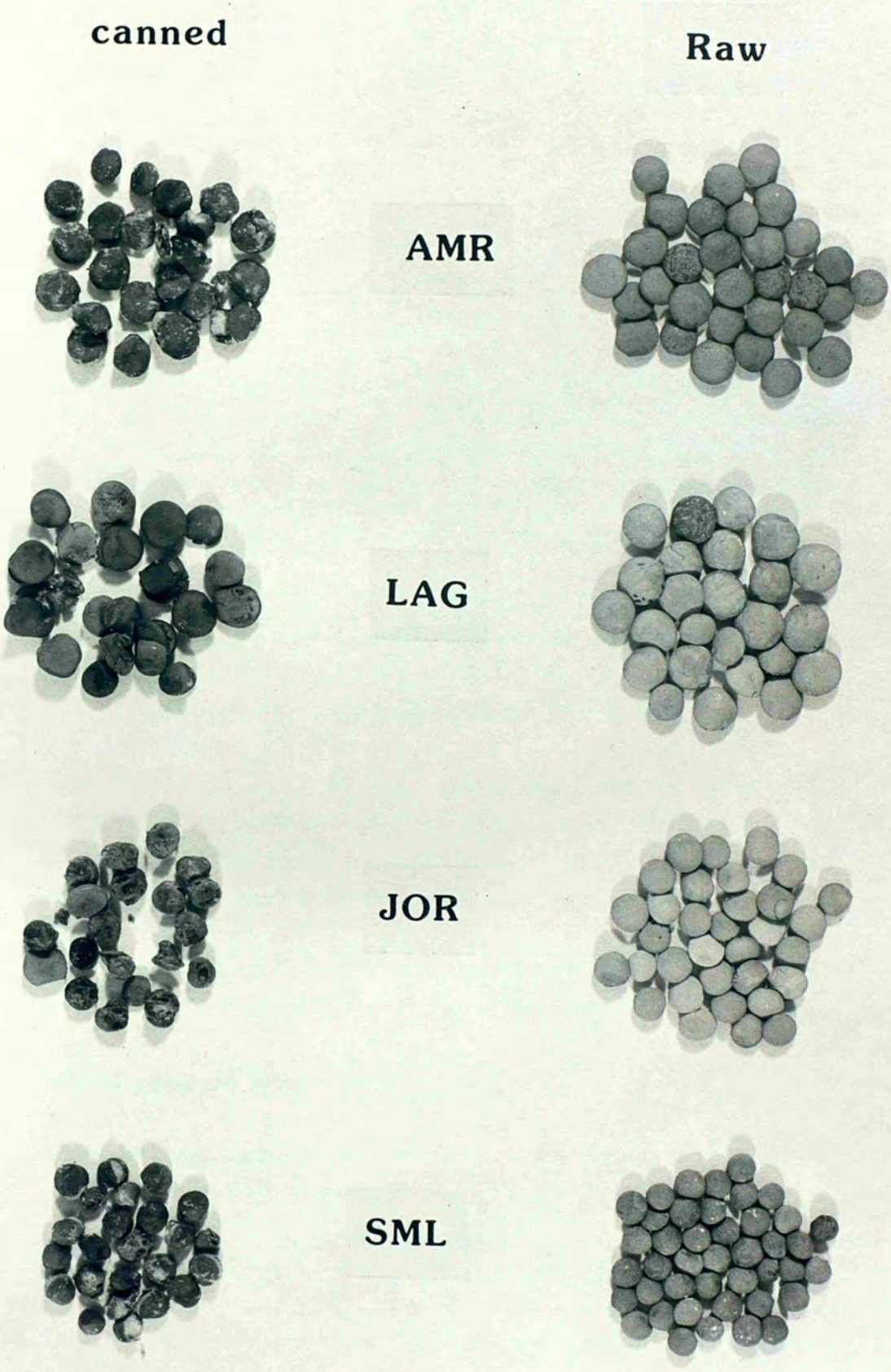
In preliminary experiments, different soaking times were tested. A relationship was found between the temperature and soaking

Fig. 1. Raw and canned lentil seeds of the four varieties used in the present study

On the right-hand raw seeds

On the left-hand canned seeds

Raw and canned seed lentils



time. At 22±1 C, the appropriate soaking time was 3-4 h which was used, while at ambient temperatures (23-27 C), the longer time of about 16 h, had a tendency to inhibit germination through anaerobiosis.

5. Germination technique

About 70-80 g of dry, clean and unbroken seeds were washed and soaked in five times their volume of water, for 3-4 h. At the end of the soaking time, the water was drained, and the seeds were washed with fresh water. For germination, FORDHAM's (1975) method was used. The soaked seeds were spread over plain cellulose sponges, which were saturated with water, and placed in clean opaque plastic pans (dimensions 12 x 8 x 3"). Chlorinated lime solution (0.001-0.002%) was used to inhibit the growth of molds.

Germination was carried out in the dark at 25 C, at 35 C, and in the light at 25 C. For the dark experiments, the pans were placed in an incubator at either 25±1 C or at 35±1 C, and in each case the relative humidity (R.H.) was between 90 and 92%.

Germination in the light was carried out with the pans being placed under a bank of 3 Universal White Mazda 40W lamps (1100 lux) in a temperature-controlled incubator; the temperature was 25±1 C and R.H. was between 85 and 92%.

Under all three conditions, the germinated seeds were collected at daily intervals and up to 5 days after germination.

Determination of percent germination

The term germination may include a fairly large number of processes. For the purpose of this work, a seed was adjudged to have germinated when the radical had just broken the testa (MAYER and POLJOKOFF-MAYBER, 1975). For percent germination, a pan was selected at random and the number of germinated seeds and the

number of total seeds in that pan were counted, and percent germination was determined.

Determination of length of germinated seeds

Hundred seedlings were selected at random. The length of each one was measured to the nearest mm.

RESULTS AND DISCUSSION

Seeds grown at 25 C in the dark

As can be seen from table 2 and fig. 2, there is hardly any difference between the moisture content of the four varieties. The moisture content of dry seeds of AMR, LAG, JOR, and SML are 9.6, 10.1, 8.5, and 8.1% respectively. The moisture content increases sharply after the first day of germination and then there is a gradual increase to the fifth day (fig. 2).

The percent germination on the fifth day was found to be 96, 97, 88, and 87% for AMR, LAG, JOR, and SML respectively (table 2).

Seeds grown at 25 C in the light

Table 3 shows the moisture content of seeds germinated at 25 C in the light. Generally the values are lower than those germinated in the dark. On the fifth day of germination, the moisture content for AMR, LAG, JOR, and SML is 72, 67, 65, and 72% respectively.

The percent seeds germinated in the light on the fifth day after germination are 92, 90, 79, and 83% respectively for AMR, LAG, JOR, and SML (table 3). Light therefore does not affect percent germination.

Table 2: The moisture content (%), percentage germination and the length (cm) of seeds germinated at 25°C inthe dark.

Variety	AMR			LAG			JOR			SML		
Days of germination	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm
o (Control)	9.60(0.52)	0	0	10.10(0.45)	0	0	8.50(0.34)	0	0	8.14(0.28)	0	0
1	56.39(1.10)	68.1	0.6	50.93(0.81)	70.0	0.8	51.53(1.20)	62.7	0.5	50.14(0.95)	55.7	0.4
2	62.51(0.70	70.3	1.3	55.85(1.61)	86.5	2.1	56.44(2.25)	70.8	1.1	58.17(1.21)	68.7	1.0
3	70.92(2.50)	86.2	2.7	60.26(1.30)	90.4	3.3	61.62(1.16)	81.5	2.3	66.76(2.46)	82.4	1.8
4	75.86(1.22)	90.5	4.1	70.69(0.78)	94.2	5.2	69.16(0.90)	84.3	3.7	72.19(1.53)	85.0	3.5
5	80.27(2.32)	96.2	6.1	76.31(1.90)	97.1	7.8	73.27(1.70)	87.9	5.8	74.85(2.13)	87.1	5.7

( ) = SE

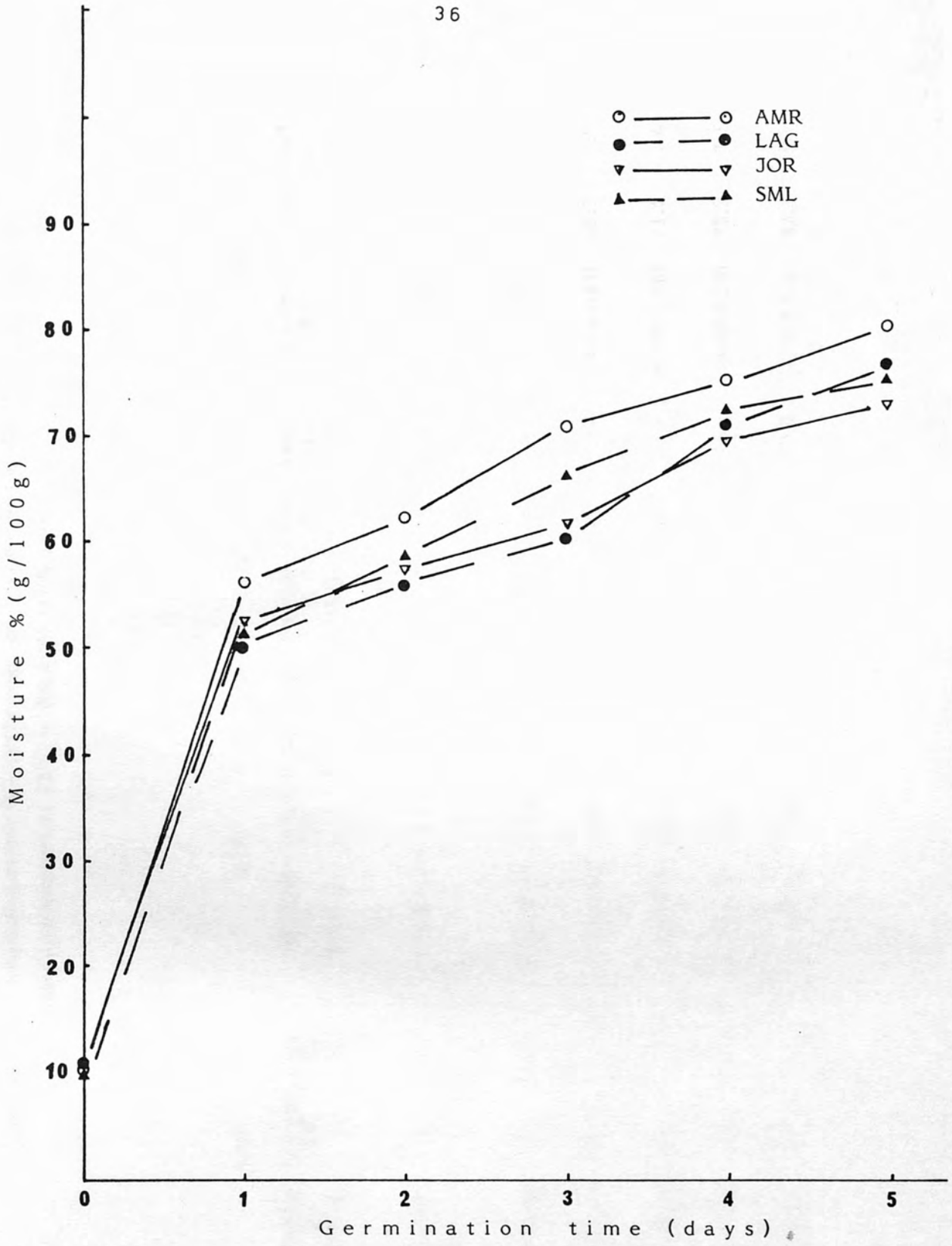


Fig. 2: Changes in the moisture content during the germination of lentil varieties at 25°C in the dark

Table 3: The moisture content (%), percentage germination and length (cm) of seeds germinated at 25°C in the light (1100 lux)

Variety	AMR			LAG			JOR			SML		
Days of germination	Moisture (%) g/100 g	Germin (%)	Length in cm	Moisture (%) g/100 g	Germin (%)	Length in cm	Moisture (%) g/100 g	Germin (%)	Length in cm	Moisture (%) g/100 g	Germin (%)	Length in cm
0 (Control)	9.6(0.52)	0	0	10.10(0.45)	0	0	8.50(0.34)	0	0	8.14(0.28)	0	0
1	52.90(1.00)	68.1	0.4	53.12(0.74)	65.84	0.4	50.57(1.26)	59.6	0.5	49.21(1.00)	57.6	0.3
2	60.55(0.90)	74.5	1.4	56.46(1.10)	76.15	1.2	56.34(0.85)	63.1	0.9	54.08(1.89)	68.3	0.8
3	64.73(1.62)	83.3	2.1	59.53(1.35)	81.07	2.7	59.03(1.80)	70.5	1.6	60.96(1.40)	71.5	1.6
4	68.21(1.23)	87.6	2.9	62.90(1.90)	85.24	3.6	62.45(1.11)	75.2	3.5	64.87(1.15)	78.2	3.1
5	72.46(2.18)	92.0	3.8	67.27(1.45)	90.32	5.5	65.47(1.42)	79.4	4.9	71.8(3.11)	83.1	4.6

( ) = SE

Seeds grown at 35 C in the dark

Table 4 gives the moisture contents of seeds germinated at 35 C in the dark. On the fifth day the moisture content of AMR, LAG, JOR, and SML was 60, 55, 62, and 61% respectively.

Table 4 also shows the percent germination of the seeds germinated at 35 C in the dark. On the fifth day, the percent germination of AMR, LAG, JOR, and SML was 79, 81, 71, and 68% respectively.

SAINT-CLAIR (1972) has reported that different sizes of lentil seeds have a different optimum temperature : for larger and medium size seeds, the optimum temperature ranges from 15-25 C, while the smaller seed varieties have a lower optimum temperature (between 18 and 20 C).

Table 4: The moisture content (%), percentage germination and the length (cm) of seeds germination at 35°C in the dark.

Variety	AMR			LAG			JOR			SML		
Days of germination	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm	Moisture (%) g/100g	Germin (%)	Length in cm
0 (Control)	9.6(0.52)	0	0	10.10(0.45)	0	0	8.50(0.34)	0	0	8.14(0.26)	0	0
1	61.10(0.96)	62.5	0.3	62.54(1.24)	65.8	0.3	65.41(1.40)	56.0	0.4	58.30(1.00)	54.7	0.3
2	68.73(1.10)	67.2	1.2	65.27(1.75)	70.2	0.8	70.38(1.64)	61.7	0.9	66.58(1.10)	60.1	0.6
3	65.92(1.60)	73.6	1.6	62.76(2.15)	73.4	1.9	67.19(1.20	66.2	1.3	64.86(1.56)	64.2	1.4
4	63.55(1.89)	75.8	2.4	57.45(1.30)	76.1	2.9	63.93(1.60)	68.4	3.2	62.54(1.30)	66.7	2.8
5	59.80(2.10)	78.5	3.2	55.34(0.88)	80.7	3.8	61.51(1.10)	70.8	4.8	60.82(1.80)	68.0	4.1

( ) = SE

CHAPTER II

CARBOHYDRATES

Nutrient composition of dry and germinated lentil seeds

INTRODUCTION

Lentils are an important food legume crop, because of their relative high protein content, which can reach 30%, and the ease with which they are digested. Lentils are widely cultivated in temperate and subtropical regions. Their susceptibility to diseases has tended to handicap their development as a food legume (KAY, 1979). Legumes contain certain nutritional inhibitors which may cause physiological disturbances when consumed in large quantities. Among the major nutrients are proteins, carbohydrates and vitamins (table 5).

Table 5. Comparative food value of lentils (SAINT-CLAIR, 1972)

Crop	g/100g			Calories /100g
	Protein	Fat	Carbohydrates	
Lentils	23.7	1.2	65.0	354
Wheat	9.3	1.9	78.6	363
Rice	6.5	0.4	82.2	356

Lentils have good nutritional qualities and they compare well with other crops, e.g. wheat, rice (table 5). The protein digestibility of lentils is 92%, the highest of all other legume proteins investigated to-date (BRESSANI et al., 1973). In legume seeds, cotyledons contribute the largest amount of protein

because of their greater weight, while seed coats contain about 5% protein. BERRY (1981) has reported that a lower quantity of protein from a given food, may actually be more nutritious if its protein is in an easily digestible form, or if it contains a good balance of the nutritionally required amino acids. EVANS and BANDEMER (1967) have studied many legumes and have found that most are deficient in tryptophan, in methionine, or in other sulphur amino acids compared to the reference value of egg (FAO, 1957). Most of the legumes are good sources of lysine and may therefore be used as supplements to lysine-deficient proteins. Many seeds including the Leguminoseae contain non-protein nitrogen which may be toxic to higher animals (BELL, 1975; VAN ETTEN et al., 1967). MILNER (1974) has emphasized that food legumes still have a long way to go before they can make a nutritional impact comparable to that of the high yielding cereal varieties, especially as regards their contribution as protein sources. The task of supplying more protein through legume grains, more specifically through lentils is in many ways not as difficult as one may imagine. What is indeed required is that more be consumed.

Lentils are also a good source of the B-vitamins, e.g. niacin; 100 g lentils provide about 7% of the U.S. Recommended Daily Allowance (RDA) (HAYTOWITZ et al., 1981). KYLEN and McCREADY (1975) have reported that dry lentils contain thiamine, riboflavin, and vitamin C in the following quantities : 0.72, 0.29, and 7 mg/100g respectively. MEINERS et al. (1976) have found the following quantities of minerals in mg/100g cooked lentils : calcium, 7.8, copper, 0.2, and iron, 0.3. Further, lentils are rich in carbohydrate which supplies energy in the diet. Certain of these organic substances and other chemical nutrients will be investigated in the course of the present study.

Chemical and Biochemical changes in lentil seedlings

Two different methods are applied to cereals and legumes to improve their nutritive values : germination and fermentation. The former is more advantageous than the latter because it is easy, simple, and quick to perform. The old practice of sprouting beans and soybeans has been advocated as a means of greatly improving the nutritional worth of many other seeds (VANDERSTOEP, 1981). Sprouting of seeds for food use is increasing in popularity. Several kinds of sprouts are sold in Eastern and Western markets and increasing amounts are being consumed in raw or cooked forms. Germinated lentil seeds may be used directly as food (KYLEN et al., 1975; HSU et al., 1980); and also germinated legume seeds may be used to fortify wheat flour (POMERANZ et al., 1977).

Changes in certain nutrients of etiolated lentil seedlings at 25 and at 35 C over a period of 5 days will therefore be investigated in the present study.

Light is another factor which improves the nutrient composition of food. It is well-known that different parts of plants react differently to the same stimulus, for example, when certain etiolated seedlings are illuminated the hypocotyls stop growth almost immediately whereas the leaves start to grow and expand. It would have been ideal then to study the different parts of lentil seedlings to observe what happens in each part as a response to light. However, the lentil seedlings used in this study were made up of thin hypocotyls, of small cotyledons, and of very small first leaves, and of radicals. For most biochemical studies, it was not convenient to separate each seedling into its different parts, and so the whole seedling was extracted except where otherwise stated.

Most of the studies concerning germination of legume seeds have been carried out in the dark and at a temperature of between 24 and 27 C. This part of the present study will be divided into two

(a) changes taking place in selected nutrients of lentils during germination at 25 C in the light (1100 lux), and

(b) changes in the same nutrients during germination at 35 C in the dark.

A temperature of 35 C was selected as it is the average summer temperature of certain tropical countries. Light has also been selected since under field conditions it is one of the most important growth factors, and lentil crops are usually subjected to light intensities in the region of 1000 lux or higher.

As mentioned previously, VANDERSTOEP (1981) has stated that it is still not clear whether temperature or light affected the protein content of germinated legume seeds, and the present study will attempt to answer this rather complex question.

This present chapter will deal with the carbohydrates of lentils, and subsequent chapters on the other nutrients mentioned previously.

CARBOHYDRATES

Carbohydrates can be defined as substances composed of polyhydroxy aldehydes or ketones, or as substances that yield these compounds on hydrolysis (HEIMANN, 1980) . They can be divided into two classes :

1. "available carbohydrates" which include those which may be utilized by humans, e.g. starch and dextrins, and soluble sugars, and

2. "unavailable carbohydrates", e.g. pectins and celluloses which cannot be utilized by humans (SOUTHGATE, 1976).

Soluble sugars

This group includes mono-, di-, and oligo-saccharides. Monosaccharides are either aldoses or ketoses, e.g. glucose or fructose respectively. Disaccharides consist of two monosaccharide units which are bound together through a glycosidic linkage, e.g. sucrose, maltose. Oligosaccharides contain three or more monomer units, e.g. the trisaccharide, raffinose (glucose, galactose and fructose or galactosylsucrose), and the tetrasaccharide, stachyose (fructose, glucose and two galactose units or galactosylraffinose). Legumes are rich in oligosaccharides, e.g. raffinose, and stachyose (JOSLYN, 1970). In the present study, determinations of total available carbohydrates, starches and dextrins, and the soluble sugars will be carried out.

MATERIALS AND METHODS

Determination of total available carbohydrates

The Clegg anthrone method (OSBORNE and VOOGT, 1978)

This method estimates the amount of starches and dextrins together with soluble sugars, i.e. total available carbohydrates. The samples are digested with perchloric acid, then determined colorimetrically by the anthrone method. An advantage of this method is that the anthrone reagent reacts with mono- and oligo-saccharides and this gives consistent results (SOUTHGATE, 1976).

Reagents

1. Perchloric acid (52%)
2. Sulphuric acid : 760 ml conc. H_2SO_4 is added to 330 ml H_2O,
3. Anthrone reagent : 0.1% anthrone made up in (2),
4. Glucose standard solution : 100 mg glucose is dissolved in 100 ml distilled water; then 10 ml of this are diluted with 100 ml distilled water.

Extraction

One g dry powder sample is transferred to a 100 ml measuring cylinder and 10 ml water added. This is stirred to disperse the sample. Thirteen ml of 52% perchloric acid is added, stirred for 20 min, then diluted to 100 ml with water. The mixture is filtered using Whatman No 542 filter paper and made up to 250 ml in a volumetric flask.

Determination

Ten ml of the extract is made up to 100 ml with distilled water. One ml of the diluted filtrate is pipetted into a test tube, and 1 ml of diluted glucose in another. One ml of water is pipetted into a third tube and used as a blank. Five ml anthrone reagent (3) are added rapidly to each, then stoppered and the contents mixed. The tubes are placed in a boiling water-bath for 12 min, and cooled to room temperature. The absorbance of the samples and of the standard are read in a spectrophotometer (LKB Biochrom, Model ULTROSPEC 4050) at 630 nm.

Calculation

Total available carbohydrates are calculated by using the following formula :

$$\text{Total available carbohydrates (as \%)} = \frac{25 \times b}{a \times w}$$

where w = weight (g) of sample

a = absorbance of diluted glucose solution

b = absorbance of diluted sample.

Starch and low molecular weight sugars

The LUFF-SCHOORL Method

Principle

The sugars are extracted from the food product by hot ethanol, hydrolysed in aqueous solution with hydrochloric acid and determined titrimetrically as glucose. Starches are isolated from the residue of the hot ethanolic extraction after treatment with potassium hydroxide solution. On hydrolysis, starch is converted to glucose which is then determined titrimetrically.

Reagents

1. Sodium hydroxide solution (150g/500ml),
2. Hydrochloric acid solution (1N),
3. Ethanolic potassium hydroxide solution. 25g KOH are dissolved in 400 ml of 95% (v/v) ethanol and diluted with ethanol to 500 ml.,
4. Deproteinising solution I. 26g potassium ferrocyanide are dissolved in water and diluted to 250 ml.,
5. Deproteinising solution II. 11.44g zinc acetate are dissolved in water. Six ml of glacial acetic acid are added and the mixture

is diluted with water to 200 ml.,

6. Copper reagent. 37.5g anhydrous sodium carbonate, 24.5g sodium hydrogen carbonate, 35g trisodium citrate dihydrate, and 12.5g powdered copper sulphate pentahydrate were mixed in a beaker and dissolved in about 400 ml cold water, stirring continuously. The mixture is made up to 500 ml with water. The pH after a 1 : to 49 dilution with freshly boiled water should be 10 ± 0.1,

7. Bromothymol blue solution. 2g in 200 ml 95% ethanol (v/v),

8. Starch indicator solution. A mixture of 1g soluble starch, 1 mg mercury (II) iodide (as a preservative) and 3 ml water are added to 100 ml boiling water and the boiling continued for 3 min before cooling,

9. Sodium thiosulphate standard solution (0.1N). 12.5g sodium thiosulphate ($Na_2S_2O_3$) and 0.1g sodium carbonate are dissolved in 500 ml water,

10. Ethanol, 80% (v/v),

11. Potassium iodide solution. 5g potassium iodide is dissolved in water and diluted to 50 ml,

12. Hydrochloric acid solution, 6N,

13. Potassium thiocyanate solution. 10g potassium thiocyanate are dissolved in water and diluted to 50 ml.

Procedure

Starch determination

Twenty g sample are weighed and transferred to a 250 ml centrifuge bottle, 150 ml of 80% ethanol are added, and the bottle covered. After heating on a steam bath for 1 h, it is centrifuged for 10 min. The supernatant is decanted, and the residue containing starch is washed with 50 ml ethanol and centrifuged for 10 min. The residue is next transferred to a beaker, and 300 ml hot ethanolic potassium hydroxide added, and the contents heated on a boiling water bath for 1 h. The solution is decanted through Whatman No 542 filter paper and the starch residue is washed directly with hot ethanol. A hole is made in

the filter paper and the starch washed through into a beaker. One hundred ml hot 1N-HCl is added and the beaker placed in a boiling water bath for two and a half h. The solution is cooled and neutralized to about pH 6.5 with NaOH (1). The mixture is transferred to a 200 ml flask and 3ml of deproteinising solution I added, mixed, 3ml of deproteinising solution II added, and diluted to 200 ml. The mixture is next made alkaline to bromothymol blue. A 25 ml aliquot is pipetted into a 250 ml conical flask, 25 ml of copper reagent (6) are added, and boiled for 10 min under a condenser. Next, the flask was cooled, 5 ml of potassium iodide (11), followed by 20 ml 6N-HCl(12) added. Ten ml thiocyanate solution (13) added, and the liberated iodine is titrated with standard solution thiosulphate. When the solution becomes pale yellow, 1 ml starch indicator is added and the titration continued until the blue colour disappears. A blank determination is carried out.

Calculation

The amount (ml) of 0.1N thiosulphate solution is calculated as follows :

$$(V_0 - V) \times T \times 10,$$

where,

V_0 = Volume (ml) of standard thiosulphate needed for blank,

V = Volume (ml) of standard thiosulphate needed for the determination,

T = Normality of the standard thiosulphate solution.

The corresponding amount of glucose is found from the conversion table found in the Appendix 1 (p.321). Let this amount in mg = g,

$$\text{then, the starch content (\%)} = \frac{g \times 80 \times 0.9}{W}$$

(0.9 is the factor used to convert glucose equivalents to starch).

MATERIALS AND METHODS

Qualitative and quantitative determinations of mono-, di-, and oligo-saccharides of lentils by TLC.

Extraction of sugars

SOUTHGATE (1976) has reported that the extreme solubility of soluble sugars in aqueous solutions makes these the obvious choice as extracting media. Most analytical approaches for the extraction of sugars have attempted to combine the twin requirements of the complete extraction of sugars and the removal of interfering substances (AOAC, 1975).

Preparation of samples

Five g of whole dry seeds are transferred to a Waring Blender, to which 100 ml 80% ethanol are added. After blending the mixture for 2 min, it is transferred to a 250 ml round bottom flask and extracted by refluxing for 1 h. The mixture is allowed to cool, filtered, and the residue washed twice with 100 ml 80% ethanol. The extracts and washings are combined, and concentrated under vacuum at 48 C.

Clarification of the alcohol extract

To the concentrated alcohol extract, 1.5 ml saturated lead acetate is added (AOAC, 1975; KLINE et al., 1970). The supernatant is separated by centrifugation.

Standards for sugar solutions

The standard solutions of sucrose, fructose, glucose, raffinose, and stachyose (all from Sigma Chemical Co., St Louis, U.S.A.) are each made to a concentration of 4 mg/ml with distilled water.

Separation of sugars

Thin-layer plates (20 x 20 cm) were plastic-backed, and coated with a layer of 0.25 mm silica gel, type polygram Sil G, supplied by Macherey Nagel and Co. Ltd., Germany. The samples are applied as a streak (1.5 cm wide), and 1 cm apart and 2 cm from the lower edge of the plate. The method used for the separation of sugars by TLC was carried out according to SUGIMOTO and VANBURN (1970) and to TANAKA et al.(1975). Five µl of each sample of lentils are applied to the plate with a microsyringe, also 5 µl of the standard mixture are applied. Applications are made in small increments (about 2 µl) under a stream of air to facilitate rapid drying and thus to minimize spot diffusion.

Developing solvent system

Three solvent systems were used.

1. n-Propanol : ethyl acetate : water :: 6 : 1 : 3 (v/v)

2. n-Butanol : ethyl acetate : isopropanol : acetic acid : water :: 35 : 100 : 10 : 35 : 30 (v/v)

3. Chloroform : acetic acid : water :: 3.0 : 3.5 : 0.5 (v/v).

It was found that the chloroform solvent system (3) gave the best separation and this was used in all subsequent experiments. Ascending chromatography was carried out according to MENZIES et al. (1978). Each plate was rolled into a 250 ml beaker, and the beaker inverted in a chromatography tank which was then closed. At the end of the run of about 5 h, the plates were dried.

Colour reaction and identification of sugars

Detecting reagents as described by DE STEFANIS and PONTE (1968) were used. One g diphenylamine and 1 ml aniline were dissolved in

100 ml acetone. Just before spraying, 10 ml of the above solution was mixed with 1 ml 85% phosphoric acid, and then this was sprayed on to the TLC plates which were heated at 130 C for 10 min. At the end of the heating period, different colours were developed. Fructose appears rosy-brown, glucose blue-green, sucrose, raffinose, and stachyose dark-grey. The sugars were also identified by comparison with the authentic standards.

Quantitative determination of the sugars

Quantitative determinations were made by using a TLC transmission densitometer. Each series of chromatographic zone was cut into 1.5 cm slices. The slices were then scanned in a Chromoscan double-beam recording and integrating densitometer (Joyce-Loebl Co. Ltd., England), with quartz iodine light source, blue filter (drive in gear ratio was 1 : 1). The sugars were shown as a series of peaks on the recorder. The areas of the peaks are proportional to the amount of sugars originally present. The area of each peak was determined by multiplying the peak height x the band width at half-peak height. Sugars were determined by comparison with the standard sugars developed simultaneously with the samples (JOSLYN, 1970), and using the following formula :

$$\frac{\text{Peak area of standard}}{\text{Peak area of sample}} = \frac{\text{Amount of sugar in standard}}{\text{Amount of sugar in sample}}$$

(FARHANGI, 1980).

CARBOHYDRATES OF DRY LENTIL SEEDS

Identification of unknown sugars in lentils

Work to-date had indicated only the presence of sucrose, stachyose, and raffinose in lentils; while verbascose was possibly present (BHATTY and SLINKARD, 1979). In the present

study, a number of sugars were observed and an attempt was made to identify them.

All the varieties have nearly the same number of between 3 and 4 unknown sugars on the TLC plates : one is above sucrose (1); the second is between sucrose and raffinose (2); the third is above stachyose but below raffinose (3); while the fourth is below stachyose (4) (fig. 3). Standard solutions of the following sugars (4mg/ml) obtained from BDH Chemicals, Poole, England were prepared : 1. rhamnose, 3. xylose, 4. ribose, 5. arabinose, 6.cellobiose; as was 2. digitose obtained from Sigma Chemical Co., St Louis, U.S.A. (fig. 4).

TLC was carried out as described on p.50 . Rf values defined as follows were used in preliminary identifications :

$$Rf = \frac{\text{distance travelled by sugar from the starting line} \times 100}{\text{distance travelled by solvent from the starting line}}$$

Rf values are called mobilities, as are Rglu values (SOUTHGATE, 1976; STAHL and KALTENBACH, 1965).

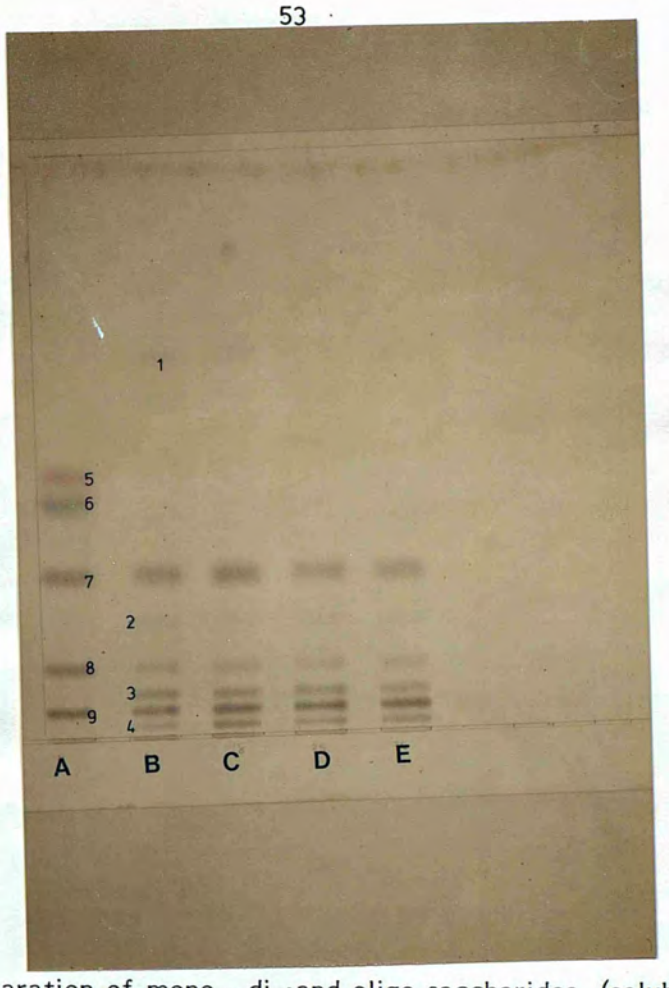


Fig. 3: TLC separation of mono-, di-;and oligo-saccharides (soluble sugars) in dry seeds of four varieties of lentils.

5 = Fructose, 6 = Glucose, 7 = Sucrose, 8 = Raffinose,
9 = Stachyose
1, 2, 3 & 4 are unidentified sugars

A = Mixture of standard sugars
B = AMR seeds, C = LAG seeds, D = SML seeds, E = JOR seeds

Developing solvent: Chloroform-acetic acid-water
(3.0 : 3.5 : 0.5 , V/V)

Table 6. Rf values of some standard, and unknown sugars from lentils (fig. 4) in chloroform : acetic acid : water :: 3.0 : 3.5 : 0.5 .

Standard Sugars	Rf value	Unknown Sugars	Rf value
Digitose	66	Unknown 1	63
Rhamnose	53		
Ribose	50		
Xylose	46		
Arabinose	40	Unknown 2	19
Cellobiose	16	Unknown 3	8
		Unknown 4	4

From table 6, unknown 1 has an Rf between that of digitose (Rf 66) and that of rhamnose (Rf 53). The Rf of unknown 2 (Rf 19) is very close to that of cellobiose (Rf 16), and therefore may be cellobiose.

Manninotriose was prepared from stachyose (Sigma Chemical Co.) either by acid hyrolysis (JOSLYN, 1970) or by enzyme hydrolysis (FORD, 1979). Hydrolysis of stachyose yields fructose and manninotriose (spot 3, fig. 5). Both acid and enzyme hydrolysed samples were run on TLC (p.56) and also co-chromatography was carried out. From fig. 5, it can be seen that acid hydrolysis (E) gives more manninotriose (fig. 5, spot 3) and this technique was used to obtain pure manninotriose. Spot 3 is therefore manninotriose, and further spot 2 was identified as cellobiose

Fig. 4: TLC for identification of unknown sugars in dry seeds of lentils varieties

5= Fructose; 6= Glucose; 7=Sucrose; 8=Raffinose; 9= Stachyose
Initially 1, 2, 3 and were unknown, but later 2 and 3 were identified as cellobiose and manninotriose respectively

A= Mixture and standards sugars; B= Mixture of standard ribose and rhamnose; C= AMR seeds; D= LAG seeds; E= Xylose; F= Rhamnose; G= Ribose; H= Arabinose; I= Cellobiose; K= Digitose

Fig. 5: TLC identification of umknown sugars

5= Xylose; 6= Fructose; 7= Glucose; 8= Sucrose; 9= Cellobiose; 10= Mellibiose; 11= Raffinose; 12= Stachyose; 3= Manninotriose

A= Mixture of above standard sugars; B= AMR seeds; C= LAG seeds; D= Enzymic hydrolysis of stachyose; E= Acid hydrolysis of stachyose; F= Standard cellobiose; G= Enzymic hydrolysis of AMR seeds; H= Acid hydrolysis of AMR seeds; I= AMR seeds + cellobiose (co-chromatography of cellobiose)

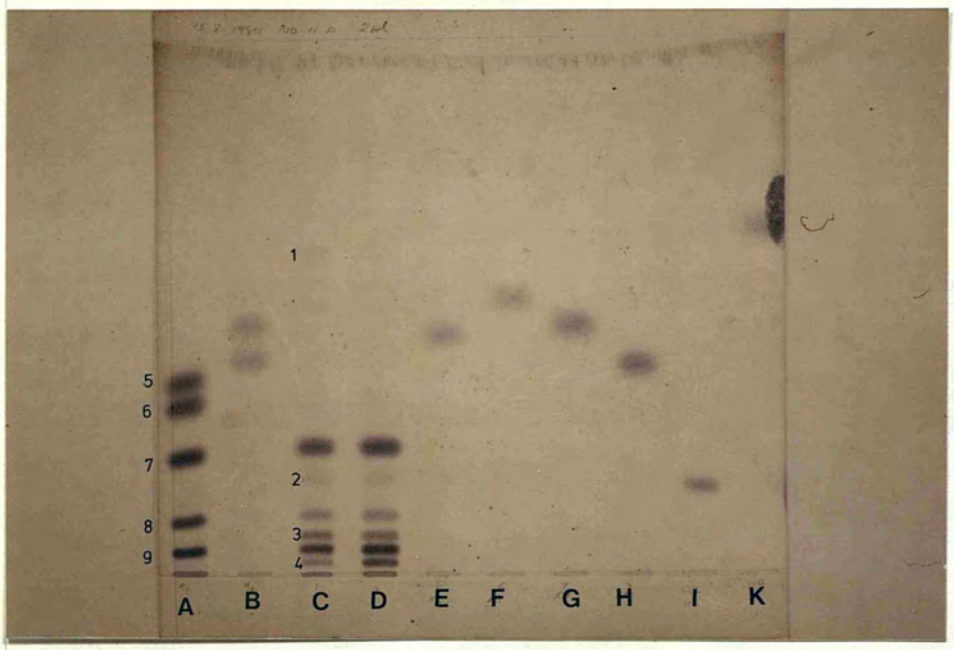


Fig.4

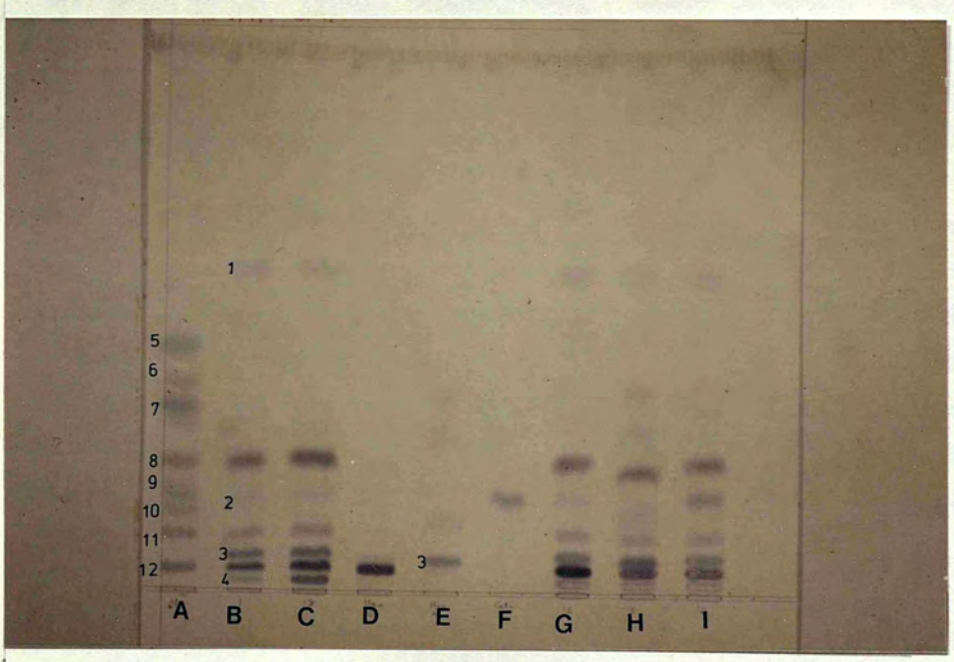


Fig.5

(fig. 5). The Rf of unknown 2 (Rf 19, fig. 4) is very close to that of cellobiose (Rf 16), and co-chromatography shows it to be cellobiose (fig.5, spot 2).

Quantitative determination of cellobiose and manninotriose

A standard solution of cellobiose was prepared and determined using the TLC method already outlined on p. 49 .

There was however, a problem in obtaining pure manninotriose as it is not available commercially. The following method was therefore used to isolate manninotriose, and thus to obtain a standard solution of this sugar.

Acid-hydrolysed stachyose was run on TLC, and manninotriose was separated as previously described (p. 49), scraped, and collected in a test tube, when 30 ml of 80% ethanol was added. After left standing for 1 h with occasional shaking, the eluate was filtered through glass wool. The alcohol mixture was reduced to near dryness in a rotary evaporator, under reduced pressure, transferred to an evaporating dish, and dried under a stream of air. Sufficient material was then available to prepare the standard solution of manninotriose.

In all the four varieties of lentils, cellobiose was found in trace amounts. In AMR, LAG, JOR, and SML manninotriose was found to be 0.15, 0.11, 0.10, and 0.17 g/100g respectively (table 7, p. 58). Cellobiose and manninotriose will not be included in subsequent studies as these two sugars are present in such small quantities. So, the more abundant sugars (including sucrose, stachyose, raffinose) only will be discussed in the present study.

In preliminary experiments, the Luff-Schoorl method of estimating starch was used. The quantitative value obtained for the latter was not significantly different (at the 5% level) from those

Table 7: Cellobiose and manninotriose of lentil varieties

The results are expressed as g/100g dry weight.

Variety	Cellobiose	Manninotriose	TOTAL
AMR	T	0.15	0.15 (0.04)
LAG	T	0.11	0.11 (0.02)
JOR	T	0.10	0.10 (0.02)
SML	T	0.17	0.17 (0.03)

obtained by the subtraction of values for soluble sugars from total available carbohydrates. Values for starch + dextrins in the present study were therefore obtained by this means.

Quantification

All experiments were carried out at least four times. The results wherever appropriate are expressed as $x \pm$ S.E., where x is the mean of four replicates, and S.E. the standard error of the mean.

All % retentions given in the tables represent increases or decreases compared to dry, unprocessed seeds.

RESULTS AND DISCUSSION

Table 8 shows that total available carbohydrates in AMR, LAG, JOR, and SML are 51.11, 49.23, 47.14, and 46.81g/100g dry weight respectively.

The starches and dextrins in AMR, LAG, JOR, and SML are 48.62, 46.26, 43.88, and 44.25g/100g respectively; soluble sugars are 2.49, 2.97, 3.26, and 2.56g/100g respectively (table 8).

The results for soluble sugars (table 8) are slightly higher than those obtained by BHATTY and SLINKARD (1979) of about 2% in their varieties Laird and Tekoa. The high content of total available carbohydrates makes lentils a good source of energy.

Mono-, di-, and oligo- saccharides of the four lentil varieties

In the present study, the following sugars were identified in the four varieties of lentil seeds : sucrose, stachyose, raffinose, cellobiose, and manninotriose. Stachyose and sucrose were the major sugars, while raffinose, manninotriose and cellobiose were the minor ones (fig. 3).

Monosaccharides

Neither fructose, nor glucose was present in dry seeds.

Disaccharides

Tables 9 and 10 show that sucrose in AMR, LAG, JOR, and SML is 0.63, 1.29, 1.18, and 0.97g/100g respectively.

Table 8: Changes in total available carbohydrates, starches and soluble sugars of four varieties of lentil during germination in the dark at 25°C.

ESS = Ethanol-soluble sugars, ST+D = Starch + dextrin, TACHO = Total available carbohydrate

(Results are expressed as g/100g seeds ± SE)

Days of germin.	AMR			LAG			JOR			SML		
	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO
Control or o	2.49 (0.15)	48.62 (1.25)	51.11 (1.15)	2.97 (0.12)	46.26 (1.18)	49.23 (1.30)	3.26 (0.18)	43.88 (1.50)	47.14 (1.90)	2.56 (0.13)	44.25 (0.88)	46.81 (1.47)
1	2.62 (0.21)	46.12 (2.45)	48.74 (1.58)	3.53 (0.20)	43.58 (1.29)	47.11 (0.90)	3.84 (0.23)	40.89 (1.24)	44.73 (0.98)	3.17 (0.15)	41.94 (1.35)	45.11 (1.35)
2	3.41 (0.11)	42.40 (1.13)	45.81 (0.80)	3.97 (0.25)	42.17 (2.10)	46.14 (1.20)	4.20 (0.28)	39.56 (1.80)	43.76 (1.15)	3.96 (0.18)	40.34 (0.80)	44.30 (1.17)
3	4.30 (0.17)	39.12 (1.06)	43.42 (1.47)	4.68 (0.13)	37.37 (1.20)	42.05 (2.55)	4.77 (0.16)	37.87 (1.25)	42.64 (2.10)	5.09 (0.12)	36.01 (1.16)	41.10 (1.00)
4	5.65 (0.15)	34.95 (1.30)	40.60 (0.95)	5.31 (0.14)	34.93 (1.65)	40.24 (1.40)	6.10 (0.19)	35.07 (1.46)	41.17 (1.17)	6.43 (0.24)	33.93 (1.09)	40.36 (1.70)
5	7.32 (0.18)	30.66 (1.19)	38.00 (1.30)	6.71 (0.30)	31.90 (1.11)	38.61 (1.10)	8.11 (0.25)	31.96 (1.10)	40.07 (1.25)	7.63 (0.16)	31.75 (1.55)	39.38 (0.90)

( ) = SE

Oligosaccharides

Raffinose in AMR, LAG, JOR, and SML is 0.26, 0.19, 0.15, and 0.28g/100g, while stachyose is 1.45, 1.38, 1.83, and 1.14g/100g respectively. The ratio of stachyose to sucrose varies from one variety to another, being 2.3:1, 1.7:1, 1.6:1, and 1.2:1 for AMR, LAG, JOR, and SML respectively (tables 9 and 10). Raffinose is present in small amounts compared to sucrose and to stachyose.

The non-identification of glucose and fructose agrees with results obtained by BHATTY and SLINKARD (1979) who have reported that no glucose was found in their two varieties of lentils (Laird and Tekoa). Fructose was not identified in pink beans (SILVA and LUH, 1979); and also soybeans contain neither glucose nor fructose (EAST et al., 1972).

The four lentil varieties investigated here were found to contain more stachyose than sucrose. These findings agree with those of JOSLYN (1970) who found that dry lentils of unspecified variety had more stachyose than sucrose.

Table 9: Changes in mono-, di- and oligo-saccharides of AMR and LAG varieties of lentil, during germination in the dark at 25°C

The results are expressed as g/100g dry weight.

Days of germin.	FRU	GLU	SUC	RAF	STA	TOTAL
AMR Control	0.00	0.00	0.63	0.26	1.45	2.34 (0.15)
1	0.32	0.25	1.08	0.19	0.78	2.62 (0.21)
2	0.67	0.58	1.67	0.14	0.35	3.41 (0.11)
3	1.10	1.22	1.81	T	0.17	4.30 (0.17)
4	1.64	1.95	2.06	0.00	T	5.65 (0.15)
5	2.26	2.75	2.31	0.00	0.00	7.32 (0.18)
LAG Control	0.00	0.00	1.29	0.19	1.38	2.86 (0.12)
1	0.30	0.49	1.77	0.07	0.90	3.53 (0.20)
2	0.80	0.63	1.93	T	0.61	3.97 (0.25)
3	1.32	0.94	2.17	0.00	0.25	4.68 (0.13)
4	1.51	1.45	2.35	0.00	0.00	5.31 (0.14)
5	1.83	2.15	2.73	0.00	0.00	6.71 (0.30)

( ) = SE
T = Traces

Table 10: Change in mono-, di- and oligo-saccharides of JOR and SML varieties of lentil during germination in the dark at 25°C.

The results are expressed as g/100g dry weight.

Days of germin.	FRU	GLU	SUC	RAF	STA	TOTAL
JOR Control or o	0.00	0.00	1.18	0.15	1.83	3.16 (0.18)
1	0.39	0.48	1.75	0.08	1.15	3.85 (0.23)
2	0.52	0.79	2.21	T	0.68	4.20 (0.28)
3	0.87	1.20	2.43	0.00	0.27	4.77 (0.16)
4	1.57	1.85	2.68	0.00	T	6.10 (0.19)
5	2.60	2.60	2.91	0.00	0.00	8.11 (0.25)
SML Control or o	0.00	0.00	0.97	0.28	1.14	2.39 (0.13)
1	0.61	0.55	1.23	0.10	0.68	3.17 (0.15)
2	0.93	1.08	1.46	T	0.49	3.96 (0.18)
3	1.45	1.58	1.81	0.00	0.23	5.07 (0.12)
4	2.18	1.98	2.27	0.00	T	6.43 (0.24)
5	2.45	2.68	2.47	0.00	0.00	7.60 (0.16)

( ) = SE
T = Traces

General Introduction

Changes in carbohydrates during germination in the dark

TSAI et al. (1975) have reported that at the end of five days germination of maize seeds, reducing sugars increased 60-fold, while starch decreased by about 30%. During germination of soybeans, raffinose disappeared after 96 h and stachyose after 144 h of germination (EAST et al., 1972). MAYER and POLJAKOFF-MAYBER (1975) have reported that changes that occur in seed carbohydrates include the breaking down or the interconversion of these carbohydrates. They have also reported that starch is normally broken down by amylases, and when amylopectin is attacked dextrins are formed, when it is amylose, the molecule is broken down more or less completely. Amylopectin however, is broken down preferentially. MAYER and POLJAKOFF-MAYBER (1975) also added that sucrose may be formed as a result of raffinose breakdown.

Changes in total available carbohydrates, starches, and soluble sugars of lentils during germination at 25 C in the dark

Changes in total available carbohydrates

There was a decrease in total available carbohydrates of all four varieties during germination. the decrease in total available carbohydrates for all four varieties are as follows : AMR from 51.1 to 38.0 (26%), LAG from 49.2 to 38.6 (21%), JOR from 47.1 to 40.1 (15%), and SML from 47.8 to 39.4g/100g (16%) (table 8, p. 61).

Changes in starches and dextrins

There was a decrease in starches and dextrins in all four varieties during germination. Table 8, p. 61 shows that the decrease in starches and dextrins was as follows : for AMR from 48.6 to 30.7 (37%), LAG 46.3 to 31.9 (31%), JOR 43.9 to 31.9

(25%), and SML 44.2 to 31.7g/100g (28%).

The results are in agreement with those of PALMIANO and JULIANO (1972) who have reported a 33% decrease in germinated rice on the third day, and with those of TSAI et al. (1975) who have reported about 50% decrease in 5-day old germinated etiolated maize seedlings. A lower value of a decrease of about 14% in mung beans has been reported by AMAN (1979) and by FARHANGI (1980). The starch of germinated legume seeds decreases in parallel with increases in reducing sugars (HSU, 1981) and these may be brought about through the action of enzymes (SILVA and LUH, 1979; AKAZAWA, 1976). Sucrose-starch interconversion processes have also been observed in developing cereal seeds (AKAZAWA, 1976).

Changes in soluble sugars

Table 8 (p.61) shows that during germination, there was an increase in the ethanol-soluble sugars in all four varieties. Ethanol-soluble sugars increased from 2.5 to 7.3 (192%), from 2.9 to 6.7 (133%), from 3.2 to 8.1 (153%), and from 2.5 to 7.6g/100g (204%) in AMR, LAG, JOR, and SML respectively. SML has the highest increase, while LAG the lowest.

These results are in agreement with those of ABRAHAMSEN and SUDIA (1966) who have reported an increase in the soluble sugars of germinated soybeans, and of PALMIANO and JULIANO (1972) an increase in those of 5 day-old germinated rice seedlings.

Changes in mono-, di-, and oligo-saccharides during germination at 25 C in the dark

Monosaccharides

There was a substantial increase in both glucose and fructose of the four varieties of germinating lentils since they were not present in the dry seeds (tables 9 and 10, p.63 & 64 ; fig. 6).

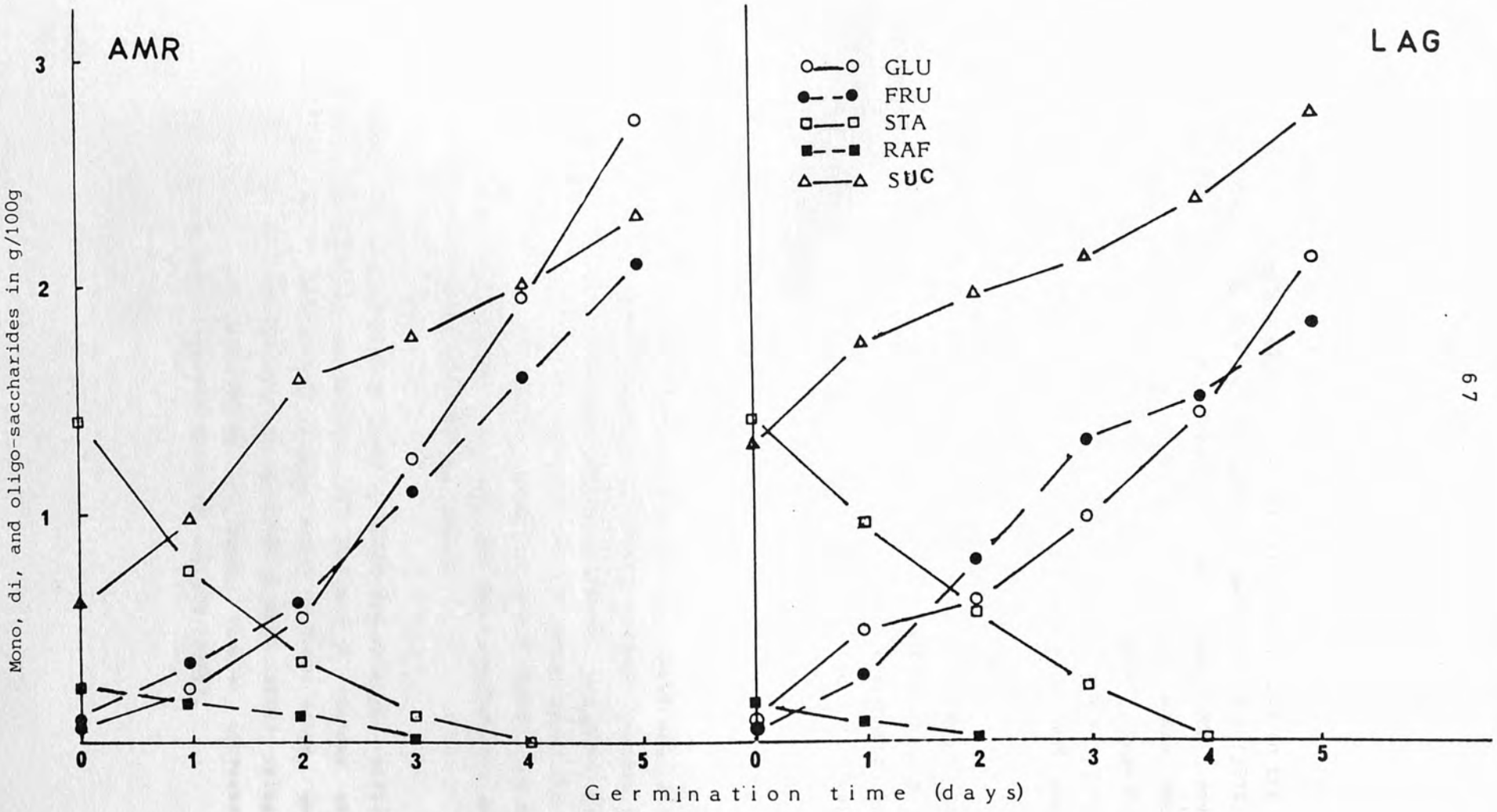


Fig 6: Changes in mono-, di-,and oligo-saccharides of lentil seeds germinated in the dark at 25°C

Fructose increased from 0.3 on the first day to 2.3 on the fifth day (666%), from 0.3 to 1.8 (500%), from 0.4 to 2.6 (550%), and from 0.6 to 2.5g/100g (316%) in AMR, LAG, JOR, and SML respectively. AMR shows the highest increase, while SML the lowest. Glucose increased from 0.25 to 2.7 (980%), from 0.5 to 2.1 (320%), from 0.5 to 2.6 (420%), and from 0.6 to 2.7g/100g (350%) in AMR, LAG, JOR, and SML respectively. AMR shows the highest increase while LAG the lowest.

A much higher increase in fructose and glucose of about tenfold has been reported on the third day of germination of mung beans by KYLEN and McCREADY (1975). The most likely explanation is that the glucose and fructose must have come from the hydrolysis of oligosaccharides (HSU et al., 1973).

Disaccharides

Sucrose also increased during germination. There was a rapid increase after the first day, and then a gradual increase until the fifth day of germination (tables 9 and 10, p.63,64). Sucrose increased from 0.6 in the dry seeds to 2.3 (283%) after the fifth day, from 1.3 to 2.7 (107%), from 1.3 to 2.9 (141%), and 0.9 to 2.5g/100g (177%) in AMR, LAG, JOR, and SML respectively. AMR has the highest increase and JOR the lowest.

These results agree with those of KYLEN and McCREADY (1975) who have reported an approximate 200% increase in sucrose on the third day of germination of mung beans; a lower value, about a 110% increse in sucrose was reported by AMAN (1979) using the same beans. In germinating pink beans, similar increases in sucrose have been reported by SILVA and LUH (1979).

Oligosaccharides

In the present study, stachyose decreased in all four varieties from the first day onwards (tables 9 and 10, p.63,64). The decrease in stachyose continued so that by the fourth day there were only traces, and even these disappeared completely by the fifth day of germination. Raffinose followed a similar pattern, but the decrease was such that by the fourth day there was none left in any of the varieties studied, and in three varieties except for AMR none by the third day. These results concerning both stachyose and raffinose are in agreement with those of most other workers (HSU et al., 1973; EAST et al., 1972). It appears that there is a relationship between the decrease in stachyose and in raffinose on the one hand and the increase in sucrose, in fructose, and in glucose on the other. Sucrose is a hydrolytic product of both stachyose and of raffinose, and so the accumulation of sucrose may be due to the rapid hydrolysis of these two oligosaccharides which occurs during this period (ABRAHAMSEN and SUDIA, 1966). Similarly, some of the sucrose may also be hydrolysed to yield glucose and fructose. The non-appearance of galactose during germination can be explained by JOSLYN's (1970) suggestion that galactose rarely occurs free in measurable concentrations in plants. This means that this sugar and also oligosaccharides are not always hydrolysed but may also be utilized through a reversal of some synthetic mechanisms, e.g. the synthesis of sucrose which may take place as follows :

Raffinose + Uridine diphosphate -->Uridine diphosphate galactose + sucrose

Uridine diphosphate galactose ---> Uridine diphosphateglucose (JOSLYN, 1970).

In the present study, it was found that the oligosaccharides (raffinose, stachyose) decreased significantly during the germination of lentil seeds, so, germination could be used as a means of decreasing or removing oligosaccharides from dry seeds, as these are believed to cause the intestinal discomfort of flatulence which follows the consumption of legumes like beans and peas (EAST et al., 1972; RAO and BELAVADY, 1978). Raffinose and stachyose are considered as the storage forms of galactosyl, glucosyl, and fructosyl residues in legumes, and the hydrolyses of these compounds will therefore cause increases in sucrose (GANDER, 1976).

Effect of light on total available carbohydrates, starches and dextrins, and ethanol-soluble sugars of lentils grown at 25 C in the light

Effect of light on total available carbohydrates

Table 11 shows a gradual decrease in total available carbohydrates during germination in the light. On the fifth day of germination, total available carbohydrates in AMR, LAG, JOR, and SML decreased from 51.1 to 43.1 (17%), from 49.2 to 39.1 (20%), from 47.1 to 37.2 (21%), and from 46.8 to 40.2 g/100g (14%) respectively.

Although total available carbohydrates were expected to increase during germination in the light, the decrease may be explained by their being utilized to supply energy to the growing organs. Also, the leaves are very small and not properly developed at this stage. So, presumably not sufficient chlorophyll had been synthesized and thus more carbohydrates were used up than actually manufactured.

Table 11: Effect of light on total available carbohydrates, starches and sugars of four varieties of lentil. The results are expressed as g/100g dry weight.

ESS = Ethanol soluble sugars, ST+D = Starch + dextrins, TACHO = Total available carbohydrates

Day of germin.	AMR			LAG			JOR			SML		
	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO
Control or o	2.49 (0.15)	48.62 (1.25)	51.11 (1.15)	2.97 (0.12)	46.26 (1.18)	49.23 (1.30)	3.26 (0.18)	43.88 (1.50)	47.14 (1.90)	2.56 (0.13)	44.25 (0.88)	46.81 (1.47)
1	2.66 (0.17)	47.55 (1.36)	50.20 (1.16)	3.27 (0.13)	45.10 (1.15)	48.37 (2.37)	3.56 (0.10)	42.16 (2.05)	45.72 (1.26)	2.87 (0.18)	42.39 (1.69)	45.26 (1.01)
2	3.22 (0.23)	45.40 (1.08)	48.62 (2.72)	3.49 (0.18)	42.80 (1.60)	46.28 (1.32)	4.10 (0.16)	39.78 (1.51)	43.88 (1.35)	3.48 (0.18)	40.47 (1.40)	43.95 (1.39)
3	3.82 (0.12)	42.70 (1.30)	46.52 (0.87)	3.94 (0.08)	39.80 (1.36)	43.79 (1.16)	4.69 (0.23)	35.85 (1.18)	40.54 (0.79)	4.02 (0.21)	38.71 (0.95)	42.73 (1.17)
4	4.69 (0.25)	40.16 (1.59)	44.85 (1.71)	4.93 (0.26)	36.30 (1.08)	41.23 (0.92)	5.40 (0.17)	33.56 (1.24)	38.96 (1.43)	5.20 (0.14)	36.40 (1.51)	41.60 (1.51)
5	5.23 (0.16)	39.00 (1.24)	43.13 (1.39)	5.23 (0.16)	33.85 (1.23)	39.08 (1.12)	6.22 (0.30)	30.99 (0.93)	37.21 (1.14)	6.71 (0.30)	33.43 (1.10)	40.20 (1.22)

( ) = SE

Effect on starches and dextrins

Table 11 shows that starches and dextrins decreased gradually during germination in the light. On the fifth day of germination, starches and dextrins in AMR, LAG, JOR, and SML decreased from 48.6 to 39.0 (18%), from 46.2 to 33.8 (27%), from 43.9 to 30.9 (29%), and from 44.2 to 33.4 (24%) respectively.

The decrease in starches and dextrins may be explained along the same lines as for total available carbohydrates above, as well as by their hydrolysis to produce smaller molecules.

Effect on ethanol-soluble sugars

Table 11 shows that soluble sugars have increased in all four varieties during germination at 25 C in the light. On the fifth day of germination, soluble sugars in AMR, LAG, JOR, and SML increased from 2.5 to 5.2 (108%), from 2.9 to 5.3 (83%), from 3.2 to 6.2 (93%), and from 2.6 to 6.8 g/100g (162%) respectively.

It may be seen from the above that soluble sugars increased gradually throughout the germination period; SML shows the highest while LAG the lowest increase in soluble sugars over the five day germination period.

Effect of light on mono-, di-, and oligo-saccharides of lentils grown at 25 C in the light

In the present study, during germination at 25 C in the light, mono-, and di-saccharides increased, while oligosaccharides decreased (tables 12 and 13).

Effect on monosaccharides

Tables 12 and 13 show that in AMR, fructose increased from 0.3 on the first day to 1.4g/100g on the fifth day (366%), in LAG from

Table 12: Effect of light on mono-, di- and oligo-saccharides of AMR and LAG varieties of lentil grown at 25°C in the light.
(The results are expressed as g/100g dry weight)

Days of germin.	AMR FRU	GLU	SUC	RAF	STA	TOTAL	Days of germin.	LAG FRU	GLU	SUC	RAF	STA	TOTAL
Control or o	0.00	0.00	0.63	0.26	1.45	2.34(0.15)	0	0.00	0.00	1.29	0.19	1.38	2.86(0.12)
1	0.31	0.22	0.98	0.15	1.00	2.66(0.17)	1	0.36	0.41	1.53	T	0.97	3.27(0.15)
2	0.67	0.55	1.20	0.10	0.78	3.30(0.23)	2	0.58	0.63	1.75	0.00	0.52	3.48(0.18)
3	0.88	0.76	1.70	T	0.48	3.82(0.12)	3	0.87	0.93	2.19	0.00	T	3.99(0.08)
4	1.14	1.27	2.08	0.00	0.20	4.69(0.26)	4	1.31	1.25	2.28	0.00	0.00	4.84(0.26)
5	1.43	1.54	2.26	0.00	0.00	5.23(0.16)	5	1.45	1.36	2.42	0.00	0.00	5.23(0.16)

( ) = SE
T = Traces

Table 13: Effect of light on mono-, di- and oligo-saccharides of JOR and SML varieties of lentil.
(The results are expressed as g/100g dry weight)

Days of germin.	JOR FRU	GLU	SUC	RAF	STA	TOTAL	Days of germin.	SML FRU	GLU	SUC	RAF	STA	TOTAL
0	0.00	0.00	1.18	0.15	1.83	3.16(0.18)	0	0.00	0.00	0.97	0.28	1.14	2.39(0.13)
1	0.38	0.46	1.63	0.10	0.97	3.54(0.10)	1	0.62	0.35	1.14	0.16	0.60	2.87(0.11)
2	0.76	0.84	1.72	T	0.78	4.10(0.16)	2	0.89	0.91	1.26	T	0.42	3.48(0.18)
3	1.15	1.23	1.86	0.00	0.45	4.69(0.23)	3	1.39	1.17	1.51	0.00	T	4.07(0.21)
4	1.74	1.58	2.08	0.00	0.00	5.40(0.17)	4	1.71	1.56	1.93	0.00	0.00	5.20(0.12)
5	2.16	1.94	2.12	0.00	0.00	6.22(0.30)	5	2.36	1.89	2.47	0.00	0.00	6.71(0.30)

( ) = SE

T = Traces

0.4 to 1.5 (275%), in JOR from 0.4 to 2.1 (425%), and in SML from 0.6 to 2.3g/100g (283%).

Glucose also increased during germination in the light. In AMR, glucose increased from 0.22 on the first day to 1.54g/100g on the fifth day of germination (600%), in LAG, in JOR, and in SML, it increased from 0.41 to 1.36 (231%), from 0.46 to 1.94 (322%), and from 0.35 to 1.89g/100g (440%) respectively (tables 12 and 13).

Effect on disaccharides

The sucrose content of lentils increased during germination at 25 C in the light. In AMR, LAG, JOR, and SML sucrose increased from 0.63 to 2.26 (276%), from 1.29 to 2.42 (87%), from 1.18 to 2.12 (80%), and from 0.97 to 2.47g/100g (154%) respectively (tables 12 and 13).

Effect on oligosaccharides

In the present study, raffinose showed a rapid decrease in illuminated germinating lentils. It disappeared completely on the third day of germination for LAG, JOR, SML, and on the fourth day for AMR. On the second day of germination, raffinose in AMR, LAG, JOR, and SML decreased from 0.26 to 0.15 (42%), from 0.19 to trace amounts, from 0.15 to 0.10 (33%), and from 0.28 to 0.16g/100g (43%) respectively.

Stachyose showed a gradual decrease during germination at 25 C in the light. In LAG, JOR, and SML it disappeared on the fourth day, while in AMR it disappeared on the fifth day of germination. In AMR, LAG, JOR, and SML stachyose decreased from 1.45 to 0.20 (86%), from 1.38 to 0.52 (62%), from 1.83 to 0.45 (75%), and from 1.14 to 0.42g/100g (63%) respectively (tables 12 and 13).

The total available carbohydrates, starches and dextrins, and ethanol-soluble sugars of four varieties of lentils grown at 35 C in the dark

Total available carbohydrates at 35 C

Table 14 shows that total available carbohydrates in all four varieties studied decreased on germination at 35 C in the dark. In AMR, LAG, JOR and SML total available carbohydrates on the fifth day of germination compared to dry seeds decreased from 51.11 to 41.92g/100g (18%), from 49.23 to 38.79 (21%), from 47.14 to 37.41 (20%) and from 46.81 to 39.05g/100g(16%) respectively.

Starches and dextrins at 35 C

Table 14 shows that starches and dextrins in all four varieties decreased over the germination period. In AMR, LAG, JOR and SML, there was a decrease on the fifth day of germination compared to dry seeds; from 48.77 to 39.32/100g (18%), from 46.37 to 35.66 (23%), from 43.88 to 33.93 (21%) and from 44.41 to 36.72g/100g (17%) respectively.

Ethanol-soluble sugars at 35 C

In the present study, the ethanol-soluble sugars in germinated lentils at 35 C in the dark increased, reached a maximum, and then started decreasing (table 14& fig. 7). In AMR, JOR, and SML on the second day after germination, soluble sugars increased maximally to 5.38g, 6.10g, and 4.81g/100g respectively, then on the fifth day they decreased to 2.60, 3.38, and 2.25g/100g respectively, while in LAG, they increased maximally to 5.05 on the third day,and then decreased to 3.13g/100g on the fifth day. Although the soluble sugars had reached a maximum, they then started to decrease gradually as shown, and on the fifth day the amounts were only slightly higher than or similar to the original content of the seeds, i.e. they were 11, 9, and 10% higher in

Table 14: The total available carbohydrate (TACHO), starch and dextrin (ST+D) and ethanol soluble sugars (ESS) of four varieties of lentil grown for five days at 35°C in the dark.

(The results are expressed as g/100g dry weight)

Days of germin.	AMR			LAG			JOR			SML		
	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO
Control or o	2.49 (0.15)	48.62 (1.25)	51.11 (1.15)	2.97 (0.12)	46.26 (1.18)	49.23 (1.30)	3.26 (0.18)	43.88 (1.50)	47.14 (1.90)	2.56 (0.13)	44.25 (0.88)	46.81 (1.47)
1	4.35 (0.16)	44.24 (1.23)	48.59 (2.26)	3.59 (0.09)	44.35 (1.32)	47.94 (1.10)	4.22 (0.12)	41.46 (2.11)	45.68 (1.35)	3.32 (0.18)	43.08 (1.10)	46.40 (1.60)
2	5.38 (0.21)	42.89 (1.70)	48.27 (1.20)	4.49 (0.24)	40.47 (2.10)	44.96 (1.90)	6.10 (0.21)	37.75 (1.48)	43.85 (2.25)	4.81 (0.29)	40.56 (1.40)	45.37 (1.10)
3	4.74 (0.15)	41.38 (1.40)	46.12 (1.30)	5.05 (0.20)	39.82 (1.60)	44.87 (1.26)	5.34 (0.15)	36.43 (1.26)	41.77 (1.10)	3.90 (0.11)	39.52 (1.77)	43.42 (1.70)
4	3.64 (0.10)	40.63 (1.10)	44.27 (1.45)	4.58 (0.11)	37.75 (1.14)	42.33 (1.47)	4.66 (0.19)	35.98 (1.35)	40.64 (1.30)	3.41 (0.17)	38.53 (1.52)	41.94 (1.12)
5	2.60 (0.14)	39.32 (1.36)	41.92 (1.15)	3.13 (0.17)	35.66 (0.87)	38.79 (1.60)	3.38 (0.13)	33.93 (1.65)	37.41 (1.80)	2.25 (0.10)	36.72 (1.22)	39.07 (1.25)

( ) = SE

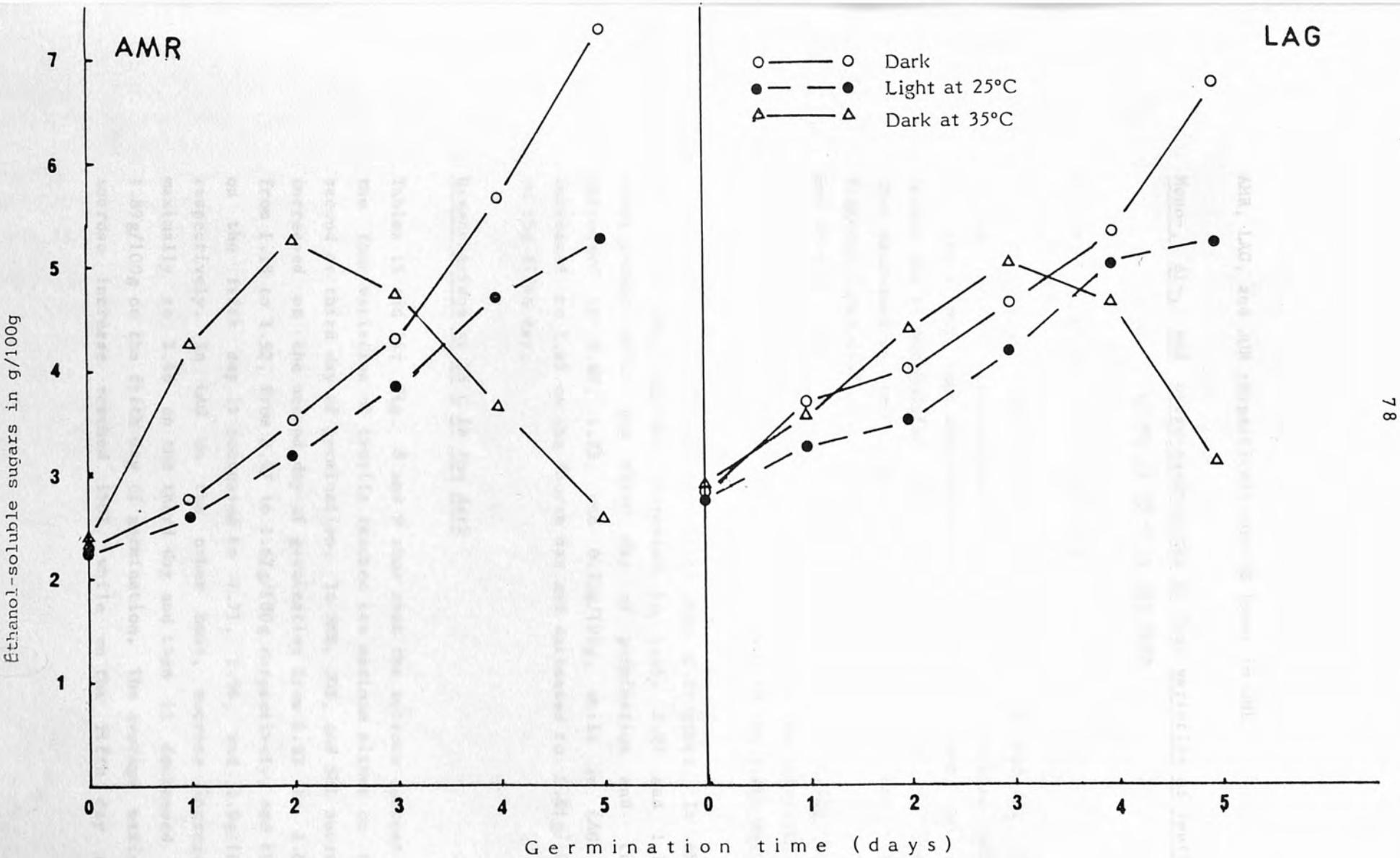


Fig. 7: Changes in ethanol-soluble sugars of lentil seeds germinated in the dark and in the light at 25°C, and in the dark at 35°C

AMR, LAG, and JOR respectively and 2% lower in SML.

Mono-, di-, and oligo-saccharides of four varieties of lentils grown at 35 C in the dark

Monosaccharides at 35 C in the dark

In this study (tables 15 and 16, fig. 8 and 9) the monosaccharides, glucose and fructose, increased rapidly until the third day, and then decreased gradually. In AMR, on the second day of germination, fructose increased from nil to 0.96, then decreased to reach 0.21 on the fifth day; in JOR, and in SML fructose increased respectively from nil to 1.89, and to 1.36, and then on the fifth day it decreased to 0.61 and to 0.52g/100g. In LAG, fructose increased from nil to 1.27g on the third day of germination, and then decreased to 0.43g/100g on the fifth day.

Glucose showed a similar pattern to that of fructose. In AMR, JOR, and SML, glucose increased to 1.46, 2.27 and 1.73g respectively after the third day of germination and then decreased to 0.68, 1.23, and 0.73g/100g, while in LAG it increased to 1.45 on the fourth day and decreased to 0.81g/100g on the fifth day.

Disaccharides at 35 C in the dark

Tables 15 and 16; fig. 8 and 9 show that the sucrose content of the four varieties of lentils reached its maximum either on the second or third day of germination. In AMR, JOR, and SML sucrose increased on the second day of germination from 0.63 to 2.85, from 1.18 to 1.92, from 0.97 to 1.67g/100g respectively, and then on the fifth day it decreased to 1.71, 1.54, and 1.0g/100g respectively. In LAG on the other hand, sucrose increased maximally to 2.46 on the third day and then it decreased to 1.89g/100g on the fifth day of germination. The average maximum sucrose increase reached 150%, while on the fifth day the

Table 15: Effect of 35°C on mono-, di- and oligo-saccharides of AMR and LAG varieties of lentils grown in the dark.
(The results are expressed as g/100g dry weight)

Days of germin.	AMR FRU	AMR GLU	AMR SUC	AMR RAF	AMR STA	AMR TOTAL	LAG FRU	LAG GLU	LAG SUC	LAG RAF	LAG STA	LAG TOTAL
Control or o	0.00	0.00	0.63	0.26	1.45	2.34(0.15)	0.00	0.00	1.29	0.19	1.38	2.86(0.12)
1	0.75	0.83	2.42	0.00	0.35	4.35(0.19)	0.88	0.56	1.63	T	0.52	3.59(0.09)
2	0.96	1.28	2.85	0.00	0.29	5.38(0.29)	1.13	0.95	2.18	0.00	0.17	4.43(0.19)
3	0.67	1.46	2.45	0.00	0.16	4.74(0.15)	1.27	1.31	2.46	0.00	T	5.04(0.20)
4	0.35	0.93	2.36	0.00	0.00	3.64(0.13)	0.97	1.45	2.16	0.00	0.00	4.58(0.11)
5	0.21	0.68	1.71	0.00	0.00	2.60(0.15)	0.43	0.81	1.89	0.00	0.00	3.13(0.17)

( ) = SE

T = Traces

Table 16: Effect of 35°C on mono-, di- and oligo-saccharides of JOR and SML varieties of lentil grown in the dark.

(The results are expressed as g/100g dry weight)

Days of germin.	JOR FRU	GLU	SUC	RAF	STA	TOTAL	Days of germin.	SML FRU	GLU	SUC	RAF	STA	TOTAL
Control or o	0.00	0.00	1.18	0.15	1.83	3.16(0.18)	Control or o	0.00	0.00	0.97	0.28	1.14	2.39(0.13)
1	0.94	1.21	1.40	0.00	0.61	4.22(0.18)	1	0.73	0.91	1.15	0.10	0.43	3.32(0.16)
2	1.89	1.82	1.92	0.00	0.47	6.10(0.25)	2	1.36	1.55	1.67	T	0.23	4.81(0.29)
3	1.32	2.27	1.75	0.00	T	5.34(0.20)	3	0.92	1.73	1.25	0.00	T	3.90(0.12)
4	1.12	1.89	1.65	0.00	0.00	4.66(0.12)	4	0.78	1.45	1.18	0.00	0.00	3.41(0.18)
5	0.61	1.23	1.54	0.00	0.00	3.38(0.19)	5	0.52	0.73	1.00	0.00	0.00	2.25(0.13)

( ) = SE

T = Traces

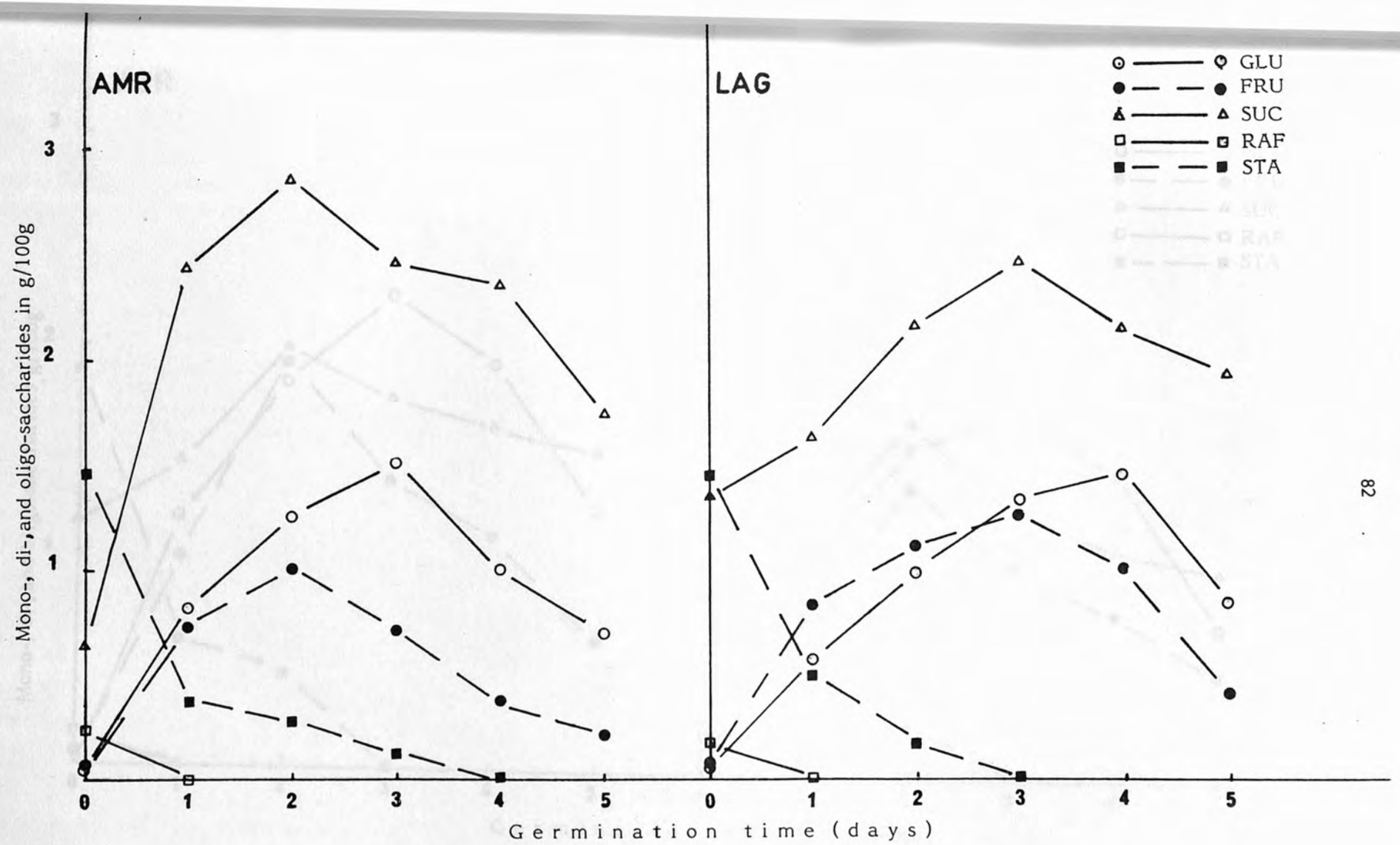


Fig. 8: Changes in mono-, di-,and oligo-saccharides of lentil varieties grown in the dark at 35°C

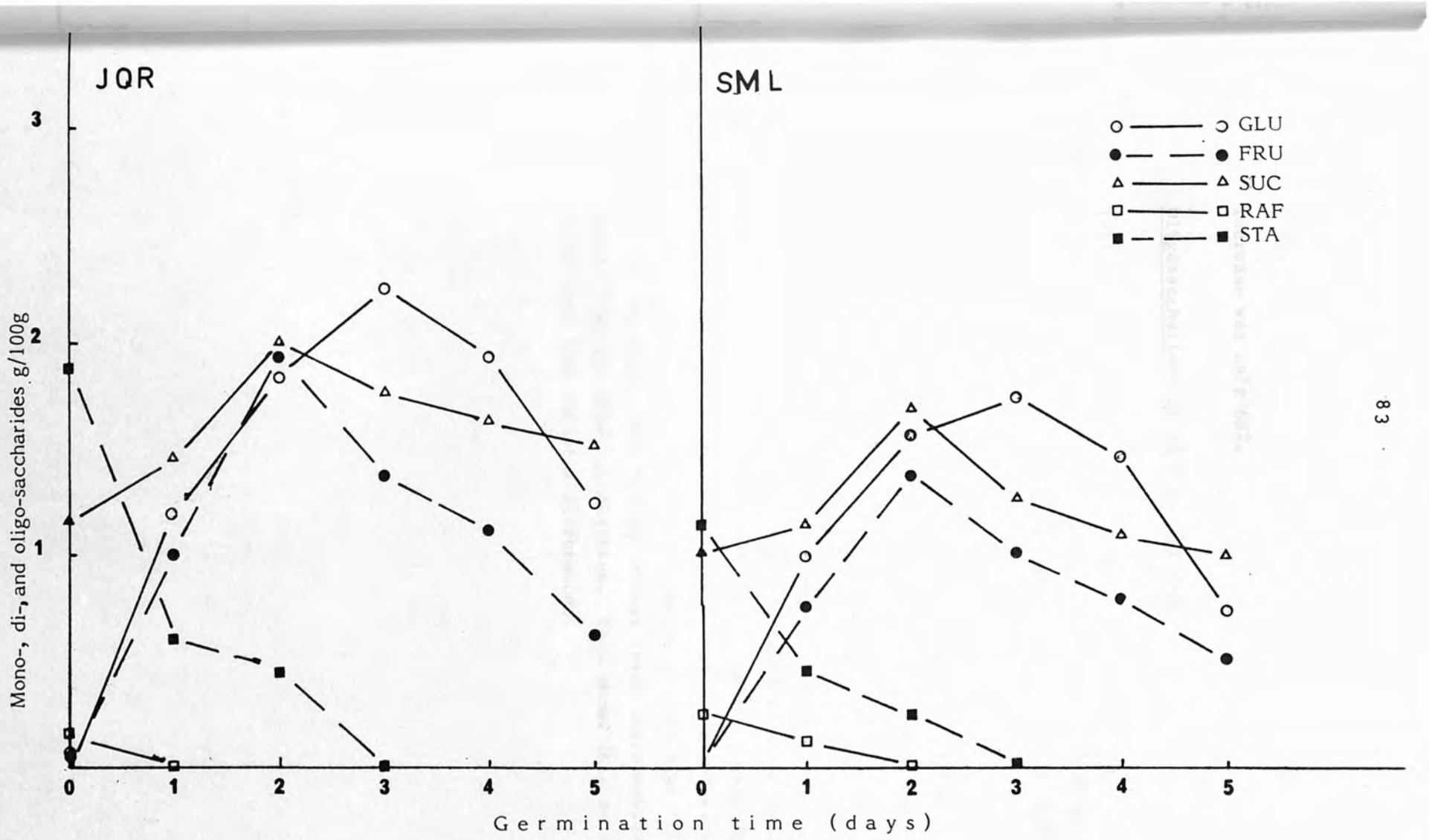


Fig. 9: Changes in mono-, di- and oligo-saccharides of lentil varieties grown in the dark at 35°C

increase was only 68%.

Oligosaccharides at 35 C in the dark

Tables 15 and 16 (fig. 8 and 9) show that there is almost complete disappearance of raffinose by the first day of germination, while stachyose disappeared completely either on the third or fourth day of germination.

Raffinose in AMR, LAG, and JOR disappeared on the first day, while it disappeared in SML on the second day of germination. In AMR, on the third day of germination, stachyose decreased from 1.45g in the dry seeds to 0.16g/100g, while in LAG, JOR, and SML, it decreased in the dry seeds from 1.38, 1.83, and 1.14g/100g respectively to 0.17, 0.47, and 0.23g/100g on the second day of germination. The different varieties tended to vary as to their changes in the various mono-, di-, and oligo-saccharides when grown at 35 C in the dark over a period of five days (fig. 8 and 9). On the whole, they did not always reach their maximum content on the same day after germination. This shows that in this case, there were some varietal differences.

CHAPTER 111

LIPIDS AND CRUDE FIBRE

INTRODUCTION

Lipids are a class of coumpounds grouped together because of similar solubility properties (OSER, 1965). The neutral fats are important because they form the bulk of the lipid material in the adipose tissue of the animal body. STUMPF (1976) has reported that in higher plants triacylglycerols (triglycerides) are the major constituents of lipids. Chemically, triglycerides are esters of the trihydric alcohol glycerol with three fatty acids. Plant triacylglycerols that are solid at room temperature are called fats, and have as their major fatty acid the saturated fatty acid : palmitic, while those liquid at room temperature are called oils and have as their major acids the unsaturated fatty acids: oleic (C18:1), linoleic and linolenic acids. Acids do hydrolyse triglycerides, since fatty acids are linked to the alcohol groups of glycerols by an ester bond. SALUNKHE et al. (1983) have reported that the distribution of total lipids in legumes, varies with the species, varieties, and location, and that the neutral lipids are the predominant class of lipids in legumes. Legume seed lipids contain substantial amounts of saturated fatty acids, especially palmitic acid (C16:0), ranging from 10 to 33% of the total lipids, also in most legumes, either of the unsaturated fatty acids, i.e. linoleic (C18:2) or linolenic acid (C18:3) is present as the major unsaturated fatty acids (SALUNKHE et al., 1983). The susceptibility of legume lipids to oxidation leads to several changes in them as well as in other food constituents, and of particular importance are the reactions between them and other compounds such as aldehydes, ketones, proteins, amino acids and vitamins. Neutral lipids (triglycerides), phospholipids, glycolipids and sterols are the major lipids in lentils.

In the present study, total lipids of the four varieties of lentils will be determined.

MATERIALS AND METHODS

The lipid fraction of a food is usually separated from the other compounds present in the food by extraction with an organic solvent such as petroleum ether, ethyl ether and reported as "ether-soluble fraction" or "crude fat" (MEYER, 1978). The initial problem in the determination of the lipid content of a plant tissue occurs in selecting the best method of extraction, because some methods suffer from incomplete extraction of cellular lipids (HITCHCOCK and NICHOLS, 1971). Another problem is in selecting the suitable solvent(s). However, quantitative extraction of lipids is obtained by maceration of fresh tissue with a solvent(s), most of which contain a fairly high percentage of alcohol to effect dehydration of the tissue. Nevertheless, care must be taken to minimize lipase activity during extraction of the tissue. CHRISTIE (1973) has shown that a mixture of chloroform-methanol (2:1, v/v) extracted more lipids exhaustively from plant, animal or bacterial tissues than most other simple solvent systems. In the present study, chloroform-methanol (2:1, v/v) was selected and the method of KATES (1970) was used.

Determination of total lipids

1. Extraction

To 10 g of seeds or 20 g of germinated seeds, 150 ml of choroform-methanol (2:1, v/v), was added, and the mixture blended in a Waring blender. The homogenate was filtered with suction, and the residue was reblended with 150 ml chloroform-methanol (2:1, v/v), followed by washing the residue with 40 ml H_2O, 70 ml chloroform-methanol (2:1, v/v). The combined filtrates were transferred to a separating funnel and 125 ml of choroform, and 150 ml of water were added, and the contents were shaken and the

phases allowed to separate. The chloroform layer was withdrawn, and then concentrated to 20-30 ml under vacuum at 30-35 C.

2. Estimation of lipids

To each of four glass vials, an aliquot of 5 ml of the concentrated chloroform solution was added. The sample was evaporated at 30 C in a stream of nitrogen. The vials were placed in a desiccator over KOH pellets and then were taken out and weighed. The sample was kept in the desiccator until the weight was constant to within $\pm$ 0.05 mg.

3. Calculation

The total extractable lipid content as per cent dry weight can be calculated from the following formula :

$$\text{Total extractable lipid (as \%)} = \frac{V_1 \times W_1 \times 100}{V_2 \times W_2}$$

where W_1 = weight (g) of the lipid in the aliquot,
W_2 = weight (g) of the sample (dry wt),
V_1 = volume of the concentrated chloroform layer,
V_2 = volume of aliquot (5 ml) concentrated chloroform.

CRUDE FIBRE

Crude fibre is defined as the organic residue which remains after the material has been treated with hot H_2SO_4, alkali, and alcohol (PEARSON, 1976). The crude fibre consists largely of cellulose with a little lignin (PEARSON, 1976; JOSLYN, 1970; VANSOEST and ROBERTSON, 1977). The fibre content of food is gaining a great deal of interest among many workers. However, the term is not a precise one and opinions vary, especially as to which minor components should be included or excluded under this term (JOHNSTON and OLIVER, 1982). Crude fibre and dietary fibre are not strictly interchangeable expression, and only one fifth to

one half of the total dietary fibre is actually crude fibre (SCALA, 1974). Crude fibre values for a given food tend to show variation with climate and degree of maturity (MEYER, 1978). Next to cereals, legumes are fibre-rich. WILSON et al. (1979) have reported that diets which are high in fibres are beneficial to humans, since lack of them causes certain diseases of the colon.

Therefore, the crude fibre content of the four varieties of lentils will be determined.

MATERIALS AND METHODS

In the present study, the AOAC method (AOAC,1975) was used. Is is based on the extraction with ether, then by boiling with H_2SO_4 , filtering and boiling with NaOH, and then filtering again, drying to constant weight, weighing and then ashing, and weighing again. The difference between the two weights is the crude fibre.

Reagents

1. H_2SO_4 : stock solution, 10% (w/v)
2. H_2SO_4: working solution, 1.25% (w/v)
3. NaOH : stock solution, 10% (w/v)
4. NaOH : working solution, 1.25% (w/v)
5. HCl solution, 1% (v/v)
6. Antifoam. 2% silicon antifoam in carbon tetrachloride.

1. Extraction

One g of the dry sample was extracted in 20 ml petroleum ether by stirring, settling and decanting three times.

2. Determination

The extracted sample (1) was air-dried, and transferred to a 500 ml conical flask. Two hundred ml boiling 1.25% H_2SO_4 were added, heated to boiling within 1 min, and a few drops of antifoam added. This was boiled gently for 30 min under reflux. The contents were filtered through a Buchner funnel No 3, using Whatman filter paper No 54. The sample was washed back into the original flask with 200 ml boiling 1.25% NaOH. The contents were again boiled for 30 min, taking the same precautions as in the previous boiling. The insoluble matter was transferred to a sintered glass crucible and then washed successively with boiling water, and with 1% HCl. The residue was washed twice with alcohol and three times with acetone, dried at 100 C to constant weight and then ashed in a furnace at 550 C for 1 h. The crucible was cooled and weighed.

3. Calculation

The fibre content (%) was calculated using the following formula:

$$\text{Fiber content (\%)} = \frac{(W_2 - W_3) \times 100}{W_1}$$

where, weight (g) of sample = W_1

weight (g) of insoluble matter = W_2

weight (g) of ash = W_3

RESULTS AND DISCUSSION

Total lipids of the dry lentils

Total lipid content in AMR, LAG, JOR, and SML are 1.17, 0.96, 1.32, and 1.21g/100g dry weight respectively. Table 17 shows that there is no significant difference in total lipids between the four varieties under investigation. The values of total lipids obtained in this study compare well with those of EL-NAHRY et al. (1980) for Egyptian varieties. The total lipid contents of all legumes, except peanuts and soybeans vary from between 1 to 7% depending on the species (SALUNKHE et al., 1983). So, total lipids of lentils are at the lower end of the range for legumes.

Crude fibre of dry lentil seeds

Crude fibre (table 17) in AMR, LAG, JOR, and SML is 4.10, 3.70, 4.54, and 4.86g/100g dry weight respectively. The crude fibre values obtained from the present study are slightly lower than those reported by VERMA et al. (1977) working with Indian varieties of lentils. Nowadays, different terms are used, e.g. fibre, crude fibre, and dietary fibre which may have different meaning (See p. 87). Dietary fibre has been defined as non-starch polysaccharides (CUMMINGS, 1980), or again as the remnants of plant cells resistant to hydrolysis by the enzymes of man and this will include lignin, cellulose, and hemicellulose (TROWELL, 1977). Some workers are of the opinion that lignin cannot be excluded because it is merely one of a number of polysaccharide-associated substances found in plant cell walls (CUMMINGS, 1980; THEANDER, 1977). Therefore, in the present context, crude fibre is defined as the the loss on ignition of dried residue of plant food left after sequential extraction with solvent, dilute acid and dilute alkali.

Table 17: Changes in total lipids and crude fibre of lentil varieties during germination in the dark at 25°C.
(The results are expressed as g/100g dry weight)

Days of germin.	Crude Fibre AMR	LAG	JOR	SML	Days of germin.	Total Lipids AMR	LAG	JOR	SML
Control or 0	4.10 (0.09)	3.70 (0.13)	4.54 (0.12)	4.86 (0.15)	0	1.17 (0.07)	0.96 (0.06)	1.32 (0.10)	1.21 (0.08)
1	4.72 (0.16)	4.25 (0.15)	5.07 (0.22)	5.54 (0.19)	1	1.00 (0.08)	0.77 (0.04)	1.14 (0.09)	0.93 (0.10)
2	5.49 (0.22)	5.36 (0.18)	5.76 (0.13)	6.46 (0.24)	2	0.83 (0.05)	0.61 (0.06)	0.88 (0.05)	0.71 (0.04)
3	6.56 (0.13)	6.40 (0.25)	7.08 (0.14)	7.29 (0.31)	3	0.50 (0.03)	0.34 (0.03)	0.59 (0.04)	0.35 (0.04)
4	7.95 (0.25)	7.80 (0.35)	8.26 (0.19)	8.50 (0.20)	4	0.23 (0.02)	0.12	0.39	0.11
5	9.25 (0.46)	8.90 (0.27)	9.76 (0.32)	10.11 (0.50)	5	T	T	0.18	T

( ) = SE ; SE is omitted when <0.02 g/100g; T = Traces

Changes in total lipids of lentil seedlings grown at 25 C in the dark

Lipids are broken down and converted both to pyruvic acid or to sugars, but this is not always so, as lipids in some seeds e.g. Citrullus are used in respiration and seem not to be converted to carbohydrates (MAYER and POLJAKOFF-MAYBER, 1975).

Changes in lipids of lentils grown at 25 C in the dark

There is a gradual decrease in total lipids in the four varieties of lentils after germination in the dark at 25 C (table 17, p. 91). On the fifth day of germination total lipids in AMR, LAG, and SML decreased to trace amounts while in JOR they decreased from 1.32 in the dry seeds to 0.18g/100g (86%).

These results agree with those of KYLEN and McCREADY (1975) who have reported that during germination of seeds, there is a rapid decrease in lipids. This decrease in lipids is explained by the action of lipases on lipids, followed by partial oxidation, and by conversion of certain intermediates to sucrose (STUMPF, 1976).

Changes in crude fibre of lentils grown at 25 C in the dark

Table 17 (p.91) shows that during germination at 25 C in the dark, there was an increase in crude fibre in the four lentil varieties under investigation. Crude fibre in AMR, LAG, JOR, and SML increased from 4.10 in the dry seeds to 9.25 (125%) on the fifth day, from 3.70 to 8.90 (140%), from 4.54 to 9.76 (115%), and from 4.86 to 10.11g/100g (108%) respectively.

Changes in lipids of lentils grown at 25 C in the light

There was a rapid decrease in total lipids of the four lentil varieties germinated in the light at 25 C (table 18). On the fourth day of germination, total lipids of AMR, LAG, and SML were

Table 18: Changes in crude fibre and total lipids of lentil varieties during germination in the light at 25°C.
(Results are expressed as g/100g dry weight)

Days of germin.	Crude Fibre AMR	LAG	JOR	SML	Days of germin.	Total Lipids AMR	LAG	JOR	SML
0	4.10 (0.09)	3.70 (0.13)	4.54 (0.12)	4.86 (0.15)	0	1.17 (0.07)	0.96 (0.06)	1.32 (0.10)	1.21 (0.08)
1	4.42 (0.21)	4.14 (0.25)	4.90 (0.80)	5.10 (0.23)	1	0.90 (0.08)	0.77 (0.05)	1.09 (0.11)	0.75 (0.06)
2	4.92 (0.29)	4.70 (0.17)	5.22 (0.32)	5.54 (0.20)	2	0.71 (0.07)	0.60 (0.06)	0.74 (0.08)	0.52 (0.06)
3	5.33 (0.18)	5.37 (0.21)	5.76 (0.25)	5.92 (0.15)	3	0.34 (0.02)	0.29	0.36 (0.03)	0.22
4	6.15 (0.22)	6.25 (0.27)	7.15 (0.14)	6.51 (0.26)	4	T	T	0.13	T
5	6.90 (0.34)	7.20 (0.20)	8.10 (0.28)	7.53 (0.40)	5	0.00	0.00	T	0.00

( ) = SE
T = Traces

found only in trace amounts, while in JOR they decreased from 1.32 to 0.13g/100g (90%).

These results agree well with those of ABRAHAMSEN and SUDIA (1966) who have reported that lipids were depleted more rapidly in soybean seedlings grown in light than in those grown in the dark.

Changes in crude fibre of lentils grown at 25 C in the light

Table 18 shows that there was an increase in crude fibre in the four lentil varieties tested during germination at 25 C in the light. On the fifth day of germination in light, crude fibre in AMR, LAG, JOR, and SML increased from 4.10 to 6.90 (68%), from 3.70 to 7.20 (95%), from 4.54 to 8.10 (78%), and from 4.86 to 7.53g/100g (55%) respectively.

Changes in total lipids of lentils grown at 35 C in the dark

There was a substantial decrease in total lipids of the four lentil varieties under investigation (table 19). On the third day of germination, total lipids in LAG, and SML decreased to traces, while in AMR, and JOR they decreased from 1.17 to 0.15 (87%), and from 1.32 to 0.25g/100g (81%) respectively.

Changes in crude fibre of lentils grown at 35 C in the dark

During germination at 35 C in the dark, there was a substantial increase in crude fibre in all four lentil varieties being tested (table 19). On the fifth day of germination compared to dry seeds, crude fibre in AMR, LAG, JOR, and SML increased from 4.10 to 11.27 (174%), from 3.70 to 10.89 (194%), from 4.54 to 11.35 (148%), and from 4.86 to 12.39g/100g (155%) respectively.

Table 19: Changes in crude fibre and total lipids of lentils during germination at 35°C in the dark.
(Results are expressed as g/100g dry wt.)

Days of germin.	Crude Fibre AMR	LAG	JOR	SML	Days of germin.	Total Lipids AMR	LAG	JOR	SML
0	4.10 (0.09)	3.70 (0.13)	4.54 (0.12)	4.86 (0.15)	0	1.17 (0.07)	0.96 (0.06)	1.32 (0.10)	1.21 (0.08)
1	5.30 (0.10)	4.89 (0.10)	5.45 (0.29)	5.83 (0.13)	1	0.70 (0.10)	0.54 (0.04)	0.86 (0.08)	0.58 (0.07)
2	6.56 (0.28)	6.10 (0.33)	6.40 (0.25)	6.80 (0.21)	2	0.45 (0.05)	0.24	0.55 (0.06)	0.30 (0.04)
3	8.40 (0.30)	7.62 (0.17)	7.80 (0.21)	8.90 (0.38)	3	0.15 (0.03)	T	0.25 (0.03)	T
4	9.96 (0.24)	9.25 (0.40)	9.53 (0.43)	10.54 (0.52)	4	0.00	0.00	T	0.00
5	11.27 (0.50)	10.89 (0.26)	11.35 (0.61)	12.39 (0.38)	5	0.00	0.00	0.00	0.00

( ) = SE
T = Traces

CHAPTER IV

PROTEINS

INTRODUCTION

BENDER (1978) has reported that the usefulness of a protein for tissue synthesis depends on the amino acid which is found as the lowest amount (limiting amino acid). VAN ETTEN et al. (1967) have studied the potential of seeds (i.e. Leguminoseae family) for food and have concluded that the seeds were a good source of low cost protein. OSER (1965) has reported the erroneous generalization that all plant proteins are incomplete or poorly balanced with respect to their essential amino acids. In fact, leafy vegetables and beans, are almost as suitable as sources of amino acids as many of the expensive animal products.

Protein in food is not actually determined as such, but conventionally protein is estimated from the total nitrogen (N) content using an empirical factor (PEARSON, 1976; MAURON, 1973). Proteins differ in amino acids and therefore in their nitrogen content, even so the factor 6.25 is commonly used (ADRIAN, 1982). The factor 6.25 has been used for lentils by many workers (FAO, 1970; HSU et al., 1980; EL-NAHRY et al., 1980).

MATERIALS AND METHODS

Determination of proteins

The protein contents are usually evaluated by multiplying the nitrogen percent by the coefficient 6.25. Besides the nitrogen determination by the KJELDAHL method, there are several analytical techniques for measuring protein. These comprise colorimetric methods e.g. dye binding capacity (MOSSE and

PERNOLLET, 1983). However, in the present study, since there were changes in the nature of the protein under the different treatments, the macro-KJELDAHL method, which basically is the recommended AOAC method was used (OSBORNE and VOOGT, 1978). It is based on the fact that oxidation by sulphuric acid causes the nitrogen to be converted to ammonium sulphate. After making the solution alkaline with concentrated sodium hydroxide solution, the ammonia is distilled into an excess of boric acid, and the ammount of NH_3, is estimated by titrating against hydrochloric acid.

Reagents

1. Sulphuric acid (concentrated).
2. Hydrochloric acid (0.1N).
3. Boric acid solution; 40 g boric acid (H_3BO_3) was dissolved in distilled water and diluted on 1 litre.
4. Sodium hydroxide solution; 500 g of sodium hydroxide (NaOH) was dissolved in 1 litre (1) distilled water.
5. Copper sulphate pentahydrate($CuSO_4.5H_20$).
6. Sodium sulphate (anhydrous Na_2SO_4).
7. Mixed indicator solution; 2 g of methyl red and 1 g of methylene blue were dissolved in 1 l ethanol (96% v/v).
8. Glass boiling beads.

Digestion

A few glass beads were placed in a KJELDAHL flask to which 8 g of Na_2SO_4 and 0.5 g $CuSO_4$ were added. One g, weighed to the nearest mg, of seeds was also transferred to the flask and 25 ml sulphuric acid was carefully poured in. Gentle swirling of the liquid ensured sufficient mixing. The flask was placed on an electric heater in an inclined position, then heated gently until foaming ceased and the contents liquefied.

Digestion was completed by boiling the mixture vigorously until the liquid became clear and of a light blue-green colour (approximately 3 hour). The flask was occasionally rotated while

heating but caution was taken to avoid any spillage. The solution was cooled and 50 ml of distilled water cautiously added.

Distillation and titration

The contents of the KJELDAHL flask were diluted with approximately 300 ml of water, transferred to 1 l distillation flask, to which fresh boiling beads were added. Fifty ml of boric acid solution were added to a conical flask, as were 4 drops of the indicator. The flask was placed under the condenser of the distillation apparatus so that the outlet tube dipped below the boric acid-indicator solution. One hundred ml of NaOH solution was added carefully to the distillation flask. The mixture was distilled to give between a minimum distillate of 150 ml and a maximum of 250 ml. If bumping was irregular after 150 ml the distillation was discontinued

Just prior to terminating distillation, the receiving flask was lowered, so that the outlet tube was above the liquid level. The condenser and the outlet tube were washed down into the receiver. The contents of the receiving flask were titrated with HCl, and the volume noted. A blank test was conducted at the same time, following the same procedure exactly, except that no sample was added here.

Calculation

Weight (g) of test sample =W

Volume (ml) of HCl used for blank $=V_1$

Volume (ml) of HCl used for sample $=V_2$

Normality of HCl solution =N

$$\text{Total nitrogen (\%)} = \frac{(V_2 - V_1) \times N \times 0.014 \times 100}{W}$$

$$\text{Crude protein (\%)} = \frac{(V_2 - V_1) \times N \times 0.014 \times 100 \times 6.25}{W}$$

PROTEIN IN DRY LENTIL SEEDS

RESULTS AND DISCUSSION

The protein values were found to be 24.14, 26.43, 25.35, and 27.57 g/100g dry weight for AMR, LAG, JOR, and SML respectively (table 20).

These results are comparable with those of EL- NAHRY et al. (1980) who observed that protein content of the Egyptian and Pakistani varieties e.g. Giza 9, family 91, and family 195 varied from 25.4 to 27.5 g/100g. Also higher contents e.g. 30.3g/100g of some lentil varieties have been reported (BHATTY et at., 1976). On the other hand, lower protein content varying between 20.2 and 22.9 g/100g has been reported for some Indian varieties (VERMA et al., 1977). Among legumes, yellow peas and lentils tend to have nearly the same protein contents (26%), while faba beans tend to have higher values (30%) (HSU et al., 1980). Lentils have a higher protein content compared to cereals, e.g. for lentils, wheat and rice it is 23.7, 9.3, and 6.5 g/100g respectively (SAINT-CLAIR, 1972).

3. Changes in protein content in the dark

The role of protein in seeds is still obscure, and germination involves varied and intense biological activities and one can therefore assume that protein provides the raw materials for such activities (BERK, 1976). Contradictory results about changes in seed protein have been reported by some workers. An increase in protein content as a result of germination has been reported by some workers (KYLEN and McCREADY, 1975; FORDHAM et al., 1975). On the other hand BEEVERS and GUERNSEY (1966) have shown that

Table 20: Effect of germination at 25°C in the dark on the protein content of four varieties of lentils.
(The results are expressed as g/100g dry weight)

Days of germin.	AMR	LAG	JOR	SML
Control or o	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
1	21.40(0.65)	23.42(0.43)	22.50(0.57)	25.30(0.85)
2	19.54(0.45)	21.56(0.71)	20.25(0.60)	23.90(0.51)
3	18.09(0.52)	19.24(0.37)	19.14(0.76)	21.60(0.75)
4	17.04(0.46)	17.94(0.48)	18.25(0.54)	20.27(0.63)
5	15.62(0.39)	16.23(0.40)	16.31(0.61)	18.00(0.48)

( ) = SE

during the germination of peas in the dark protein content declined. MAYER and POLJAKOFF-MAYBER (1975) have reported that metabolic changes that occur to storage proteins include the breakdown of proteins and the synthesis of new ones, but the exact time of protein synthesis seems to be rather variable.

4. Changes in protein amino acids

YOUNG and VARNER (1959) have reported that a net synthesis of enzyme protein during germination possibly accounts for a major portion of the protein increase. During germination, there is probably a turnover of protein and amino acids, with the balance between synthetic and degradative process determining the resultant pattern (CHEN and THACKER, 1978). However, it is well known that germination increases the protein quality of cereals (WALKER and KOHHAR, 1982; WANG and FIELD, 1978).

Effect of germination at 25 C in the dark on protein contents

RESULTS AND DISCUSSION

There is a decrease in protein content in all four varieties during germination at 25 C in the dark (table 20, p.100).
On the fifth day after germination compared to dry seeds, the decrease was as follows : AMR from 24.1 to 15.6 (35%), LAG from 26.4 to 16.2 (38.6), JOR from 25.4 to 16.3 (36%), and SML from 27.6 to 18.0g/100g (34%). SML lost less protein (34%) while LAG lost the most (38.6%).

During germination, the protein is depleted, and amino acids are released from protein by hydrolysis (BEEVERS and GUERNSEY, 1966). The above suggestion may explain the reduction in protein content during germination.

The results obtained from the present study agree with those

reported by AMAN (1979) who has reported a 14% decrease in protein on the third day of germination of mung beans, and PALIANO and JULIANO (1972) a 52% decrease in 5 day-old rice seedlings. Other workers have also reported a reduction in protein content in other germinated seed legumes (BEEVERS, 1968; DESHMUSHI and SOHONIE, 1966; CHEN and THACKER, 1978).

On the other hand, opposite contradictory results, an increase in protein as a result of germination in the dark, have been reported by some workers. On a dry weight basis, the protein content of germinated peas and beans ranges from 21.2 to 32.6% which is comparable to 20.0 to 29.5% in dry seeds (CHEN et al., 1975).

KYLEN and McCREADY (1975), have found that the protein content of all sprouts was higher than that of seeds e.g. from 106.6% of the original in lentils to 119% in mung beans after the third day of germination.

Of the above, those who have reported an increase in protein, have suggested that the increase in protein may have been due to a net synthesis of enzymic protein as has been previously suggested by YOUNG and VARNER (1959). But even though there is some synthesis of new proteins, their rates of formation may be slower than those of its decrease through hydrolysis, thus showing an overall decrease. Some of the breakdown proteins may be used directly for the synthesis of new amino acids or may be converted to other amino acids or may be used as a carbon source for carbohydrate and lipid syntheses (HURST, 1970). Since there are several steps in the synthesis and in the metabolism of protein, it is not surprising that different results are obtained with different seeds. Eventually, it is the balance between synthesis and metabolism which is important: with higher synthesis/metabolism ratio there is an increase and the reverse ratio a decrease in protein. It should not be assumed however, that there is no protein synthesis in the dark.

Effect of light on protein content of lentils grown at 25 C in the light

RESULTS AND DISCUSSION

In this study, the protein content of all four varieties was quantified during germination at 25 C in the light (table 21). On the 5th day of germination the protein content of AMR, LAG, JOR, and SML decreases from 24.14 to 17.30(28%), from 26.43 to 19.24(27%), from 25.35 to 18.25(28%) and from 27.57 to 20.25g/100g (25%) respectively. The average protein content of all lentil varieties on the fifth day of germination in light is approximately 74%.

Different effects of light on protein during germination have been reported by different workers. The results of this study agree with those of PALMIANO and JULIANO (1972) who have reported that protein content of rice grains germinated for 5 days in light decreased from 1.36 to 0.92 mg/grain. RACUSEN and ARONOFF (1954) have reported that CO_2 was assimilated into the protein of soy beans leaves in the light due to resynthesis of protein, even though the total protein was on the decline. So, what is important is the balance between synthesis and hydrolysis (breakdown) of protein during illumination. Also the results of the present study agree with those of HAMILTON and VANDERSTOEP (1979) who have reported that the protein of alfalfa seeds germinated for 5 days decreasd by 8%.

Illumination increases the activity of some enzymes (CHEN et al., 1967) and this results in greater protein synthesis, and therefore the protein appears to be greater in illuminated than in dark grown seedlings.

Table 21: Effect of light during germination on the protein content of four varieties of lentil grown at 25°C.
(The results are expressed as g/100g dry weight)

Days of germin.	AMR	LAG	JOR	SML
Control or o	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
1	22.08(0.64)	23.60(0.57)	23.50(0.48)	25.65(0.51)
2	21.10(0.75)	22.88(0.66)	22.40(0.82)	24.57(0.69)
3	19.83(0.48)	21.58(0.51)	21.25(0.45)	23.22(0.36)
4	18.72(0.58)	20.54(0.48)	20.15(0.61)	21.60(0.49)
5	17.30(0.41)	19.24(0.29)	18.25(0.58)	20.25(0.29)

( ) = SE

Protein content of lentils germinated at 35 C in the dark

Table 22, fig. 10 show that the high temperature (35 C) during germination has decreased the protein of all four varieties studied compared to dry seeds.

In AMR, on the fifth day of germination, protein had decreased from 24.14 to 12.86 g/100g (47%), in LAG it decreased from 26.43 to 13.10g/100g (50%), in JOR from 25.35 to 14.64 (42%) and in SML from 27.57 to 16.25g/100g (40%).

From the above results two conclusions can be drawn:

1) during the first two days of germination, the higher temperature (35 C) has a more influential effect on protein hydrolysis and

2) for the rest of the germination period the rate of decrease in protein in the four varieties under investigation is higher than that at 25 C in the dark.

Protein amino acids

INTRODUCTION

Protein utilization is a function of the amino acids present. The amino acids play an important role in determining the nutritive value of a protein. There are eight essential amino acids, tryptophan, lysine, threonine, valine, leucine, isoleucine, methionine and phenylalanine. Histidine is also considered essential for infants. OSER (1965) has reported that "essential" and " non-essential" amino acids are important in one way or another for the metabolic processes of the body. A protein of high nutritive value is one which supplies an adequate amount, not only of essential but also of non-essential ones to minimize the requirement for their synthesis. The presence of low

Table 22: The protein content of four varieties of lentils germinated for five days at 35°C in the dark.
(The results are expressed as g/100g dry weight)

Days of germin.	AMR	LAG	JOR	SML
Control or o	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
1	21.65(0.60)	22.78(0.34)	21.50(0.55)	23.18(0.41)
2	18.70(0.72)	19.73(0.50)	19.45(0.61)	21.52(0.78)
3	16.30(0.24)	17.60(0.30)	18.05(0.48)	19.63(0.40)
4	14.45(0.36)	15.38(0.68)	16.90(0.29)	18.40(0.60)
5	12.86(0.42)	13.10(0.38)	14.64(0.58)	16.25(0.35)

( ) = SE

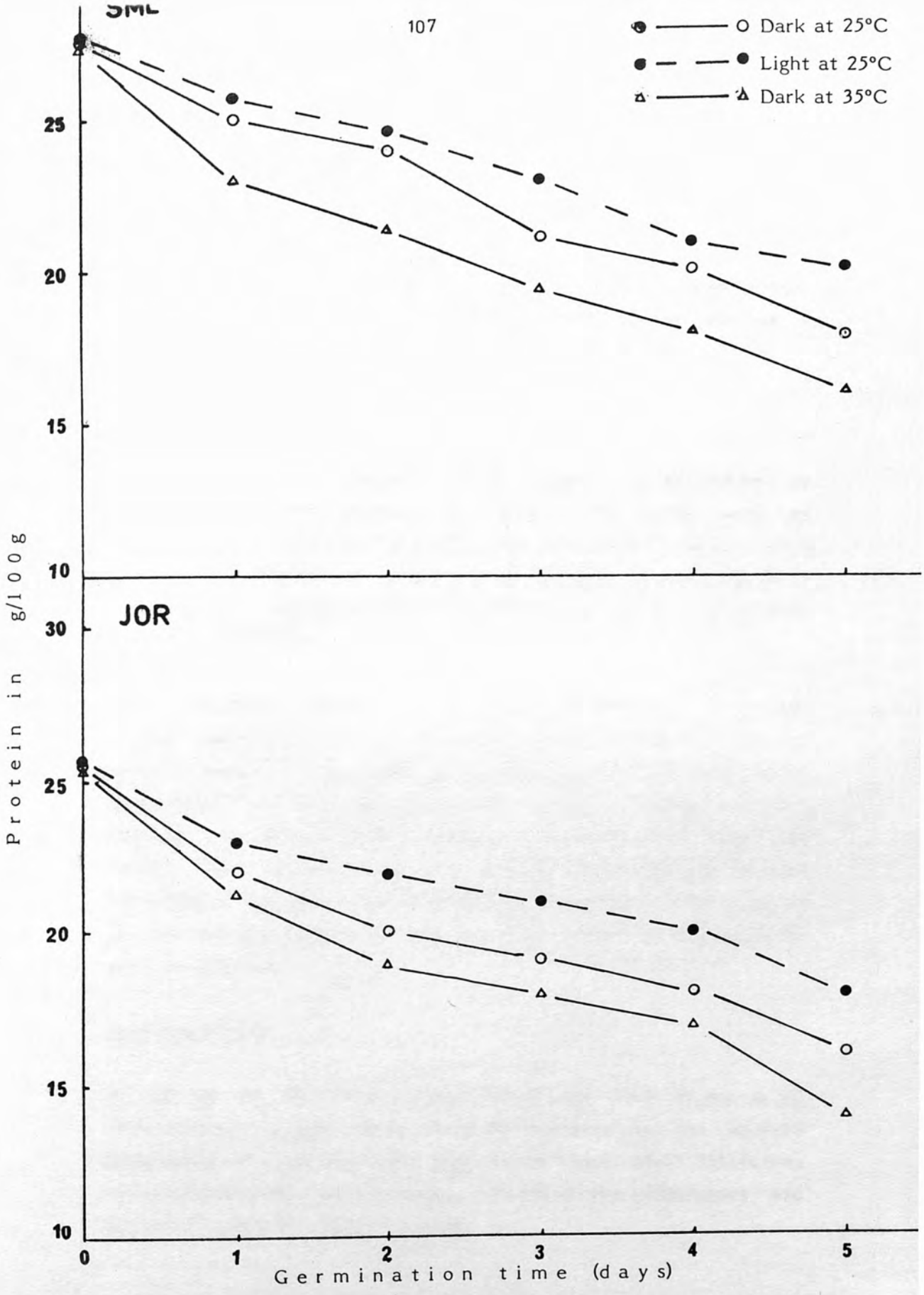


Fig. 10: Changes in protein of lentil varieties germinated in the dark and in the light at 25°C, and in the dark at 35°C

inadequate amount of even one of the essential amino acids may be a limiting factor in the biological value of a protein.

Most legume seeds appear to be slightly deficient in isoleucine, tryptophan or valine, but histidine is usually very limiting (EVANS and BANDEMER, 1967).

Protein hydrolysis

The amino acid composition of a protein is determined by hydrolysis of the protein to a mixture of amino acids by splitting the peptide linkages. The mixture of amino acids accurately reflects the amino acid of the hydrolysates and will allow the composition of the original protein to be readily obtained (BLACKBURN, 1968).

Acid hydrolysis (using HCl) gives good results for the acid-stable amino acids, e.g. all those commonly occurring in food protein except cystine, cysteine, methionine and tryptophan which are labile under acid hydrolysis, and require a separate method (OSBORNE and VOOGT, 1978). However, the amino acids that are labile under acidic conditions can be determined by alkali hydrolysis, in particular tryptophan (BLACKBURN, 1968), and so in the present study both acid and alkali hydrolyses of proteins were carried out.

Acid hydrolysis

To 25 mg of the dried residue (from the free amino acids determination p126), 10 ml of 6N HCl was added and the mixture hydrolysed in a boiling water bath for 20 hours. After filtration using Whatman No 1 filter paper, the pH of the hydrolysate was adjusted to 2 $\pm$ 0.1 with 5 N NaOH.

Alkaline hydrolysis

To 25 mg of the dried residue (p126), 10 ml of 5N-NaOH was added. This was hydrolysed in a boiling water bath for 15 hours. After filtration through a Whatman No 1 filter paper, the pH of the solution was adjusted to 2±0.1 with 5N HCl.

Estimation of the individual amino acids

The original separation procedure of SPACKMAN et al. (1958) is still the most widely used. The separation and quantitative determination of the amino acids was carried out using an amino acid analyser (JOEL Model JLC 6AH). The sample is pumped down through the column filled with ion-exchange resin (LCR-2). The resin retards the amino acids and each one in a buffer solution emerges after a characteristic length of time. The effluent is met by a capillary stream of ninhydrin reagent. The colour is developed by passing the mixture of reagent and effluent through a spiral, capillary Teflon tubing immersed in a boiling water bath. The absorbance of the resulting solution is measured continuously at 570 and 440 nm as it flows through a cylindrical glass cell. The peaks on the curves can be integrated with a precision of 100 ±3% for loads from 0.1 to 3 µmole for each amino acid (SPACKMAN et al., 1958).

The amount of amino acids in each sample is calculated by comparison of peak areas with those obtained using a calibration mixture as described by EVELEIGH and WINTER (1979) (fig.11). The calibration mixture of amino acids was expressed in µmole. In order to convert the values for amino acids into g weight, a modified formula was used to include the molecular weights of individual amino acids.

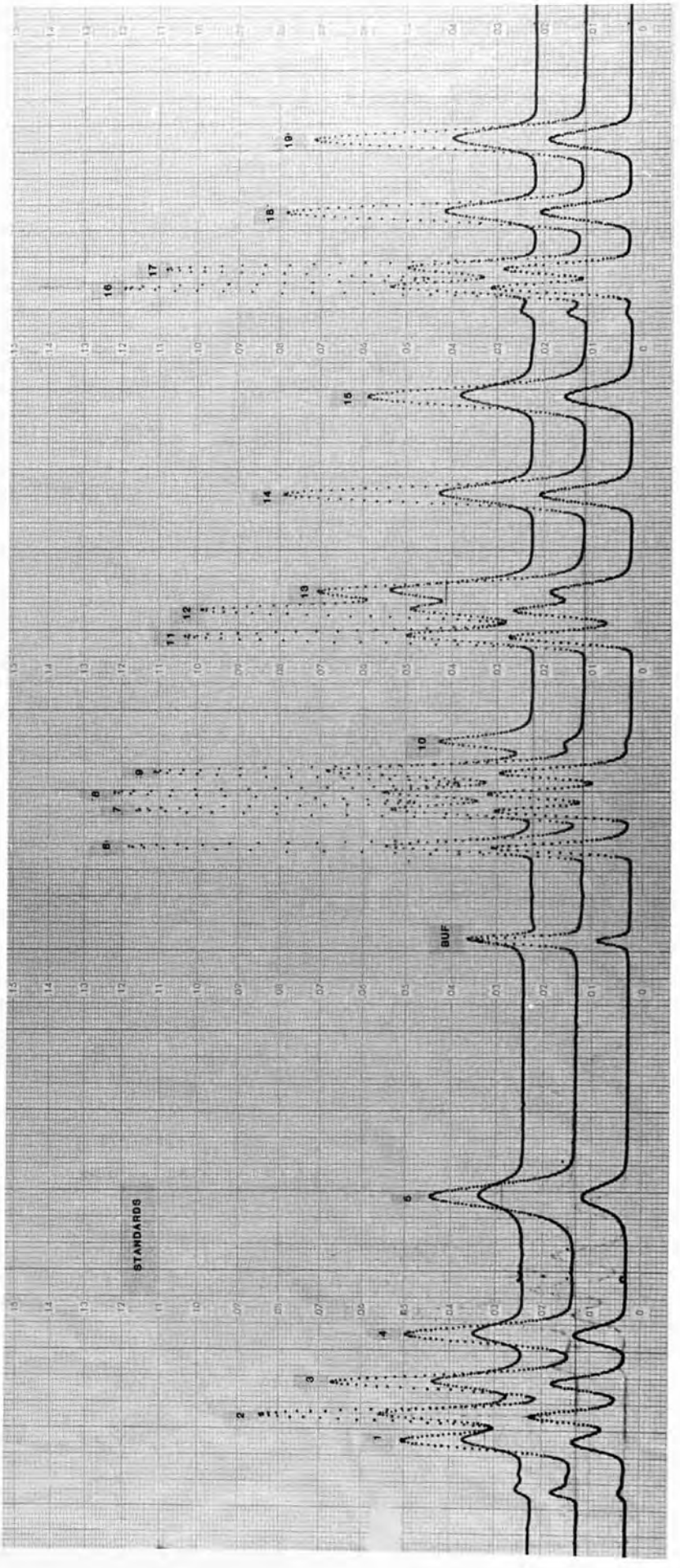
Weight of amino acid (g) in sample = µmoles amino acid x M $\times 10^{-6}$

where M = Molecular weight of each amino acid.

Fig. 11. Amino acid analysis by automatic amino acid analyser (Standards)

Following key applies to fig. 11 and 12

1. Tryptophan
2. Lysine
3. Histidine
4. Ammonia
5. Arginine
6. Aspartic acid
7. Threonine
8. Serine
9. Glutamic acid
10. Proline
11. Glycine
12. Alanine
13. Cysteine
14. Valine
15. Methione
16. Isoleucine
17. Leucine
18. Tyrosine
19. Phenylalanine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PROTEIN AMINO ACIDS OF DRY LENTIL SEEDS

In the present study (table 23) eighteen animo acids plus ammonia were identified, and the amino acids were: tryptophan, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine. The total protein amino acids of AMR, LAG, JOR and SML are 80.8, 83.5, 81.9, and 82.6 g/100g protein respectively. LAG has the highest, while AMR the lowest.

Aspartic acid, glutamic acid and arginine are the most abundant ones. Tryptophan content (table 23) ranged from 0.78 to 0.91g/100g protein, a slightly higher value of 1.14g/100g protein has been reported by VERMA et al. (1977) for lentils. The methionine content varied from 0.50 to 0.70g/100g protein; similar results having been reported by BHATTY et al. (1976) and by VERMA et al. (1977). Histidine content (table 23) ranged from 1.9 to 2.0 g/100g protein and therefore lentils are not very deficient in this amino acid. This result is in disagreement with that of EVANS and BANDEMER (1967) who have reported that legume seeds are generally very deficient in histidine.

In the present study, the differences in total amino acids of the four varieties were not significant. This is in agreement with the suggestion of MOSSE and PERNOLLET (1983) who have reported that the seed protein content of different varieties does not vary so much, and this may explain partially why the differences in total amino acids are often not significant.

Changes in protein amino acids during germination at 25 C in the dark

Table 23 (p.113) shows the changes in protein amino acids during germination at 25 C in the dark. On the fifth day of germination compared to dry seeds, protein amino acids in AMR decreased from

Table 23: Changes in protein-amino acids of four varieties of lentils during germination at 25°C in the dark as a function of time. The results are expressed as g amino acid/100g protein.

Amino Acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan	0.78	0.60	0.44	0.36	0.90	0.72	0.58	0.50	0.80	0.66	0.41	0.36	0.85	0.67	0.56	0.31
Lysine	6.45	6.64	6.85	7.01	6.38	6.45	6.67	6.83	6.29	6.41	6.66	6.73	6.12	6.25	6.47	6.60
Histidine	1.93	1.61	1.42	1.13	1.87	1.53	1.17	0.84	1.68	1.28	1.06	0.77	2.10	1.67	1.24	0.90
Ammonia	2.23	2.45	2.78	2.96	2.44	2.65	2.92	3.17	2.00	2.27	2.48	2.60	2.58	2.83	3.05	3.35
Arginine	6.72	5.36	4.36	2.85	7.05	5.95	4.34	3.12	6.86	5.64	4.28	3.08	6.53	5.47	3.98	2.93
Aspartic acid	10.19	8.58	7.37	5.13	10.56	8.92	6.93	4.73	10.82	9.17	7.23	5.15	10.46	9.15	7.06	5.25
Threonine	2.46	2.05	1.56	1.22	2.64	2.15	1.69	1.36	2.10	1.62	1.32	1.14	2.25	1.98	1.53	1.23
Serine	3.00	2.40	1.78	1.15	3.29	2.56	1.96	1.31	3.17	2.48	1.86	1.39	3.30	2.81	1.95	1.48
Glutamic acid	11.18	11.45	11.73	12.05	11.25	11.45	11.63	11.80	11.60	11.85	12.18	12.41	11.06	11.28	11.45	11.70
Proline	4.06	3.24	2.48	1.60	4.43	3.52	2.81	1.50	4.26	3.46	2.64	1.91	4.12	3.48	2.80	1.70
Glycine	4.50	3.42	2.85	2.02	4.90	3.92	3.18	2.25	5.08	4.32	3.49	2.25	5.20	4.57	3.48	2.34
Alanine	4.88	4.12	3.16	2.18	5.07	4.10	3.27	2.06	5.29	4.27	3.22	2.30	5.30	4.66	3.44	2.12
Cysteine	1.06	0.85	0.71	0.60	0.80	0.68	0.53	0.41	0.74	0.61	0.55	0.40	0.90	0.78	0.59	0.49
Valine	4.10	3.19	2.74	2.18	4.36	3.74	3.20	2.29	4.15	3.56	2.75	2.30	4.45	3.73	2.99	2.31
Methionine	0.54	0.42	0.32	0.25	0.60	0.44	0.34	0.28	0.50	0.40	0.29	0.21	0.70	0.59	0.44	0.38
Isoleucine	2.96	2.49	2.17	1.74	3.10	2.71	2.10	1.70	3.00	2.67	2.14	1.65	2.80	2.40	1.87	1.56
Leucine	5.28	4.57	4.06	2.80	5.20	4.26	3.32	2.91	5.42	4.69	3.45	3.05	4.92	4.31	3.28	2.69
Tyrosine	4.17	3.48	2.87	2.25	4.31	3.56	2.71	2.35	3.87	3.23	2.85	2.43	4.23	3.52	2.68	2.25
Phenylalanine	4.48	3.65	2.94	2.42	4.43	3.52	3.10	2.33	4.30	3.61	3.08	2.70	4.80	3.84	3.26	2.81
TOTAL	80.88	70.57	62.59	51.90	83.58	72.84	62.45	51.74	81.91	72.20	60.95	52.81	82.67	73.89	62.05	52.40
	(2.80)	(2.52)	(1.79)	(2.09)	(2.15)	(3.00)	(2.35)	(2.11)	(1.90)	(2.59)	(1.78)	(2.17)	(2.46)	(2.99)	(2.30)	(1.85)

( ) = SE, 0,1,3,5 = Days

81.9 to 51.9, LAG from 83.6 to 51.7, JOR from 81.9 to 52.8 and SML from 82.6 to 52.4 g/100g protein. In all the varieties a few amino acids increased e.g. lysine and glutamic acid, while all the other amino acids decreased.

These results are in agreement with those of HSU *et al.* (1980) and of CHEN and THACKER (1978) who have reported an increase in some amino acids in germinated lentils and peas. On the other hand a decrease in some or all essential amino acids in germinated soybeans and green grams has been reported by BAU and DEBRY (1979) and by KRISHNAMURTHY *et al.* (1982).

As can be seen from the above results, studies of changes in amino acids in legumes during seed germination tend to show a great deal of variation.

CHANGES IN PROTEIN AND AMINO ACIDS OF LENTILS DURING GERMINATION IN THE LIGHT AT 25 C AND IN THE DARK AT 35 C

GENERAL INTRODUCTION

STEWARD and BIDWELL (1962) have pointed out that the environment in which the plant grows has a determining effect on the course of nitrogen metabolism and on the soluble compounds which are formed and stored. LITENBERGER (1973) has reported that amino acid composition studies are underway in some countries e.g. U.S.A. and India to study the individual amino acids and their interaction with both protein and environmental condition.

McALISTER and KROBER (1951) grew soybean seedlings under natural light (the seedlings were dependent upon the reserves of the cotyledons throughout the growing period) and have reported that protein showed a rapid decrease until nine days after germination, followed by a steady but much less rapid decrease. HAMILTON and VANDERSTOEP (1979) have found that the protein content of germinated alfalfa seeds was higher at 72 h light

compared to 72 h in the dark, while it was lower at 120 h light than at 120 h dark. Mint plant tends to promote glutamine synthesis in the light and that of asparagine in the dark. This is associated with the general trend toward protein synthesis in the light and toward breakdown in the dark (STEWARD and BIDWELL, 1962).

Effect of light on protein amino acids of lentil seeds grown at 25 C

In this study (table 24), total protein amino acids of the four varieties studied decreased during germination in the light.

On the fifth day of germination total amino acids in AMR decreased from 80.9 to 56.8 g/100g protein, in LAG from 83.6 to 58.3, in JOR from 81.9 to 57.4, and in SML from 82.7 to 60.1 g/100g protein. During germination in light lysine and glutamic acid decreased, as did the other 16 amino acids. FARHANGI (1980) has reported that amino acids of dark-grown mung beans seedlings subjected to 24 h illumination decreased slightly from 96.4 to 94.3 g/100g protein.

Changes in protein amino acids of lentils during germination at 35 C in the dark

Table 25 shows that during germination at the higher temperature (35 C) in the dark total amino acids of the four varieties of lentils tested has decreased.

In AMR, on the fifth day of germination compared to dry seeds, total amino acids decreased from 80.9 to 43.3 g/100g protein, in LAG they decreased from 83.6 to 45.2, in JOR from 81.9 to 44.5, and in SML from 82.7 to 46.7 g/100g protein.

During germination at 35 C all protein amino acids decreased, while when germinated at lower temperature (25 C) in the dark,

Table 24: Changes in protein amino acid of four varieties of lentils during germination in light at 25°C. The results are expressed as g amino acid/100g protein.

Amino acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan	0.78	0.66	0.54	0.46	0.90	0.77	0.67	0.54	0.80	0.70	0.60	0.48	0.85	0.73	0.54	0.44
Lysine	6.45	6.00	5.37	5.16	6.38	5.98	5.60	5.42	6.29	5.76	5.45	5.34	6.12	5.85	5.49	4.89
Histidine	1.93	1.69	1.42	1.25	1.87	1.54	1.38	1.20	1.68	1.52	1.43	1.30	2.10	1.85	1.63	1.40
Ammonia	2.23	2.53	2.75	3.12	2.44	2.80	3.00	3.23	2.00	2.21	2.43	2.71	2.58	2.75	2.97	3.13
Arginine	6.72	6.10	5.69	4.36	7.05	6.00	5.32	4.20	2.86	5.98	5.30	4.45	6.58	6.00	5.25	4.27
Aspartic acid	10.19	9.20	8.24	7.13	10.56	9.87	9.10	7.44	10.82	9.68	8.48	7.25	10.46	9.90	8.95	8.19
Threonine	2.46	2.08	1.83	1.59	2.64	2.39	2.18	1.84	2.10	1.85	1.65	1.47	2.25	2.00	1.82	1.64
Serine	3.06	2.55	2.19	1.81	3.29	2.80	2.49	2.00	3.17	2.85	2.48	2.20	3.30	2.97	6.64	2.31
Glutamic acid	11.18	10.56	9.46	8.94	11.25	10.52	9.63	9.07	11.60	11.08	10.22	9.15	11.06	10.34	9.80	9.35
Proline	4.10	3.48	3.09	2.72	4.43	3.87	3.30	2.87	4.26	3.91	3.48	2.95	4.12	3.77	3.40	2.95
Glycine	4.50	4.00	3.55	2.97	4.90	4.06	3.57	2.95	5.08	4.15	3.65	3.16	5.20	4.46	3.90	3.28
Alanine	4.88	4.17	3.64	3.17	5.07	4.30	3.87	3.32	5.29	4.26	3.38	2.90	5.30	4.39	3.97	3.54
Cysteine	1.06	0.81	0.64	0.53	0.80	0.69	0.56	0.48	0.74	0.60	0.46	0.31	0.90	0.76	0.65	0.50
Valine	4.10	3.40	2.95	2.62	4.36	3.52	3.10	2.87	4.15	3.69	3.10	2.53	4.45	3.82	3.38	2.78
Methionine	0.54	0.44	0.32	0.21	0.60	0.48	0.37	0.23	0.50	0.42	0.31	0.20	0.70	0.59	0.50	0.40
Isoleucine	2.96	2.41	2.11	1.89	3.10	2.67	2.37	2.10	3.00	2.71	2.55	2.21	2.80	2.52	2.10	1.95
Leucine	5.28	4.47	3.95	3.32	5.20	4.57	3.90	3.44	5.42	4.53	3.90	3.52	4.92	4.40	4.16	3.78
Tyrosine	4.17	3.56	3.19	2.79	4.30	3.98	3.35	2.58	3.87	3.26	2.77	2.40	4.23	3.78	3.25	2.80
Phenylalanine	4.48	3.78	3.25	2.82	4.43	3.77	2.99	2.56	4.31	3.74	3.25	2.70	4.80	4.32	3.75	3.20
TOTAL	80.88	71.88	64.18	56.86	83.58	74.58	66.75	58.34	81.91	72.90	64.98	57.44	82.67	75.20	72.15	60.11
	(2.80)	(2.55)	(3.0)	(1.15)	(2.15)	(2.85)	(1.68)	(2.49)	(1.90)	(3.17)	(1.76)	(1.19)	(2.46)	(1.65)	(2.90)	(1.78)

( ) = SE, 0,1,3,5 = Days

Table 25: Changes in protein amino acids of four varieties of lentils during germination at 35°C in the dark for 5 days. (Results are expressed as g amino acid/100g protein)

Amino acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan	0.78	0.68	0.53	0.31	0.90	0.77	0.57	0.42	0.80	0.67	0.53	0.30	0.85	0.61	0.46	0.25
Lysine	6.46	5.76	5.16	4.51	6.38	5.98	5.67	5.42	6.29	5.97	5.27	5.00	6.12	5.79	5.46	5.20
Histidine	1.93	1.57	1.29	0.96	1.87	1.69	1.45	1.00	1.68	1.45	1.30	1.17	2.10	1.70	1.50	1.35
Ammonia	2.23	2.45	2.64	2.87	2.44	2.50	2.66	2.90	2.00	2.19	2.35	2.71	2.58	2.70	2.85	3.00
Arginine	6.72	5.69	4.42	2.68	7.05	5.74	4.34	2.87	6.86	5.64	4.28	2.94	6.53	5.46	4.16	2.61
Aspartic acid	10.19	8.80	6.56	5.50	10.56	8.95	6.82	4.55	10.82	9.46	7.15	5.00	10.46	9.00	6.89	5.15
Threonine	2.46	2.16	1.60	1.10	2.64	2.28	1.76	1.21	2.08	1.70	1.35	0.95	2.25	1.80	1.36	0.86
Serine	3.00	2.60	1.83	1.05	3.29	2.56	1.90	1.15	3.17	2.58	1.92	1.26	3.30	2.70	1.98	1.35
Glutamic acid	11.18	9.76	8.87	8.00	11.25	10.64	10.08	9.86	11.60	10.09	7.76	8.56	11.06	10.23	9.68	9.20
Proline	4.06	3.20	2.75	1.36	4.43	3.35	2.46	1.30	4.26	3.45	2.56	1.74	4.12	3.40	2.70	1.65
Glycine	4.50	3.96	2.90	1.75	4.90	3.95	2.84	1.96	5.08	4.25	3.27	2.00	5.20	4.57	3.53	2.15
Alanine	4.88	3.82	3.07	1.95	5.07	3.80	2.77	1.81	5.29	4.16	3.15	2.20	5.30	4.55	3.50	2.28
Cysteine	1.06	0.84	0.65	0.55	0.80	0.68	0.51	0.30	0.74	0.57	0.46	0.20	0.90	0.73	0.58	0.30
Valine	4.10	3.40	2.58	2.17	4.36	3.78	2.90	2.10	4.15	3.40	2.54	2.00	4.45	3.75	2.86	2.45
Methionine	0.54	0.41	0.34	0.20	0.60	0.45	0.34	0.20	0.50	0.41	0.37	0.19	0.70	0.54	0.45	0.30
Isoleucine	2.96	2.49	1.88	1.39	3.10	2.65	2.17	1.55	3.00	2.58	2.10	1.45	2.80	2.46	2.16	1.76
Leucine	5.28	4.47	3.43	2.69	5.20	4.36	3.35	2.50	5.42	4.37	3.29	2.75	4.92	4.10	3.00	1.85
Tyrosine	4.17	3.52	2.70	2.12	4.31	3.67	2.80	2.10	3.87	3.26	2.50	1.90	4.23	3.40	2.85	2.40
Phenylalanine	4.48	3.78	2.95	2.16	4.43	3.80	2.90	2.00	4.30	3.56	2.70	2.19	4.80	4.25	3.10	2.45
TOTAL	80.88	69.36	56.15	43.32	83.58	71.60	58.29	45.21	81.91	69.76	54.85	44.51	82.67	71.74	59.07	46.56
	(2.80)	(2.35)	(1.64)	(1.83)	(2.15)	(2.66)	(1.54)	(0.85)	(1.90)	(2.30)	(1.48)	(1.10)	(2.46)	(2.35)	(1.46)	(1.80)

( ) = SE ,0,1,3,5 = Days

all amino acids decreased except for lysine and glutamic acid which increased. The decrease in total amino acids at 35 C is higher than at 25 C in the dark and higher than seeds germinated in the light e.g. the average total amino acids left at 25 C in the dark is 64%, while that at 35 C in the dark is 54%.

The results of the present study agree well with those of GEERVANI and THEOPHILUS (1980) who have reported a decrease in methionine, cysteine, threonine, tryptophan and lysine in green gram germinated at 31 C for 24h compared to dry seeds.

Contradictory results of the effects of high temperatures (i.e. above 25 C) during germination on the protein amino acids of legumes and cereals have been reported, for example, WANG and FIELD (1978) have reported a large increase in lysine, tryptophan and methionine of corn seeds germinated in the dark for 6 days at 30 C compared to dry seeds. TSAI et al. 1975) have reported an increase in lysine and tryptophan in germinated maize seeds. On the other hand, KRISHNAMURTHY et al. (1982) have reported a decrease in the essential amino acids of germinated greem gram.

This discrepancy about the changes in individual amino acids during germination of legume and cereal seeds, may therefore be due to the different materials: corn in the one case and legumes in the other.

GENERAL INTRODUCTION

Protein quality of lentils

The nutritive value of a protein or its quality is defined as protein utilization (KAKADE, 1974). Growth and nitrogen balance are among parameters used to evaluate the quality or the nutritive value of a protein (BERRY, 1981). BENDER (1972) has reported that inadequate methods of assessing the nutritive value

of a protein can lead to false conclusions, thus biological value (BV), net protein utilization (NPU) and protein efficiency ratio (PER) will reveal changes only when the limiting amino acids are affected.

The chemical methods are based on the chemical analysis of proteins for their essential amino acids, and these will disclose any excess or deficit of individual essential amino acids (SHEFFNER, 1967). The most common method is the "chemical score". The essential amino acids are taken for the evaluation of protein quality relative to the quality of a reference protein e.g. of egg protein.

A/T Method

The content of each essential amino acid in a food protein (Ax) is expressed as a percentage of the content of the same amino acid in the same quantity of egg (Ae). The amino acid showing the lowest percentage is called the limiting amino acid and this percentage is the chemical score of the protein (FAO, 1970).

$$\text{Chemical score} = \frac{(\text{Ax}) \times 100}{(\text{Ae})}$$

In this study, the protein quality used is expressed in terms of chemical score which is the score of the limiting amino acid in the protein. Amino acid scores (chemical scores) were calculated by comparing the essential amino acids (EAA) in lentil protein with the amino acid pattern of egg (whole) as reference protein according to FAO (1973) and of OSBORNE and VOOGT (1978) as shown in Appendix 2.

PROTEIN QUALITY OF DRY LENTIL SEEDS

RESULTS AND DISUSSION

From table 26 it can be seen that the chemical score of the four varieties varied from 22 to 28. The most limiting amino acids are the sulphur containing amino acids, methionine and cysteine. Next to these come tryptophan, isoleucine and valine. A chemical score of 31 for lentils has been reported by FAO (1970), and of 35 by BHATTY et al. (1976). The AMR and SML have the highest protein quality, while JOR has the lowest.

Similar results about deficiency of sulphur amino acids in lentil proteins have been reported by AL-NAHRY et al. (1980). On the other hand, the values for lysine, phenylalanine, leucine and isoleucine obtained from this study, are lower than those of AL-NAHRY et al. (1980) who have shown that in their lentil cultivars lysine and phenylalanine were in excess, while leucine and isoleucine were almost the same as those of FAO (1957).

In general, animal proteins are found to have better quality e.g. higher biological value than plant proteins because of the more satisfactory distribution in species and amounts of the various amino acids present.

Nevertheless, as has been shown in the present study, all essential amino acids have been found in the four varieties of lentils and that although some of them are limiting, lentils are still a good source of a number of essential amino acids. The best quality plant proteins are found in legumes, such as beans, peas and lentils.

Table 26: Chemical score of essential amino acids of lentil varieties germinated at 25°C in the dark.
Results are expressed as % of the same EAA in reference protein (whole egg)

Essential Amino acids	AMR				LAG				JOR				SML			
(EAA)	CON	1	3	5	CON	1	3	5	CON	1	3	5	CON	1	3	5
Lysine	98	100	103	106	97	98	101	103	95	97	101	102	93	95	98	100
Tryptophan	46	35	26	21	53	42	34	29	47	39	24	21	50	39	33	18
Isoleucine	51	43	37	30	53	47	36	29	52	46	37	28	48	41	32	27
Leucine	59	51	47	31	58	48	37	33	61	53	39	34	55	48	37	30
Valine	55	43	37	29	59	51	43	31	56	48	37	31	60	50	40	31
Methionine + Cysteine	28	22	18	15	25	20	15	12	22	18	15	11	28	24	18	15
Threonine	49	41	31	24	53	43	39	27	42	32	26	23	45	40	31	25
Phenylalanine + Tyrosine	93	77	62	50	94	76	62	50	88	74	64	55	96	79	64	54
Histidine	88	73	64	51	85	70	53	38	76	58	48	35	95	76	56	41
Lowest Chemical Score	28	22	18	15	25	20	15	12	22	18	15	11	28	24	18	15

1, 3, 5 = Days

Chemical score of essential amino acids of lentils germinated at 25 C in the dark

Table 26 (p.121) shows the protein quality i.e. chemical scores of individual amino acids of lentil protein during germination at 25 C in the dark. On the fifth day of germination, compared to dry seeds, the protein chemical score in AMR, LAG, JOR and SML decreased from 28 to 15, from 25 to 12, 22 to 11 and from 28 to 15 respectively.

These results agree with those of KRISHNAMURTHY et al. (1982) who have reported a decrease in the chemical score from 45 to 41 on the third day of germination of green gram. NOOR et al. (1980) have reported loss of protein quality of germinated mung beans, and this is in part due to loss of methionine.

The results obtained from the present study and those mentioned above disagree with those of HOFSTEN (1979) who has reported that during germination of legume seeds e.g. mung beans, the amino acid profile does not change dramatically, and the chemical score never decreases although it may sometimes increase.
Relatively few studies have been carried out on protein quality of germinated lentil seeds, while protein quality of other germinated legume seeds e.g. soybeans and peas has already been reported by BATES et al. (1977) and by BAU and DEBRY (1979).

Changes in the chemical score of essential amino acids of lentils during germination at 25 C in the light

RESULTS AND DISUSSION

Table 27 shows that light causes a decrease in protein quality in terms of chemical score of protein in the four varieties of germinated lentils.

Table 27: Changes in chemical scores of essential amino acids of four lentil varieties during germination in light conditions.

Results are expressed as % of the same EAA in reference Protein (whole egg)

Essential Amino acids (EAA)	AMR				LAG				JOR				SML			
	CON	1	3	5	CON	1	3	5	CON	1	3	5	CON	1	3	5
Lysine	98	91	81	78	97	91	85	82	95	87	83	81	93	87	83	74
Tryptophan	46	39	32	27	53	45	39	32	47	41	35	28	50	43	32	26
Isoleucine	51	42	36	33	53	46	41	36	52	47	44	38	48	43	36	34
Leucine	59	50	44	37	58	51	44	39	61	51	44	40	55	49	46	42
Valine	55	46	40	35	59	48	42	39	56	50	42	34	60	52	46	38
Methionine + Cysteine	28	22	17	13	25	21	16	12	22	18	14	9	28	24	20	16
Threonine	49	42	37	32	53	48	44	37	42	37	33	29	45	40	36	33
Phenylalanine + Tyrosine	93	79	69	60	94	83	68	55	88	75	64	55	96	87	75	65
Histidine	88	76	65	57	85	70	63	55	76	69	65	59	95	84	74	65
Lowest Chemical Score	28	22	17	13	25	21	16	12	22	18	14	9	28	24	20	16

1, 3, 5 = Days

On the fifth day of germination, the chemical score for AMR, LAG, JOR and SML decreased from 28 to 13, from 25 to 12, from 22 to 9 and from 28 to 16 respectively.

Changes in the chemical score of essential amino acids of four lentil varieties during germination at 35 C in the dark

Table 28 shows that the protein quality i.e. protein chemical score and chemical scores of the individual amino acids decreased during germination of the four lentil varieties at 35 C in the dark for five days.

On the fifth day of germination at 35 C, the protein chemical score of AMR, LAG, JOR and SML decreased from 28 to 13, from 25 to 9, from 22 to 7 and from 28 to 11 respectively.

The decrease in protein quality of lentils grown at 35 C in the dark is higher when compared to those grown at the lower temperature (25 C) in the dark or under illumination.

FREE AMINO ACIDS

General Introduction

It is well known that there is no correlation between the relative proportions of free amino acids and of protein amino acids (SMITH, 1968). The free amino acids which exist in plants are not to be regarded as precursors of protein (KASAI et al. (1966).

WEAVER et al. (1978) have reported that the free amino acid content differs among cultivars of potato tubers. However, free amino acids are nutritionally as important as their counterparts found in protein (DESBOROUGH and WEISER, 1974). Very little

Table 28: Changes in the chemical score of essential amino acids of four varieties of lentil during germination at 35°C in the dark.

Results are expressed as % of the same EAA in reference protein (whole egg)

Essential Amino acids (EAA)	AMR				LAG				JOR				SML			
	CON	1	3	5	CON	1	3	5	CON	1	3	5	CON	1	3	5
Lysine	98	87	78	68	97	91	86	82	95	90	80	76	93	88	83	79
Tryptophan	46	40	31	18	53	45	34	25	47	39	31	18	50	36	27	15
Isoleucine	51	43	32	24	53	46	37	27	52	44	36	25	48	42	37	30
Leucine	59	50	39	30	58	49	38	28	61	49	37	31	55	46	34	21
Valine	55	46	35	29	59	51	39	28	56	46	34	27	60	51	39	33
Methionine + Cysteine	28	22	17	13	25	20	15	9	22	17	15	7	28	22	18	11
Threonine	49	43	32	22	53	46	35	24	42	34	27	19	45	36	27	17
Phenylalanine + Tyrosine	93	78	61	46	94	80	61	44	88	73	56	44	96	82	64	52
Histidine	88	71	59	45	85	77	65	45	76	66	59	53	95	77	68	61
Lowest Chemical Score	28	22	17	13	25	20	15	9	22	17	15	7	28	22	18	11

1,3 ,5 = Days

information, concerning the free amino acids in lentils is available compared to that of other chemical compounds.

This study, will investigate the effect of different treatments i.e. germination, cooking and canning on free amino acids of the four varieties of lentils.

MATERIALS AND METHODS

Identification and determination of free amino acids

Extraction of free amino acids

Trichloroacetic acid (TCA), is the most widely used solvent to precipitate protein. Many workers have reported that TCA is more effective than alcohol (SING, 1981; DESBOROUGH and WEISER, 1972). Therefore, in the present study, the TCA method was used.

1.Procedure

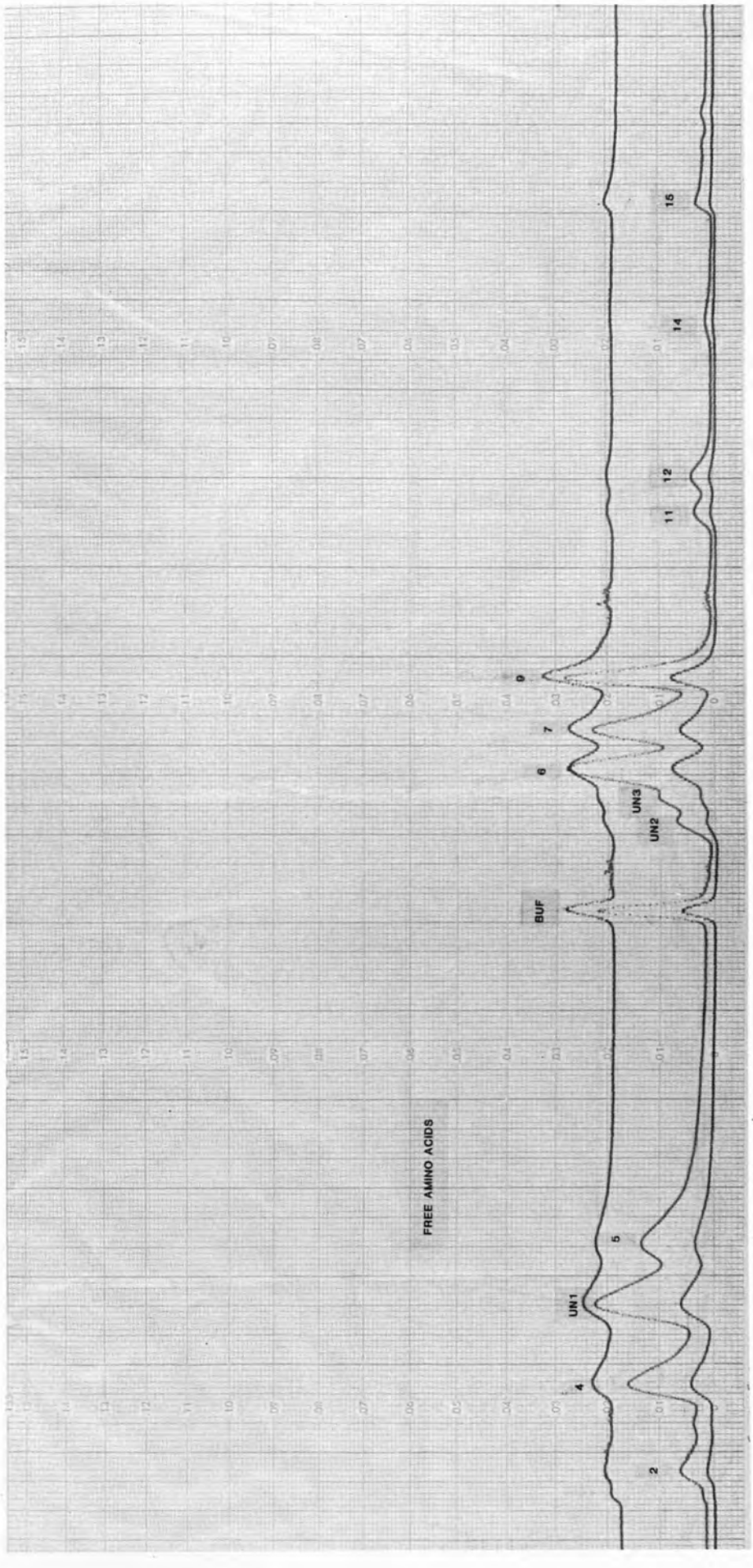
The samples were dried at 50 C overnight. The dried material was ground to pass a sixty-mesh screen. To 100 mg of this powder, 5 ml of 2% phenol and 10 ml of 30% TCA were added. The mixture was shaken for three hours using an arm shaker at low speed (MAZZA et al., 1983). The sample was then filtered and the supernatant which contained the free amino acids had its pH adjusted to 2±0.1 (BLACKBURN, 1968). The residue was used for protein amino acid determination.

2. Identification and determination of free amino acids

The method of separation, identification and estimation of protein amino acids was used (p109).

Fig.12. Free amino acids of lentil seeds

2. Lysine
4. Ammonia
UN. Unknown
5. Arginine
6. Aspartic acid
7. Threonine
9. Glutamic acid
11. Glycine
12. Alanine
14. Valine
15. Methionine


FREE AMINO ACIDS
BUF
UN1
UN2
UN3
2
4
5
6
7
9
11
12
14
15

FREE AMINO ACIDS OF DRY LENTIL SEEDS

RESULTS AND DISCUSSION

All four varieties of lentils have the same 8 free amino acids, namely lysine, arginine, aspartic acid, threonine, glutamic acid, glycine, alanine and valine, and ammonia (although this is not an amino acids), with var SML having an extra one (methionine in trace amounts) (table 29). The free amino acid contents in AMR, LAG, JOR and SML were found to be 1.39, 1.14, 1.30 and 1.56 g/100g seeds respectively.

The results of the free amino acids obtained in the present study, are in agreement with those of ALTSCHUL and YATSU (1966) who reported that free amino acids made up 2% of seed legumes. All 4 varieties have three unknown amino acids found in small amounts: a peak between histidine and arginine, 2 and 3 peaks appearing just before aspartic acid. In all the four varieties arginine, aspartic acid and glutamic acid are the most abundant ones. SULSER and SAGER (1976) have investigated the free amino acids in five varieties of lentils and their findings were that lysine and arginine were 0.06 and 0.41 g/100g seed respectively (for their American variety) compared to 0.16 and 0.21 g/100g seeds in the American (AMR) variety used in the present study.

Changes in free amino acids during germination at 25 C in the dark

RESULTS AND DISCUSSION

In the present study (table 29 p130), there was an increase in free amino acids during germination at 25 C in the dark, increasing with germination time.

On the fifth day of germination compared to dry seeds, free amino

Table 29: Changes in free amino acids of lentils during germination in the dark at 25°C.

(Results are expressed as g/100g seeds)

Amino Acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan																
Lysine	0.16	0.29	0.42	0.60	0.14	0.28	0.40	0.56	0.12	0.25	0.45	0.60	0.15	0.30	0.50	0.70
Histidine	0.00	0.30	0.43	0.53	0.00	0.26	0.50	0.65	0.00	0.35	0.50	0.67	0.00	0.22	0.37	0.56
Ammonia	0.18	0.28	0.65	0.85	0.11	0.32	0.48	0.79	0.11	0.27	0.40	0.54	0.19	0.25	0.40	0.60
Arginine	0.21	0.40	0.59	0.80	0.30	0.50	0.80	1.10	0.41	0.65	0.97	1.24	0.39	0.55	0.80	1.10
Aspartic acid	0.19	0.45	0.78	0.90	0.17	0.43	0.75	1.00	0.20	0.40	0.86	1.17	0.22	0.40	0.60	0.80
Threonine	0.17	0.33	0.50	0.67	0.11	0.25	0.31	0.47	0.10	0.25	0.38	0.50	0.13	0.20	0.33	0.45
Serine	0.00	0.00	0.11	0.23	0.00	0.00	0.14	0.30	0.00	0.00	0.13	0.35	0.00	0.06	0.14	0.28
Glutamic acid	0.22	0.54	0.87	1.34	0.12	0.45	0.83	1.15	0.18	0.36	0.80	1.25	0.23	0.70	1.00	1.30
Proline	0.00	0.08	0.20	0.30	0.00	0.09	0.21	0.35	0.00	0.12	0.25	0.45	0.00	0.13	0.27	0.41
Glycine	0.08	0.20	0.29	0.41	0.06	0.20	0.30	0.49	0.08	0.23	0.39	0.52	0.09	0.18	0.30	0.47
Alanine	0.18	0.25	0.39	0.50	0.13	0.25	0.45	0.61	0.10	0.26	0.47	0.66	0.16	0.30	0.45	0.70
Cysteine	0.00	0.00	0.07	0.14	0.00	0.00	0.11	0.23	0.00	0.00	0.11	0.17	0.00	0.00	0.00	0.12
Valine	T	0.13	0.26	0.35	T	0.19	0.30	0.45	T	0.16	0.38	0.50	T	0.15	0.32	0.45
Methionine	0.00	0.00	0.00	0.15	0.00	0.00	0.13	0.21	0.00	0.08	0.13	0.22	T	T	0.08	0.14
Isoleucine	0.00	0.10	0.19	0.28	0.00	0.14	0.29	0.38	0.00	0.00	0.33	0.45	0.00	0.08	0.14	0.25
Leucine	0.00	0.09	0.20	0.29	0.00	0.15	0.28	0.42	0.00	0.10	0.20	0.31	0.00	0.10	0.20	0.39
Tyrosine	0.00	0.00	0.10	0.20	0.00	0.00	0.13	0.25	0.00	0.09	0.17	0.25	0.00	0.11	0.18	0.32
Phenylalanine	0.00	0.18	0.27	0.38	0.00	0.21	0.30	0.42	0.00	0.16	0.30	0.47	0.00	0.08	0.23	0.36
TOTAL	1.39 (0.16)	3.62 (0.19)	6.34 (0.28)	8.96 (0.45)	1.14 (0.09)	3.72 (0.25)	6.71 (0.37)	9.83 (0.56)	1.30 (0.08)	3.73 (0.20)	7.22 (0.40)	10.32 (0.48)	1.56 (0.10)	1.81 (0.15)	6.31 (0.24)	9.40 (0.54)

( ) = SE, T = Traces , 0,1,3,5 = Days

acids in AMR, LAG, JOR and SML increased from 1.39 to 8.96, from 1.14 to 9.83, from 1.30 to 10.32, and from 1.56 to 9.4 g/100g respectively.

New free amino acids appeared daily even after one day germination in the dark so that by day 5 the same 18 amino acids were observed in all four varieties, the same found in protein amino acids.

Generally the increase in most amino acids ranged from a 2- to 4-fold increase after the fifth day of germination, and this result agrees closely with those reported by HURST (1970) and by DESHMUSHI and SOHONIE (1966). Also it is to be noted in the present study, that the most abundant amino acids at the end of the germination period of five days, were glutamic acid, aspartic acid, glycine and arginine.

Higher increases have been reported in other legumes. A 7-fold increase in free amino acids on the third day of germinating soy beans has been reported by KASAI et al. (1966), also an 8-fold increase after 5 days germination of peas has been reported by CHEN and THACKER (1978).

Generally though, most studies have reported an increase in the content of free amino acids in etiolated germinating seedlings. The increase has generally been attributed to the hydrolysis of storage protein. The formation of free amino acids depends upon the external conditions as well as on the synthetic activity of the plant (DESHMUSHI and SOHONIE, 1966).

Changes in free amino acids of lentils during germination in the light at 25 C

RESULTS AND DISCUSSION

Table 30 shows that free amino acids increased in all four varieties during germination at 25 C in the light.

In AMR, LAG, JOR and SML, the free amino acids on the fifth day of germination compared to dry seeds increased from 1.39 to 5.63 g/100g, from 1.14 to 5.73, from 1.3 to 5.86 and from 1.56 to 5.70 g/100g respectively.

During germination in the light some amino acids increased appreciably e.g. lysine, arginine, aspartic acid and glutamic acid, while others increased slightly e.g. isoleucine and leucine were in measurable amounts only on the fifth day of germination, and cysteine was found in trace amounts on the fifth day only. Although free amino acids increased during germination in the light, their increases ranged from 2.6- to 4.0-fold, a much lower increase than in the dark.

These results agree with those of PALMIANO and JULIANO (1972) who have reported that the increase in free amino acids during germination of rice grains was much less in the light than in the dark. HURST (1970) has reported that free amino acids of soybean seedlings germinated for 5 days in the dark and for 3 days dark + 2 days light increased by 8- and 11-fold respectively, and the patterns of changes during germination in the dark and in the light differed only in their quantities.

Table 30: Changes in free amino acids of lentils during germination in the light

(Results are expressed as g/100g germinated seeds)

Amino acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan																
Lysine	0.16	0.20	0.27	0.38	0.14	0.22	0.31	0.40	0.12	0.18	0.27	0.34	0.15	0.23	0.31	0.40
Histidine	0.00	0.00	0.21	0.40	0.00	0.17	0.35	0.50	0.00	0.00	0.21	0.38	0.00	0.00	0.24	0.37
Ammonia	0.18	0.26	0.39	0.50	0.11	0.19	0.26	0.32	0.11	0.15	0.22	0.29	0.19	0.25	0.33	0.45
Arginine	0.21	0.28	0.33	0.43	0.30	0.40	0.55	0.65	0.41	0.57	0.68	0.84	0.39	0.51	0.60	0.71
Aspartic acid	0.19	0.30	0.40	0.54	0.17	0.28	0.37	0.50	0.20	0.35	0.51	0.65	0.22	0.29	0.35	0.45
Threonine	0.17	0.25	0.30	0.37	0.11	0.16	0.20	0.27	0.10	0.27	0.35	0.48	0.13	0.19	0.28	0.35
Serine	0.00	0.00	0.10	0.22	0.00	0.00	0.13	0.24	0.00	0.00	0.09	0.19	0.00	0.00	0.08	0.15
Glutamic acid	0.22	0.45	0.78	0.98	0.12	0.31	0.67	1.00	0.18	0.42	0.60	0.88	0.23	0.40	0.52	0.80
Proline	0.00	0.00	0.11	0.19	0.00	0.00	0.00	0.16	0.00	0.00	0.12	0.25	0.00	0.00	0.10	0.21
Glycine	0.08	0.10	0.15	0.20	0.06	0.15	0.22	0.30	0.00	0.00	0.17	0.30	0.09	0.18	0.27	0.38
Alanine	0.18	0.22	0.27	0.32	0.13	0.18	0.26	0.37	0.10	0.16	0.24	0.35	0.16	0.23	0.30	0.37
Cysteine	0.00	0.00	0.00	T	0.00	0.00	0.00	T	0.00	0.00	0.00	T	0.00	0.00	0.00	T
Valine	T	T	0.11	0.20	T	T	0.09	0.21	T	T	0.13	0.25	T	T	T	0.22
Methionine	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.13	T	T	T	0.18
Isoleucine	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.12
Leucine	0.00	0.00	0.00	0.18	0.00	0.00	0.08	0.17	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.15
Tyrosine	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.17
Phenylalanine	0.00	0.09	0.15	0.28	0.00	0.12	0.19	0.30	0.00	0.00	0.00	0.17	0.00	0.00	0.14	0.26
TOTAL	1.39 (0.16)	2.15 (0.11)	3.57 (0.25)	5.63 (0.36)	1.14 (0.09)	2.18 (0.14)	3.47 (0.28)	5.73 (0.23)	1.30 (0.08)	2.10 (0.09)	3.59 (0.26)	5.86 (0.41)	1.56 (0.10)	2.28 (0.19)	3.52 (0.35)	5.70 (0.30)

( ) = SE

T = Traces, 0, 1, 3, 5 = Days

Changes in free amino acids of lentils during germination at 35 C in the dark

RESULTS and DISCUSSION

In the present study (table 31) there is an increase in free amino acids of all varieties studied during germination at 35 C in the dark.

In AMR, LAG, JOR and SML, on the fifth day of germination compared to dry seeds, the free amino acids increased from 1.39 to 6.71 g/100g seeds, from 1.14 to 7.79 g, from 1.30 to 8.64, from 1.56 to 7.29 g/100g seeds respectively.

The increase in free amino acids of the four varieties tested ranged from 3.7- to 5.8-fold.

Table 31: Changes in free amino acids of lentils during germination at 35°C in the dark.

(Results are expressed as g/100g seeds)

Amino acids	AMR				LAG				JOR				SML			
	0	1	3	5	0	1	3	5	0	1	3	5	0	1	3	5
Tryptophan																
Lysine	0.16	0.24	0.35	0.49	0.14	0.29	0.35	0.47	0.12	0.20	0.31	0.46	0.15	0.29	0.38	0.54
Histidine	0.00	0.15	0.27	0.53	0.00	0.16	0.24	0.53	0.00	0.14	0.22	0.65	0.00	0.18	0.32	0.60
Ammonia	0.18	0.40	0.60	0.72	0.11	0.24	0.45	0.64	0.11	0.21	0.30	0.40	0.19	0.22	0.34	0.56
Arginine	0.21	0.32	0.45	0.61	0.30	0.62	0.83	1.06	0.41	0.60	0.78	1.18	0.39	0.53	0.64	0.87
Aspartic acid	0.19	0.35	0.41	0.54	0.17	0.28	0.42	0.86	0.20	0.47	0.81	1.05	0.22	0.30	0.40	0.50
Threonine	0.17	0.22	0.29	0.35	0.11	0.19	0.28	0.40	0.10	0.30	0.45	0.60	0.13	0.20	0.26	0.39
Serine	0.00	0.00	0.00	0.17	0.00	0.00	0.10	0.22	0.00	0.00	0.00	0.20	0.00	0.00	0.12	0.25
Glutamic acid	0.22	0.51	0.87	1.15	0.12	0.39	0.70	1.00	0.18	0.48	0.79	1.13	0.23	0.43	0.67	1.00
Proline	0.00	0.00	0.08	0.20	0.00	0.00	0.12	0.25	0.00	0.00	0.15	0.29	0.00	0.00	0.11	0.21
Glycine	0.08	0.17	0.23	0.30	0.06	0.11	0.19	0.32	0.08	0.19	0.27	0.41	0.09	0.15	0.23	0.39
Alanine	0.18	0.25	0.36	0.45	0.11	0.15	0.34	0.50	0.10	0.27	0.35	0.57	0.16	0.26	0.37	0.58
Cysteine	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.16
Valine	T	T	0.11	0.21	T	0.07	0.20	0.32	T	T	0.21	0.35	T	T	0.13	0.26
Methionine	0.00	0.00	0.07	0.12	0.00	0.00	0.09	0.19	0.00	0.00	0.00	0.15	T	T	T	0.17
Isoleucine	0.00	0.00	0.10	0.16	0.00	0.00	0.14	0.26	0.00	0.00	0.14	0.22	0.00	0.00	0.09	0.20
Leucine	0.00	0.00	0.06	0.11	0.00	0.00	0.08	0.21	0.00	0.00	0.11	0.20	0.00	0.00	0.00	0.15
Tyrosine	0.00	0.00	0.09	0.15	0.00	0.00	0.10	0.16	0.00	0.09	0.18	0.25	0.00	0.00	0.08	0.24
Phenylalanine	0.00	0.08	0.19	0.35	0.00	0.00	0.14	0.30	0.00	0.07	0.13	0.24	0.00	0.00	0.13	0.22
TOTAL	1.39 (0.16)	2.69 (0.17)	4.53 (0.26)	6.71 (0.43)	1.14 (0.09)	2.50 (0.12)	4.77 (0.18)	7.79 (0.36)	1.30 (0.08)	3.02 (0.20)	5.20 (0.15)	8.64 (0.51)	1.56 (0.10)	2.56 (0.15)	4.27 (0.22)	7.29 (0.48)

( ) = SE, T = Traces , 0,1,3, 5 = Days

CHAPTER V

VITAMINS

Vitamins are a group of coupounds that are essential in relatively minute quantities for body growth and maintenance. They perform specific and vital functions in the cells and in the tissues. They cannot be synthesized by the organism, and their absence or inadequate utilization results in specific deficiency diseases.

Thiamine is essential for the normal activity of the nervous system (SALUNKHE, 1974); while riboflavin is for healthy eyes (WILSON et al., 1979). Two forms of vitamin C are found in nature: the reduced form (ascorbic acid) and the oxidised form (dehydroascorbic acid), and both forms exist in plant and animal tissues. It is believed that vitamin C has an important role in collagen formation, in protein metabolism and in fat metabolism (WILSON et al., 1979). Nutritionlly, vitamin C can be supplied as either ascorbic acid or as dehydroascorbic acid.

In the present study, estimation of total vitamin C, thiamine and riboflavin was carried out.

MATERIALS AND METHODS

Determination of "total" vitamin C

The vitamin C activities of food and biological materials are associated with their L-ascorbic acid contents. For determination of "total vitamin C", the procedure described by FREED (1966) was used. It is based upon the oxidation of ascorbic acid to dehydroascorbic acid. Subsequent transformation of dehydroascorbic acid to diketogulonic acid, followed by coupling with 2,4-dinitrophenylhydrazine gives red-coloured osazones. The advantage of

this method is that it is specific for vitamin C.

Reagents

1. 85% sulphuric acid
2. 2% 2,4-dinitrophenylhydrazine in 9N sulphuric acid
3. 2% thiourea in 5% metaphosphoric acid (HPO_3)
4. 10% metaphosphoric acid
5. Bromine
6. Ascorbic acid standard: 100 mg ascorbic acid dissolved in 100 ml of 5% metaphosphoric acid. This solution contained 1 mg ascorbic acid per ml. Standard solutions were prepared in the range 1-12 µg/ml.

1. Extraction

(a) Five g of seeds are homogenised with 100 ml 10% metaphosphoric acid in a Waring blender, the homogenate is then centrifuged.

2. Oxidation to dehydroascorbic acid

(a) To the solution of 1(a), a few drops of bromine (liquid) are added until the solution appears slightly yellow. The excess bromine is decanted and nitrogen gas is passed through the solution until it becomes colourless.

(b) To a 10 ml aliquot of the oxidised extract from 2(a), 10 ml of 2% thiourea in 5% HPO_3 are added and mixed.

3. Formation of osazone

Aliquots (4 ml each) of the sample dilution 2(a) are pipetted into four test tubes: one of the tubes is set aside to serve as a blank, and to each of the remaining tubes 1 ml of 2% 2,4-dinitrophenylhydrazine is added. Then all the tubes are placed in

a water bath at 37±1 C for exactly 3 hours after which they are removed and placed in an ice bath.

4. Treatment with 85% H_2SO_4 (formation of soluble pigment)

While the tubes are in the ice bath, 5 ml of 85% H_2SO_4 are added to each tube, and in the blank only, one ml of 2% 2,4-dinitrophenylhydrazine is added. The tubes are shaken, removed from the ice bath and allowed to stand for exactly 30 min at room temperature.

5.Measurement of colour

The colours produced in the samples and in the standard solutions of ascorbic acid are measured against the blank, by recording the percentage transmittance at 540 nm using a spectrophotometer (LKB,BIOCHEM Model ULTROSPEC, 4050).

6. Standard solution and calculation

Standard ascorbic acid solutions are treated in the same way as samples in Steps 2(a) through 5(a). A calibration chart is obtained by plotting percentage transmittance against concentration of vitamin C on semi logarithmic paper. The total vitamin C content of each aliquot is obtained from this chart according to the formula:

$$\frac{R \times 100}{W \times 1000} = \text{mg total vitamin C (per 100 g of sample)}$$

Where R = mg of total vitamin C per ml of solution obtained by reading from the chart.

W = g of sample per ml of the diluted solution.

2. THIAMINE (B_1)

Thiamine consists of a substituted pyrimidine linked to a substituted thiazole by a methylene group (ARCHER and TANNENBAUM, 1979). It exists in foods in a number of forms, including free as thiamine or in a combined form as a protein complex. The free form of thiamine occurs in large amounts in plants. Thiamine is synthesized by all higher plants (OSER, 1965).

MATERIALS AND METHODS

Determination of thiamine

Among the chemical methods, the fluorometric determination, is the recommended AOAC method (AOAC, 1975), and is based on the oxidation of thiamine hydrochloride to thiochrome. It is reproducible and sensitive (GLICK, 1958) and was used in the present study.

Reagents

1. Oxidizing agent: 4 ml of 1% potassium ferrocyanide are made to 100 ml with 15% NaOH.
2. Thiamine stock: 1. 50 mg of thiamine is dissolved in 500 ml of 20% alcohol.
3. Thiamine stock solution: 2. 100 ml of thiamine stock 1 are diluted to 1 l with 20% alcohol.
4. Thiamine standard solution. To 10 ml of thiamine stock soltion 2, 50 ml of 0.1 N HCl are added: this solution contains 1 µg thiamine per ml.

1. Extraction

To 5 g of seeds, 50 ml of 0.1 N HCl is added, the sample is blended in a Waring blender for 2 min.

2.Digestion of sample

The sample is autoclaved for 30 min at 121- 123 C. After that the mixture is centrifuged at 4,000rpmfor 10 min.

3. Oxidation of thiamine to thiochrome

(a) To each of four tubes, 1.5 g NaCl and 5 ml of thiamine standard solution are added, followed by 3 ml of potassium ferrocyanide (oxidizing agent). Each tube is shaken, and immediately 13 ml of isobutyl alcohol added. The tube is shaken again, and this treatment is called oxidised standard.

(b) Another 4 tubes are treated exactly in the same manner except that 15% NaOH is used instead of potassium ferrocyanide. This treatment is called standard blanks

(c) To another 4 tubes, 5 ml of the assay solution is added, followed by 1.5 g NaCl, 3 ml of potassium ferrocyanide (oxidizing agent), then 13 ml of isobutyl alcohol. This treatment is called oxidized assay solution.

(d) To the fourth set of 4 tubes, 5 ml of the assay solution is added, followed by 1.5 g NaCl, 3 ml of 15% NaOH and 13 ml of isobutyl alcohol, and this treament is called the assay blank.

4. Thiochrome fluorescence measurement

The thiamine content was determined by comparing the intensity of fluorescence of the extract of the oxidised assay solution with that of the oxidised standard solution. An input filter of trans mittance of approximately 365 nm (LF 2), and an output filter of approximately 435 nm (LF 5) were used. A single sided fluorimeter model LOCARTE MK4 with mercury lamp and digital voltmeter was used.

5. Calculation

Thiamine content in µg in 5 ml of assay solution is estimated using the following formula:

Thiamine in µg = (A-B)/ (S-D)

Where A= The fluorescence of the oxidized assay solution.
B= The fluorescence of the assay blank.
S= The fluorescence of the oxidized standard.
D= The fluorescence of the standard blank.

3. RIBOFLAVIN (B_2)

Riboflavin consists of the flavin pigment lumichrome and the reduced form of ribose (PRIESTLEY, 1979). It is heat stable in acid solution, but very sensitive to light, to both in the U.V. and visible regions.

It occurs normally combined with protein. The AOAC fluorometric method (AOAC, 1975) was used for riboflavin, and it is very sensitive when compared to other methods (GYORGY and PEARSON, 1967).

MATERIALS AND METHODS

Determination of riboflavin

Reagents

1. Riboflavin stock solution: 1. 50 mg of riboflavin is dissolved in 500 ml of 0.02 N-acetic acid.
2. Riboflavin stock solution: 2. 100 ml of stock 1 is diluted to 1 l with 0.02 N-acetic acid.
3. Riboflavin standard solution. 10 ml of stock 2 is diluted to

100 ml with water, this contains 1 mg riboflavin/ml.

4. Sodium hydrosulfite (dithionite, $Na_2S_2O_4$).

1. Extraction

To 10 g of sample, 50 ml of 0.1N HCl acid is added, then autoclaved for 30 min at 120-123 C.

2. Precipitation of interfering impurities

The pH of the sample is adjusted to 6 with NaOH. Immediately, the pH is brought to 4.5 with 0.1N HCl, then the sample is diluted to 100 ml with water, and filtered (a). To a 50 ml filtrate (a), 0.1N HCl is added until no precipitate is formed, then diluted with 100 ml water.

3. Acidification of extract

Ten ml of sample solution and 1 ml of glacial acetic acid are added to each of 8 tubes. To four of these tubes, 1 ml of standard riboflavin solution is added, while to the remaining four, 1 ml of water is added.

4. Oxidation

To each of the 8 tubes, 0.5 ml of 3% $KMnO_4$ solution is added, and after 2 min 0.5 ml of 3% H_2O_2 , and mixed until the red colour disappears.

5. Fluorimetry measurement

A riboflavin solution at pH 6-7 exhibits a yellowish green fluorescence with a maximum at 530 nm.

A fluorimeter model LOCARTE MK4 was used. Input filter (FL 5 about 440 nm) and output filter (FL 10 about 575 nm) were used.

The fluorescence of the tube containing the sample solution and 1 ml of water was measured, and the reading called B., then 20 mg of $Na_2S_2O_4$ was added to the tube, and the measured fluorescence is called C. The fluorescence of the tubes containing the sample and the standard was measured and called X.

6. Calculation

The amount of riboflavin in mg per ml of final solution is calculated from the following formula

$$\frac{(B-C) \times 0.1 \times 0.001}{(X-B)} = \text{mg of riboflavin per ml final sample solution}$$

Where B = fluorescence of sample and 1 ml water.

C = fluorescence of sample and water after the addition of $Na_2S_2O_4$.

X = fluorescence of sample and 1 ml standard.

RESULTS AND DISCUSSION

1. VITAMIN C OF DRY LENTIL SEEDS

From table 32 , it can be seen that vitamin C of the four varieties of lentils ranged from 2.2 (LAG) to 5.6 mg/100g dry weight (JOR).

The values are slightly lower than those of KYLEN *et al.*(1975) who have reported 7 mg/100g, but are higher than those of HSU *et al.* (1980) who have reported 0.9 mg/100g of lentils. Legumes in general and lentils in particular are not considered a good source of vitamin C.

Table 32: Changes of vitamin C, thiamine and riboflavin of lentil varieties during germination at 25°C in the dark.
(Results are expressed as mg/100g)

Days of germin.	AMR			LAG			JOR			SML		
	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo.
0	4.35 (0.14)	0.57 (0.06)	0.27 (0.03)	2.17 (0.08)	0.45 (0.06)	0.38 (0.04)	5.60 (0.25)	0.50 (0.05)	0.34 (0.03)	5.42 (0.19)	0.39 (0.03)	0.21 (0.02)
1	9.26 (0.35)	0.65 (0.06)	0.36 (0.03)	16.68 (0.56)	0.54 (0.05)	0.47 (0.04)	17.47 (0.65)	0.56 (0.06)	0.40 (0.04)	13.26 (0.35)	0.42 (0.03)	0.38 (0.03)
2	13.15 (0.49)	0.72 (0.08)	0.45 (0.05)	25.46 (0.79)	0.61 (0.07)	0.60 (0.03)	29.62 (0.84)	0.63 (0.04)	0.49 (0.03)	25.78 (0.57)	0.49 (0.05)	0.45 (0.06)
3	48.27 (1.36)	0.80 (0.07)	0.56 (0.06)	39.03 (1.15)	0.69 (0.06)	0.72 (0.06)	37.14 (0.72)	0.70 (0.08)	0.61 (0.06)	35.30 (0.75)	0.57 (0.07)	0.53 (0.04)
4	63.18 (1.78)	0.88 (0.09)	0.67 (0.07)	41.57 (1.47)	0.73 (0.05)	0.84 (0.09)	39.22 (1.60)	0.76 (0.09)	0.78 (0.08)	42.15 (1.26)	0.65 (0.10)	0.61 (0.05)
5	85.21 (2.85)	0.95 (0.12)	0.79 (0.09)	45.88 (1.80)	0.78 (0.08)	1.10 (0.13)	47.31 (1.75)	0.84 (0.10)	0.89 (0.12)	50.92 (2.00)	0.71 (0.08)	0.73 (0.09)

( ) = SE

2. THIAMINE OF DRY LENTIL SEEDS

The content of thiamine of the lentil seeds tested are given in table 32, (p.144), and ranges from 0.39 (SML) to 0.57 mg/100g (AMR). Similar results of 0.46 mg/100g have been reported by EL-NAHRY et al. (1980), while a higher value of 0.72 mg has been reported by KYLEN and McCREADY (1975).

3. RIBOFLAVIN OF DRY LENTIL SEEDS

The riboflavin content of the varieties used ranged from 0.21 (SML) to 0.38 mg/100g (LAG) (table 32, p.144). These results are in good agreement with those of EL-NAHRY et al. (1980) and of those of KYLEN and McCREADY (1975) who have found 0.28 and 0.29 mg riboflavin/100g respectively in their varieties of lentils.

CHANGES IN VITAMINS DURING GERMINATION

WAI et al. (1947) have reported an increase in vitamin C during the germination of soybeans. Other vitamins e.g. riboflavin and niacin also increased through 54 h germination, while thiamine showed alternate increases and decreases throughout the same period of germination. WU and FENTON (1953) have reported an increase in vitamin C from 4 to 16 mg/100g, and a decrease of thiamine from 1.1 to 0.25 mg/100g during the germination of soybeans. Thiamine in germinated beans and peas was as much as 6 times higher as in dry seeds (FORDHAM et al., 1975). Vitamin C can be synthesized either by glucose or galactose in plants (MAPSON, 1967) (fig. 13), while HARROW and MAZUR (1968) have reported the biosynthesis of ascorbic acid from glucose in both plants and in animals.

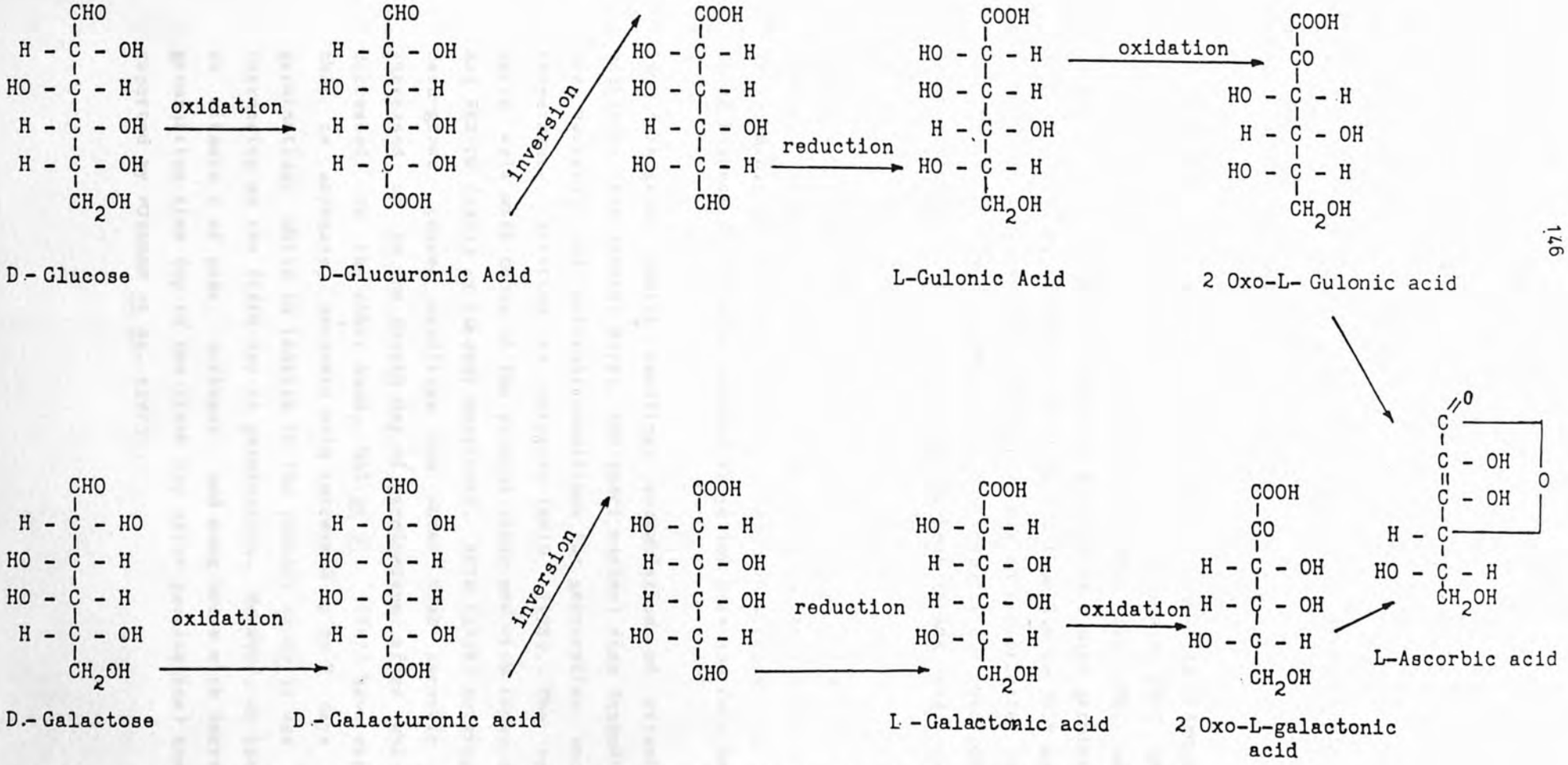


Fig.13. Pathways in the conversion of D-glucose or D-galactose to L-ascorbic acid (MAPSON, 1967)

CHANGES IN VITAMINS DURING GERMINATION AT 25 C IN THE DARK

1. VITAMIN C OF LENTILS GROWN AT 25 C IN THE DARK

In the present study, there was a large increase in vitamin C in all varieties studied during their germination at 25 C in the dark (table 32, p.144). Vitamin C in AMR, LAG, JOR, and SML increased from 4.3 in dry seeds to 85.2 after 5 days germination, from 2.2 to 45.9, from 5.6 to 47.3, and from 5.4 to 50.9 mg/100g respectively. This means that there was an approximate 20-fold and 9-fold increase in AMR and in SML respectively when compared to ungerminated seeds. Increase was highest in AMR, and lowest in SML.

These results are not unlike those of HSU et al. (1980) who have reported an 85-fold increase of vitamin C on the fourth day of the germination of lentils. This increase in vitamin C was found to be highest in lentils compared to yellow peas and faba beans.

In dark-grown lentil seedlings accumulation of vitamin C continues for several days, the exact maximal time depending on the variety, and external conditions for germination such as temperature, aeration and moisture (REID, 1938). The results agree well with those of the present study and with those of WU and FENTON (1953) as already mentioned. REID (1938) working with dark-grown cowpea seedlings has shown that ascorbic acid increased up to the fourth day of germination after which it decreased. On the other hand, WAI et al. (1947) have reported that in soybeans, ascorbic acid increased up to 2 days after germination; while in lentils in the present study it was still increasing on the fifth day of germination. However, an increase in vitamin C of peas, soybeans, and mung beans with increasing germination time (up to the sixth day after germination) has been reported by FORDHAM et al. (1975).

2. THIAMINE OF DARK-GROWN LENTILS AT 25 C

There was an increase in the thiamine content of all four varieties during germination at 25 C in the dark (table 32, p. 144). On the fifth day of germination compared to dry seeds, thiamine in AMR, LAG, JOR, and SML increased from 0.57 to 0.95 (66%), from 0.45 to 0.78 (73%), from 0.50 to 0.84 (68%), and from 0.39 to 0.71 mg/100g (82%) respectively.

These results are in good agreement with those of WU and FENTON (1953), but not with those of CHEN *et al*. (1975) who have reported that in dark-grown germinated pea seeds, the thiamine content was the same as in dry seeds.

3. RIBOFLAVIN OF DARK-GROWN LENTILS AT 25 C

Riboflavin also increased in all four varieties during germination at 25 C in the dark (table 32, p.144). On the fifth day of germination compared to dry seeds, AMR, LAG, JOR, and SML increased from 0.27 to 0.79 (192%), from 0.38 to 1.1 (189%), from 0.34 to 0.89 (161%), and from 0.21 to 0.73 mg/100g (247%) respectively.

These results showing an increase in the riboflavin content of germinated lentils agree well with those of KYLEN and McCREADY (1975) but not with those of HSU *et al*. (1980) who showed that riboflavin remained unchanged after germination.

Effect of light on vitamins

Most of the studies to-date have been on changes in vitamins in general and vitamin C in particular during germination of legumes in the dark. Few studies have been done on changes in vitamin C during seed germination in the light, although FARHANGI and VALADON (1983) have shown that illumination of mung bean seedlings for 24 h did not significantly affect total vitamin C content of etiolated seedlings. On the other hand, KASKI et al. (1944) have shown that light has a beneficial influence on the ascorbic acid content of tomatoes. The increase in vitamin C in the light may be due directly or indirectly to the influence of light on the activity of chloroplasts (REID, 1938). Very few investigations have been carried out on the effect of light and germination on vitamins other than ascorbic acid. GUSTAFSON (1948) has investigated the effect of light on thiamine and riboflavin in peas, beans and other plants and found that the thiamine content in these species is greater in plants subjected to high light intensity than in plants exposed to darkness or to low light intensity and this was also true for riboflavin.

Effect of temperature on vitamins

SOMERS et al. (1948) have reported that in the dark as the temperature increased, the ascorbic acid increased as well. REID (1941) has shown that the temperature of germination influences the ascorbic acid content of dark-grown cow-pea seedlings, e.g. higher vitamin C at 29 C than at 22 C, and points out that plants vary with respect to vitamin C contents. This is dependent on the temperature which is most favourable for germination.

GUSTAFSON (1950) has reported that not all plants respond to the same extent to temperature in their vitamin synthesis, nor are

all vitamins affected in the same way. Thiamine and riboflavin were found to be more abundant in beans, soybeans, and tomatoes grown at 28 to 30 C than between 10 and 15 C. In peas, the thiamine content was higher at between 28 to 30 C, than at the lower temperatures though riboflavin was more abundant at the latter temperatures.

CHANGES IN VITAMINS IN LENTILS GROWN AT 25 C IN THE LIGHT

VITAMIN C OF LENTILS GROWN AT 25 C IN THE LIGHT

Table 33 shows that the vitamin C content of germinating lentil seeds increased enormously. Vitamin C in AMR, LAG, JOR, and SML increased after 5 days germination when compared to dry seeds from 4.35 to 64.28 mg, from 2.17 to 38.2, from 5.6 to 38.1, and from 5.40 to 40.3 mg/100g respectively. The rate of vitamin C increase was highest on the second day of germination (table 33, p.151). These results are in agreement with those of REID (1938) who has reported that light has a remarkable effect on the accumulation of ascorbic acid during germination, and the increase could be due to photosynthesis or to the conversion of glucose to ascorbic acid. HAMMER and PARKS (1944) using fluorescent type lamps have reported a quantitative relationship between light and ascorbic acid level, and this relationship is almost identical in both young and old plants. Other compounds, e.g. carotenoids (vitamin A precursors), under light conditions behave in a similar way to vitamin C, i.e. in wheat seedlings the biosynthesis of carotenoids which can occur in total darkness is greatly stimulated in light (WOLF, 1963).

THIAMINE IN LENTILS GROWN AT 25 C IN THE LIGHT

Thiamine increased in all four lentil varieties during germination in the light.

In AMR, LAG, JOR, and SML thiamine increased after 5 days

Table 33: Effect of light on vitamin C, thiamine and riboflavin of four varieties of lentils during germination at 25°C in the light.
(Results are expressed as mg/100g dry weight)

Days of germin.	AMR			LAG			JOR			SML		
	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo	Vit.C	Thia.	Ribo.
Control or o	4.35 (0.14)	0.57 (0.06)	0.27 (0.03)	2.17 (0.08)	0.45 (0.06)	0.38 (0.04)	5.60 (0.25)	0.50 (0.05)	0.34 (0.03)	5.42 (0.19)	0.39 (0.03)	0.21 (0.02)
1	24.26 (0.60)	0.66 (0.05)	0.32 (0.03)	19.59 (0.60)	0.58 (0.07)	0.43 (0.03)	18.11 (0.36)	0.60 (0.07)	0.39 (0.04)	12.74 (0.36)	0.47 (0.03)	0.29 (0.04)
2	29.50 (0.90)	0.74 (0.10)	0.38 (0.05)	25.11 (0.81)	0.67 (0.05)	0.50 (0.05)	29.32 (0.87)	0.71 (0.05)	0.44 (0.05)	27.12 (1.10)	0.59 (0.04)	0.33 (0.04)
3	37.58 (1.00)	0.82 (0.09)	0.49 (0.03)	32.00 (1.00)	0.73 (0.08)	0.57 (0.08)	32.63 (0.70)	0.79 (0.08)	0.52 (0.07)	30.00 (0.82)	0.75 (0.07)	0.39 (0.05)
4	48.31 (1.42)	0.98 (0.09)	0.58 (0.04)	34.30 (0.89)	0.80 (0.06)	0.61 (0.06)	36.40 (0.95)	0.93 (0.10)	0.67 (0.05)	36.54 (1.25)	0.81 (0.07)	0.42 (0.06)
5	64.28 (2.15)	1.22 (0.11)	0.65 (0.07)	38.20 (1.40)	0.87 (0.10)	0.68 (0.08)	38.15 (1.36)	1.15 (0.12)	0.72 (0.06)	40.35 (1.60)	0.88 (0.09)	0.51 (0.05)

( ) = SE

germination when compared to dry seeds from 0.57 to 1.22, from 0.45 to 0.87, from 0.50 to 1.15, and from 0.39 to 0.88 mg/100g respectively (table 33, p.151). These results agree well with those of GUSTAFSON (1948) who has reported that the concentration of thiamine in beans and peas is greater in plants exposed to light than in those in the dark. He provided evidence that even though light destroyed some thiamine, or that it may be used up more rapidly in the light than in the dark, yet as a result of photosynthesis, a much larger amount was found in the light.

RIBOFLAVIN IN LENTILS GROWN AT 25 C IN THE LIGHT

Riboflavin also increased in the four lentil varieties. In AMR, LAG, JOR, and SML riboflavin of 5-day germinated seeds increased from 0.27 to 0.65, from 0.38 to 0.68, from 0.34 to 0.72, and from 0.21 to 0.51 mg/100g respectively (table 33). HAMILTON and VANDERSTOEP (1979) have reported an increase in riboflavin of light-grown alfalfa seeds from 0.74 to 2.3 mg/100g on the fifth day of germination. These results are not the same for all species tested, since the riboflavin content was not higher in light germinated seedlings of mung beans, soybeans, and lentils (VANDERSTOEP, 1981).

From the present study, the pattern of behaviour (showing an increase with time in light) of the three vitamins in the four lentil varieties are the same, but they differ from each other in the degree of response.

RESULTS AND DISCUSSION

CHANGES IN VITAMIN C OF LENTILS GROWN AT 35 C IN THE DARK

In the present study (table 34, fig. 14) the vitamin C content of lentils germinated at 35 C in the dark increased and reached its

Table 34: Vitamin C, thiamine and riboflavin content of four varieties of lentils grown at 35°C in the dark.
(Results are expressed as mg/100g on a dry weight basis)

Days of germin.	AMR			LAG			JOR			SML		
	Vit. C.	Thia.	Ribo.	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo.	Vit.C	Thia.	Ribo.
Control or o	4.35 (0.14)	0.57 (0.06)	0.27 (0.03)	2.17 (0.08)	0.45 (0.06)	0.38 (0.04)	5.60 (0.25)	0.50 (0.05)	0.34 (0.03)	5.42 (0.19)	0.39 (0.19)	0.21 (0.02)
1	28.00 (1.10)	0.65 (0.06)	0.30 (0.04)	23.72 (0.69)	0.59 (0.07)	0.42 (0.05)	21.30 (0.75)	0.55 (0.08)	0.37 (0.05)	31.58 (1.35)	0.51 (0.05)	0.27 (0.03)
2	41.19 (1.59)	0.78 (0.08)	0.34 (0.05)	34.50 (0.90)	0.65 (0.05)	0.48 (0.04)	39.85 (1.20)	0.68 (0.07)	0.48 (0.06)	38.74 (1.22)	0.58 (0.07)	0.35 (0.03)
3	37.29 (0.85)	0.61 (0.07)	0.37 (0.04)	29.60 (1.19)	0.48 (0.06)	0.54 (0.07)	42.53 (1.05)	0.61 (0.05)	0.56 (0.05)	31.43 (0.79)	0.63 (0.08)	0.40 (0.06)
4	28.67 (0.56)	0.52 (0.04)	0.29 (0.02)	21.30 (0.78)	0.36 (0.05)	0.41 (0.06)	33.11 (1.17)	0.52 (0.05)	0.39 (0.04)	27.65 (0.65)	0.49 (0.06)	0.26 (0.03)
5	21.51 (0.94)	0.35 (0.05)	0.22 (0.03)	17.42 (0.77)	0.25 (0.03)	0.31 (0.04)	26.80 (0.98)	0.41 (0.03)	0.28 (0.03)	25.56 (0.96)	0.31 (0.04)	0.19 (0.02)

( ) = SE

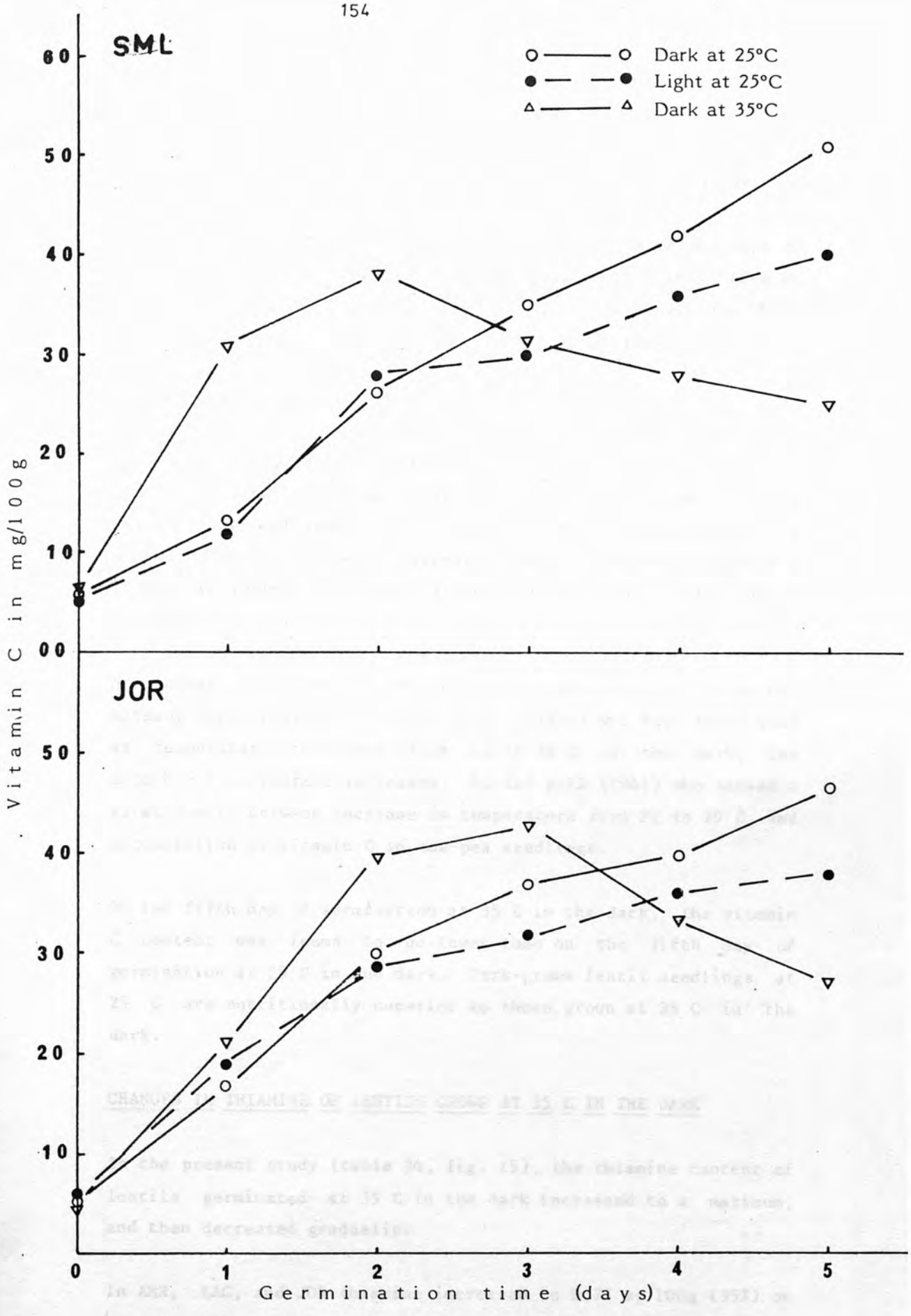


Fig. 14: Changes in vitamin C of lentil varieties germinated in the dark and in the light at 25°C, and in the dark at 35°C

maximum content either on the second or the third day of germination, and then started decreasing gradually.
In AMR, LAG, and SML vitamin C increased on the second day of germination to 41.1, 34.5, and 38.7 mg/100g respectively, then on the fifth day it decreased to 21.5, 17.4, and 25.5 mg/100g; while in JOR on the third day it increased maximally to 42.5, decreasing to 26.8 mg/100g.

On the fifth day of germination, vitamin C in the four varieties ranged from 370 to 577% (table 34). From the present study, it can be seen that during germination at 35 C in the dark, in the early stages of germination, higher rates of accumulation of vitamin C are obtained and vitamin C content reaches its maximum in most varieties on the second day of germination, after which it starts to decline.

An increase in vitamin C content as the temperature increases has already been reported by SOMERS et al. (1948) who have shown that as temperature increases from 24 to 30 C in the dark, the ascorbic acid content increases. So did REID (1941) who showed a relationship between increase in temperature from 22 to 29 C and accumulation of vitamin C in cow-pea seedlings.

On the fifth day of germination at 35 C in the dark, the vitamin C content was found to be lower than on the fifth day of germination at 25 C in the dark. Dark-grown lentil seedlings at 25 C are nutritionally superior to those grown at 35 C in the dark.

CHANGES IN THIAMINE OF LENTILS GROWN AT 35 C IN THE DARK

In the present study (table 34, fig. 15), the thiamine content of lentils germinated at 35 C in the dark increased to a maximum, and then decreased gradually.

In AMR, LAG, and JOR thiamine increased to 0.78 mg/100g (35%) on

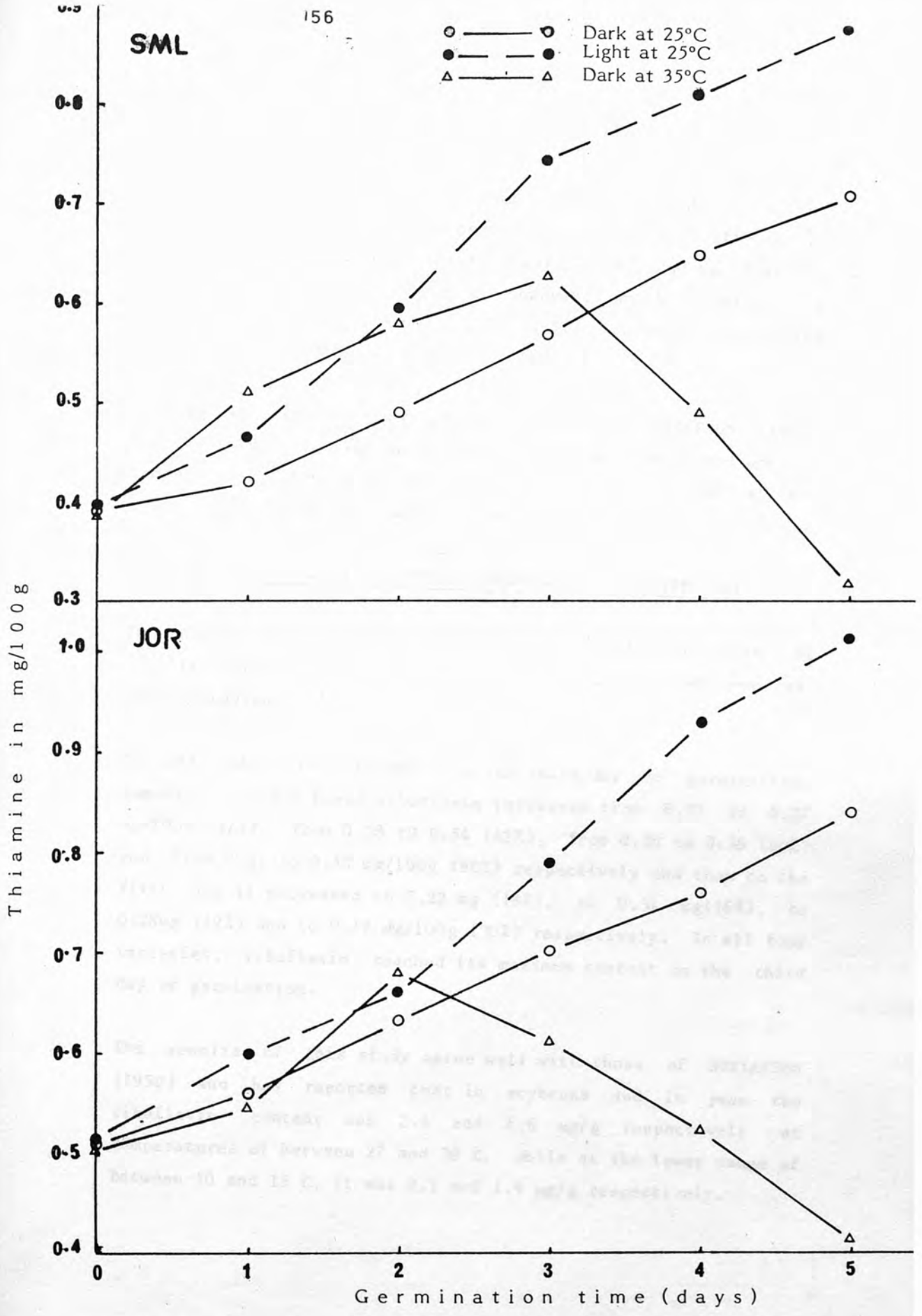


Fig. 15: Changes in thiamine of lentil varieties germinated in the dark and in the light at 25°C, and in the dark at 35°C

the second day of germination, from 0.45 to 0.65 (31%), and from 0.50 to 0.68 mg/100g (36%) respectively, then on the fifth day it decreased to 0.35 mg/100g (38%), to 0.25 (44%) and to 0.41 mg /100g (18%) respectively. In SML however, on the third day it increased from 0.39 to 0.63 mg/100g (61%), and then on the fifth day it decreased to 0.31 mg/100g (21%).

The results of this study agree with those of GUSTAFSON (1950) who observed that thiamine content of soybeans, beans and peas at temperatures of between 27 to 30 C was higher than that at the lower temperatures of between 10 and 15 C .

CHANGES IN RIBOFLAVIN IN LENTILS GROWN AT 35 C IN THE DARK

In this study (table 34, fig. 16), the riboflavin content of lentils during germination at 35 C increased to a maximum and then decreased gradually.

In AMR, LAG, JOR and SML, on the third day of germination, compared to dry seeds riboflavin increased from 0.27 to 0.37 mg/100g (37%), from 0.38 tO 0.54 (42%), from 0.34 to 0.56 (64%) and from 0.21 to 0.40 mg/100g (90%) respectively and then on the fifth day it decreased to 0.22 mg (18%), to 0.31 mg(16%), to 0.28mg (19%) and to 0.19 mg/100g (30%) respectively. In all four varieties, riboflavin reached its maximum content on the third day of germination.

The results of this study agree well with those of GUSTAFSON (1950) who has reported that in soybeans and in peas the riboflavin content was 2.4 and 1.6 µg/g respectively at temperatures of between 27 and 30 C, while at the lower range of between 10 and 15 C, it was 2.1 and 1.9 µg/g respectively.

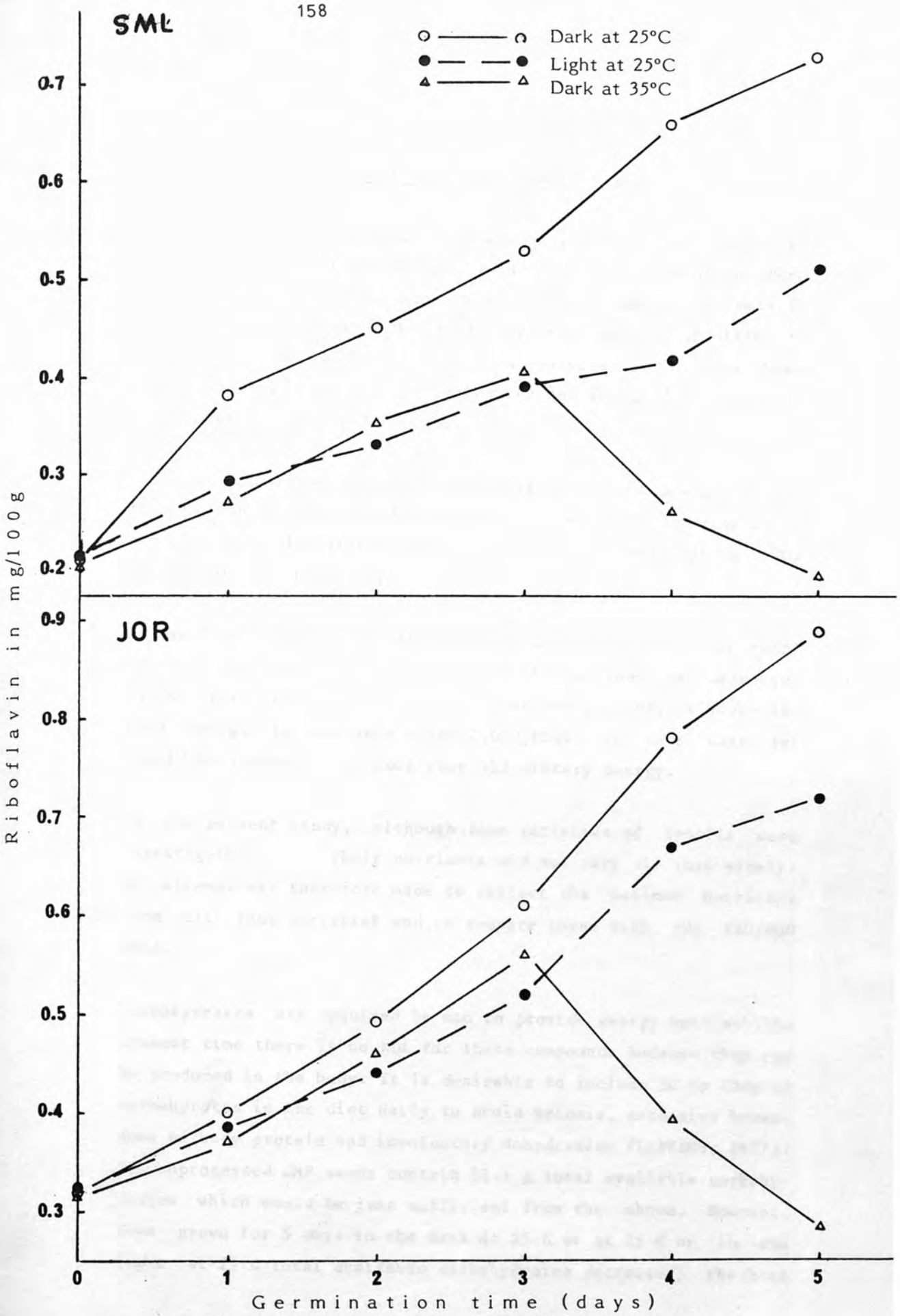


Fig. 16: Changes in riboflavin of lentils germinated in the dark and in the light at 25°C, and in the dark at 35°C

General Discussion On Section 1

Food is required by man for metabolic processes and physical growth. The nutritive components investigated in lentils were carbohydrates, lipids, proteins and three vitamins: vitamin C, thiamine and riboflavin. Man needs different amounts of different nutrients. Recommended Dietary Allowances (RDA) have been suggested and these help in evaluating the adequacy of food in meeting man's nutritional needs.

Different countries have different RDAs and in the present study, the FAO/WHO Recommended Intakes for Nutrients (RDA) for an adult man was used. The results will be based on an adult eating 100g dry weight of lentils per day.

It has been suggested by HANSEN and WYSE (1980) that 35% of total calories must be supplied as fat and the remainder as carbohydrates. This is especially so in developing countries where the food energy is sometimes below 2,000 KCal, in such case fat should be reduced to no more than 35% dietary energy.

In the present study, although four varieties of lentils were investigated, their nutrients did not vary all that widely. An attempt was therefore made to collect the maximum nutrients from all four varieties and to compare these with the FAO/WHO RDAs.

Carbohydrates are required by man to provide energy but at the present time there is no RDA for these compounds because they can be produced in the body. It is desirable to include 50 to 100g of carbohyrates in the diet daily to avoid ketosis, excessive breakdown of body protein and involuntary dehydration (LAPEDES, 1977). Dry unprocessed AMR seeds contain 51.1 g total available corbohydrates which would be just sufficient from the above. However, when grown for 5 days in the dark at 25 C or at 35 C or in the light at 25 C total available carbohydrates decreased; the best

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amount obtained was 50.2g (still within the range 50-100g) in AMR on the first day of germination in the light: so, dry lentil seeds are good at providing carbohydrates.

Legume seeds on the whole do not contain large amounts of lipids and it is not unexpected to find that JOR seeds contained the maximal lipid content of 1.3g which is very insufficient in terms of the carbohydrate : lipid ratio of 65 : 35. Total lipids decreased during germination; the best that was obtained (1.1g in JOR on the first day of germination in the dark at 25 C) is not as good as dry seeds.

SML dry seeds contain 4.8g crude fibre. At the present time there is no RDA for it. Diets which are high in crude fibre are beneficial to humans, since lack of them causes certain diseases of the colon. However, when seeds are germinated under the above mentioned conditions crude fibre increased: the best obtained was 12.4g SML on the fifth day of germination in the dark at 35 C. Dry unprocessed SML seeds contain the maximum protein (27.5g) which provides 74% RDA. However, when grown under the above conditions protein decreased: the best that was obtained (25.6g in SML on the first day of germination at 25 C in the light), supplies only 69% RDA.

Dry unprocessed lentil seeds contain eighteen amino acids. Most amino acids decreased during germination under the previously mentioned conditions except for lysine, an essential amino acid which increased slightly during germination at 25 C in the dark in AMR.

Dry unprocessed JOR seeds contain 5.6mg vitamin C, which represents 19% RDA. Ascorbic acid deficiency can cause scurvy and this can be prevented by a higher intake of this vitamin. However, when seeds are grown for 5 days in the dark at 25 C and 35 C or at 25 C in the light, vitamin C increased: the best that was obtained (85.0mg in AMR on the fifth day of germination at 25 C

in the dark) provides substantially more than the RDA of 30mg.

Dry AMR seeds contain 0.57mg thiamine, and this accounts for 47% RDA. Thiamine deficiency will cause beriberi. Seeds when germinated under the above mentioned conditions showed an increase in thiamine : the best that was obtained (1.22mg in AMR on the fifth day of germination at 25 C in the light) supplies 79% RDA.

Dry LAG seeds contain 0.38mg riboflavin; this provides 21% RDA. However, when grown under the above mentioned conditions riboflavin increased: the best that was obtained (1.1mg in LAG on the fifth day of germination at 25 C in the dark) provides 69% RDA.

On the whole therefore, germination increases vitamins substantially and they may thus be sufficient for the daily requirements. Germinated lentils are a good source of the three vitamins studied here. Unlike other larger sprouts , which are palatable only in the etiolated form, lentils may be eaten etiolated or as light-grown seedlings. This is an added advantage of lentils over other legumes e.g. mungbean sprouts.

Finally, in spite of variations in the nutrients under the growth conditions discussed above, dry lentil seed still represents one of the most nutritious forms of food, is readily available and is not expensive, therefore, in Section 11 of the present study only lentil seeds will be used for further study.

Changes in nutritional value of lentil seeds after two different processing techniques:

1) home processing (soaking + cooking) and

2) canning will be investigated as well as the effects of storing the canned products at 25 C and 35 C over a period of twelve months, which will be the subject of the next Section.

SECTION II

CANNING AND STORAGE AND SCANNING ELECTRON MICROSCOPE STUDIES OF LENTILS

CHAPTER VI

EFFECT OF HOME PROCESSING ON CERTAIN NUTRIENTS OF LENTILS

Importance, function and beneficial aspects of cooking

Cooking may be defined as the art and science of heating food to make it more palatable, nutritious and free from natural toxic constituents. Cooking processing makes hard seeds soft by improving the plasticity of the cell , thus facilitating cell expansion and reduction of the intercellular adhesion. BENDER (1972) has reported that the nutritive value of most legumes is improved by cooking, and this is partly due to the destruction of trypsin inhibitors and toxic substances and partly to improved digestibility. Destruction of certain substances which occur in most legumes, that inhibit human digestive enzymes increases the nutritive value of the food; heat treatment e.g. cooking beyond that needed for this purpose destroys these substances and causes a reduction in their nutritive value (BENDER, 1978).

Losses in nutrients that occur when foods are cooked depend upon the freshness of the food, the size of the product, type of the equipment, amount of water during cooking, length of time and temperature of cooking (LACHANCE, 1975). Nutrient losses are caused in part by loss in cooking water and in part by destruction due to heat. The following are examples of the effect of cooking on various nutritional components and vitamins in vegetables. Retention of riboflavin in cabbage depends on the volume of water used : a large volume tends to cause a large decrease of this vitamin. If a cooking pan is covered, this gives advantages in that more ascorbic acid may be retained (SALUNKHE, 1974). Also the time of boiling is very important since retention of ascorbic acid is greatly reduced when broccoli is boiled for long periods of time. The amounts of some essential amino acids retained are different due to the different methods of cooking.

SMITH (1968) has reported that amounts of tryptophan varied according as to whether potatoes were boiled, fried, or mashed. Although other legume processing techniques (e.g. fermentation, sprouting) are used as alternative procedures for utilizing legumes in many areas of the World, soaking and boiling is probably the most widely used (ROCKLAND, 1978).

Aims of this part of the study

A large number of studies on the effect of processing on grain legumes especially soybeans have been carried out. Until recently, only those legumes of primary importance to industrialized countries such as beans, and peas have received much attention from plant breeders (WALKER and KOCHHAR, 1982), while other legumes e.g. lentils have received little or no attention. So, very little is known concerning the effects of processing on lentils. The home processing (soaking and cooking) of lentils, like that of other legumes makes them more acceptable to the consumer, but it also may alter the bioavailability of certain nutrients. Very little is known concerning the nutrients of lentils during home processing although in some cases, changes in specific nutrients have been investigated, but the cooking conditions were not always specified. Most of these investigations have dealt mainly with changes in amino acids and in proteins (AHMAD and SHAH, 1975; MORCOS et al., 1976).

Changes in the vitamin contents of some legumes have already been reported (AUGUST et al., 1981), and these have centred largely on the changes of vitamin C during cooking (LACHANCE, 1975), with very little on other vitamins e.g. thiamine and riboflavin. The present study will investigate the effect of cooking on vitamin C, on two other vitamins : thiamine and riboflavin, on carbohydrates, on protein, and amino acids of four varieties of lentils.

MATERIALS AND METHODS

Cooking (home processing)

The method of MEINERS et al. (1976) was used with slight modification. One hundred g of each of the four varieties were soaked in 500 ml water for 2 hours, when they were washed with fresh water, and transferred to a glass cooking pan. Five hundred ml of water was added to each pan, then cooked for 1 h at 100 C (1:5 lentil to water ratio).

This ratio was found to be best for lentils which absorb x3 of their weight : therefore, water in less than 300 ml/100g is not sufficient, and 1 l/100g is far too large a volume.

From now on, this procedure of cooking lentils will be referred to as home processing or simply to cooking.

Effect of home processing on total available carbohydrates, starches, and ethanol-soluble sugars of lentils

As a result of changes in cellular structure, all plant foods are softened when cooked. The cells may either be separating, rupturing, shrinking, or a combination of these changes may occur. MEYER (1978) has reported that in general changes in carbohydrates after cooking, may be summarised as follows :

(i) solubility, for during the cooking process soluble carbohydrates are dissolved, and

(ii) hydrolysis, for during the cooking process some polysaccharides are hydrolysed.

Further, pectic substances undergo hydrolysis, as do starches but to a limited extent.

The nutritional value of food carbohydrate is an integral part of its chemical, physical, and biological properties. BIRCH (1977) has reported that heat affects different carbohydrates to different extent in food.

The following physical changes occur during the cooking of starch containing materials : starch granules swell and eventually burst, releasing their polysaccharide components into the surrounding aqueous environment. Also the process of cooking softens cellulosic and pectic tissues so that mastication and digestion are facilitated.

One other change that occurs during cooking is starch gelatinisation. PRIESTLEY (1979) has reported that on boiling potatoes in water, the starch gelatinises and cell walls become weakened by the degradation and removal of pectic substances. Stickiness may occur owing to the rupture of starch-granules.

RESULTS AND DISCUSSION

Table 35 shows that cooking has decreased the total available carbohydrates, starches, and ethanol-soluble sugars in all four varieties.

In AMR, total available carbohydrates, starches, and ethanol-soluble sugars decrease from 51.11 to 41.97 (Retn 82%), from 48.62 to 40.45 (Retn 83%), and from 2.49 to 1.50 (Retn 60%) respectively, while in LAG they decrease from 49.23 to 41.58 (Retn 84%), from 46.26 to 39.88 (Retn 86%), and from 2.97 to 1.71g/100g (Retn 57%). In JOR, total available carbohydrates decrease from 47.14 to 38.20 (Retn 80%), starches from 43.88 to 36.06 (Retn 82%) and soluble sugars from 3.26 to 1.65g/100g (Retn 65%), while in SML the same components were found to be 36.52, 35.09, and 1.43g/100g corresponding to retention values of 78,

Table 35: Effect of cooking on total available carbohydrates (TACHO), Starches + dextrin (ST+D) and ethanol soluble sugars (ESS) of four varieties of lentil.

(Results are expressed as g/100g dry weight)

Variety	AMR			LAG			JOR			SML		
	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO	ESS	ST+D	TACHO
Control	2.49 (0.15)	48.62 (1.25)	51.11 (1.15)	2.97 (0.12)	46.26 (1.18)	49.23 (1.30)	3.26 (0.18)	43.88 (1.50)	47.14 (1.90)	2.56 (0.13)	44.25 (0.88)	46.81 (1.47)
Cooked	1.50 (0.07)	40.45 (0.80)	41.95 (1.10)	1.71 (0.13)	39.88 (1.20)	41.59 (0.95)	1.65 (0.11)	36.06 (1.26)	38.20 (1.45)	1.43 (0.10)	35.09 (1.57)	36.52 (0.90)
Retn. (%)	60.2	83	82	57.6	86	84	65.5	82	80	55.9	79	78

( ) = SE

79, and 56% respectively.

Effect of home processing on mono-, di-, and oligo-saccharides

RESULTS AND DISCUSSION

Table 36 shows that there was a significant decrease in mono-, di-, and oligo-saccharides after cooking lentils for 1 h. The following have already been identified as major sugars in all four varieties of lentils : sucrose, raffinose and stachyose (table 8, p.61). As a result of cooking, sucrose decreased from 0.63 to 0.33g/100g (Retn 55%) in AMR, from 1.29 to 0.81 (Retn 63%) in LAG, from 1.18 to 0.53 (Retn 45%) in JOR, and from 0.97 to 0.48g/100g (Retn 49%) in SML. Raffinose also decreased in all four varieties from 0.26 to 0.12 (Retn 46%), from 0.19 to 0.08 (Retn 41%), from 0.15 to 0.05 (Retn 36%), and from 0.28 to 0.11g/100g (Retn 40%) in AMR, LAG, JOR, and SML respectively. Stachyose also decreased from 1.45 to 1.05g/100g (Retn 72%), from 1.38 to 0.92 (Retn 67%), from 1.83 to 1.07 (Retn 59%), and from 1.14 to 0.84g/100g (Retn 74%) respectively in AMR, LAG, JOR, and SML.

These results are in agreement with those of SILVA and BRAGA (1982) who have reported that in whole beans (Phaseolus vulgaris) cooked at 14 p.s.i., at 120 C for 1 h in 1:10 bean to water ratio, sucrose decreased as did raffinose and stachyose. On the other hand, RAO and BELAVADY (1978) do not agree with these results as they have shown an increase of approximately 100% in sucrose, raffinose and stachyose of red gram, chick pea, black gram, and red gram subjected to cooking for 15 min at 15 p.s.i., when the legume water ratio was 1:2.

The reason for these differences is probably varietal, and also possibly due to the length of time used in cooking.

GREENWOOD and MUNRO (1979) have reported that the polyhydroxyl character of mono- and oligo-saccharides leads to extensive

Table 36: Effect of cooking on mono-, di- and oligo-saccharides of four varieties of lentil.
(Results are expressed as g/100g dry weight)

Sugars	AMR			LAG			JOR			SML		
	Control	Cooked	% Retn.	Control	Cooked	% Retn.	Control	Cooked	% Retn.	Control	Cooked	% Retn.
FRU	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-
GLU	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-	0.00	0.00	-
SUC	0.63	0.33	55	1.29	0.81	63	1.18	0.53	45	0.97	0.48	49
RAF	0.26	0.12	46	0.19	0.08	41	0.15	0.05	36	0.28	0.11	40
STA	1.45	1.05	72	1.38	0.92	67	1.83	1.07	59	1.14	0.84	74
TOTAL	2.34 (0.15)	1.50 (0.07)		2.86 (0.12)	1.81 (0.13)		3.16 (0.18)	1.65 (0.11)		2.39 (0.13)	1.43 (0.10)	

( ) = SE

inter- and intra-hydrogen bonding.

Effect of home processing on lipids and crude fibres

When lipids are thermally heated, two types of reaction occur :

(i) hydrolysis which results in the formation of free fatty acids, and,

(ii) oxidation which leads to the formation of hydroperoxides and hydroxides.

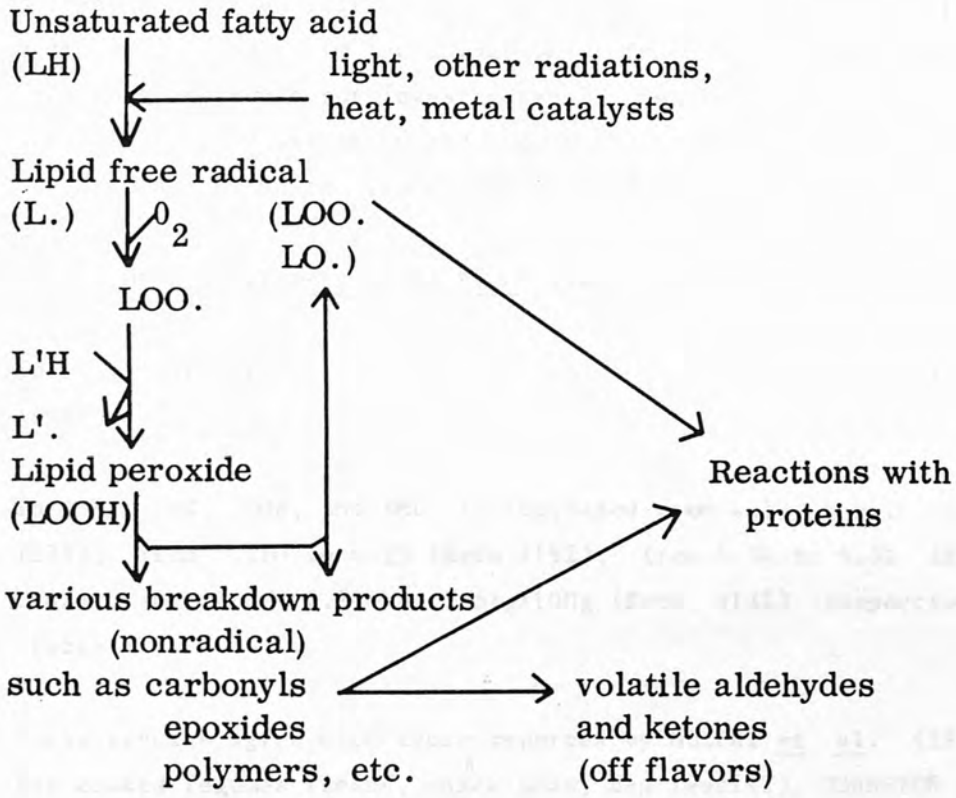
The nature and relative proportions of various products formed during oxidation will depend on the temperature used, and the degree of aeration. Auto oxidation (reaction with oxygen) proceeds more rapidly in the presence of a critical residual amount of water, and storage time and temperature are important as to the extent of oxidative damage (WITTING et al., 1979). Lipids and their oxidation products (especially oxidizing lipids and free radicals, fig. 17) can react with other food components such as proteins (CHEFTEL, 1979). Free radicals formed in unsaturated fat under heat treatment can react directly with protein, causing extensive cross-linking of the protein chain via either protein-lipid cross-links or protein-protein cross-links (HURRELL, 1980).

The change that occurs in pectic substances during the cooking of vegetables is the hydrolysis of large molcules, of insoluble protopectins to smaller substances (pectin) (MEYER, 1978).

RESULTS AND DISCUSSION

Effect of home processing on total lipids

There is a decrease in the lipid content of all four varieties



Schematic representation of reactions linked to lipid oxidation.

Fig. 17: (CHEFTEL, 1979)

tested. Table 37 (p.173) shows that in AMR the lipid content decreased from 1.17 to 0.88 (Retn 75%), in LAG from 0.96 to 0.79 (Retn 82%), in JOR from 1.32 to 1.19 (Retn 90%), and in SML from 1.21 to 1.07g/100g (Retn 87%).

These results are in agreement with those of NOOR et al. (1980) who reported a decrease in the fat content of cooked beans, and with those of OSBORNE and VOOGT (1978) of cooked lentils. These decreases may be correlated with the fact that in heated fats hydrolysis results in the formation of free fatty acids (MORTON, 1977)(but this was not investigated in the present study), and also that lipid oxidation and protein-lipid interactions may be taking place in heated lipids (BENDER, 1978; CHEFTEL, 1979).

Effect of home processing on crude fibre

In all four varieties tested, the crude fibre increased after cooking.

In AMR, LAG, JOR, and SML, it increased from 4.10 to 5.29 (Retn 129%), from 3.70 to 4.25 (Retn 115%), from 4.54 to 5.31 (Retn 118%), and from 4.86 to 5.52g/100g (Retn 113%) respectively (table 37).

These results agree with those reported by MURPHY et al. (1975) for cooked legumes (beans, chick peas, and lentils). JOHNSTON and OLIVER (1982) have reported that cooking (boiling) increased the fibre content of potatoes. Also the crude fibre of carrots, cabbages, and okras increased after cooking (boiling) (MATTHEE and APPLEDORF, 1978). On the other hand, just the opposite was observed by NOOR et al. (1980) who showed that the crude fibre of cooked beans decreased, as did that of boiled lentils (OSBORNE and VOOGT, 1978). These differences may have been due to the different legume grains used, and also possibly due to the time of boiling.

Table 37: Effect of home processing on crude fibre and total lipids of four varieties of lentils.
(Results are expressed as g/100g dry weight)

Variety	CRUDE FIBRE			TOTAL LIPIDS		
	Control	Cooked	% Retn.	Control	Cooked	% Retn.
AMR	4.10(0.09)	5.29(0.14)	129	1.17(0.07)	0.88(0.05)	75
LAG	3.70(0.13)	4.25(0.16)	115	0.96(0.06)	0.79(0.05)	82
JOR	4.54(0.12)	5.31(0.10)	118	1.32(0.10)	1.19(0.08)	90
SML	4.86(0.15)	5.52(0.22)	113	1.21(0.08)	1.07(0.10)	87

( ) = SE

Effect of home processing on protein content

The changes that take place on heating have been investigated both in isolated protein systems and in food. It has been found to consist of two types of reactions:

1) the reaction of protein with carbohydrates, resulting in the destruction of some amino acids and a change in the digestibility of the protein by proteolytic enzymes (p221);

2) reaction of protein in the absence of carbohydrates which results in a decrease in the availability of amino acids.

Also heat inactivation of enzyme inhibitors.

It is well established that heat (under constant conditions) can alter the nutritive value of a protein in a number of species. Many proteins show a decrease, but in a few there is an increase (MEYER, 1978).

Various proteins respond differently to processing depending on the composition and properties of the protein and the state of the food. Also lipid oxidation (p171) and protein-lipid interactions take place and damage the proteins.

DESBOROUGH and WEISER (1974) have reported a 54% reduction in potato protein during cooking (boiling) for 30 min, while LACHANCE (1975) reported that boiling beans (for 2 h) lost about 29% protein. KAKADE and EVANS (1965) have reported that cooking navy beans at 121 C for 30 min decreased some essential amino acids e.g. phenylalanine and arginine.

The effect of home processing (soaking + cooking) on proteins of lentils will be investigated in this part of the present study.

RESULTS AND DISCUSSION

Table 38 shows that cooking decreases the protein content of all varieties tested but such decreases were not substantial.

In AMR, protein decreased from 24.1 to 21.9 (Retn 91%), in LAG from 26.4 to 24.5 (Retn 93%), in JOR from 25.3 to 24.5 (Retn 97%), and in SML from 27.5 to 26.2g/100g (Retn 95%). So, on the whole, protein loss is slightly less than in uncooked seeds, except for JOR which shows no significant difference between cooked and uncooked seeds.

AHMAD and SHAH (1975) have reported a small decrease (3%) in protein after lentils have been boiled for 30 min (100 C), while OSBORNE and VOOGT (1978) showed a much greater decrease (70%) when lentils were cooked (conditions not specified).

On the other hand, ADRIAN (1981) has reported that at moderate heat treatment in a wet medium, total proteins seldom decrease even after lengthy treatments. NOOR et al. (1980) have reported a low decrease in protein content of mung beans, while MURPHY et al. (1975) reported that protein retention of cooked lentils is not significantly different from controls. All these results show quite clearly that cooking seeds may cause them to lose some protein, although this decrease is not always significant when compared to uncooked seeds.

Effect of home processing on protein amino acids

Proteins and protein amino acids are the most reactive of the major food components and reactions between food proteins and amino acids on the one hand and other food components on the other hand are the major chemical reactions that occur during processing. Certain amino acids such as lysine, cysteine, and tryptophan can react with reducing sugars and are therefore destroyed during what is known as Maillard reactions (fig. 24; p.

Table 38: Effect of home processing on protein of lentil varieties
(Results are expressed as g/100g dry weight)

Variety	AMR	LAG	JOR	SML
Control	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
Cooked	21.96(0.78)	24.55(0.66)	24.58(0.40)	26.19(0.35)
% Retention	91	93	97	95

( ) = SE

221). They can also react with fats and their oxidation products, also with vitamins e.g. vitamin B (HURRELL, 1980). All these reactions lead to a reduction in the nutritive value of such foods.

BENDER (1978) has reported that a heated casein-glucose mixture lost arginine, threonine, methionine, histidine, and isoleucine in that order, as well as lysine, but when milk was heated under the same conditions, only lysine was lost. This shows differences between model systems and the food itself. As a result of this,

BENDER (1978) has proposed that investigation of each food is necessary in order to find the exact effect of cooking. The effect of cooking on protein amino acids is therefore to be investigated here.

RESULTS AND DISCUSSION

Table 39 shows that amino acids decreased after cooking. In AMR, protein amino acids decreased from 80.9 to 74.1 (Retn 92%), in LAG from 83.6 to 75.7 (Retn 90%), in JOR from 81.9 to 72.8 (Retn 88%), and in SML from 82.7 to 73.8 g/100g protein (Retn 89%). Also the nineteen protein amino acids all decreased to a greater or lesser extent : the ones with most losses being lysine, cysteine, histidine, tryptophan, and isoleucine.

MORCOS et al. (1976) have reported that whole lentil seeds cooked in boiling water for one hour resulted in losses of most amino acids except for lysine and tryptophan which increased, and further, that as the cooking temperature increased, amino acids decreased.

Similarly AHMAD and SHAH (1975) have reported that Bengal gram cooked at 100 C for 30 min showed losses in leucine, isoleucine, valine, methionine, and lysine. Further, they showed that when lentils were cooked under the same conditions, losses in

Table 39: Effect of home processing (cooking) on amino acids of lentils
(Results are expressed as g amino acid/100g of protein)

Amino acids	AMR			LAG			JOR			SML		
	Control	Cooked	Retentn.	Control	Cooked	Retentn.	Control	Cooked	Retentn.	Control	Cooked	Retentn.
Tryptophan	0.78	0.69	88	0.91	0.75	82	0.80	0.70	87	0.85	0.68	80
Lysine	6.45	5.16	80	6.38	5.48	85	6.29	5.22	82	6.12	5.24	85
Histidine	1.93	1.67	86	1.87	1.57	83	1.68	1.34	79	2.05	1.73	85
Ammonia	2.23	2.43	109	2.44	2.85	117	2.00	2.21	111	2.58	2.94	114
Arginine	6.72	6.18	91	7.05	6.63	94	6.86	6.12	89	6.53	6.05	92
Aspartic acid	10.19	9.48	93	10.56	9.60	91	10.82	10.15	93	10.46	9.12	87
Threonine	2.46	2.31	94	2.64	2.45	93	2.10	1.76	83	2.25	1.84	82
Serine	3.00	2.79	93	3.29	2.92	88	3.17	2.72	85	3.30	3.00	90
Glutamic acid	11.18	10.73	96	11.25	10.55	94	11.60	11.00	94	11.06	10.34	93
Proline	4.06	3.84	95	4.43	3.98	89	4.26	3.70	86	4.12	3.64	88
Glycine	4.50	4.05	90	4.90	4.51	92	5.08	4.64	91	5.20	4.94	95
Alanine	4.88	4.29	87	5.07	4.53	89	5.29	4.76	89	5.30	5.05	95
Cysteine	1.06	0.85	80	0.80	0.68	85	0.74	0.65	88	0.90	0.75	83
Valine	4.10	3.89	94	4.36	3.96	90	4.15	3.85	92	4.45	3.87	86
Methionine	0.54	0.47	87	0.60	0.53	88	0.50	0.40	80	0.70	0.59	84
Isoleucine	2.96	2.66	89	3.10	2.88	91	3.00	2.61	87	2.80	2.54	90
Leucine	5.28	4.85	91	5.20	4.62	88	5.42	4.48	82	4.92	4.31	87
Tyrosine	4.17	4.00	96	4.31	4.00	92	3.87	3.42	88	4.23	3.63	85
Phenylalanine	4.48	4.08	91	4.43	3.96	89	4.30	3.52	81	4.80	4.36	90
TOTAL	80.88 (2.80)	74.16 (2.96)		83.58 (2.15)	75.65 (2.64)		81.91 (1.90)	72.80 (3.27)		82.67 (2.46)	73.75 (2.55)	

( ) = SE

histidine, tryptophan, and methionine were observed as were increases in arginine. On the other hand, GEERVANI and THEOPHILUS (1980) have reported that methionine, cysteine, threonine, tryptophan, and lysine all decreased when green gram was boiled for 30 min at 100 C. These latter results are not unlike the ones observed in the present study, and taking all these results into consideration, it can be seen that amino acids are affected differently in different foods subjected to cooking : the same protein amino acid either increasing or decreasing depending on the food used and the conditions of cooking.

Effect of home processing on protein quality

Most of the studies dealing with the effect of processing on protein quality have used biological methods in order to observe their effects if any. ROCKLAND and RADE (1981) have reported a decrease in protein quality as measured by protein efficiency ratio (PER) of cooked beans, while a decrease in protein quality has been reported by KAKADE and EVANS (1965).

In the present atudy, protein quality (in terms of the chemical score) as defined on p. 119 has been investigated after the four lentil varieties were home processed.

RESULTS AND DISCUSSION

From table 40, it can be seen that cooking has decreased the nutritive value (chemical score) of protein of the four lentil varieties under study.

The chemical score of all individual amino acids for all four cooked varieties are lower than those for uncooked ones. The chemical score of the proteins (i.e. the amino acid with the lowest chemical score) of AMR, LAG, JOR, and SML have decreased significantly from 28 to 23, from 25 to 19, from 22 to 16, and

Table 40: Effect of home processing on chemical score of essential amino acids of four lentil varieties.

(Results are expressed as % of the same EAA in reference to protein (whole egg))

Essential Amino acids (EAA)	AMR		LAG		JOR		SML	
	CON Uncooked	Cooked	CON Uncooked	Cooked	CON Uncooked	Cooked	CON Uncooked	Cooked
Lysine	98	78	97	83	95	79	93	80
Tryptophan	46	41	53	44	47	41	50	40
Isoleucine	51	46	53	49	52	45	48	43
Leucine	59	54	58	52	61	50	55	48
Valine	55	52	59	53	56	52	60	52
Methionine + Cysteine	28	23	25	19	22	16	28	22
Threonine	49	46	53	49	42	35	45	37
Phenylalanine + Tyrosine	93	75	94	87	88	75	96	88
Histidine	88	76	85	71	76	61	95	71
Lowest Chemical Score	28	23	25	19	22	16	28	22

from 28 to 22.

The results of this study are in close agreement with those of NOOR et al. (1980) who have reported loss in protein quality due to cooking beans. ABU-SHAKRA and TANNOUS (1981) have reported that autoclaving lentils at 121 C decreased the amino acid content, and so also the chemical score, and as autoclaving time increased there was a greater decrease in the chemical score.

Effect of home processing on free amino acids

Cooking has caused a considerable decrease in all free amino acids so that only trace amounts were still present in the seeds of all four varieties. There was therefore no need to express these results in a table.

These results are in agreement with those of LUH and WOODROOF (1975) who have reported that the free amino acid content of mushroom decreased substantially after boiling.

Effect of home processing on vitamins

It has often been suggested that home processing generally leads to much greater losses than those encountered in industrial processing. Also, the general rule is that most vitamins break down to a greater or lesser extent on heating (PRIESTLEY, 1979; BENTERUD, 1977).

Studies of the effects of home processing on the following vitamins (vitamin C, thiamine, and riboflavin) have been carried out and are discussed below.

1. Effect of home processing on vitamin C

Vitamin C is one of the most readily destroyed of the vitamins, and its retention is often used as an index of the severity of

processing. LACHANCE (1975) has reported that the retention of vitamin C is used as a measure of the relative desirability of a cooking procedure for foods. BENDER (1966) has reported that peas cooked before drying, retained only half the vitamin C content of uncooked peas. Losses here may be due to leaching. BENTERUD (1977) has reported that the retention of vitamin C during household cooking ranged from 25 to 45, and from 55 to 75% depending upon the conditions of boiling.

RESULTS AND DISCUSSION

Table 41 shows that cooking caused a decrease in the vitamin C content of all four varieties.

Vitamin C decreased from 4.35 to 1.96 (Retn 45%) in AMR, from 2.17 to 0.78 (Retn 36%) in LAG, from 5.60 to 2.24 (Retn 40%) in JOR, and from 5.42 to 1.79 mg/100g (Retn 33%) in SML.

LACHANCE and ERDMAN (1975) have reported that vitamin C retention in some boiled vegetables is about 50% which is slightly higher than the values obtained with lentils in this study. So, more vitamin C is lost in lentils after boiling than in certain other vegetables.

BIRCH et al. (1974) have reported that loss of vitamin C in processed fruit and vegetable is due to leaching, oxidation (chemical and enzymic) and thermal degradation. Higher oxidation should occur at the maximum cell disruption with minimum enzyme inactivation.

2. Effect of home processing on thiamine

Thiamine is next to vitamin C as an index of the severity of heat treatments. The greatest source of its loss from most processed food too is leaching (BENDER, 1978). It is stable at high temperatures but certain enzymes e.g. thiaminase can destroy this

Table 41: Effect of cooking on vitamin content of lentil varieties
(Results are expressed as mg/100g dry weight)

Variety	Vitamin C			Thiamine			Riboflavin		
	Uncooked	Cooked	% Retn.	Uncooked	Cooked	% Retn.	Uncooked	Cooked	% Retn.
AMR	4.35 (0.14)	1.96 (0.10)	45	0.57 (0.06)	0.31 (0.04)	54	0.27 (0.03)	0.17	63
LAG	2.17 (0.08)	0.78 (0.07)	36	0.45 (0.06)	0.23 (0.03)	50	0.38 (0.04)	0.23	61
JOR	5.60 (0.25)	2.24 (0.15)	40	0.50 (0.05)	0.22 (0.03)	44	0.34 (0.03)	0.20	59
SML	5.42 (0.19)	1.79 (0.09)	33	0.39 (0.03)	0.19 (0.02)	49	0.21 (0.02)	0.14	65

( ) = SE

SE is omitted when $\leq$0.02 mg/100g

vitamin. The thiamine (B_1) retention in boiled chicken is about 58% (BENTERUD, 1977), while the boiling of vegetables generally results in a retention of over 75%; thiamine being the most heat labile of the B vitamins (PRIESTLEY, 1979).

RESULTS AND DISCUSSION

In the present study, there was a decrease in the thiamine content of all four varieties used (table 41).

Thiamine content decreased in AMR from 0.57 to 0.31 (Retn 54%), in LAG from 0.45 to 0.23 (Retn 50%), in JOR from 0.50 to 0.22 (Retn 44%), and in SML from 0.39 to 0.19 mg/100g (Retn 49%).

AUGUSTIN et al. (1981) have reported that there was a 27% decrease in the thiamine content of cooked beans (Phaseolus vulgaris) as did FARRER (1955) who found boiling, or any treatment at or near 100 C also caused a low decrease in thiamine. However, GEERVANI and THEOPHILUS (1980) have reported a large decrease of this vitamin in boiled red gram as was found in the present study. This suggests once more that the vitamins of different legumes respond differently to the same or to similar treatments.

So, on the whole in the present study there is a decrease of about 50% in thiamine of lentils subjected to home processing.

3. Effect of home processing on riboflavin

Like other vitamins, a number of studies have suggested that riboflavin is affected by heat in different food. Heat alone (even at 130 C) does not damage riboflavin, which is relatively stable at neutral pHs (BENDER, 1978). ARCHER and TANNENBAUM (1979) have reported that riboflavin is stable in food under most processing and cooking conditions. The boiling of vegetables generally results in a retention of over 60% riboflavin compared

to uncooked ones. HARRIS (1975) has reported that riboflavin is stable to heat if in the dry form.

RESULTS AND DISCUSSION

Riboflavin was shown to decrease in all four varieties of lentils tested (table 41).

Riboflavin in AMR decreased from 0.27 to 0.17 (Retn 63%), in LAG from 0.38 to 0.23 (Retn 61%), in JOR from 0.34 to 0.20 (Retn 59%), and in SML from 0.21 to 0.14 mg/100g (Retn 65%).

The results from the present study are slightly lower than those of AUGUSTIN et al. (1981) who have reported a retention of 76% riboflavin in cooked beans compared to uncooked ones. LACHANCE (1975) has reported that beans cooked (boiling for 30 min) retained 64-96% of the original riboflavin content. However, the decrease in the present study (app. 40%) is not as high as that reported for boiled red gram by GEERVANI and THEOPHILUS (1980) who showed an approximate 80% loss.

So the results of the present study (100 C at pH 7) and those cited above are not in agreement with the generalization put forward by BENDER (1978) that when foods at neutral pH are heated, there is relatively no effect on riboflavin. Therefore, riboflavin may also be affected in some cases by cooking. As riboflavin is water-soluble, the effect of cooking may be due partly to its being leached into the cooking water.

THE COOKABILITY OF LENTILS

INTRODUCTION

Reducing the cooking time of legumes is of great importance to the consumer. Studies are already in progress for preparing quick cooking legumes. ELIAS et al. (1973) have prepared pre-cooked dehydrated whole beans, while KARKANAWI (1974) has also prepared pre-cooked, refrigerated and frozen legume seeds. The following figure (fig. 18) shows the steps in the preparation of dehydrated and frozen pre-cooked legumes as suggested by KARKANAWI (1974), and by ELIAS et al. (1973).

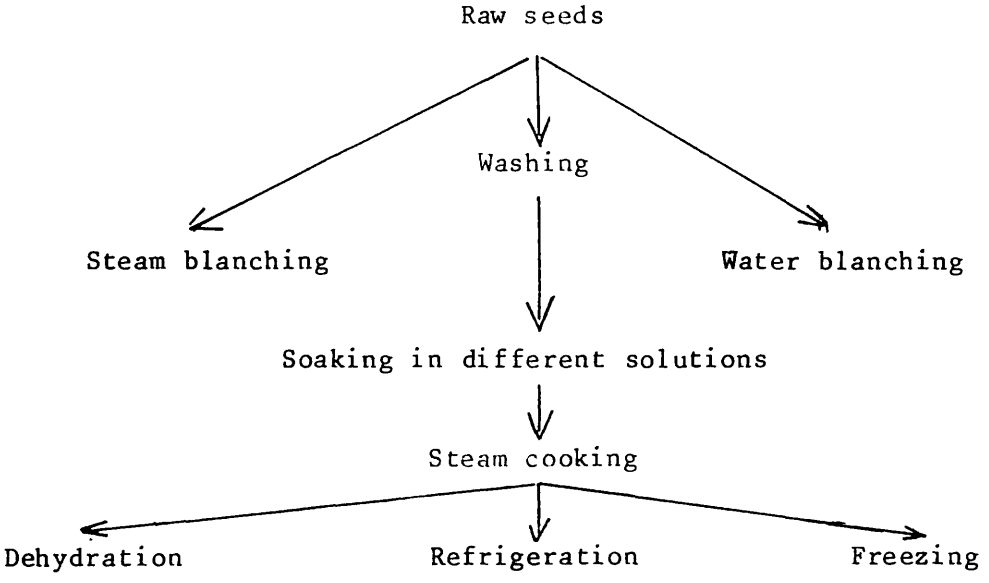


Fig. 18. Steps for preparing different forms of pre-cooked legume seeds after ELIAS et al. (1973) and after KARKANAWI (1974).

Reducing the cooking time of legumes by soaking and/or cooking them in chemicals is receiving a great deal of attention, because it is less expensive compared to other forms of processing e.g. dehydration or freezing as shown in fig. 18. Several steps are involved between the raw seeds and the final dehydrated or

refrigerated or frozen end products and in every step energy is involved which may turn the final product into an expensive or uneconomic commodity.

When seed legumes are heated in boiling water, a large gain in volume or in weight or in both is an indicator that the seeds are cooked (SHINDE and SHIRALKAR, 1980; MEINERS et al. 1976). The increase in weight or volume is limited to the water holding capacity of the legume beyo nd which the seed coat breaks.

Legume seeds are difficult to cook, possibly due to the presence of insoluble pectins in the cell wall (ABU-SHAKRA and TANNOUS, 1981). Other factors affecting the cooking times of legumes e.g. of peas and beans are the contents of lignin and cellulose in the seed-coat or in the seed-coat layer (MULLER, 1967). Also cooking time of peas is influenced by factors such as electrolytes, pH, isoelectric point, presence of pectins and minerals.

ROCKLAND and METZLER (1967) have futher reported that differences in cooking properties may be related to the protein and fibre content of the seeds. They suggested that certain soaking solutions designed to disperse or solubilize proteinaceous materials are very effective in reducing the cooking time of legumes. SCHOCH and MAYWALD (1968) have reported that the cooking qualities of various peas and beans are probably influenced by the pasting characteristic of their starch components.

The effect of some chemicals on the cooking times will be investigated in this part of the present study.

Effect of some chemicals on the cooking of legumes

Many chemicals including neutral salts, acids, alkalis, oxidizing and reducing agents and surface active materials have been investigated. ROCKLAND (1978) has reported that a 2% NaCl

solution decreases the cooking times slightly as does re-hydration of whole beans in dilute alkali solution. Also various phosphates have been tested, because of their recognized metal chelating properties and the premise that cotyledonous cells may be held together by calcium or other bivalent cation bridges in the protein-carbohydrates matrix of the middle lamella (ROCKLAND, 1978).

Another possible explanation of how these solutions affect the cooking time is that by wetting the inner membrane it hydrates rapidly, plasticising the seed and causing it to expand to its maximum dimension within a short time (ROCKLAND and METZLER, 1967).

In the present study, a chemical solution was used during the soaking period only and its advantages over cooking in the chemical solution is that any change in the organoleptic properties i.e. colour and flavour that takes place during the soaking period may be annulled by washing the seeds in fresh water.

Measuring the cooking time of seeds

There are methods either objective or subjective for measuring cooking time.

1.The objective method

This method is based on the use of specialized equipment to measure the pressure needed for a probe to penetrate the seed. The tenderness of cooked or partially cooked legumes can be estimated by shearpress measurements (ROCKLAND and METZLER, 1967).

2. The subjective methods

Under this category different ways have been reported.

(a) By the feeling of softness when cooked seeds are pressed between the thumb and the first finger (SHINDE and SHIRALKAR, 1980).

(b) Pressing the cooked seeds between two glass slides until no hard material is left.

(c) When a trained person(s) agrees that the product is cooked and uniform in texture.

(d) The tenderness and texture of boiled seeds may be assessed by feel after pressing individual seeds with the back of a spoon, which when well cooked produce a smooth uniform paste, while when still uncooked do not form a paste (ABU-SHAKRA and TANNOUS, 1981).

This method (d) has been used in the present work as it has obvious advantages over the other three methods just described.

Preliminary studies on the water-uptake during cooking of lentil seeds

NARASIMHA and DESIKACHAR (1978) have reported that water-uptake after a definite period of cooking, could present a measure of cookability of legumes. This method (water-uptake) has been used in the present study over a period of 60 min (after the seeds had been soaked for 2 h in water) in an attempt to find the maximum rate of water uptake in lentils. The higher the increase in water uptake, the quicker the seeds cooked (hence a decrease in cooking time).

Procedure

Three aliquots, 10 g each, from each variety, were soaked in 50 ml of tap water for 2 h. They were drained and then cooked in 50 ml water as in the previous method (p.165). The first aliquot was cooked for 20 min, the second for 40 min, and the third for 60 min. For the determination of water uptake, the method of NARASIMHA and DESIKACHAR (1978) was used. The cooked samples were centrifuged at 3000 rpm for 15 min and after decanting the supernatant, and removing excess surface water, the residue was weighed, and increase in weight was determined and water uptake was determined in each case.

Table 42 (fig. 19 ;appendix 3) shows the water uptake at different times. The rate of water uptake increased maximally between 20 and 40 min of boiling. Lentils cooked for 1 h showed an increase in water uptake from 260% (SML) to 281% (AMR) (table 42).

The preliminary results show that when soaking the seeds for 2 h and then cooking them in water, the highest rate of water uptake is between 20 and 40 min. In subsequent studies, a cooking time of 20 min was used as it was within the above observed range.

Effect on cooking time after soaking the seeds in different chemical solutions

For the purpose of this part of the study, a number of chemicals have been used, and they include salts, alkalis and acids, e.g. ammonium carbonate, sodium bicarbonate, trisodium phosphate, amino acids (e.g. glutamic acid and arginine) and hydrochloric acid.

These chemicals were used separately as follows :

1.$(NH_4)_2CO_3$ (1%)
2.$NaHCO_3$ (1%)
3.Na_3PO_4 (1%)
4.Glutamic acid (1%)
5.Arginine (1%)
6.Hydrochloric acid (1%)

Acids were tested, because it is thought that the beneficial effect (reducing cooking time) of other chemicals is due to their raising the pH of the solution (CAROBUENO et al, 1980). Therefore, salts and acids have been tested to find (1) if they affect the cookability of lentils and (2) their effect, i.e. whether they enhanced or delayed the rehydration rate.

In the present study, the cookability (cooking time) of lentils was investigated. This was done by soaking the seeds in different solutions for 2 h and then cooking them for various times in water. While other investigators have added the chemicals directly to the cooking water, the reason why this was not done in the present study has been mentioned ealier (p.188).

MATERIALS AND METHODS

1.Determination of time taken to cook lentil seeds

For time taken to cook, the method as described on p165was used. Twenty g of each of the four varieties were soaked in 100 ml of water for 2 h. At the end of the soaking period, they were washed with fresh water and 100 ml was added and then boiled gently (simmered) for 50 min.

The tenderness and texture of boiled seeds were assessed by the method of ABU-SHAKRA and TANNOUS (1981) (p189) which depends on the mouth feel after pressing seeds with a spoon. The boiling was continued and the test for cooked seeds was repeated every five

minutes using the same procedure of tenderness and texture as above.

2.Effect of chemicals on time to cook

Three samples 10 g each, from each variety, were soaked for 2 h in 50 ml of water (control) and another set of three samples were soaked in 50 ml of each of the following solutions:

1. $(NH_4)_2CO_3$ (1%)
2. $NaHCO_3$ (1%)
3. Na_3PO_4 (1%)
4. Glutamic acid (1%)
5. Arginine (1%)
6. Hydrochloric acid (1%)

At the end of the soaking time, the solution was drained, and seeds were rinsed once with water. The first sample was used to determine the water uptake at the end of the soaking period. The second sample was boiled in 50 ml of tap water for 20 min. Boiling and water uptake were carried out as described previously (p.190). The third sample was boiled in 50 ml water, and time needed for cooking was assessed by mouth feel of spoon pressed seeds (p.189).

RESULTS AND DISCUSSION

In the present study, lentils cooked for 1 h showed a range of increases in water uptake from 260% (SML) to 280% (AMR) (table 42), and time for cooking ranges from 60 min (JOR) to 72 min (SML) (table 44).

Lower values of 200% of water uptake for split lentils have been reported by MEINERS et al. (1976). The differences obtained by the latter and those in the present study, may partly be due to differences in the starting material, i.e. either split or whole

Table 42: Increase in water uptake (g/100g) after cooking time (min.)

Variety	20 min	40 min	60 min
AMR	102	265	281
LAG	105	259	275
JOR	116	252	273
SML	90	247	260

lentil seeds, and in cooking times, since their cooking time was for 30 min. It is also well known that time to cook may vary within a single variety coming from different location (SHINDE and SHIRALKAR, 1980).

Table 43 shows that soaking lentil seeds in the six chemical solutions of p.191 have increased the water uptake of all four varieties tested.

It was observed that during soaking the seeds in ammonium carbonate and in trisodium phosphate, changes in the colour of the seeds from olive to dark brown took place, and when the soaked seeds were washed with fresh water, the colour improved. It can be seen (table 44) that the effects of soaking in the various chemicals on the cookability of lentils vary from one chemical to another. Ammonium carbonate is the most effective, followed by trisodium phosphate and sodium bicarbonate, while HCl is the least effective. Both HCl and glutamic acid show no significant effect when compared to water controls generally, except for a slight significant decrease in cooking time after the glutamic acid treatment of var. LAG.

On the whole, results show a good correlation with the water uptake experiments (table 44) : the higher the water uptake, the lower the cooking time.

In cell walls of legume seeds, there are varying amounts of calcium in the form of calcium pectate. The latter is difficult to break down and therefore a long soaking and/or prolonged cooking time are required.

Two suggestions have been put forward to account for the reduction in cooking times of legumes soaked in chemicals :

1. that the cookability of legumes is influenced by some factors

Table 43: Effect of soaking lentils for 2 hours in various chemicals on water uptake

Soaking Solution	AMR	LAG	JOR	SML
	Increase in% water uptake over control	Increase in% water uptake over control	Increase in% water uptake over control	Increase in% water uptake over control
Na_3PO_4	29	18	15	27
$(NH_4)_2CO_3$	40	35	24	31
$NaHCO_3$	25	19	18	23
Glutamic acid	21	13	11	20
Arginine	18	10	5	8
HCl acid	10	6	4	5

Table 44: Effect of chemicals on cookability of lentils

Cooking time in min ± SE (Nearest 0.5 min)

Soaking solution	AMR		LAG		JOR		SML	
	% water uptake at 20 min g/100g	Cooking time min	% water uptake at 20 min g/100g	Cooking time min	% water uptake at 20 min g/100g	Cooking time min	% water uptake at 20 min g/100g	Cooking time min
Water (Control)	97.0	69(4.0)	98.0	65(3.0)	109	60(5.0)	85.0	72(3.6)
Na_3PO_4	129.0	53(2.0) (23%)	132.0	45(4.0) (30%)	133.0	45(2.5) (25%)	119.0	55(3.0) (23%)
$(NH_4)_2CO_3$	141.0	48(3.5) (30%)	150.0	40(3.5) (38%)	145.0	40(3.0) (33%)	126.0	45(4.5) (37%)
$NaHCO_3$	122.0	55(2.5) (20%)	134.0	45(5.0) (30%)	129.0	45(3.5) (25%)	106.0	60(2.5) (17%)
Glutamic acid	114.0	62(3.0) (10%)	120.0	55(3.0) (15%)	119.0	55(2.5) (8%)	97.0	65(3.5) (9%)
Arginine	109.0	65(2.5) (6%)	113.0	60(4.0) (8%)	117.0	55(3.0) (8%)	92.0	65(4.0) (10%)
HCl acid	102.0	65(2.0) (5%)	106.0	60(3.5) (8%)	115.0	55(3.5) (8%)	94.0	68(3.5) (6%)

( ) = SE

such as pH, and electrolytes. HOLDSWORTH (1979) has reported that ions and pH affect the release of pectic substances during the cooking of potatoes, and so presumably also in legumes. MULLER (1967) has reported that the Na^+/ K^+ salts react with the insoluble Ca^{2+}/ Mg^{2+} pectate present in the cell wall (especially those in the seed coats), which converts the Ca^{2+}/ Mg^{2+} pectate to the soluble Na^+ / K^+ pectate.

2. that certain compounds act as chelating agents and that softening of the tissue is due in part to extraction of calcium and/or magnesium and dissociation of the complexes during cooking.

In the present context, the three compounds that give the best results are the three alkalis, ammonium carbonate, trisodium phosphate, and sodium carbonate, and therefore all three may solubilise the protein components. Only one is a chelating agent (trisodium phosphate). So, it seems that those chemicals which are effective in reducing cooking times are alkalis.

The conclusion from this study is therefore that alkalis and solubilisers of protein are important in reducing the cooking time of lentils.

The overall results indicate that soaking lentils in certain chemicals helps in reducing the cooking time. This is especially important in food deficient areas which also suffer from lack of fuel. Consequently this treatment not only offers a means of saving cooking time but also of saving fuels which would otherwise be wasted.

CHAPTER V11

EFFECT OF CANNING AND STORAGE ON NUTRIENTS OF LENTILS

INTRODUCTION

1.Development of canning

The Greek Civilization had a wide range of foods inherited from the East. Different factors influenced food production and processing by their demands for certain kind of foods or their prohibition of others. STEWART and AMERINE (1973) have reported that Napoleonic wars stimulated research for new methods of processing food in a stable and nutritious condition and stated that Appert successfully preserved variou food in sealed jars heated in boiling water as early as 1795. The commercial development of canning processing was one of the most important scientific developments of the food industry. To-day, the latest development in Science and Engineering finds its application in food processing.

2. Reasons, functions, and significance of canning

Canning is carried out for a variety of purposes including preservation, improvement of palability and texture of food. There is a great deal of effort from International Organisations to increase food production by decreasing post-harvest losses and also improving storage conditions. Processing, therefore, is essential to ensure that surplus crops will be sufficient and fit for human consumption. DOBIE (1982) has reported that legume seeds, during storage may be attacked by a number of agents including (1) micro-organisms, (2) insects, and (3) rodents, which result in loss in the quality and in the quantity of stored seeds. In tropical and subtropical countries, insects account for the greatest weight loss of legumes after harvest.

The main functions of food processing as summarised by STEWART and AMERINE (1973) are :

1) prevention of undesirable changes in food which are caused by the invasion and growth of micro-organisms, or by chemical, physical, and biochemical reactions of the compounds present in the food itself,

2) conversion of raw materials into consumer items, and

3) the production of an efficient handling unit.

Canning forms the major method of preserving foods, and is founded on the basic premise of destruction of micro-organisms by heat, and the prevention of recontamination (LUH and WOODROOF, 1975). In modern legume technology, other forms of processing, mainly precooked dehydrated or precooked frozen legume seeds are also used (ROCKLAND and METZLER, 1967). ELIAS et al. (1973) have pointed out that in selecting the type of processing for legumes the following aspects should be considered :

1) they should have the advantages of being constantly available throughout the year,

2) from an industrial point of view, they must be economical since processing involves an increase in the final cost of the product, and

3) processing must be developed to the dietary habit of the people.

Canning was selected in the present study, as it is one of the forms that can fulfill the above considerations. To-day, a larger proportion of vegetables is being processed commercially than ever before, and food combinations in preserved forms that retain their nutritive value, have improved man's health, added variety

to his diet and reduced the time for food preparation.

3. Principles of canning

Canning is defined as a process for preserving food which is achieved by the application of a thermal sterilisation procedure to products packed in hermetically sealed containers. This includes the following steps (HERSOM and HULLAND, 1980; LUH and WOODROOF, 1975) :

i.Preparation of the food. In the case of vegetables, they are blanched. The aim of blanching is to expel the air from the product so as to reduce internal can pressure during heat sterilisation, to inactivate enzymes, and thus to arrest changes in flavour, and to soften the product so that more may be filled into the can (LUH and WOODROOF, 1975).

ii. Filling of the container with food material. The amount of food to be added depends upon the size of the can, and this is also governed by the Code of Practice for each country. Brine or syrup is added. Apart from the economic aspect, the filling of the correct weight of material has an important bearing on other operations in the canning process.

iii. Exhausting which is essential for the removal of oxygen which accelerates the internal corrosion of the can. Heat or mechanical exhaust is used to release air or gas trapped in the product and to displace air in the headspace by water vapour.

iv. Sealing of the container. This is essential since the maintenance of sterility is dependent on the efficiency of operations such as seaming and closing of cans. The cans are hermetically sealed.

v. Processing/Sterilisation. This is the heart of the canning factory (MOSS, 1979). The containers must be heated for an accurately predetermined time and temperature in an atmosphere of saturated steam or in heated water. Micro-organisms which cause spoilage are destroyed by heat. The temperature and time of processing vary with the nature of the product and size of the can, e.g. a No. 303 can of peas sealed at 140 F may be processed in 35 min at 240 F.

vi. Cooling. The cans are cooled as rapidly as possible. Pressure cooling may also be carried out by maintaining the retort pressure during the cooling period by replacing steam with compressed air; the air pressure being maintained while the retort is flooded with cold water. Cans consist primarily of tin-coated steel.

4. Quality of canned vegetables

LUH and WOODROOF (1975) have reported that the quality of canned vegetables is affected not only by the heat process, but also by the method of preparation. All vegetable products are unstable to some extent, regardless of whether they are dried, frozen, or canned, since changes may occur in flavour, colour, texture, vitamin content and other nutrients. Specific changes in a particular food depend upon time and temperature of storage. Ascorbic acid has become a criterion of quality in many canned products. The quality changes may include among others, the sensory quality, the corrosion of cans, and the loss of nutrients.

5. Nutrition evaluation of canned lentils

LUND (1975) has reported a commom misconception among consumers that commercially sterilised products remain unchanged during storage, but this is not usually the case. To evaluate any process on nutrients, the determination of nutrients before and

after processing must be carried out, and the results expressed as a percentage change (LUND, 1975; MAURON, 1980; THOMPSON, 1982). The amino acid composition of food protein is a fundamental quality (FORD, 1973). Ion-exchange chromatography of an acid hydrolysis of processed protein remains the standard method to detect processing damage to proteins, since it measures amino acid losses (MAURON, 1980). THOMPSON (1982) has reported that processing can have indirect beneficial effects on the nutritional quality of food, e.g. by the destruction of certain enzymes.

Aims of this part of the study

GUERRANT et al. (1945) have reported many conditions which may affect the chemical composition of canned foods. These include the food variety and different methods of blanching and processing. Although some workers have reported the vitamin content of canned legumes, yet they have not always reported the original content of vitamins (HALABY et al., 1981). SALUNKHE (1974) has reported that the influence of storage of canned foods on the amount and quality of protein in terms of essential amino acids has received little attention. In tropical countries, canned foods are usually stored at high temperatures.

In the present study, investigations into the effects of canning and storage conditions, i.e. temperature and time, on the chemical composition (carbohydrates, proteins, protein amino acids, and vitamin C, thiamine, and riboflavin) of four lentil varieties will be carried out. So the theme for this part of the present study, is to investigate the effect of canning, and of storage of canned lentils at 25 C and at 35 C for a period of six months and of one year on the various above-mentioned nutrients. Generally speaking, although the results after six-month storage are given in the tables, only those of one-year storage will be

discussed thoroughly.

Canning of lentil seeds

Canning of lentil seeds was carried out according to LUH and KEAN's (1975) method, at the Food Processing Unit of the Department of Food Technology at Reading University.

Dry seeds were soaked from between 4-6 h, and the water was then drained. Seeds were blanched for 5 min in boiling water, drained and cooled with a spray of water. A 4oz (dimension 200 x 211), tin-plate, lacquered can was filled with 75 g of seeds, 40 ml of brine (NaCl 1.5% and citric acid 0.05%) was added. The cans were exhausted and then sealed. The sealed cans were heat-processed in a still retort at 118.3 C (245 F) for 30 min. The cans were cooled in the retort with water to 50 C, then dried, labelled, and stored. The pH of the final product was 4.5.

Processing parameters and quality attributes

Processing parameters of canning include blanching time, processing time and temperature. Quality attributes most affected by canning are texture and colour. Texture may be defined as firmness or softness of tissues. Processing tends to damage cells. The nature of the cell wall of processed vegetables also affects the texture. The cell wall, its structure and composition appears to be responsible for many different textural qualities of vegetables (HALL, 1981).

Canning and cooking cause breakdown of the pectic substances of the middle lamella that leads to the weakening of the intercellular cement, resulting in the softening of the tissues which occurs at both low and high pHs. The processing time and temperature for other legumes are well established (LUH and WOODROOF, 1975), but this is not the case with lentils. As

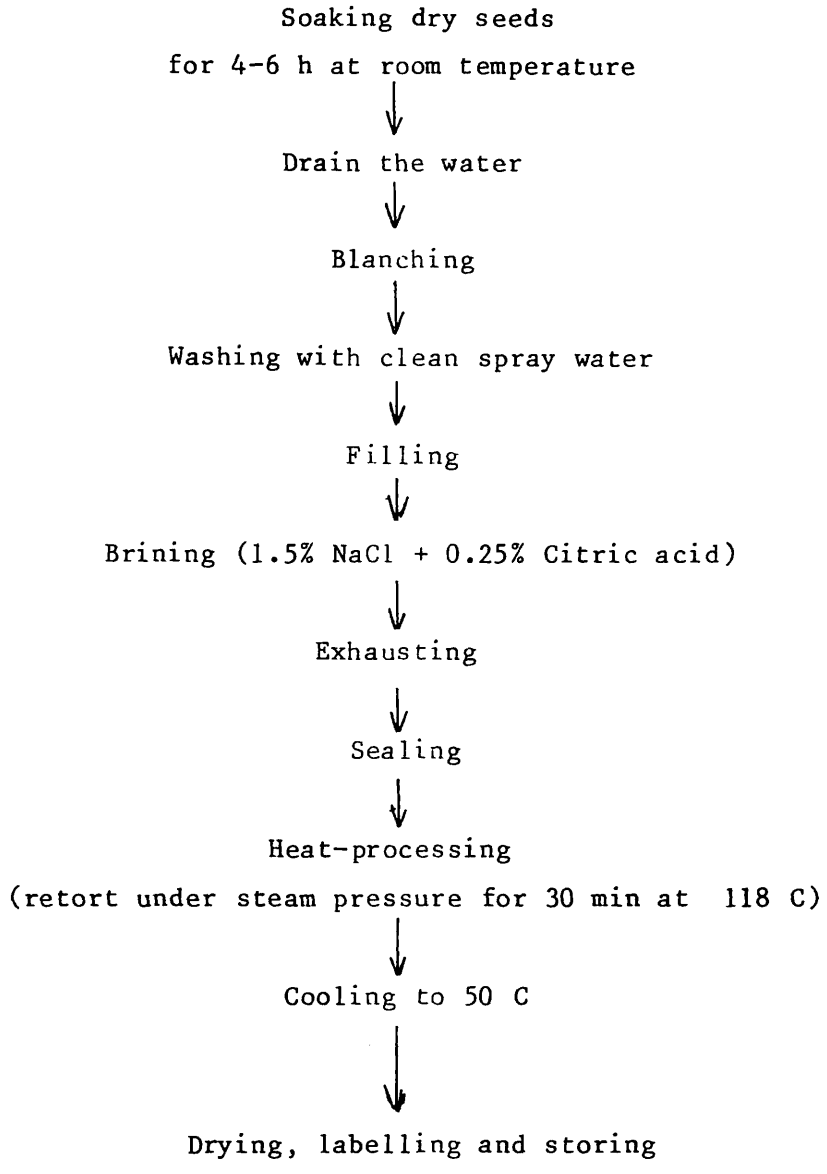


Fig. 20. Flow diagram for canned lentils

lentils are softer than other legumes, so it is important to use the most suitable processing parameters. The factor responsible for damage in foods with respect to texture during the sterilization step is length of time rather than temperature in low acid foods. The most heat resistant pathogen in foods is Clostridium botulinum. Foods e.g. legumes and lentils having a pH of above 4.5 require thermal processing to destroy these organisms.

The following time-temperature combinations were found equally effective in the destruction of C. botulinum in low acid media (POTTER, 1978):

(a) 330 min at 100 C (212 F)
(b) 10 min at 116 C (240.8 F)
(C) 5.3 min at 118 C (245 F)
(d) 2.78 min at 121 C (250 F)

In the case of lentils in order to produce seeds that are soft and ready to eat, the sealed cans had to be heat-processed at 245 F for 30 min.

To minimize the effect of canning on texture, other processing parameters may be suggested. Soaking time may be reduced from 4-6 h to 1-2 h, and the normal blanching time in the range 1 to 3 instead of 5 min.

The processing parameters used in the present study were those of LUH and KEAN (1975) who designed them for beans.

In the present study, the effect of blanching on the various nutrients was not studied separately as it was considered part of the canning process. For the purpose of this work, blanching followed by canning will be referred to simply as canning.

RESULTS AND DISCUSSION

Effect of canning, and storage on total available carbohydrates, starches and dextrins, and ethanol-soluble sugars

1. Effect of canning

Table 45 shows that canning has decreased the total available carbohydrates, starches and dextrins, and ethanol-soluble sugars of all four varieties tested.

In AMR, as a result of canning, total available carbohydrates, starches, and soluble sugars decreased from 51.11 to 45.92 (Retn 89%), from 48.62 to 43.89 (Retn 90%), and from 2.49 to 2.03g/100g (82%) respectively. In LAG, these compounds decreased from 49.23 to 46.48 (Retn 94%), from 46.26 to 44.23 (Retn 95%), and from 2.97 to 2.25g/100g (Retn 76%) respectively. In JOR, they also decreased from 47.14 to 43.50 (Retn 92%), from 43.83 to 40.95 (Retn 93%), and from 3.26 to 2.55g/100g (Retn 78%) respectively, while in SML, they decreased from 46.81 to 41.82 (Retn 89%), from 44.25 to 39.71 (Retn 89%), and from 2.56 to 2.15g/100g (Retn 84%).

2. Effect of storage

In the present study, the effect of one year storage for 12 months at 25 C and at 35 C on total available carbohydrates was so similar that only the results of storage at 25 C will be discussed.

In AMR after storage for 12 months at 25 C, total available carbohydrates, starches, and soluble sugars decreased from 51.11 to 40.54 (Retn 79%), from 48.62 to 39.03 (Retn 82%), and from 2.49 to 1.51g/100g (Retn 61%) respectively. In LAG, they also decreased from 49.23 to 41.08 (Retn 83%), from 46.26 to 39.41 (Retn 85%), and from 2.97 to 1.67g/100g (Retn 56%) respectively. In JOR, they decreased from 47.14 to 39.70 (Retn 84%), from 43.88

Table 45: Effect of canning and storage at 25°C for twelve months on total available carbohydrates, starches and soluble sugars of lentils.
(Results are expressed as g/100g)

Variety	AMR			LAG			JOR			SML		
	Soluble sugars	Starch +dextrin	Total avail. carbo.	Soluble sugars	Starch +dextrin	Total avail. carbo.	Soluble sugars	Starch +dextrin	Total avail. carbo.	Soluble sugars	Starch +dextrin	Total avail. carbo.
Control	2.49 (0.15)	48.62 (1.25)	51.11 (1.15)	2.97 (0.12)	46.26 (1.18)	49.23 (1.30)	3.26 (0.18)	43.88 (1.50)	47.14 (1.90)	2.56 (0.13)	44.25 (0.88)	46.81 (1.47)
Canned	2.03 (0.10)	43.89 (1.32)	45.92 (0.70)	2.25 (0.23)	44.23 (1.45)	46.48 (1.10)	2.55 (0.16)	40.95 (1.26)	43.50 (1.85)	2.15 (0.19)	39.71 (1.09)	41.82 (0.76)
Retn. %	81.7	90	89	75.8	95.4	94.3	78.2	93.0	92.2	84	89.4	89
Six months	1.73 (0.18)	41.45 (1.08)	43.18 (1.20)	1.88 (0.09)	41.87 (0.80)	43.75 (1.39)	2.21 (0.20)	39.45 (0.86)	41.66 (1.10)	1.69 (0.12)	38.56 (1.30)	40.25 (1.60)
Retn. %	69.5	85.40	84.5	63.4	90.3	88.7	67.8	89.7	88.3	66	86.8	85.9
Twelve months	1.51 (0.13)	39.03 (1.56)	40.54 (0.95)	1.67 (0.07)	39.41 (1.29)	41.08 (1.70)	1.80 (0.11)	37.91 (1.40)	39.70 (1.12)	1.39 (0.16)	36.69 (1.55)	38.08 (1.15)
Retn. %	60.6	81.7	79.3	56.2	85.0	83.5	55.2	86.2	84.3	54.3	82.6	81.4

( ) = SE

to 37.91 (Retn 86%), and from 3.26 to 1.80g/100g (Retn 55%) respectively. In SML, they decreased to 38.08, 36.69, and 1.39g/100g respectively, which corresponds to retention values of 81%, 83%, and 54% respectively.

MEYER (1978) has reported that hydrolysis of starches occurs during processing, and this is also what has obviously taken place in the present study.

Effect of canning, and storage on mono-, di-, and oligo-saccharides of lentils

INTRODUCTION

When foods are heated or processed, several reactions take place. The most common reaction in food processing is the Maillard reaction or non-enzymic browning which occurs between proteins, amino acids, and amines with carbonyl compounds, particularly reducing sugars (Fig. 24). The non-reducing disaccharide sucrose will only induce browning after it has been inverted to glucose and fructose (ADRIAN, 1982).

RESULTS AND DISCUSSION

1. Effect of canning on individual sugars

The sugars present in canned lentils were sucrose, raffinose, and stachyose which all decreased immediately after canning (table 46, fig. 21). As a result of canning, sucrose decreased from 0.63 to 0.54g/100g (Retn 85%) in AMR, from 1.29 to 1.00 (Retn 79%) in LAG, from 1.18 to 0.94g/100g (Retn 80%), and from 0.97 to 0.88g/100g (Retn 90%) in SML.

Also as a result of canning, raffinose decreased from 0.26 to 0.19 (Retn 69%) in AMR, from 0.19 to 0.15 (Retn 78%) in LAG, from

0.15 to 0.10 (Retn 61%) in JOR, and from 0.28 to 0.21g/100g (Retn 75%) in SML.

Stachyose decreased from 1.45 to 1.30 (Retn 90%) in AMR, from 1.38 to 1.10g/100g (Retn 80%) in LAG, from 1.83 to 1.61 (Retn 88%) in JOR, and from 1.14 to 1.06g/100g (Retn 93%) in SML.

2. Effect of storage on individual sugars

Tables 46 and 47, and fig. 21 show that there was an increase in the monosaccharides, fructose and glucose, while there was a decrease in sucrose, in raffinose and stachyose during storage for 12 months both at 25 and at 35 C. The retention of fructose and of glucose was calculated compared to their first appearance after 6 months storage. As a result of storage for 12 months at 25 C, fructose increased from 0.11 to 0.17 (Retn 154%), glucose increased from 0.09 to 0.13 (Retn 140%), while sucrose, raffinose, and stachyose decreased from 0.63 to 0.25 (Retn 40%), from 0.26 to 0.10 (Retn 41%), and from 1.45 to 0.86g/100g respectively in AMR. In LAG, fructose and glucose increased from 0.13 to 0.21 (Retn 160%), and from 0.10 to 0.15 (Retn 135%) respectively, while sucrose, raffinose, and stachyose decreased from 1.29 to 0.50 (Retn 39%), from 0.19 to 0.06 (Retn 31%), and from 1.38 to 0.75g/100g (Retn 55%) respectively. In JOR, fructose and glucose increased from 0.12 to 0.20 (Retn 165%), and from 0.07 to 0.11g/100g (Retn 150%) respectively, while sucrose, raffinose, and stachyose decreased from 1.18 to 0.40 (Retn 33%), from 0.18 to trace amounts, and from 1.83 to 1.09g/100g (Retn 60%) respectively. In SML, fructose, and glucose increased from 0.08 to 0.14 (Retn 175%), and from 0.06 to 0.10g/100g (Retn 158%) respectively, while sucrose, raffinose, and stachyose decreased from 0.97 to 0.36 (Retn 37%), from 0.28 to 0.12 (Retn 42%) and from 1.14 to 0.67g/100g (Retn 59%) respectively.

At 35 C, the contents of fructose, glucose, sucrose, raffinose, and stachyose of AMR were found to be 0.24, 0.18, 0.19, 0.07, and

Table 46: Effect of canning and storage at 25°C for twelve months on mono-, di- and oligo-saccharides of lentils (Results are expressed as g/100g)

Variety	FRU	GLU	SUC	RAF	STA	Total	Variety	FRU	GLU	SUC	RAF	STA	Total
AMR							JOR						
Control	0.00	0.00	0.63	0.26	1.45	2.34(0.15)	Control	0.00	0.00	1.18	0.15	1.83	3.16(0.18)
Canned	T	T	0.54	0.19	1.30	2.03(0.13)	Canned	T	T	0.94	0.10	1.61	2.65(0.16)
Retention(%)			85	69	90	86.7	Retention(%)			80	61	88	79
6 months	0.11	0.09	0.39	0.14	1.00	1.73(0.22)	6 months	0.12	0.07	0.69	0.05	1.28	2.21(0.20)
Retention(%)			62	57	69	74.0	Retention(%)			59	33	70	69
12 months	0.17	0.13	0.25	0.10	0.86	1.51(0.17)	12 months	0.20	0.11	0.40	T	1.09	1.80(0.11)
Retention(%)	154	140	40	41	60	64.5	Retention(%)	165	150	33	-	60	57
LAG							SML						
Control	0.00	0.00	1.29	0.19	1.38	2.86(0.12)	Control	0.00	0.00	0.97	0.28	1.14	2.39(0.13)
Canned	T	T	1.00	0.15	1.10	2.25(0.12)	Canned	T	T	0.88	0.21	1.06	2.15(0.19)
Retention(%)			79	78	80	78.7	Retention(%)			90	75	93	89.9
6 months	0.13	0.10	0.70	0.10	0.85	1.88(0.20)	6 months	0.08	0.06	0.57	0.18	0.80	1.69(0.12)
Retention(%)			54	52	62	65.7	Retention(%)			60	64	71	71.7
12 months	0.21	0.15	0.50	0.06	0.75	1.67(0.15)	12 months	0.14	0.10	0.36	0.12	0.67	1.39(0.16)
Retention(%)	160	135	39	31	55	58.4	Retention(%)	175	158	37	42	59	58.2

( ) = SE, T = Traces

Table 47: Effect of canning and storage at 35°C for 12 months on mono-, di- and oligo-saccharides of lentils.
(Results are expressed as g/100g)

Variety	FRU	GLU	SUC	RAF	STA	Total	Variety	FRU	GLU	SUC	RAF	STA	Total
AMR							JOR						
Control	0.00	0.00	0.63	0.26	1.45	2.34(0.15)	Control	0.00	0.00	1.18	0.15	1.83	3.16 (0.18)
Canned	T	T	0.54	0.19	1.30	2.03(0.13)	Canned	T	T	0.94	0.10	1.61	2.65 (0.15)
Retention(%)	-	-	85	69	90	86.7	Retention(%)	-	-	80	61	88	79.2
6 months	0.15	0.12	0.35	0.15	0.85	1.62(0.15)	6 months	0.17	0.10	0.60	0.05	1.15	2.05 (0.17)
Retention(%)	-	-	55	58	59	67.5	Retention(%)	-	-	51	33	63	62.7
12 months	0.24	0.18	0.19	0.07	0.65	1.33(0.13)	12 months	0.28	0.16	0.30	T	0.88	1.62 (0.12)
Retention(%)	166	152	30	27	45	54.3	Retention(%)	170	158	27	-	48	50.3
LAG							SML						
Control	0.00	0.00	1.29	0.19	1.38	2.86(0.12)	Control	0.00	0.00	0.97	0.28	1.14	2.39(0.13)
Canned	T	T	1.00	0.15	1.10	2.25(0.12)	Canned	T	T	0.88	0.21	1.06	2.15(0.19)
Retention(%)	-	-	79	78	80	**78.7**	Retention(%)	-	-	90	75	93	89.9
6 months	0.14	0.13	0.60	0.06	0.71	1.64(0.14)	6 months	0.10	0.08	0.40	0.14	0.79	1.51(0.15)
Retention(%)	-	-	47	35	63	64.3	Retention(%)	-	-	41	50	70	63
12 months	0.25	0.19	0.27	T	0.57	1.28(0.11)	12 months	0.18	0.14	0.24	0.06	0.52	1.14(0.08)
Retention(%)	175	147	25	-	42	44.7	Retention(%)	186	170	26	21	46	48

( ) = SE, T = Traces

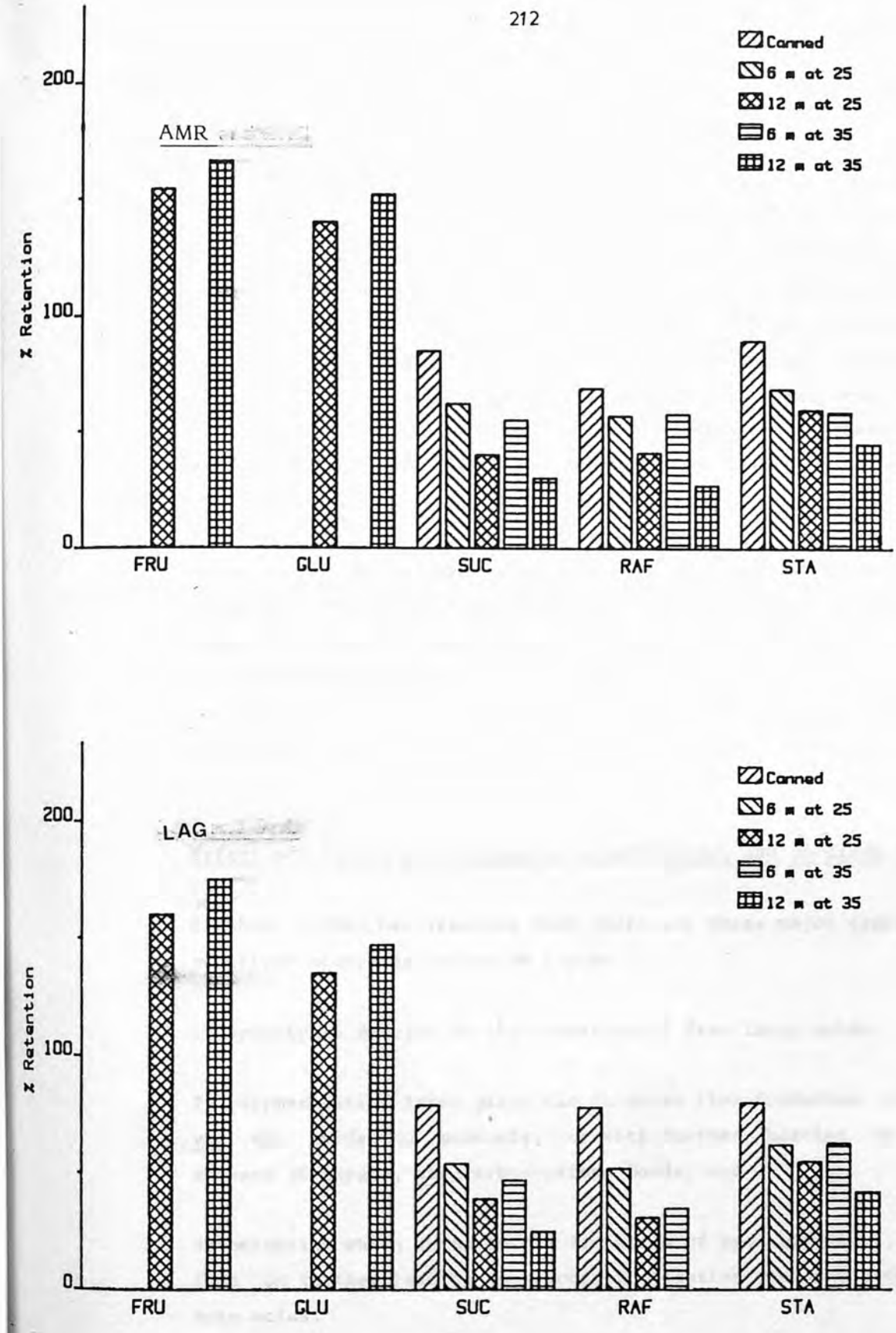


Fig. 21: Effect of canning and storage for one year at 25°C and 35°C on mono-, di-,and oligo-saccharides of four lentil varieties.

0.65g/100g respectively. These results correspond to retention values of 166%, 152%, 30%, 27%, and 45% respectively. In LAG, they were found to be 0.25, 0.19, 0.27, traces, and 0.57g/100g, corresponding to retention values of 175%, 147%, 25%, nil, and 42% respectively. In JOR, they were found to be 0.28, 0.16, 0.30, traces, and 0.88g/100g, corresponding to retention values of 170%, 158%, 27, nil, and 48% respectively. In SML, they were 0.18, 0.14, 0.24,0.06, and 0.52g/100g, corresponding to retention values of 186%, 170%, 26%, 21%, and 46% respectively.

The increase in glucose, and in fructose due to time and temperature of storage agrees with the results of DALAL and SALUNKHE (1968) who have shown an increase of these sugars in canned apricots, in canned cherries, and also as did MANII (1984) in canned orange juice. These results may be explained in that sucrose is hydrolysed into its monomers under these storage conditions, hence an increase in glucose and in fructose.

Effect of canning and storage on total lipids, and on crude fibre

HURRELL (1980) has reported that there are three major types of reactions occurring in heated lipids :

1) hydrolysis results in the formation of free fatty acids,

2) polymerisation takes place due to cross link formation either via the oxidation products, or with further heating in the absence of oxygen, via carbon-carbon bonds, and

3) oxidation which leads to the formation of hydroperoxides, and then on further heating to secondary oxidation products such as keto acids.

Heating may cause cross-linking to form new carbon-carbon bonds between two glyceride molecules, particularly in the absence of

oxygen. New bonds between two different fatty acids lead to the formation of dimeric acids either within one triglyceride molecule or between two molecules, and this reaction is the first step in the polymerization of lipids (MORTON, 1977).

The mixture of reaction products obtained during anaerobic heat treatment is less complex than that obtained under aerobic conditions; the products formed will vary with the degree of unsaturation of the fatty acids. Polymerization by carbon-carbon formation has been noted at 100 C or at higher temperatures (WITTING et al., 1979). Oxidative compounds such as peroxides and free radicals may occur as a result of non-enzymic browning (CHEFTEL, 1979).

The free radicals can interact with vitamins, and proteins to give a variety of different products and to inactivate nutrients by means of the oxidation of vitamins and amino acids (MAURON, 1977). Hydroperoxides formed from unsaturated fat may be broken down to acids, alcohols, and ketones which in turn react with various groupings in proteins (BENDER, 1978), thus causing a loss in protein.

RESULTS and DISCUSSIONS

1.Effect on total lipids

In this part of the present study, the effects of one year storage at 25 and 35 C on total lipids and crude fibres were so similar that only the results of storage at 25 C are discussed.

Canning decreases the total lipids of all four varieties tested (table 48, fig. 22).

After canning, total lipids of AMR, LAG, JOR and SML were 0.79, 0.70, 1.05 and 0.90 g/100g, and retentions were 68, 75, 83 and 76% respectively.

Table 48: Effect of canning and canned storage at 25°C on crude fibre and total lipids of four varieties of lentil.

(Results are expressed as g/100g dry weight)

Variety	AMR		LAG		JOR		SML	
	Crude fibre	Total lipids	Crude fibre	Total lipids	Crude fibre	Total lipids	Crude fibre	Total lipids
Control	4.10(0.09)	1.17(0.07)	3.70(0.13)	0.96(0.06)	4.54(0.12)	1.32(0.10)	4.86(0.15)	1.21(0.08)
Canned	4.75(0.14)	0.79(0.06)	4.00(0.12)	0.70(0.08)	5.08(0.18)	1.05(0.11)	5.20(0.22)	0.90(0.06)
Retention %	115	68	109	75	112	83	107	76
6 months	5.16(0.17)	0.70(0.05)	4.25(0.16)	0.58(0.06)	5.44(0.10)	0.90(0.07)	5.40(0.11)	0.80(0.07)
Retention %	126	60	115	60	120	70	112	67
12 months	5.69(0.23)	0.59(0.05)	4.66(0.13)	0.50(0.07)	5.97(0.30)	0.79(0.08)	5.80(0.15)	0.68(0.05)
Retention %	140	52	126	51	132	60	119	57

( ) = SE

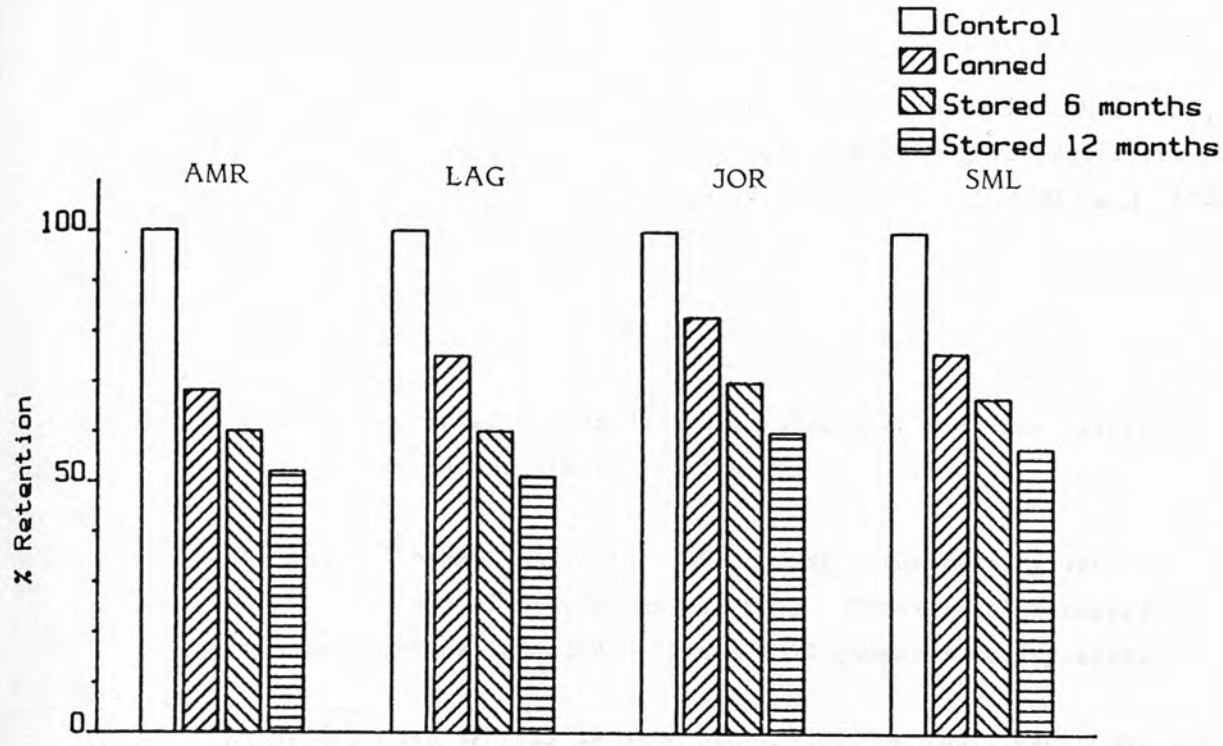


Fig. 22: Effect of canning and one year storage at 25°C on percentage of total lipids of four lentil varieties.

After storage for 12 months at 25 C, total lipids in AMR, LAG, JOR and SML were 0.59, 0.50, 0.79 and 0.68 g/100g respectively. These results correspond to retention of 52, 51, 60 and 57% respectively.

2.Effect on crude fibre

Canning increased the crude fibre content of all four varieties under investigation (table 48, fig. 23).

The crude fibres of AMR, LAG, JOR and SML after canning were 4.75, 4.0, 5.0 and 5.20 g/100g respectively. These results correspond to retentions of 115, 109, 112 and 107% compared to dry seeds.

After one year storage at 25 C crude fibre in AMR, LAG, JOR and SML were found to be 5.69, 4.66, 5.97 and 5.80 g/100g. These results correspond to re tentions of 140, 126, 132 and 119% respectively.

Effect of canning and storage on proteins

The essential building blocks for protein are the amino acids, and all amino acids possess both an amino and carboxylic group. The alpha amino acids differ in the nature of the side-chain attached to a central carbon atom (LEDWARD, 1979). Proteins may exist in a number of forms e.g. primary, secondary and tertiary configurations. Food processing may have an effect on individual amino acid components, which in turn may lead to an alteration in the structure of the protein molecules.

In broad terms, only two kinds of links occur between protein molecules:

1) covalent link, of which the only probable kind is the disulphide, and

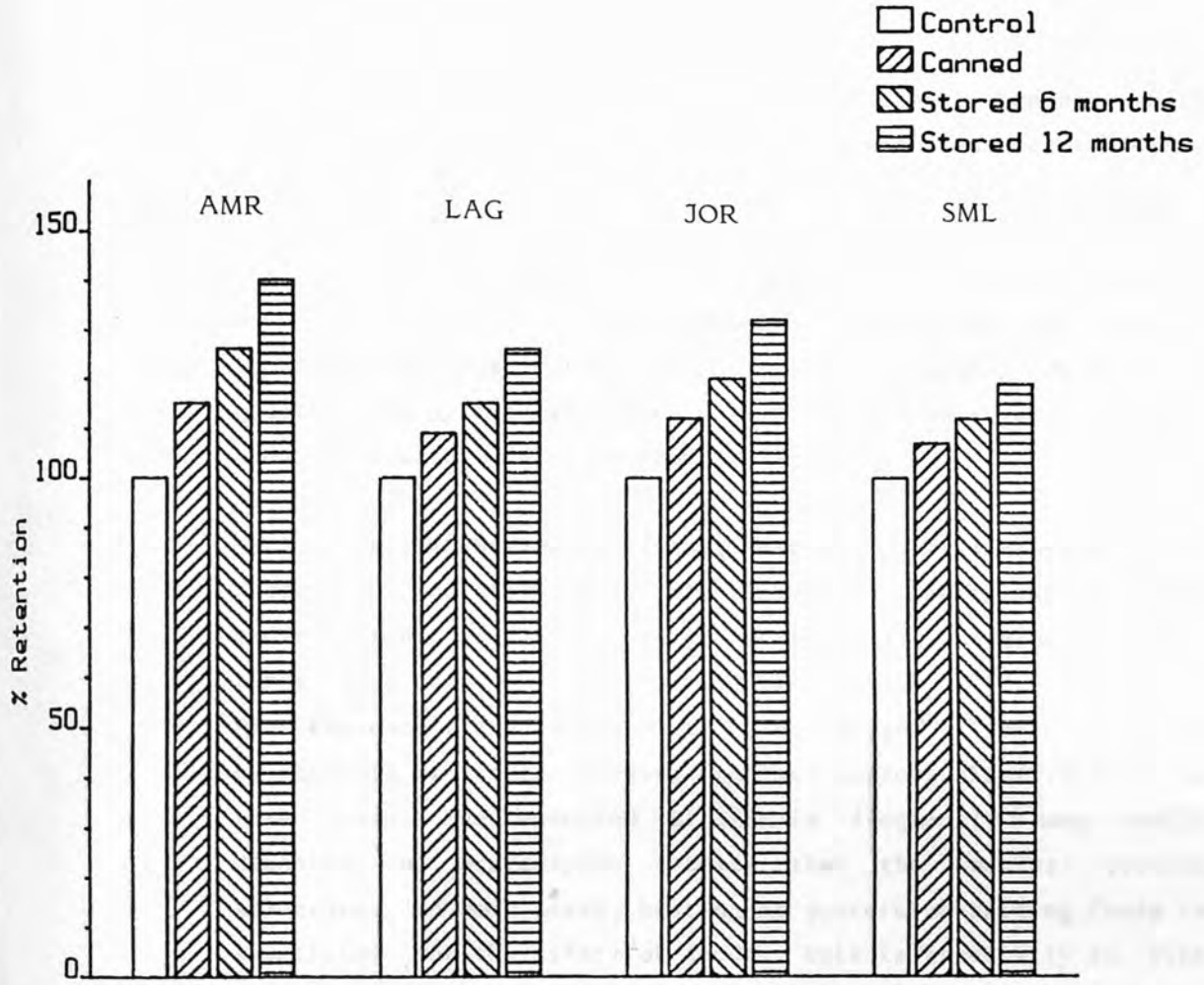


Fig. 23: Effect of canning and one year's storage at 25°C on percentage retention of crude fibre of four lentil varieties.

2) non-covalent link involving electrostatic, hydrogen bond and hydrophobic interactions.

Heating protein foods brings about conformational changes, which alter the way in which protein-protein interactions occur (TOMBS, 1970).

Two apparently contradictory conclusions can be drawn from the evidence in the literature, namely , that except in special circumstances most processes have little effect on protein quality and on the other hand, proteins can be readily damaged even under mild conditions (BENDER, 1978).

CHEFTEL (1979) has reported that heat and other factors of food processing and storage also affect protein configuration. Such modifications of the secondary, tertiary and quaternary structures of proteins may be of great importance.
From the nutritional standpoint "denaturation" of food proteins by heat is of little concern, but can improve digestibility in some cases. The unfolded protein is frequently more easily digested by proteolytic enzymes than the native protein structure. In some cases, heating of protein-containing foods is beneficial for the safety of foods. This is especially so with many vegetables (e.g.pulses and oilseeds) which contain heat-labile factors such as trypsin inhibitors.

From the standpoint of the nutritive value of proteins, only the chemical modifications brought about by processing and storage may have detrimental effects.

In heat treatment, interactions between protein and the products of lipid oxidation take place. The mechanism of interaction involves propagation of the free radical chain to the protein system. Various groups in the protein molecule are capable of converting to free radical by losing one hydrogen atom to a free radical of lipid origin.

Processing, or even lengthy storage, may oxidize various food components, including the amino acid residues of proteins. The detrimental effects of increasing the heat treatment of cotton seed protein meals are due to the presence of some oligosaccharides such as raffinose and the reaction of these compounds with lysine residues (CHEFTEL, 1979). In addition to reducing carbohydrates and carbonyl compounds resulting from Maillard-type reactions or lipid oxidation, a number of aldehydes may find themselves in contact with proteins during processing or storage. Four distinct types of processing damage to proteins (MAURON, 1977) are listed below:

1. Mild reactions; the first type occurs under mild heating conditions with carbonyl groups taking part in early Maillard reactions (fig. 24a).

2. Severe conditions; the second type occurs under more severe heating conditions either in the absence or presence of sugars e.g. reducing or non-reducing (late Maillard reactions) (fig. 24b).

3. Alkali treatment; the third type is prominent when protein is exposed to alkali treatment. Different proteins respond differently to alkali treatment depending on the number and relative position of the various reactive groups within the protein (BENDER, 1978).

4. Oxidation; the fourth type comprises several oxidation reactions e.g. lipid oxidation products like hydroperoxides and free radicals are involved.

The main steps of Maillard as pointed out by CHEFTEL (1979) (fig. 24b) are summarised below:

i) the first step involves the condensation reactions between the

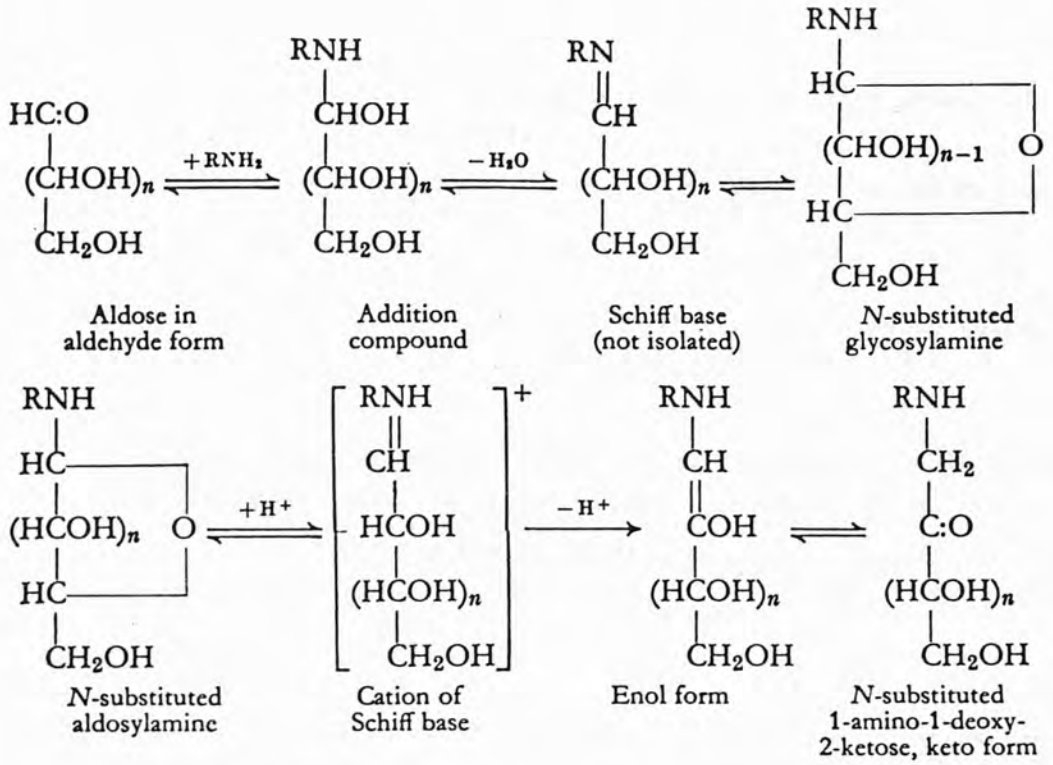


Fig. 24a: The initial stages of the Maillard reaction (FENTON, 1973)

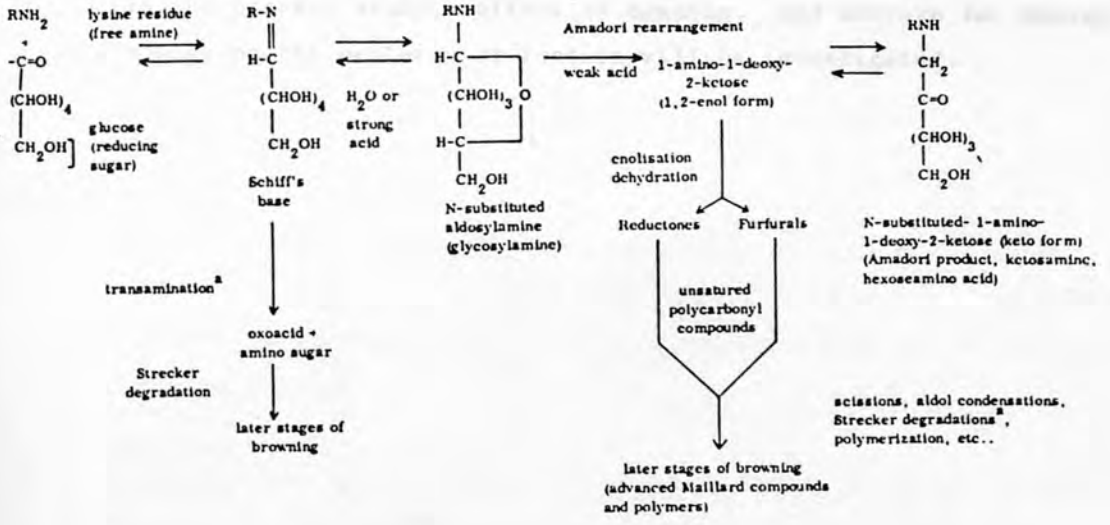


Fig. 24b: Simplified scheme of Maillard reaction (CHEFTEL, 1979)

carbonyl group of the reducing sugar and the amino group of an amino acid to form a Schiffs base,
ii) this is converted into the N-substituted aldosylamine (glycosylamine).

iii) and this is immediately converted to the N substituted 1-amino-1-deoxy-2-ketose (Amadori product).

HURRELL and CARPENTER (1977) have reported that among carbohydrates only reducing sugars can take place in the reactions, and the reactivity of glucose is much higher than that of fructose.

In the later stages of the Maillard reaction, amino acids can be degraded directly by reactions with other carbonyl compounds which are formed during these reactions.

A number of investigators have discussed the effects of processing (heat) on proteins (TOMBS, 1979; HURRELL, 1980).

In the present study, effect of canning, and storage for twelve months on the proteins of lentils will be investigated.

RESULTS AND DISCUSSION

1.Effect of canning

Table 49, fig. 25 shows that canning has decreased slightly the proteins of the four lentil varieties under study.

As a result of canning, proteins in AMR, LAG, JOR and SML decreased from 24.14 to 22.23 g/100g (Retn 92%), from 26.43 to 23.62 (Retn 89%), from 25.35 to 23.07 (Retn 91%) and from 27.57 to 25.21 (Retn 90%) respectively.

2. Effect of storage

Storage of canned lentils resulted in a small change in protein (tables 49 and 50 fig. 25).

After twelve month storage at 25 C, proteins in AMR, LAG, JOR, and SML decreased from 24.14 to 20.0 (Retn 83%), from 26.43 to 21.80 (Retn 82%), from 25.35 to 19.89 (Retn 79%) and from 27.57 to 22.61 g/100g (Retn 82%) respectively (table 49). Also after 12 month storage at 35 C proteins in AMR, LAG, JOR and SML decreased from 24.14 to 18.90 (Retn 78%), 26.43 to 21.10 (Retn 80%), from 25.35 to 18.72 (Retn 74%) and from 27.57 to 21.18 g/100g (Retn 77%) respectively (table 50).

These results are in agreement with those of MEHROTRA (1983) who has reported a decrease in the protein of canned chickpeas and those of GANGAL and MAGAR (1967) who have shown a decrease in canned meat.

Effect of canning and storage on the protein amino acids of lentils

When certain protein foods are heated Maillard reaction occurs, and in this reaction most of the amino acids especially lysine decrease,

Table 49: Effect of canning and storage for twelve months at 25°C on protein content of four varieties of lentil.

(Results are expressed as g/100g)

Variety	AMR	LAG	JOR	SML
Control	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
Canned	22.23(0.55)	23.62(0.63)	23.07(0.39)	25.21(0.59)
Retention %	92.1	89.4	91.3	90.3
6 months	21.24 (0.47)	22.45(0.51)	21.00(0.60)	23.84(0.85)
Retention %	87.8	85.7	82.7	86.7
12 months	20.04(0.68)	21.80(0.35)	19.89(0.45)	22.61(0.42)
Retention %	83.0	82.5	79	82

( ) = SE

Table 50: Effect of canning and storage for twelve months at 35°C on protein content of four varieties of lentil.

(Results are expressed as g/100g)

Variety	AMR	LAG	JOR	SML
Control	24.14(0.45)	26.43(0.39)	25.35(0.53)	27.57(0.77)
Canned	22.23(0.55)	23.62(0.63)	23.07(0.39)	25.21(0.59)
Retention %	92.1	89.4	91.3	90.3
6 months	19.79(0.37)	22.20(0.56)	20.03(0.45)	22.27(0.61)
Retention %	82.4	84.4	79	80.8
12 months	18.90(0.28)	21.10(0.36)	18.72(0.47)	21.18(0.54)
Retention %	78	80.2	74	77.4

( ) = SE

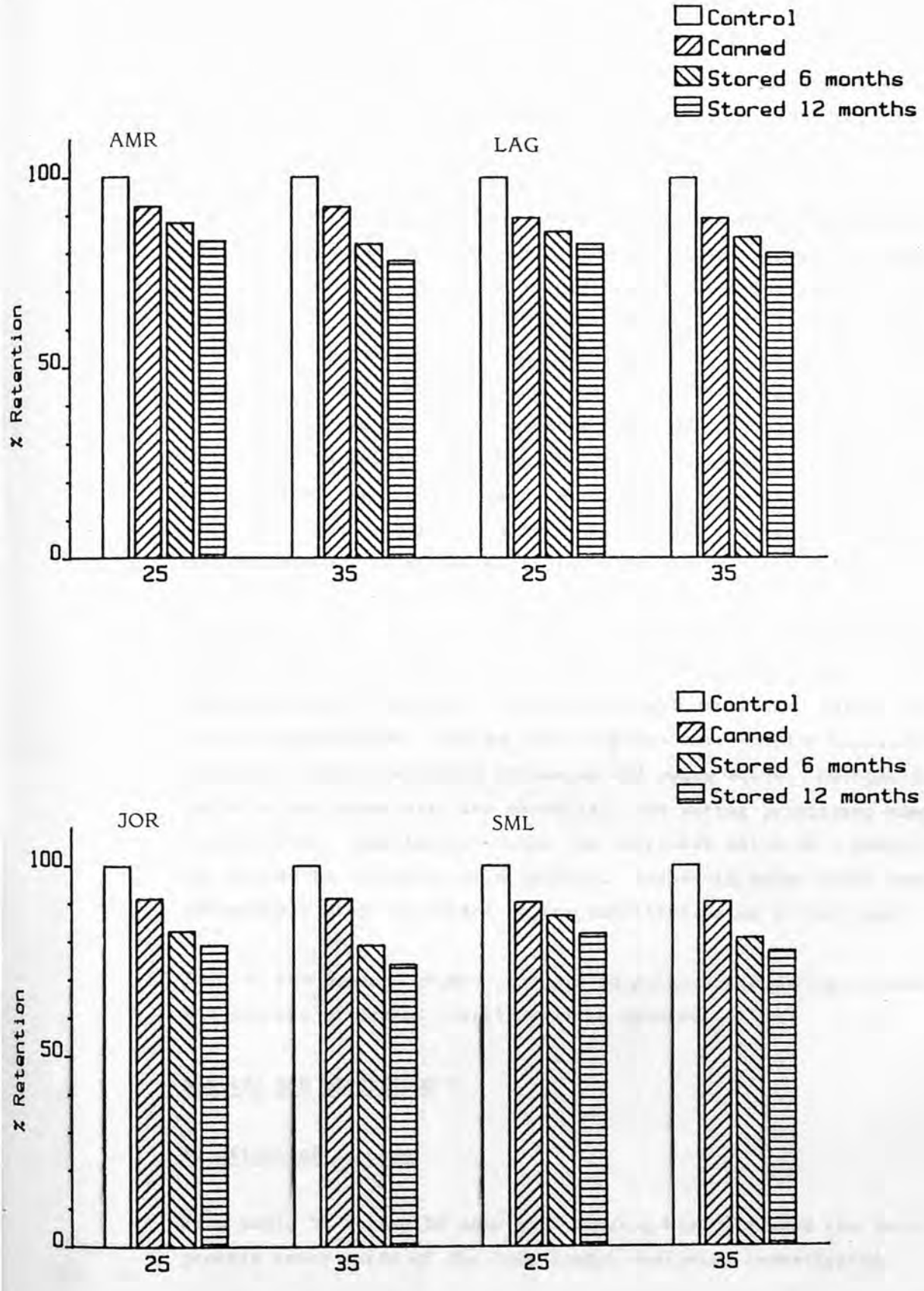


Fig. 25: Effect of canning and storage over twelve months at 25°C and 35°C on percentage retention of proteins of four lentil varieties.

and this reaction may occur during storage at ordinary or ambient temperatures or at prolonged storage time (CHEFTEL, 1979; HURRELL, 1980).

Canning peas causes a small decrease in the amount of methionine while tryptophan and phenylalanine are less damaged (BENDER, 1972). Threonine, lysine, methionine and arginine are the most thermolabile amino acids (YAMAGUCHI and WU, 1975). Autoclaving decreased most amino acids, including the chemically inert leucine, a 50% decrease was noted in both lysine and in tryptophan, and 30% in methionine (CHEFTEL, 1979). Changes in proteins and amino acids occur in stored processed food, i.e. freeze-dried mushroom stored at 30 C for 9 months showed a decrease in aspartic acid, threonine, serine, valine, phenylalanine, lysine and histidine (LUH and WOODROOF, 1975).

LEDWARD (1979) has reported that lysine destruction probably occurs due to Maillard reaction involving the amides of asparagine and glutamine or the carbonyl compounds formed by cystine degradation, and as heat increases more complex reactions occur and there are marked losses in all amino acids. Changes in amino acids, especially the essential ones during processing have a nutritional importance, since the nutritive value of a protein is related to its amino acid content. Losses in amino acids have therefore a profound effect on the nutritive value of the food.

So, in the present study, changes in amino acids during canning and storage of canned lentils will be studied.

RESULTS AND DISCUSSION

1. Effect of canning

From table 51 it can be seen that canning has decreased the total protein amino acids of the four lentil varieties investigated.

Table 51: Effect of canning and storage for one year at 25°C on amino acids of lentil varieties

(Results are expressed as g/100g protein)

	AMR						LAG						JOR						SML					
	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret
Tryptophan	0.78	0.62	80	0.52	0.41	52	0.91	0.70	78	0.60	0.50	56	0.80	0.67	84	0.56	0.45	56	0.85	0.65	77	0.52	0.42	49
Lysine	6.45	4.96	77	4.70	4.45	69	6.38	6.17	81	4.84	4.46	69	6.29	4.97	79	4.65	4.40	70	6.12	5.00	82	4.80	4.50	74
Histidine	1.93	1.56	81	1.46	1.33	68	1.87	1.50	80	1.40	1.28	68	1.68	1.29	77	1.18	1.05	63	2.04	1.72	83	1.58	1.40	71
Ammonia	2.23	2.51	113	2.61	2.72	122	2.44	2.90	119	3.00	3.14	126	2.00	2.32	116	2.44	2.60	130	2.58	3.09	120	3.11	3.30	128
Arginine	6.72	5.78	86	5.64	5.42	81	7.05	6.35	90	6.16	5.95	85	6.86	6.65	97	6.39	6.18	90	6.53	5.80	89	5.55	5.34	82
Aspartic acid	10.19	9.07	89	8.56	8.38	83	10.56	9.39	89	9.00	8.82	84	10.82	9.61	89	9.37	9.07	84	10.46	8.89	85	8.56	8.37	80
Threonine	2.46	2.21	90	2.09	1.90	79	2.64	2.37	90	2.26	2.13	80	2.10	1.68	80	1.57	1.45	69	2.25	1.77	79	1.65	1.52	71
Serine	3.00	2.67	89	2.52	2.41	81	3.29	2.79	85	2.66	2.53	77	3.17	2.56	82	2.47	2.35	74	3.30	2.84	86	2.73	2.60	79
Glutamic acid	11.18	10.40	93	10.11	9.87	89	11.25	10.13	90	9.78	9.56	85	11.60	10.44	90	10.20	9.86	85	11.06	9.95	90	9.62	9.40	85
Proline	4.06	3.69	91	3.52	3.40	85	4.43	3.85	86	3.65	3.49	79	4.26	3.53	83	3.40	3.25	76	4.12	3.54	86	3.43	3.32	80
Glycine	4.50	4.00	89	3.90	3.78	84	4.90	4.36	89	4.24	4.11	84	5.08	4.57	90	4.35	4.20	84	5.20	4.68	90	4.59	4.42	85
Alanine	4.88	4.15	85	4.00	3.80	78	5.07	4.31	85	4.10	3.9	78	5.29	4.55	86	4.39	4.32	79	5.30	4.77	91	4.66	4.50	84
Cysteine	1.06	0.85	76	0.74	0.63	61	0.80	0.64	80	0.53	0.40	50	0.74	0.62	84	0.50	0.39	53	0.90	0.72	80	0.60	0.47	52
Valine	4.10	3.73	91	3.52	3.36	82	4.36	3.75	86	3.61	3.48	80	4.15	3.69	89	3.56	3.40	82	4.45	3.69	83	3.58	3.41	77
Methionine	0.54	0.42	78	0.33	0.24	45	0.60	0.48	81	0.40	0.29	48	0.50	0.41	81	0.31	0.20	40	0.70	0.56	81	0.45	0.31	45
Isoleucine	2.96	2.54	87	2.42	2.30	78	3.10	2.72	88	2.60	2.50	81	3.00	2.55	85	2.42	2.30	77	2.80	2.41	86	2.28	2.18	81
Leucine	5.28	4.59	86	4.43	4.22	80	5.20	4.37	84	4.20	4.10	79	5.42	4.33	80	4.21	4.06	74	4.92	4.17	84	4.05	3.09	80
Tyrosine	4.17	3.79	91	3.66	3.54	86	4.31	3.88	90	3.69	3.56	83	3.87	3.37	87	3.21	3.13	81	4.23	3.60	85	3.46	3.32	79
Phenylalanine	4.48	3.94	88	3.85	3.67	82	4.43	3.76	85	3.60	3.48	78	4.30	3.39	79	3.22	3.09	72	4.80	4.46	88	4.13	3.98	83
TOTAL	80.88	70.84	-	67.74	64.76	-	83.58	73.44	-	68.55	65.74	-	81.91	70.58	-	67.55	64.56	-	82.67	71.22	-	68.14	64.33	-
	(2.80)	(2.10)		(2.54)	(1.98)		(2.15)	(2.92)		(1.83)	(2.17)		(1.90)	(2.25)		(2.31)	(1.95)		(2.46)	(2.10)		(2.86)	(2.24)	

( ) = SE, 6, 12 = Months

As a result of canning, total amino acids in AMR, LAG, JOR and SML decreased from 80.88 to 70.84 g/100g protein (Retn 85%), from 83.58 to 73.44 (Retn 88%), from 81.91 to 70.58 (Retn 86%) and from 82.67 to 71.22 g/100g protein (Retn 86%) respectively. The amino acids most affected were lysine, cysteine, methionine, tryptophan, valine, histidine, leucine and isoleucine. Their retentions ranged from 77 to 86% compared to dry seeds.

2. Effect of storage

On storage, changes in amino acids took place (tables 51 and 52). After one year storage at 25 C, the amino acids which decreased were lysine, methionine, tryptophan, cysteine, histidine and alanine and their retention values are 69, 45, 52, 61, 68, and 78% in AMR stored product.

After one year storage at 35 C the amino acids which decreased appreciably were lysine, methionine, tryptophan, cysteine, histidine and glycine and their retention values are 59, 47, 52, 50, 68 and 74% in AMR compared to dry seeds. The pattern of behaviour of amino acids storage of the other varieties e.g. LAG, JOR, and SML is similar to that of AMR.

Storage temperatures in this study, have little effect on the retention of amino acids.

The losses in amino acids due to canning and storage may be the result of Maillard reaction or non-enzymic browning which has been suggested by some workers to cause losses in most amino acids (LEDWARD, 1979; CHEFTEL, 1979; BENDER, 1978). Losses in some amino acids especially methionine and cysteine may be as a result of the fourth type of damage i.e. oxidation (p.220) as suggested by MAURON (1977).

Cysteine may react with reducing sugars, but to a much less extent than does lysine. Methionine is destroyed, while tryptophan is also used in the Maillard reaction, as are sulphur containing

Table 52: Effect of canning and storage for one year at 35°C on amino acids of lentil varieties

(Results are expressed as g/100g protein)

	AMR						LAG						JOR						SML					
	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret	Con	Can	Ret	6	12	Ret
Tryptophan	0.78	0.62	80	0.52	0.41	52	0.91	0.70	78	0.50	0.40	44	0.80	0.67	84	0.43	0.32	40	0.85	0.65	77	0.45	0.30	35
Lysine	6.45	4.96	77	4.32	3.80	59	6.38	6.17	81	4.33	3.95	62	6.29	4.97	79	4.61	4.00	64	6.12	5.00	82	4.50	4.00	65
Histidine	1.93	1.56	81	1.46	1.33	68	1.87	1.50	80	1.38	1.27	68	1.68	1.29	77	1.10	0.96	45	2.04	1.72	83	1.50	1.30	63
Ammonia	2.23	2.51	113	2.69	2.85	128	2.44	2.90	119	3.15	3.31	135	2.00	2.32	116	2.40	2.80	140	2.58	2.98	116	3.12	3.40	132
Arginine	6.72	5.78	86	5.64	5.42	81	7.05	6.35	90	5.74	5.60	80	6.86	6.65	97	6.24	5.97	87	6.53	5.80	89	5.39	5.20	80
Aspartic acid	10.19	9.07	89	8.56	8.38	83	10.56	9.39	89	8.87	8.55	81	10.82	9.61	89	9.28	8.74	81	10.46	8.89	85	8.47	8.26	79
Threonine	2.46	2.21	90	2.09	1.90	79	2.64	2.37	90	2.21	2.05	77	2.10	1.68	80	1.54	1.36	65	2.25	1.77	79	1.60	1.47	65
Serine	3.00	2.67	89	2.52	2.41	81	3.29	2.79	85	2.49	2.36	72	3.17	2.56	82	2.35	2.23	72	3.30	2.84	86	2.65	2.50	77
Glutamic acid	11.18	10.40	93	10.11	9.87	89	11.25	10.13	90	9.86	9.30	82	11.60	10.44	90	9.97	9.39	81	11.06	9.95	90	9.35	9.13	83
Proline	4.06	3.69	91	3.52	3.40	85	4.43	3.85	86	3.55	3.28	75	4.26	3.53	83	3.27	3.00	72	4.12	3.54	86	3.36	3.25	78
Glycine	4.50	4.00	89	3.60	3.36	74	4.90	4.36	89	4.16	3.90	80	5.08	4.57	90	4.20	3.95	79	5.20	4.68	90	4.47	4.05	78
Alanine	4.88	4.15	85	3.90	3.66	75	5.07	4.31	85	4.00	3.65	73	5.29	4.55	86	4.16	4.00	77	5.30	4.77	91	4.50	4.21	81
Cysteine	1.06	0.85	76	0.63	0.53	50	0.80	0.60	80	0.45	0.30	38	0.74	0.62	84	0.40	0.29	40	0.90	0.72	80	0.48	0.37	42
Valine	4.10	3.73	91	3.30	3.11	76	4.36	3.75	86	3.52	3.27	76	4.15	3.69	89	3.36	3.20	76	4.45	3.69	83	3.50	3.34	75
Methionine	0.54	0.40	78	0.30	0.19	47	0.60	0.48	81	0.30	0.20	33	0.50	0.41	81	0.26	0.15	30	0.70	0.56	81	0.35	0.20	28
Isoleucine	2.96	2.54	86	2.45	2.36	78	3.10	2.72	88	2.60	2.26	74	3.00	2.55	85	2.39	2.20	74	2.90	2.41	86	2.20	2.10	75
Leucine	5.28	4.59	87	4.38	4.22	80	5.20	4.37	84	4.16	3.95	75	5.42	4.35	80	4.50	3.83	71	4.92	4.17	84	3.95	3.82	78
Tyrosine	4.17	3.79	91	3.48	3.30	81	4.31	3.88	90	3.65	3.39	79	3.81	3.37	87	3.10	2.90	75	4.23	3.60	85	3.32	3.21	76
Phenylalanine	4.48	3.94	88	3.76	3.49	78	4.43	3.76	85	3.47	3.25	74	4.30	3.39	79	3.10	3.00	70	4.80	4.46	68	4.00	3.80	80
TOTAL	80.88 (2.80)	70.84 (1.26)	-	66.32 (1.56)	62.79 (2.57)	-	83.58 (2.15)	73.49 (2.92)	-	66.99 (2.31)	62.61 (1.55)	-	81.91 (1.90)	70.58 (2.10)	-	65.30 (2.27)	60.86 (1.50)	-	82.67 (2.46)	71.22 (2.70)	-	65.81 (3.25)	62.16 (2.27)	-

( ) = SE , 6 & 12 = Months

amino acids, tyrosine and other basic amino acids due to the Maillard reaction.

HEIMANN (1980) has reported that among the sulphur containing amino acids, methionine within the natural protein structure is sensitive to heat and this sensitivity is explained by the loss of methionine on heating and during storage of foodstuffs at higher temperatures.

Effect of canning and storage on the chemical score of essential amino acids of lentils

1. Effect of canning

Canning has decreased the chemical score of lentil proteins (table 53).

As a result of canning, chemical scores of AMR, LAG, JOR and SML decreased from 28 to 22, from 25 to 20, from 22 to 18 and from 28 to 22 respectively.

2. Effect of storage

Table 53 shows that after twelve month-storage at 25 C, the chemical scores of AMR, LAG, JOR and SML decreased from 28 to 17, from 25 to 12, from 22 to 11 and from 28 to 15 respectively. There is a negligible difference in the chemical score between those stored at 25 and 35 C. The most limiting amino acids in one year stored canned lentils were found to be methionine, cysteine and tryptophan in all the four varieties investigated.

Due to the importance of canned foods in man's life, the effect of canning on protein quality has been investigated by some workers. POWRIE and LAMBERTS (1964)have reported that canning of navy beans decreased digestibility of protein, protein efficiency ratio (PER), and biological value was also reduced. BENDER (1978)

Table 53: Effect of canning and one year storage at 25°C on the chemical score of essential amino acids of four varieties of lentil.
(Results are expressed as % of the same EAA in reference protein (whole egg)

Essential Amino acids	AMR				LAG				JOR				SML			
(EAA)	CON	Canning	6	12	CON	Canning	6	12	CON	Canning	6	12	CON	Canning	6	12
Lysine	98	75	71	67	97	93	74	68	95	95	70	66	93	76	72	68
Tryptophan	46	36	30	24	53	41	35	29	47	39	33	26	50	38	30	24
Isoleucine	51	43	41	38	53	46	44	43	52	43	41	39	48	41	39	37
Leucine	59	51	49	46	58	49	47	45	61	48	46	45	55	47	45	43
Valine	55	50	47	45	59	50	48	46	56	50	48	45	60	50	48	46
Methionine + Cysteine	28	22	20	17	25	20	16	12	22	18	15	11	28	22	18	15
Threonine	49	44	41	38	53	47	45	42	42	34	31	29	45	35	33	30
Phenylalanine + Tyrosine	93	83	81	77	94	82	78	76	88	73	69	66	96	87	82	79
Histidine	88	71	66	60	85	68	63	58	76	59	54	47	95	78	71	63
Lowest chemical score	28	22	20	17	25	20	16	12	22	18	15	11	28	22	18	15

6 & 12 = Months

has reported that canning decreases the net protein utilization (NPU) of canned pork.

Effect of canning and storage on free amino acids

RESULTS AND DISCUSSION

1. Effect of canning

From table 54 it can be seen that canning has decreased the free amino acids of the four varieties used in this study.

Free amino acids decreased as a result of canning in AMR, LAG, JOR and SML from 1.39 to 0.86 g/100g (Retn 62%), from 1.14 to 0.66 (Retn 58%), from 1.30 to 0.78 (Retn 60%) and from 1.56 to 1.0 g/100g (Retn 64%) respectively.

In all the four varieties tested aspartic acid and arginine were the most retained, their retentions ranging from between 70 and 78%, while alanine and glycine were least retained, their retentions varying between a trace amount and 50%.

2. Effect of storage

Table 54 shows that at the end of twelve month-storage at 25 C all free amino acids, except arginine, decreased to trace amounts. These results agree with those of other workers. A decrease in free amino acids of stored potatoes has been reported (WEAVER et al.,1978; TALLEY and PORTER, 1970).

There are two ways that canning and storage can affect free amino acids in foods:

1. It has been suggested by SALUNKHE (1974) that dehydroascorbic acid and 2,3-diketogulonic acid, formed from ascorbic acid during storage, react non-enzymatically with free amino acids to cause

Table 54: Effect of canning and storage at 25°C for twelve months on free amino acids of lentils.

(Results are expressed as g amino acid/100g)

	AMR					LAG					JOR					SML				
Amino acids	Con	Canning	Retn	6mon	12mon	Con	Canning	Retn	6mon	12mon	Con	Canning	Retn	6mon	12mon	Con	Canning	Retn	6mon	12mon
Tryptophan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine	0.16	0.11	69	0.06	T	0.14	0.08	0.57	T	0.00	0.12	0.06	50	T	0.00	0.15	0.09	60	T	0.00
Histidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ammonia	0.18	0.13	72	0.07	T	0.13	0.06	0.46	T	0.00	0.11	0.05	45	T	0.00	0.19	0.13	68	0.05	T
Arginine	0.21	0.15	70	0.10	T	0.30	0.23	77	0.13	0.06	0.41	0.32	78	0.21	0.12	0.39	0.29	74	0.19	0.11
Aspartic acid	0.19	0.14	74	0.06	0.00	0.17	0.11	65	0.05	0.00	0.20	0.14	70	T	0.00	0.22	0.15	68	0.08	T
Threonine	0.17	0.11	65	T	0.00	0.11	0.05	45	T	0.00	0.10	0.05	50	T	0.00	0.13	0.07	59	T	0.00
Serine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glutamic acid	0.22	0.12	55	0.05	0.00	0.12	0.06	50	T	0.00	0.18	0.10	56	T	0.00	0.23	0.17	74	0.10	T
Proline	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycine	0.08	T	-	0.00	0.00	0.06	T	-	0.00	0.00	0.08	T	-	0.00	0.00	0.09	0.00	-	0.00	0.00
Alanine	0.18	0.10	56	T	0.00	0.13	0.07	53	0.00	0.00	0.10	0.05	50	T	-	0.16	0.10	63	T	0.00
Cysteine																				
Valine	T	0.00	-	0.00	0.00	T	0.00	-	0.00	0.00	T	0.00	-	0.00	0.00	T	0.00	-	0.00	0.00
Methionine	0.00	0.00	-	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00	-	0.00	0.00	T	0.00	-	0.00	0.00
TOTAL	1.39	0.86	62	0.34	T	1.14	0.66	58	0.18	0.06	1.30	0.78	60	0.21	0.12	1.56	1.00	64	0.42	0.11

T = Traces

brown discoloration.

2. It has also been suggested by HURRELL and CARPENTER (1977) that since free amino acids have free alpha amino groups they can take part in Maillard reactions so that they are either bound or destroyed.

The losses of free amino acids observed in the present study may be due to both the above suggestions.

Effect of canning and storage on vitamins

Vitamin C exists in nature in the reduced form, L-ascorbic acid, and in the oxidized form, L-dehydroascorbic acid. So, generally, vitamin C designates both ascorbic (AA) and dehydroascorbic acid (DHA). PRIESTLEY (1979) has reported that the most important compound possessing vitamin C activity is L-ascorbic acid, which is readily oxidized to a diketo group, and the resulting L-dehydroascorbic acid (DHA) forms a redox system with L-ascorbic acid and has full vitamin C activity.

The rate of oxidation of ascorbic acid in foods is influenced by several factors including pH, trace metal, oxygen, time and temperature. The aerobic oxidation of ascorbic acid produces DHA and then other compounds. The anaerobic decomposition of ascorbic acid produces furfural (BENDER, 1978). Ascorbic acid degradation takes place in the presence or absence of oxygen as suggested by BAUERNFEIND and PINKERT (1970) (fig. 26).

In canned foods, the loss of ascorbic acid tends to be a rapid initial reaction which is oxygen-dependent and proceeds until the available oxygen is completely exhausted, followed by anaerobic degradation (ARCHER and TANNENBAUM, 1979).

Food processing causes vitamin destruction (THIJSSEN and KERKHOF,

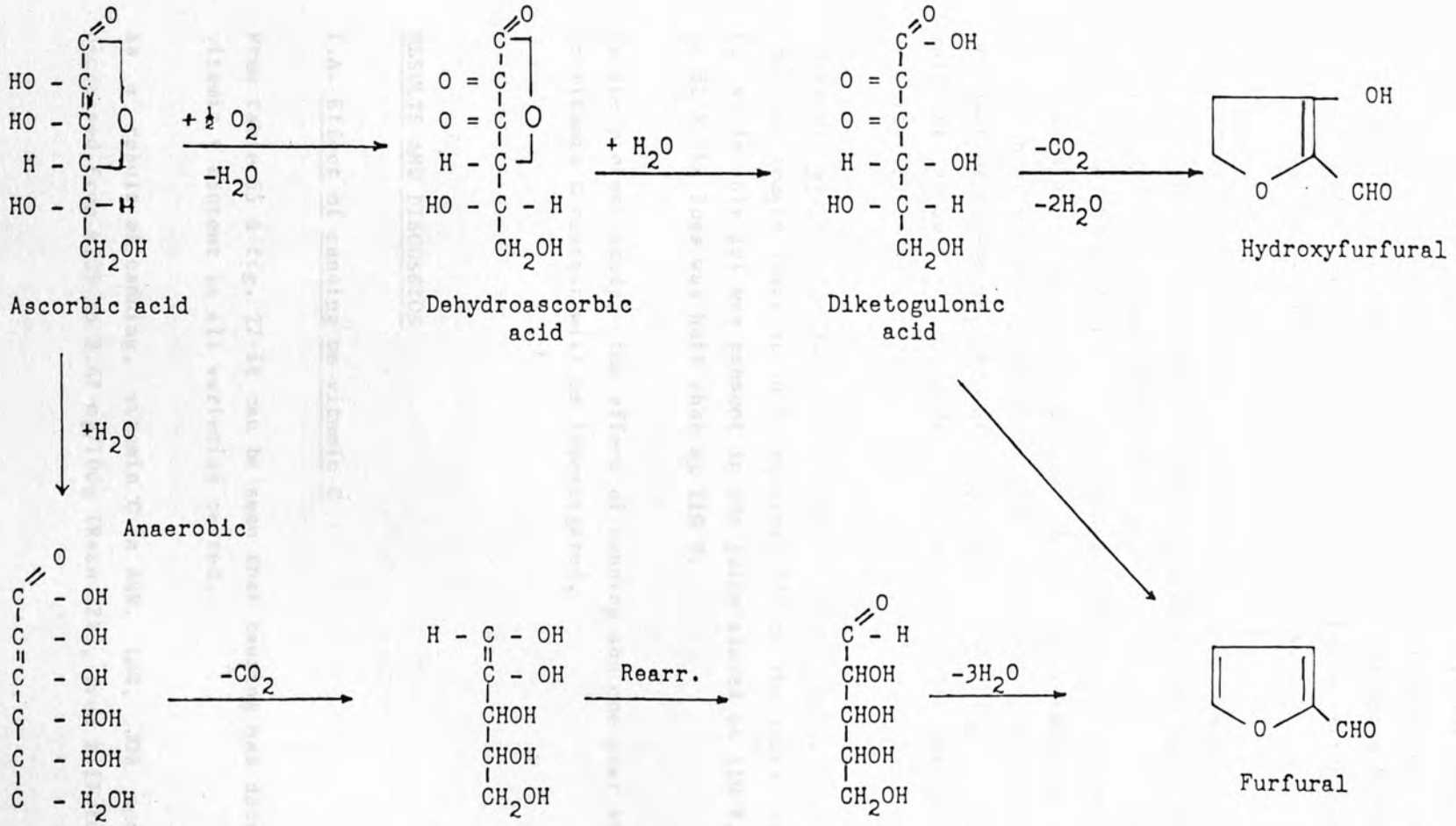


Fig.26. Proposed ascorbic acid degradation pathways in the presence or absence of oxygen (Bauernfeind and Pinkert, 1970)

1977). Vitamin C is often used as a criterion in processing and during storage. The vitamin C retention was 63% for canned peas (BENDER, 1966). Vitamin C is lost during processing and is affected by storage temperatures, e.g. processed beans stored for 6 months at 1 F, retained 30% of the vitamin after cooking compared to the vitamin content of fresh beans.

The stability of vitamin C, in canned products, depends upon the type of the can (CLIFCORN, 1948). KRAMER (1974) has reported that, canned foods when packed in less nutritive media, gradually suffer a leaching effect of this vitamin. Incomplete inactivation of enzymes and incomplete expulsion of air are believed to permit oxidation of vitamin C (CAIN, 1967). Vitamin C is an important precusor of non-enzymic browning (ADRIAN, 1982). The destruction rate of vitamin C in foods increases with increasing water activity (PRIESTLEY, 1979).

GUERRANT et al., (1945) have reported that, after one year storage, tomato juice at 30 F, retained 92% of the initial vitamin C, while only 20% was present in the juice stored at 110 F, and at 80 F the loss was half that at 110 F.

In the present study, the effect of canning and one year storage on vitamin C content will be investigated.

RESULTS AND DISCUSSION

1.A. Effect of canning on vitamin C

From table 55 & fig. 27 it can be seen that canning has decreased vitamin C content in all varieties tested.

As a result of canning, vitamin C in AMR, LAG, JOR and SML decreased from 4.35 to 2.47 mg/100g (Retn 57%), from 2.17 to 1.36

Table 55: Effect of canning and storage at 25°C for twelve months on % retention of vitamin C, thiamine and riboflavin of four varieties of lentil.

(Results are expressed as mg/100g)

Variety	Vitamin C	Thiamine	Riboflavin	Variety	Vitamin C	Thiamine	Riboflavin
AMR				JOR			
Control	4.35 (0.14)	0.57 (0.06)	0.27 (0.03)	Control	5.60 (0.25)	0.50 (0.05)	0.34 (0.03)
Canned	2.47 (0.11)	0.42	0.20	Canned	3.30 (0.19)	0.34	0.27
Retention(%)	57	75	78	Retention(%)	59	68	79
6 months	2.17 (0.13)	0.37	0.20	6 months	3.05 (0.15)	0.30	0.22
Retention(%)	50	66	78	Retention(%)	54	60	65
12 months	1.78 (0.08)	0.32	0.17	12 months	2.58 (0.18)	0.25	0.18
Retention(%)	41	57	64	Retention(%)	46	50	54
LAG				SML			
Control	2.17 (0.08)	0.45 (0.06)	0.38 (0.04)	Control	5.42 (0.19)	0.39 (0.03)	0.21 (0.02)
Canned	1.36 (0.07)	0.32	0.30	Canned	3.29 (0.21)	0.29	0.15
Retention(%)	63	70	81	Retention(%)	61	74	71
6 months	1.23 (0.08)	0.28	0.30	6 months	2.81 (0.16)	0.25	0.15
Retention(%)	57	64	81	Retention(%)	52	65	71
12 months	1.10 (0.06)	0.24	0.25	12 months	2.26 (0.13)	0.21	0.11
Retention(%)	51	54	65	Retention(%)	43	53	56

( ) = SE ; SE is omitted when <0.02 mg/100g

Table 56: Effect of canning and storage at 35°C for twelve months on % retention of vitamin C, thiamine and riboflavin of four varieties of lentil.

(Results are expressed as mg/100g)

Variety	Vitamin C	Thiamine	Riboflavin	Variety	Vitamin C	Thiamine	Riboflavin
AMR				JOR			
Control	4.35 (0.14)	0.57 (0.06)	0.27 (0.03)	Control	5.60 (0.25)	0.50 (0.05)	0.34 (0.03)
Canned	2.47 (0.11)	0.42	0.20	Canned	3.30 (0.19)	0.34	0.27
Retention(%)	57	75	78	Retention(%)	59	68	79
6 months	1.74 (0.09)	0.35	0.20	6 months	2.52 (0.15)	0.28	0.19
Retention(%)	40	62	78	Retention(%)	45	56	57
12 months	1.26 (0.12)	0.28	0.15	12 months	1.96 (0.12)	0.22	0.15
Retention(%)	29	49	56	Retention(%)	35	44	47
LAG				SML			
Control	2.17 (0.08)	0.45 (0.06)	0.38 (0.04)	Control	5.42 (0.19)	0.39 (0.03)	0.21 (0.02)
Canned	1.36 (0.07)	0.32	0.30	Canned	3.29 (0.21)	0.29	0.15
Retention(%)	63	70	81	Retention(%)	61	74	71
6 months	1.06 (0.06)	0.23	0.25	6 months	2.48 (0.15)	0.22	0.15
Retention(%)	49	51	66	Retention(%)	46	57	71
12 months	0.71	0.19	0.20	12 months	1.46 (0.10)	0.18	0.10
Retention(%)	33	46	55	Retention(%)	25	47	50

( ) = SE ; SE is omitted when <0.02 mg/100g

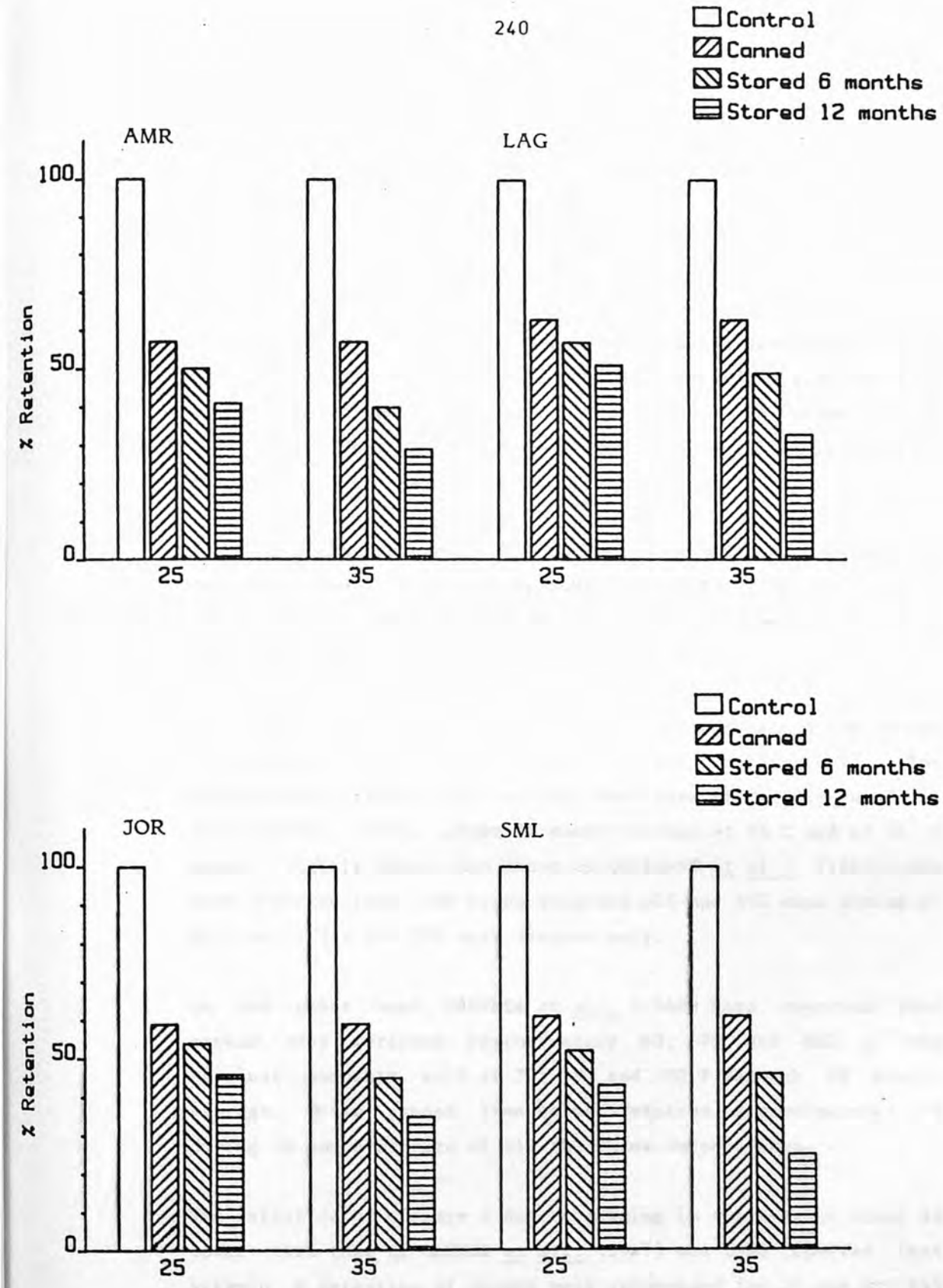


Fig. 27: Effect of canning and one year storage at 25°C and 35°C on % retention of Vitamin C of four lentil varieties.

(Retn 63%), from 5.60 to 3.30 (Retn 59%) and from 5.42 to 3.42 to 3.29 mg/100g (Retn 61%) respectively.

1.B. Effect of storage on vitamin C

Table 55 & fig.27 show that after twelve month-storage at 25 C vitamin C in AMR, LAG, JOR and SML decreased from 4.35 to 1.78 mg/100g (Retn 41%), from 2.17 to 1.10 (Retn 51%), from 5.60 to 2.58 (Retn 46%) and from 5.42 to 2.26 mg/100g (Retn 43%) respectively.

After 12 month-storage at 35 C vitamin C in AMR, LAG, JOR and SML decreased from 4.35 to 1.26 mg/100g (Retn 29%), from 2.17 to 0.71 (Retn 33%), from 5.60 to 1.96 (Retn 35%) and from 5.42 to 1.46 (Retn 25%) respectively (table 56, & fig. 27).

Information about vitamin C retention in foods canned and stored at different temperatures seems to vary considerably. The retention of vitamin C of the four varieties of lentils found in the present study, after 12 month-storage at 25 C and at 35 C seems slightly lower than those of GUERRANT et al., (1945) who have reported that lima beans retained 83% and 65% when stored at 30 F and 110 F for 270 days respectively.

On the other hand, BRENNER et al., (1948) have reported that canned peas retained approximately 80, 75 and 66% of the original ascorbic acid at 70, 90 and 100 F through 18 month-storage, while canned lima beans retained approximately 70% during 18 month-storage at all the three temperatures.

The retention of vitamin C due to canning in the present study is lower than that of WAGNER et al., (1947) who have reported that vitamin C retention of canned peas (processed for 25 min at 245 F) is 80%, and that of canned lima beans is 98%. The results from the present study, agree to some extent with those of KRAMER (1974) who has reported that canned green beans and lima beans

lost 15% vitamin C when stored for upto one year at 80 F.

Although some investigators believe that little destruction of vitamin C occurs in canned foods (PRIESTLEY, 1979), yet it is likely that losses do take place due to leaching and oxidation (BENTERUD, 1977). There may be a loss immediately after canning or bottling due to dissolved head space air. As a consequence, there is generally greater loss of vitamin C in lacquered cans than in plain ones (BENDER, 1978). HENSHALL (1974) has reported that each product presents its own problem and that the retention of vitamin C activity is product dependent.

2.Effect of canning and storage on thiamine

Like vitamin C, the B vitamins are greatly affected by heat. The processing time and temperature as well as pH and the can size are factors which determine thiamine stability in food. The vitamin retention in canned green beans (90min at 121 C) was about 45% (BENTERRUD, 1977). PRIESTLEY (1979) has reported that thiamine reacts with the free NH_2 groups of proteins and it participates in a Maillard-type reaction with glucose. KRAMER (1974) has reported that losses in thiamine in canned beans were 10% but a greater loss of 25% is observed if stored for up to 12 months at ambient temperature (80 F); about 35% is however lost if stored for longer than one year at this temperature. The low-acid canned vegetables retain less thiamine than do canned fruits (CAIN, 1967).

RESULTS AND DISCUSSION

2.A. Effect of canning on thiamine

From table 55 & fig. 28 it can be seen that canning has decreased the thiamine content in all the lentil varieties under investigation.

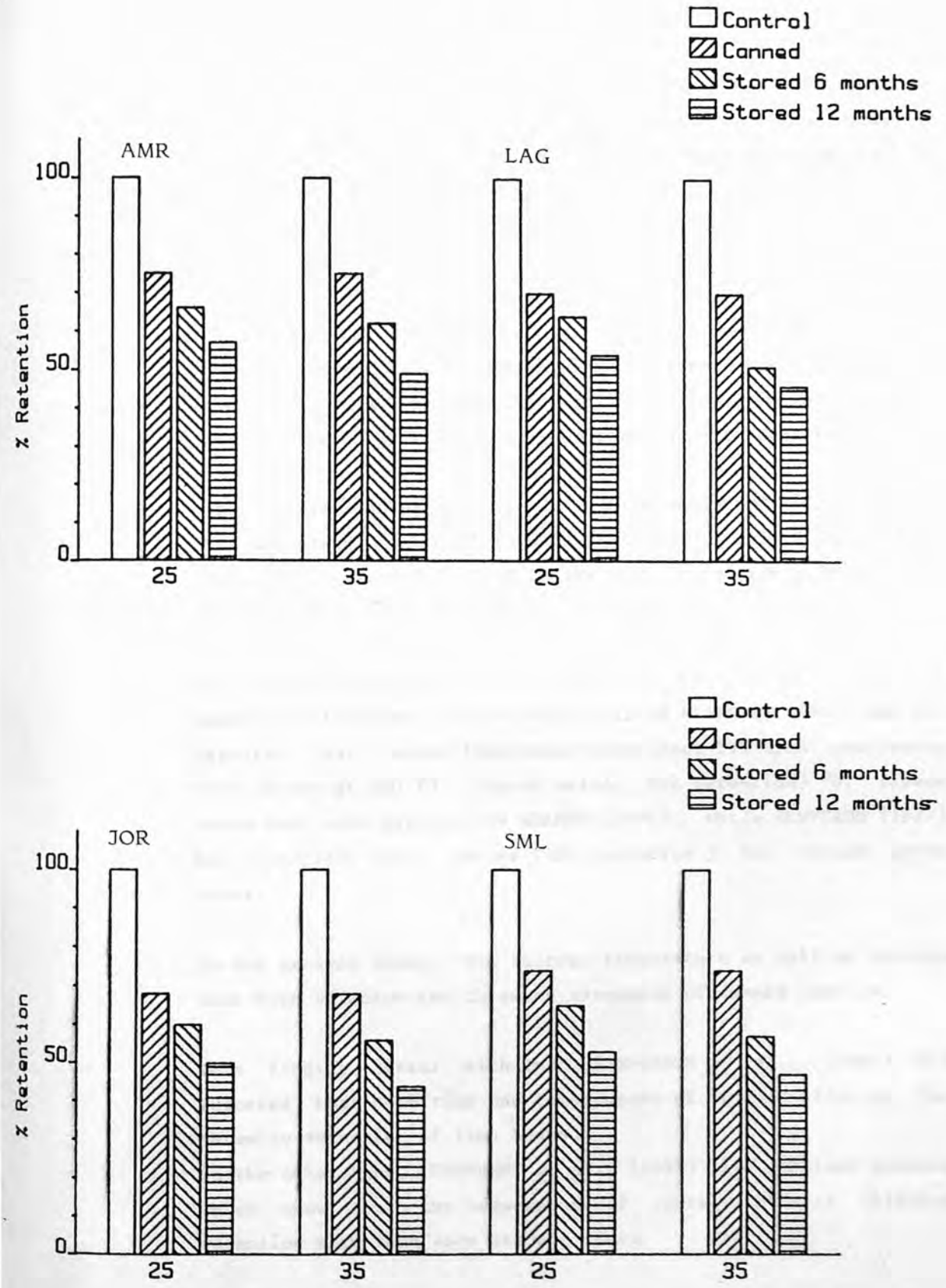


Fig. 28: The effect of canning and one year storage at 25°C and 35°C on percentage retention of thiamine of four lentil varieties.

As a result of canning, thiamine in AMR, LAG, JOR and SML decreased from 0.57 to 0.42 mg/100g (Retn 75%), from 0.45 to 0.32 (Retn 70%), from 0.50 to 0.34 (Retn 68%) and from 0.39 to 0.29 mg/100g (Retn 74%) respectively.

2.B. Effect of storage on thiamine

Table 55 & fig. 28 show that after 12 month-storage at 25 C, thiamine in AMR, LAG, JOR and SML decreased from 0.57 to 0.32 (Retn 57%), from 0.45 to 0.24 (Retn 54%), from 0.50 to 0.25 (Retn 50%) and from 0.39 to 0.21 mg/100g (Retn 53%) respectively.

After twelve month-storage at 35 C thiamine in AMR, LAG, JOR and SML decreased from 0.57 to 0.28 (Retn 49%), from 0.45 to 0.19 (Retn 46%), from 0.50 to 0.22 (Retn 44%) and from 0.39 to 0.18 mg/100g respectively (table 56, fig. 28).

The results obtained from the present study, on the effect of canning on thiamine, agree with those of CLIFCORN (1947) who has reported that mature lima beans lose about 29% upon processing (for 30 min at 240 F). Higher values (90% retention) for canned beans have been reported by KRAMER (1974), while BENTERUD (1977) has reported lower values (45% retention) for canned green beans.

In the present study, the storage temperature as well as storage time both affected the thiamine retention of canned lentils.

This finding agrees with that of EVERSON et al., (1964) who reported that both time and temperature of storage affected the thiamine retention of lima beans.
On the other hand, GUERRANT et al., (1945) have obtained results which show that the temperature of storage affects thiamine retention more than does storage time.

The results obtained in the present study concerning the effect

of storage time and temperature on thaimine retention of canned lentils agree to some extent with those of GUERRANT et al., (1945) who have reported that lima beans stored at 110 F for 12 month, retained 25% of the original thiamine content. Higher retained values, have been reported by EVERSON et al., (1964) who have found that thiamine lost during 9 month-storage of lima beans at 68 and 86 F varied from 10 to 20%.

3.Effect of canning and storage on riboflavin

Riboflavin (B_2) has low solubility in water. BENTERUD (1977) has reported that negligible losses of riboflavin occur in food during thermal processing. Other investigators, have reported losses in canned vegetables ranging from 25 to 70% (PRIESTLEY, 1979; YAMAGUCHI and WU, 1975).

GANGAL and MAGAR (1967) have reported that meat packed in brine and stored at 80 F lost about 75% of its riboflavin, and the loss seems to be due to leaching and not by being destroyed. GUERRANT et al., (1945) have reported a 63% retention in tomato juice after storage for one year at 110 F, compared to 90% if the storage was at 30 F. Futhermore, canned lima beans lost 30% riboflavin when stored for 24 months at 80 F.

RESULTS AND DISCUSSION

3.A.Effect of canning on riboflavin

From table 55 & fig. 29 it can be seen that canning has decreased riboflavin in all the four varieties tested.

In AMR, LAG, JOR and SML riboflavin decreased as a result of canning from 0.27 to 0.20 (Retn 78%), from 0.38 to 0.30 (Retn 81%), from 0.34 to 0.27 (Retn 79%) and from 0.21 to 0.15 mg/100g (Retn 71%) respectively.

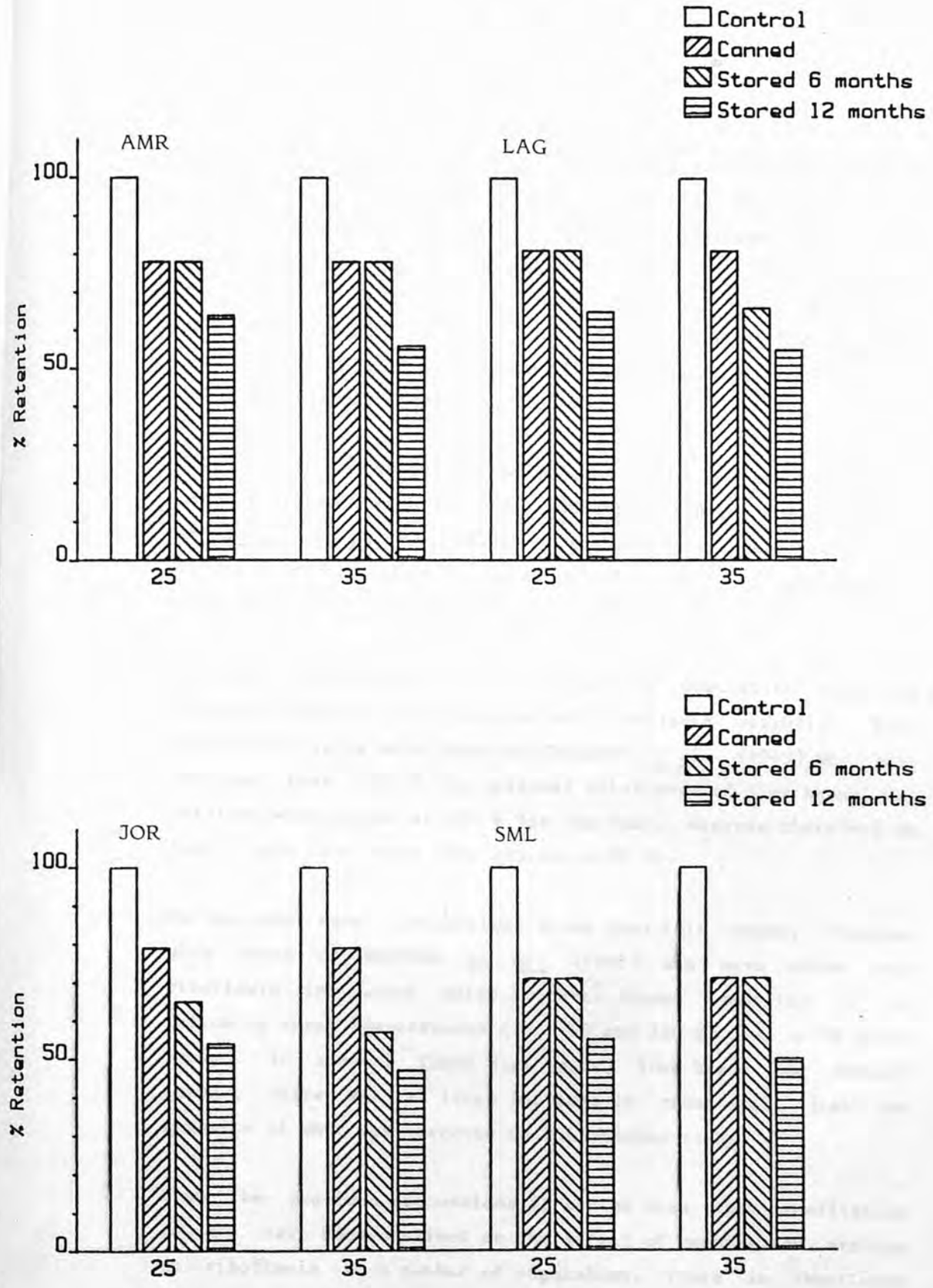


Fig. 29: The effect of canning and one year storage at 25°C and 35°C on percentage retention of riboflavin of four lentil varieties.

3.B. Effect of storage on riboflavin

After twelve month-storage at 25 C , riboflavin in AMR, LAG, JOR and SML decreased from 0.27 to 0.17 (Retn 64%), from 0.38 to 0.25 (Retn 65%), from 0.34 to 0.18 (Retn 54%) and from 0.21 to 0.11 mg/100g (Retn 56%) respectively (table 55 & fig. 29). Also after twelve month storage at 35 C riboflavin in AMR, LAG, JOR and SML decreased from 0.27 to 0.15 (Retn 56%), from 0.38 to 0.20 (Retn 55%), from 0.34 to 0.15 (Retn 47%) and from 0.21 to 0.10 (Retn 50%) respectively (table 56, fig. 29).

The results obtained in the present study on the effect of canning agree with those of AUGUSTIN (1981) for canned beans (Phaseolus vulgaris). WAGNER et al.(1947) have reported higher values for riboflavin retention in canned peas and lima beans of 79 and 104% respectively.

In the present study, it was found that temperature and time of storage affected the retention of riboflavin slightly. These conclusions agree with those of GUERRANT et al. (1945) who have reported that 60% of the original riboflavin of lima beans was retained when stored at 110 F for one year, whereas there was no loss if the lima beans were stored at 85 F.

On the other hand, conclusions drawn from this study, disagree with those of BRENNER et al. (1948) who have shown that riboflavin in canned dairy products showed stability at the following three temperatures (70, 90 and 100 F) over an 18 month period. In several foods e.g. peas, lima beans and spinach leaves, there was a large increase in riboflavin, just the opposite of what was observed in the present study.

From the previous discussions it can be seen that conflicting results have been obtained on the effect of canning and storage on riboflavin in a number of vegetables. There is thus more variation on riboflavin when compared to the other vitamins which

have been reported either by different workers, or by the same worker using different foods. Therefore, this is one of the reasons for using vitamin C and not riboflavin as a marker for studying canning and storage conditions.

CHAPTER V111

SCANNING ELECTRON MICROSCOPE STUDIES OF RAW AND PROCESSED LENTILS

Different legumes have been viewed under the scanning electron micoscope (S.E.M.) . Electron microscopy studies on red kidney beans and bean sprouts have been conducted by SILVA and LUH (1978), on dry beans by ROCKLAND et al. (1977), and on faba bean seeds by McEWEN et al. (1974). BHATTY and SLINKARD (1977) have conducted S.E.M. studies on isolated starch granules on some varieties of lentils e.g. laird and tekoa, but as far as the author is aware no other such studies on whole lentil seeds (under different conditions) have been carried out to-date.

Aim of the work

The cell contents of these four varieties of dry lentil seeds e.g. starch and protein were investigated using scanning electron microscopy (SEM). Ideally, similar studies of the changes that occur in the cell contents of all four varieties after different treatments e.g. germination, cooking and canning would also have been investigated. However, due to several technical problems SEM of only one variety, namely AMR, was carried out after various treatments. Since there were no wide differences in the four varieties, it may be assumed that the other three varieties would present somewhat similar pictures to AMR which was investigated thoroughly.

MATERIALS AND METHODS

Scanning electron microscope (SEM)

1. Fixation of sample

Reagents

a) Fixative: 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4.

b) Phosphate buffer is made from:

- Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) (9.5 ml of 31.2 g l^{-1})
- Disodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$) (40.5 ml of 35.6 g l^{-1})

made up to 100 ml.

Procedure

Dry seeds, and samples of germinated seedlings were collected at one day interval for 5 days. The seed coats of germinated seeds as well as the seed coats of seeds from other processings e.g. cooking and canning were removed by using a scalpel. Then the samples were put into glass vials. To each glass vial about 15 ml of 3% glutaraldehyde in 0.1 M buffer (pH 7.4) was added and left in the fridge (4-7 C) for about four hours.

2. Dehydration

Acetone was selected as a dehydrating agent. The following concentrations of acetone were prepared:20, 50, 70, 90 and 100%. At the end of the fixation period, glutaraldehyde was poured off. Each sample was dehydrated by staying for about 1 to 1.5 h in each of the above acetone concentrations.

3. Critical point drying (CPD)

This process dries the material without causing deformation of the cell membranes due to water surface tension effects. The critical point of a liquid is reached at a certain temperature

and pressure when the gas and liquid phases of the substances reach instant equilibrium. Cold water was run through the E3000 Polaron, the critical point dryer to reduce temperature. Specimens were loaded in the boat. The CO_2 inlet valve was opened. The vent valve was closed. The drain was opened to remove the acetone. The CO_2 was flushed to remove unseen acetone. Then all valves were closed, and machine (dryer) was left for 2 hours. At the end of the 2 h CO_2 was vented.

4. Mounting and coating

The dried samples were fractured by using a scalpel. The fracturing was done according to SILVA and LUH's (1979) method so that the cross section included the embryo. Then the specimen was attached to 13 mm stubs provided with double sided sticky tapes. The stubs were placed in the specimen holder of the Polaron E1500 Series 11, "Cool Sputter Coater". They were coated with gold palladium 50 mm thick.

5.Viewing under the SEM

The samples were examined and photographed using a "Joel SM 25" SEM at an accelerating potential of 15 Kilovolts.

RESULTS AND DISCSSION

Scanning electron microscopy (SEM) has been used to show the changes that occur to the cell constituents, and which may be related to chemical changes (HSU, 1981). The fracturing of the dried cotyledons permitted the viewing of the cell contents of the sample.

Cell component of raw seeds

1. LAG seeds

The cells measured about 52.4 µm. The starch granules are nearly spherical and measured about 18.6 µm (fig. 30, C).

2. JOR seeds

The majority of starch granules in this variety are semi oval in shape and measured 25.7 µm in length and 20.8 µm in width (fig. 31, B and C).

3. SML seeds

The majority of starch granules are kidney-shaped. The dimensions of the granules are 21.5 µm long and 8.5 µm wide (fig. 32, C).

4. AMR seeds

The dimensions of the cell are 70.3 X 78.8 µm (fig. 33, C). The starch granules are spherical and measure about 20.9 µm (fig. 33, B and C).

From the observations of the present study, the lentil cells (52.4 to 78.8 µm) are obviously smaller than those of peas (diam. 200 µm) (BRYANT, 1981).

In varieties AMR, LAG, JOR, and SML of the present study, the starch granules are bigger (20.9, 18.6, 25.7, and 21.5 µm) than those (10-15 µm) of other lentil varieties which have been investigated previously by BHATTY and SLINKARD (1979).

Cell walls of raw seeds are clearly recognisable. The cells appear well separated from each other. In all varieties of the present study, proteinaceous material occupied the spaces between

Fig. 30: Scanning electronmicrographs of raw LAG lentils. The following abbreviations are to be used in all S.E.M. figures:

A	A group of cells
P	Proteinaceous materials
S	Starch granules
W	Cell wall
M	Cell membrane
COT	Cotyledons

(A) A group of cells (x 147)

(B) Two cells (x 515)

(C) Starch granules (x 515)

S
S
M
W
P
M
S
W
P

Fig. 31: Scanning electronmicrographs from cotyledons of raw JOR lentils

(A) Top surface cells of the cotyledon (x 50)

(B) Cell with starch granules (x500)

(C) Starch granules (x 5040)

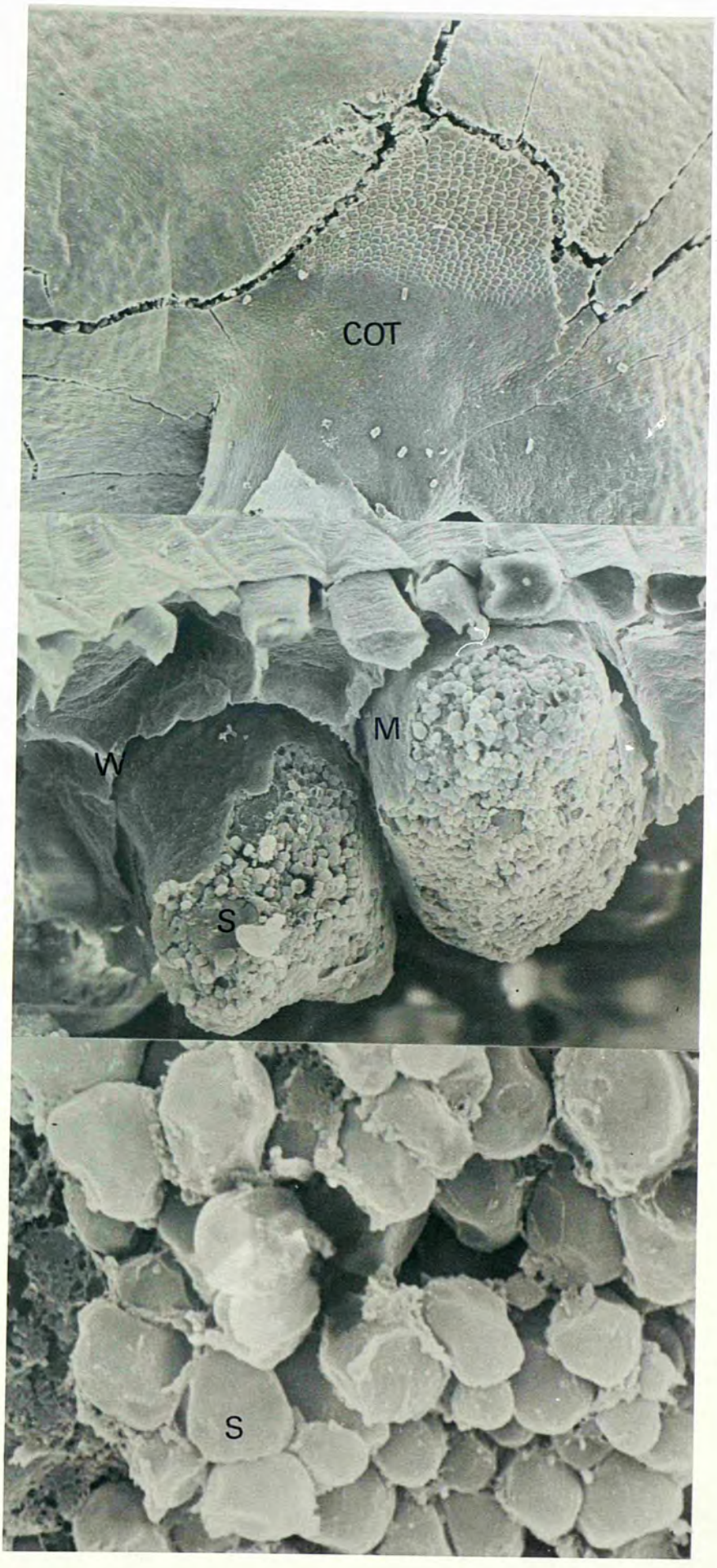
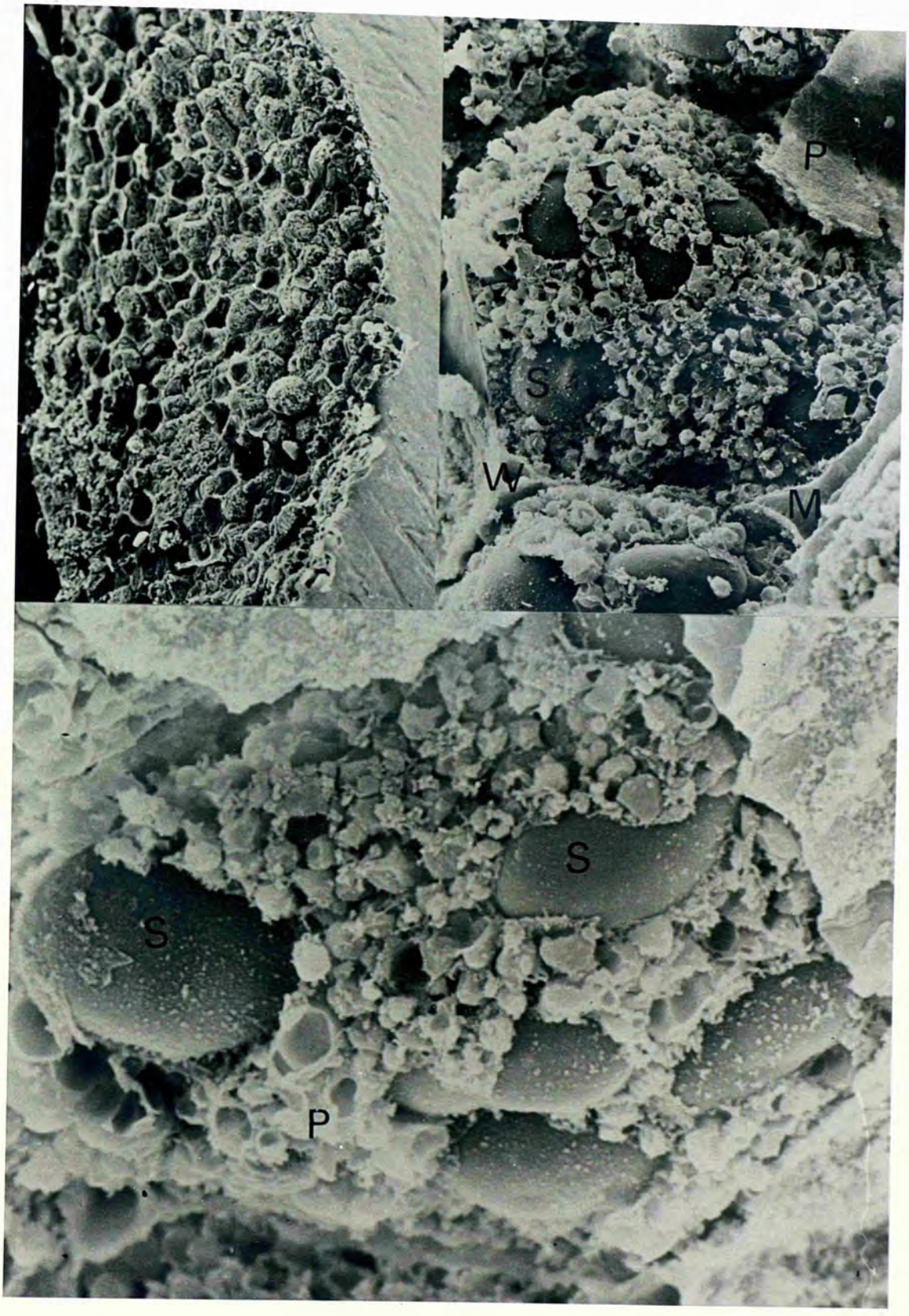

COT
M
W
S
S

Fig. 32: Scanning electronmicrographs from cotyledonous cells of raw SML lentils

(A) A group of cells (in raw lentil cotyledons) (x 1000)

(B) Starch granules (x 1000)

(C) Starch granules in a single cell (x 1500)


P
S
W
M
S
S
P

the starch granules. This is similar to results reported by SILVA and LUH (1978) and by McEWEN et al. (1974) who have observed membrane like material possibly of a proteinaceous nature in kidney and in faba beans respectively. In a few cases, proteinaceous material appears as small bodies ranging from 4.8 to 7.1 µm (fig. 32A and B). Starch granules in most plants are surrounded by thin strands of cytoplasm (MEYER, 1978).

SEM of fractured, raw cells, suggests that a thin, perhaps protein film, encapsulated the starch granules (ROCKLAND, 1978). 'Cell wall' is used to describe those portions of the plant cell laying outside the plasma membrane. The cell wall plays an important role in cell growth and differentiation, since, these events involve changes in cell size and shape (KARR, 1976). Also a cell is surrounded by a membrane (cell membrane) that separates it from its environment and enables it to control the entry and egress of substances selectively. Moreover, virtually all of the subcellular organelles are made of or surrounded by membranes or pieces of membranes (BIDWELL, 1979).

Effect of treatments on cell components

1. Effect of germination in AMR variety

a) Germination at 25 C

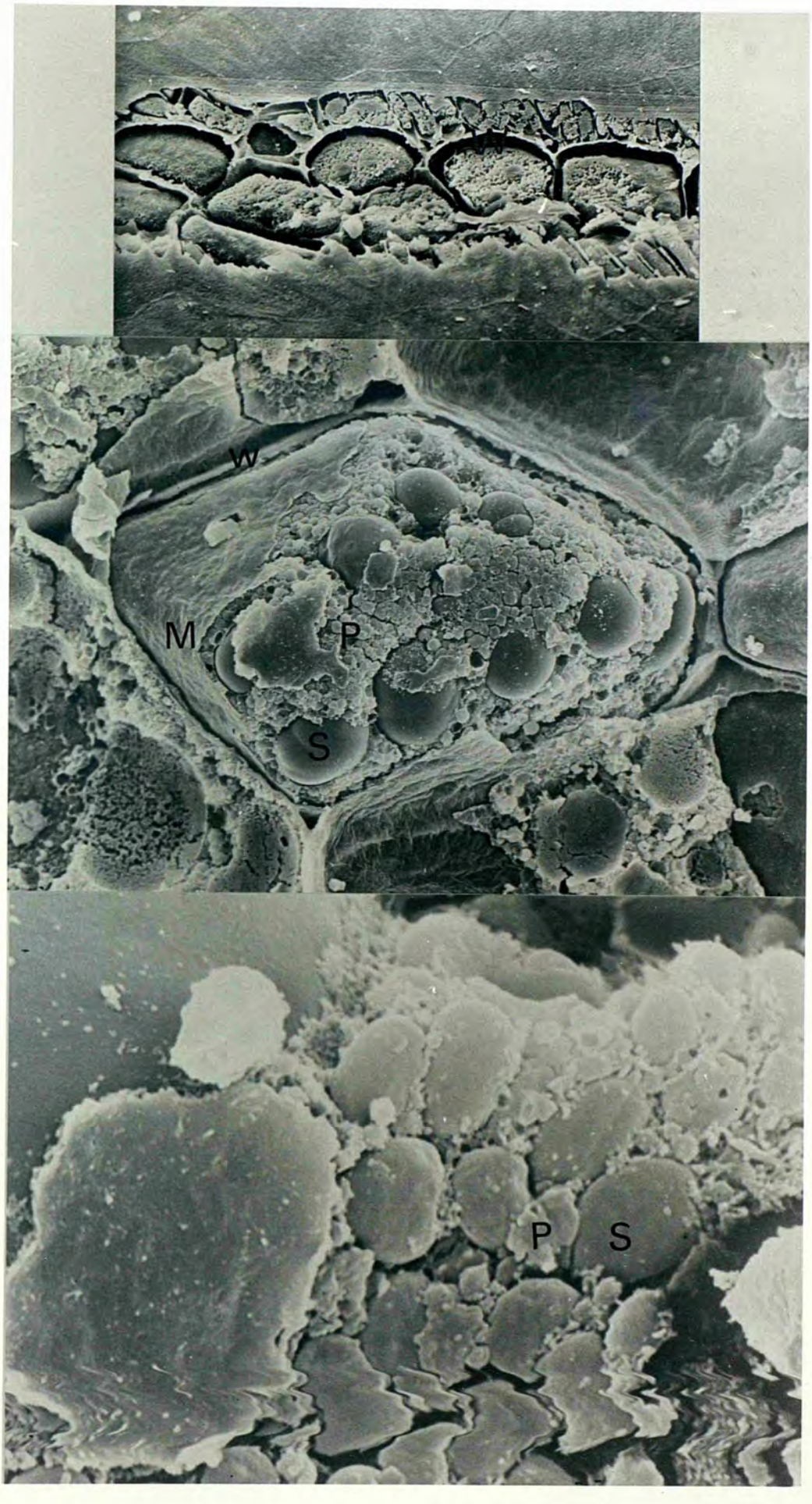
On the first day of germination, there are visible changes in the shape of starch granules: the oval-shaped ones measure about 17.8 µm, while the kidney-shaped ones about 20.7 µm (fig. 34B). On the third day of germination, further changes are observed in the starch granules: the oval-shaped ones tend to become spherical. As germination time increases, the starch granules become more easily breakable, and there are more granules with shapes in between kidney and spherical. The surface of the granules become rough (porous). The dimensions of starch granules after 5 days are between 18.4 and 20.6 µm(fig. 36, B). During germination at

Fig. 33: Scanning electronmicrographs from cotyledons of raw AMR

(A) The cells beneath the whole cells in dry lentil cotyledon (x 450

(B) A single cell contains starch granules and proteinaceous material (x 700)

(C) A group of cells (x 700)


W
M
P
S
P
S

25 C some starch granules show a hollow (fig. 36, A) and some show degradation (fig. 36, B). Similar observations have been reported by DUFFUS (1984) in germinated barley. Cell wall has been affected. Changes in cell fine structure in the cotyledons of beans (Phaseolus vulgaris) during germination have been reported (OPIK, 1966).

Electron microscopic observations indicate that as germination time increases, the gaps between starch granules become larger. This suggests an agreement between the SEM observations and the chemical analysis which shows a decrease in starch content.

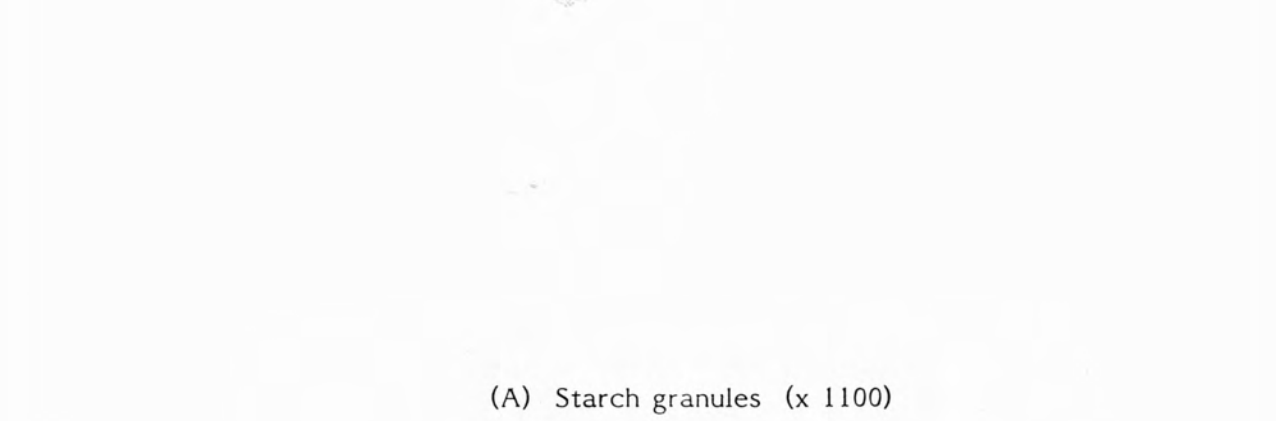
b) Germination at 35 C

On the third day of germination at 35 C, the oval-shaped starch granules measure 16.2 um, kidney-shaped ones 23.8 μm (fig. 37 A). More spaces are observed between the starch granules of seeds germinated at 35 C. At the higher temperature, the starch granules are no longer whole and slight fractures appear on the surface of the granules (fig. 37B). Also oval and kidney granules become semi oval and semi kidney respectively and measure between 18.2 and 22.9 μm (fig. 37B). The surface of the granules becomes more fragile and porous compared to the granules at the ealier stages of germination. On the fifth day of germination at 35 C, dimensions of the starch granules range from 16.5 to 25.0 μm (fig. 38B). Roughness of the surface of starch granules has also been observed by SILVA and LUH (1978) in germinated kidney bean seedlings as they age.

2.Canned seeds

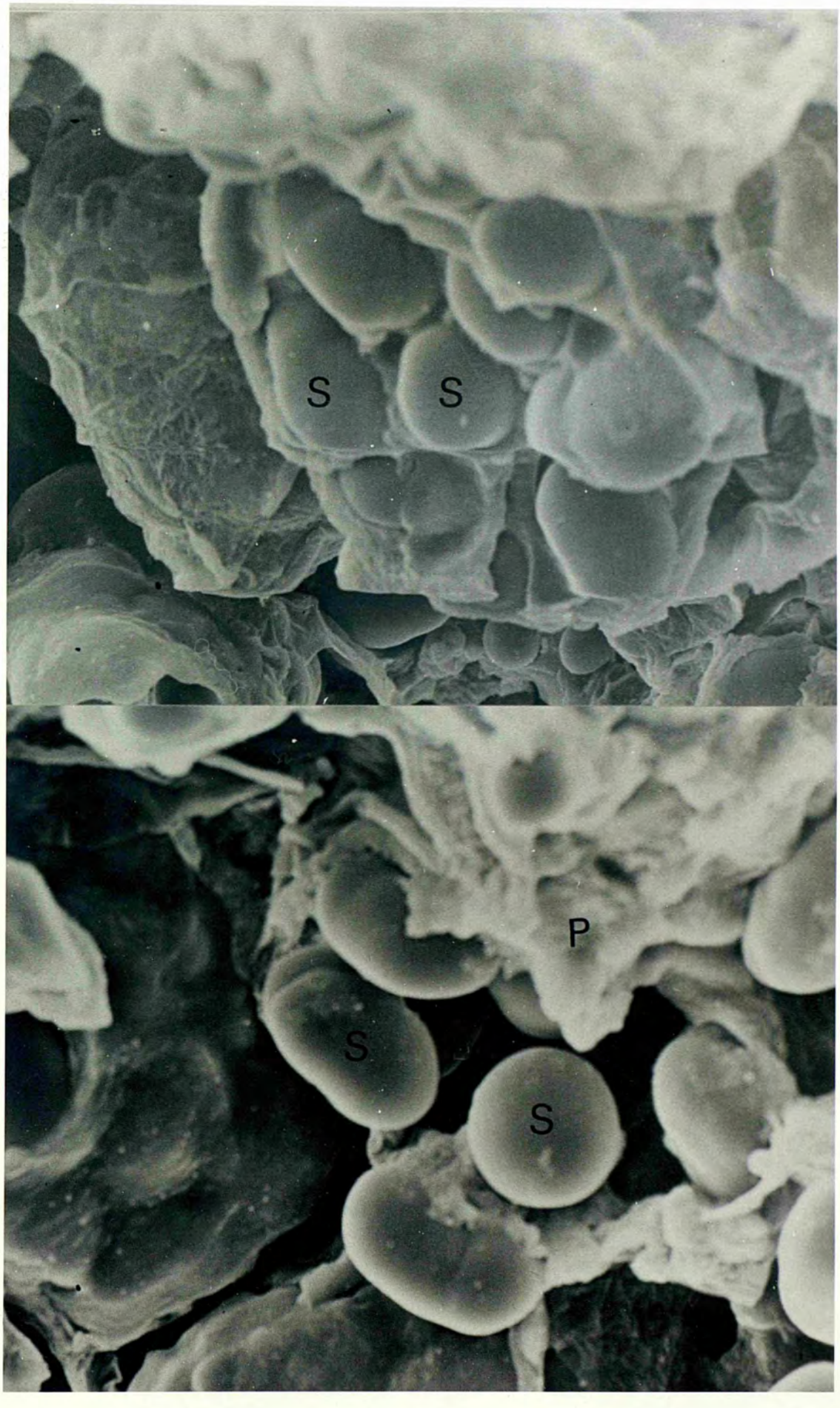
In canned seeds, the cell dimensions increased from 70.3 to 80.6 μm in diameter. Starch granules lose their shape, become rough, and show a loss of birefringence with gelatinization of starch taking place, while their dimensions change from being circular (20.9 μm in diameter) in dry seeds to being oval shaped (32.5 x 17.0 μm)

Fig. 34: Scanning electronmicrographs of AMR germinated for one day at 25°C.



(A) Starch granules (x 1100)

(B) Starch granules (x 1100)


S
S
P
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S

Fig. 35: Scanning electronmicrographs from cotyledons of cells of AMR germinated for three days.

(A) A group of cells (x 70)

(B) Starch granules (x 1000)

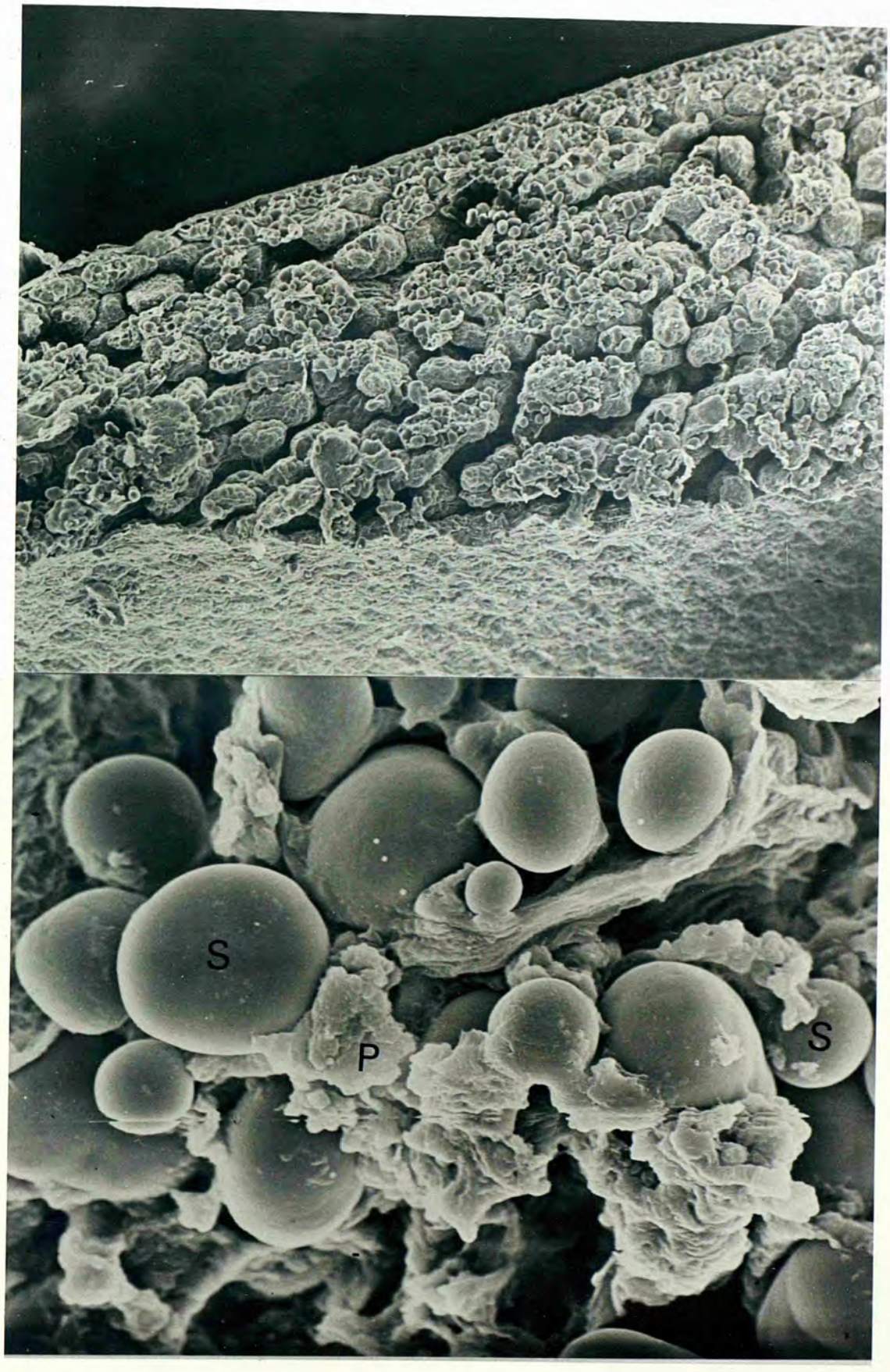

S
P
S

Fig. 36: Scanning electronmicrographs of AMR germinated for 5 days at 25°C

(A) A group of cells (x 700)

(B) A cell starch granules and proteinaceous material (x 1000)

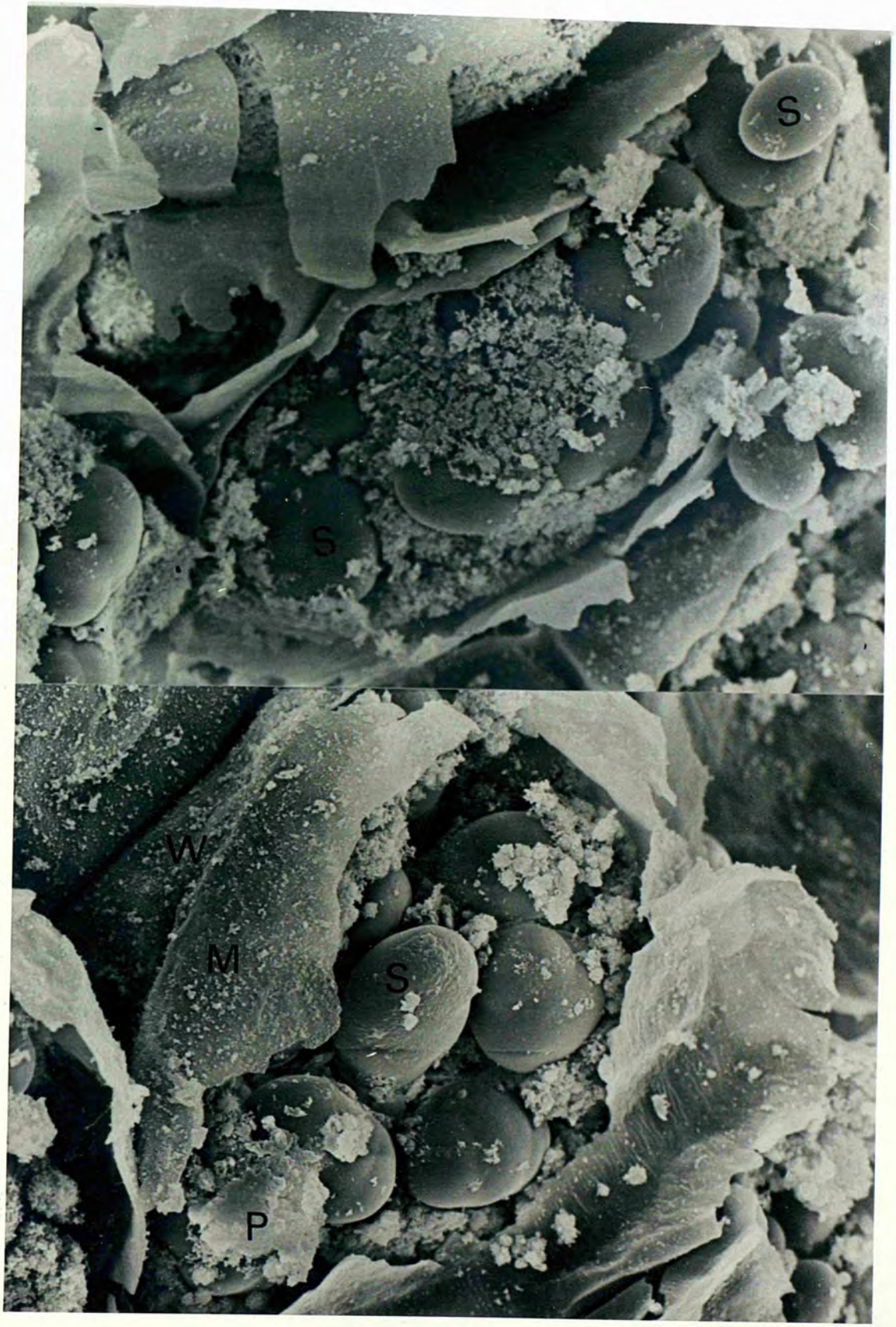

S
S
W
M
S
P

Fig. 37: Scanning electronmicrographs from cotyledon cells of AMR germinated for three days at 35°C

(A) A number of cells (x 720)

(B) The surface of a strach granule (x 3085)

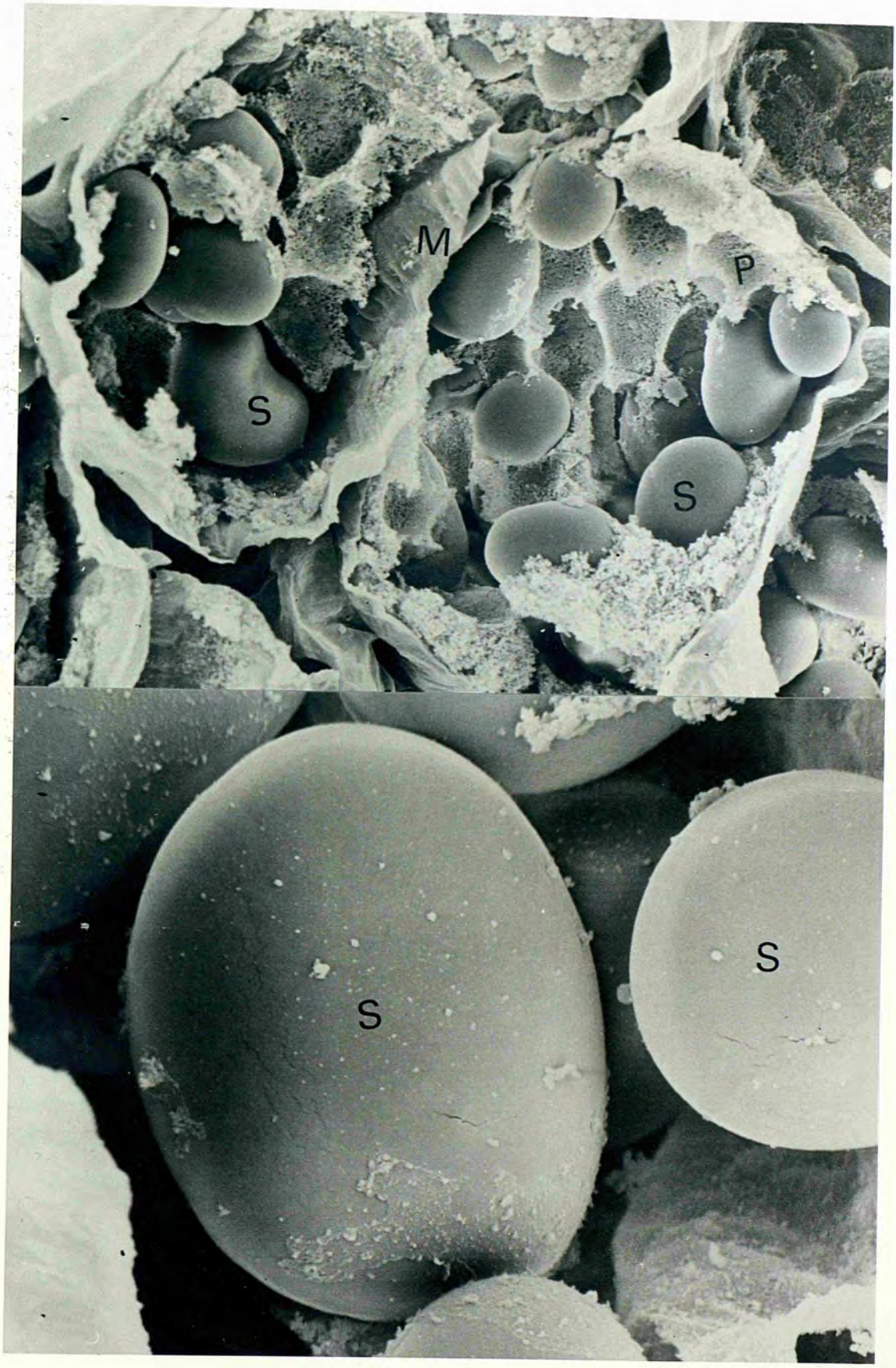
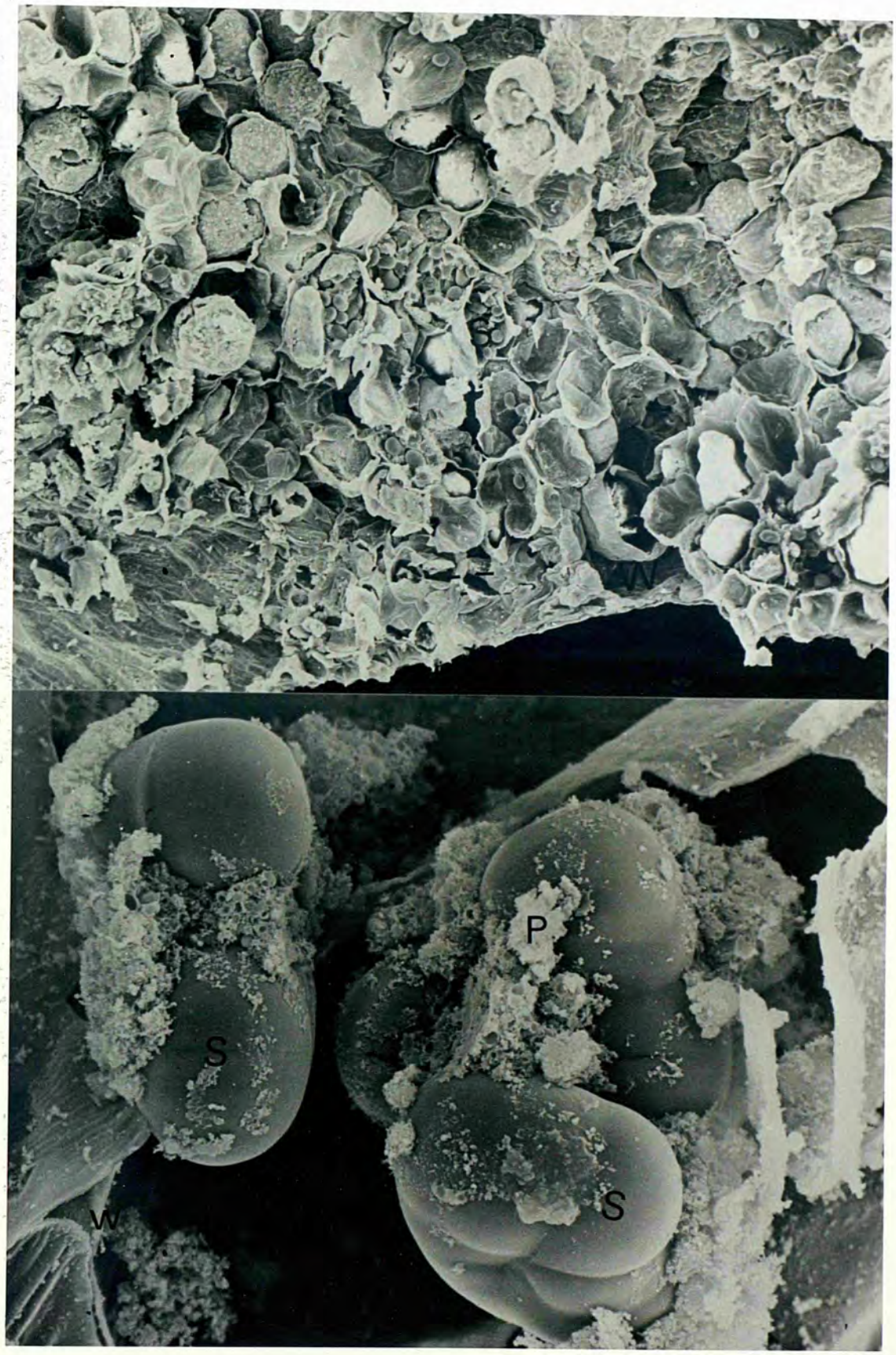

M
P
S
S
S
S

Fig. 38: Scanning electronmicrographs from cotyledon cells of AMR germinated for five days at 35°C

(A) A group of cells (x 100)

(B) Starch granules and proteinaceous material (x 1500)


P
S
S
W

(fig.39C).

3.Cooked seeds

The starch granules in cooked cells (fig. 40A) are compared here with those of raw seeds (fig. 33C). The diameter of starch granules in the latter are (20.9 µm) and the size of starch granules in cooked ones is 28.3 x 24.6 µm. Due to the increase in temperature, deformation and configurational changes have taken place and the cells become separated.

Similar observations have already been mentioned by MEYER (1978) who has reported that when vegetables are cooked, cells may be observed separating, rupturing, shrinking or a combination of these may take place.

4.Stored seeds

Storage for one year at 25 C has little effect on cell size and shape of starch granules (fig. 41C).

Storage for one year at 35 C has affected the proteinaceous material which now looks like strands instead of the amorphous layer it was before (fig. 42B).

The most prominent components of cotyledon cells of lentils are starch and protein. From the previous discussions, SEM could be used as a useful tool or technique to reveal the structure of the contents of the cotyledonous cells of lentils and later the changes in these due to different treatments e.g. germination, cooking and canning may be elucidated. Alongside with chemical and biochemical analysis, SEM studies may provide further information, and in some cases will detect some qualitative changes in the major components of the cell as was found in the present study. In lentils stored at the higher temperature (35 C), the shape of the proteinaceous material has changed, and such

changes cannot be detected by chemical analysis.

From the present study, it may be concluded that the storage of lentils at higher temperatures are to be avoided.

Fig. 39: Scanning electronmicrographs of canned AMR seeds

(A) Changes in starch (x 515)

(B) A group of cells (x 736)

(C) A cell containing starch granules (x 1105)

P
S
P
M
M
S
P

Fig. 40: Scanning electronmicrographs of cooked AMR

(A) A group of cells (x 338)

(B) A cell containing starch granules (x 750)

(C) A single cell (x 1125)

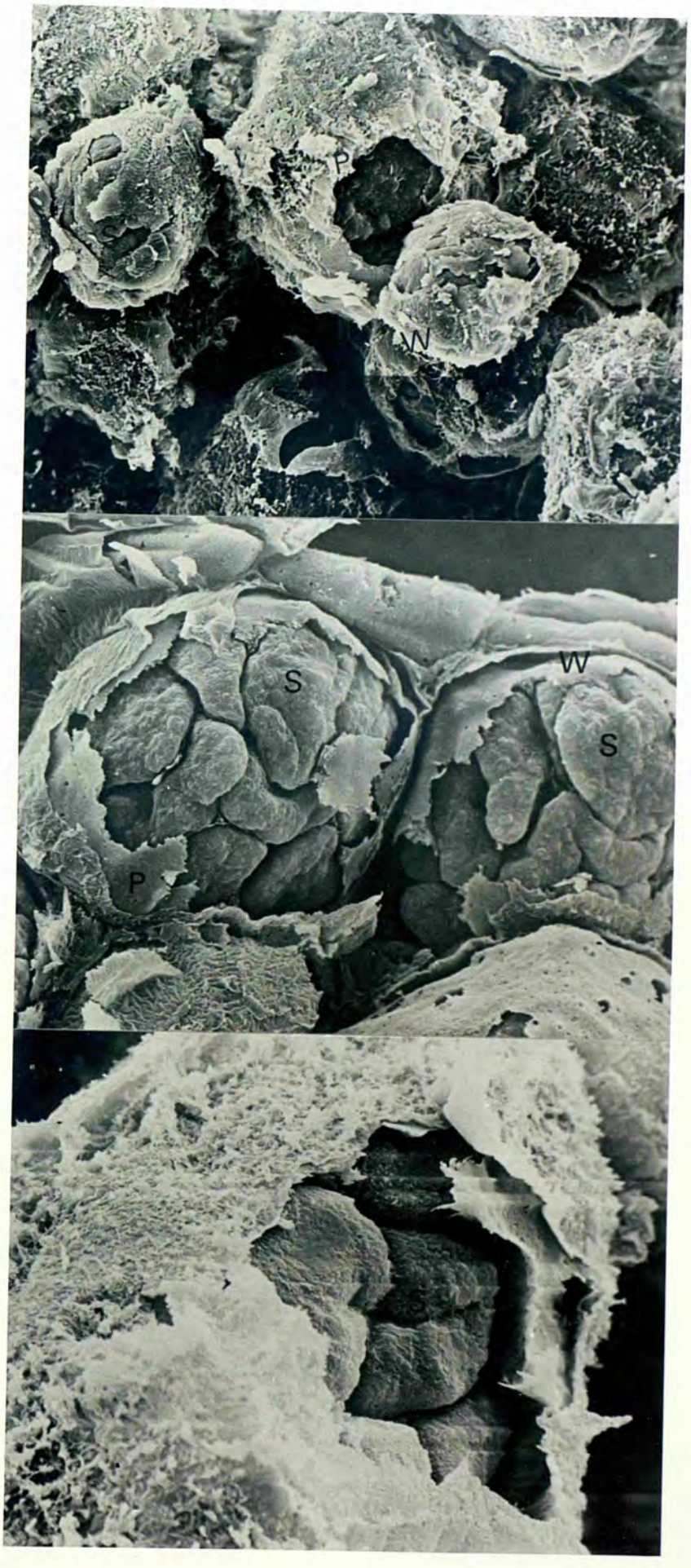

P
W
S
W
S
S
P

Fig. 41: Scanning electronmicrographs from cotyledons cells of AMR stored for 1 year at 25°C

(A) The whole view (x 110)

(B) Starch granules (x 1600)

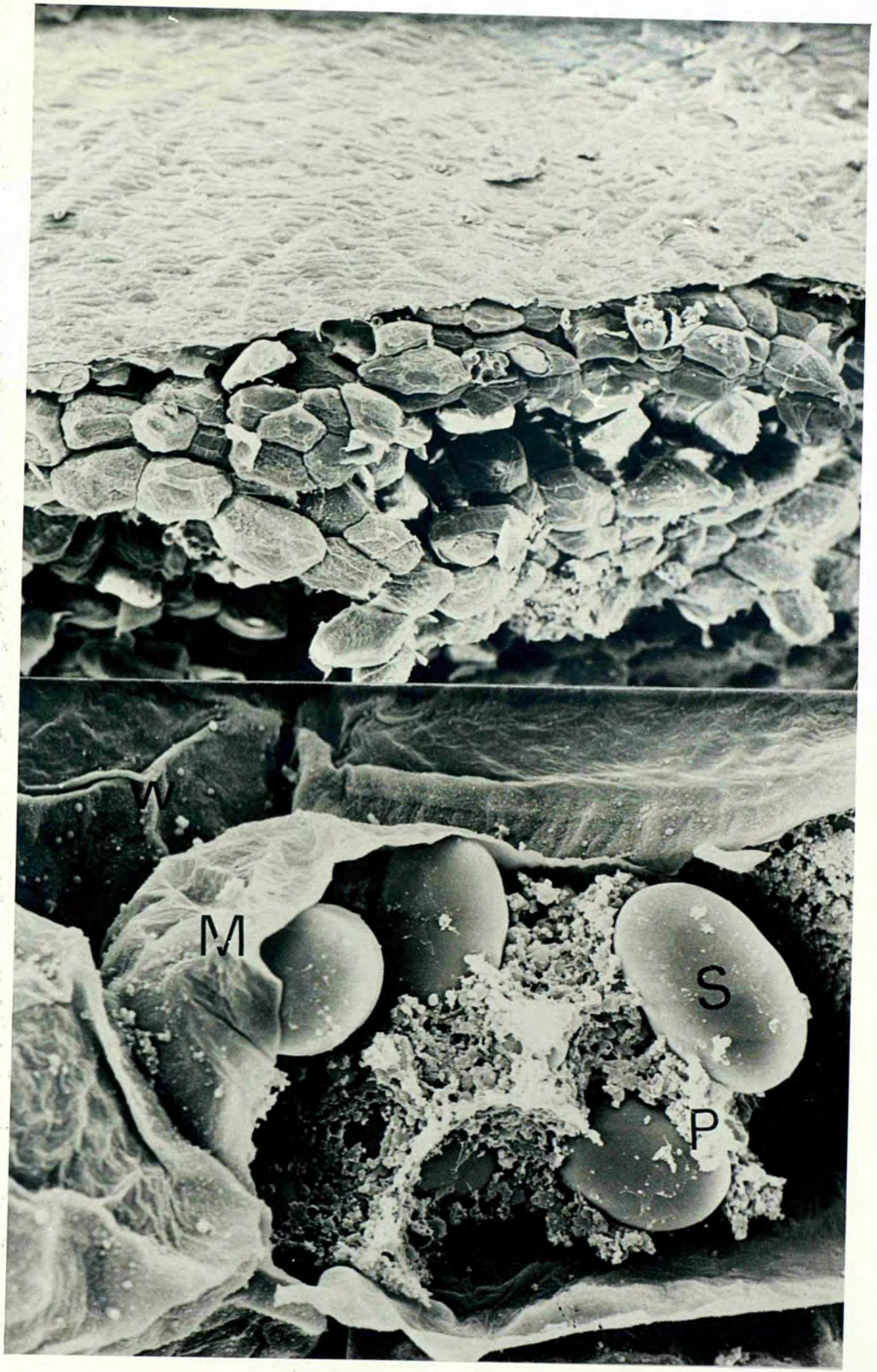

W
M
S
P

Fig. 42: Scanning electronmicrographs from cotyledon cells of AMR stored for 1 year at 35°C

(A) AMR dry seeds at 35°C, whole view (x 70)

(B) The whole (x 450)

(C) A cell containing starch granules and proteinaceous material (x 700)

S
S
P

General Discussion on Section II

The previous nutrients namely, carbohydrates, lipids, proteins and vitamins: vitamin C, thiamine and riboflavin were investigated after home processing (soaking + cooking), after canning and after storage of canned lentil seeds.

The results will be expressed on a per 100 g dry weight basis, and the variety producing the maximal amount of each nutrient discussed further.

1. Home processing

Dry unprocessed AMR seeds contain 51.1 g total available carbohydrates, and when cooked there was a significant decrease to 41.9 g. Dry SML seeds contain 4.8 g crude fibre, which increased to 5.5 g after cooking. JOR dry seeds contain 1.3 g total lipids which decreased to 1.1 g on cooking.

SML seeds contain 27.5 g (74% RDA) protein which decreased to 26.2 g (70% RDA) on cooking. Home processing therefore resulted in a slight loss in protein amino acids.

Dry JOR seeds contain 5.6 mg vitamin C (19% RDA), which decreased to 2.2 mg (7% RDA) after cooking. The thiamine content of dry AMR seeds is 0.57 mg (47% RDA), decreasing to 0.30 mg (25% RDA) after cooking. Dry LAG seeds contain 0.38 mg riboflavin (21% RDA) which decreased to 0.23 mg (13% RDA) after cooking.

Two of the chemicals ammonium carbonate and trisodium phosphate when added to the soaking solution were successfully used in the present study to reduce the cooking time of unprocessed lentil seeds.

2. Canning and storage of canned product

In the canned products, total available carbohydrates of AMR were found to be 46.0 g which decreased to 40.5 g at the end of one year storage at 25 C. Total lipids in JOR were found to be 1.0 and after one year storage at 25 C it decreased to 0.8 g. In total lipids, higher losses took place during the storage period compared to the losses due to canning. Crude fibre of canned SML product was 4.8 g, which increased to 5.8 g after twelve month storage at 25 C.

Canned SML seeds were found to contain 25.2 g (67% RDA) protein. After one year storage at 25 C it decreased to 22.6 g (61% RDA). A slightly greater loss (57% RDA) occurred during storage for one year at 35 C. Losses in protein amino acids especially after canning and storage for one year at 25 C took place. Storage for one year at 35 C caused slight increased losses in protein amino acids.

Vitamin C of canned JOR seeds was found to be 3.3 mg (11% RDA) which after twelve month storage at 25 C decreased to 2.6 mg (8% RDA) and at 35 C decreased further to 1.9 mg (6% RDA). Thiamine in canned AMR fell to 0.4 mg (33% RDA). After one year storage at 25 C it decreased to 0.32 mg (26% RDA). In canned SML riboflavin decreased from 0.38 mg (21% RDA) to 0.30 mg (16% RDA).

After one year storage at 25 C it decreased to 0.25 mg (13% RDA). Storage for one year at 35 C caused more losses in thaimine than in riboflavin.

From the above discussion it appears that storage for 12 months at 35 C resulted in more losses of most nutrients under investigation. It is strongly recommended therefore not to store canned lentils at 35 C, if at all possible.

From the present study it can be concluded, that canning followed by one year storage at 25 C or 35 C decreased the nutritive value of lentils compared to home processing. Furthermore, canning adds futher expenses to the price of dry unprocessed lentil seeds, therefore from the nutritional point of view, it is highly desirable that lentils are consumed after home processing.

CHAPTER IX

GENERAL CONCLUSIONS

Four lentil varieties: two Syrian , one Jordanian and the fourth American were used in the present study. The weight of 100 seeds in g ranged from 2.7 (SML) to 7.1 (LAG); LAG and AMR are macrosperma while JOR and SML are microsperma. The moisture content ranged from 8.1 to 10.1%. The major sugars identified in the dry seeds were stachyose (most abundant), sucrose (next most abundant), raffinose, with manninotriose in small amounts, and cellobiose in trace amounts.

In lentil seedlings grown in the dark at 25 C, monosaccharides (glucose and fructose) increased, disaccharides (sucrose) also increased, while the oligosaccharides (stachyose and raffinose) decreased at different rates, but both had disappeared by the fifth day of germination. Total available carbohydrates of lentils ranged from 46.8 to 51.1 g/100 g dry wt., ethanol-soluble sugars of the four varieties ranged from 2.49 to 3.26 g/100 g dry wt. The ethanol-soluble sugars of all four varieties were slightly higher (app. 2%) than those of other varieties as reported by BHATTY and SLINKARD (1979). During germination at 25 C in the dark total available carbohydrates decreased, starches and dextrins decreased, and soluble sugars increased.

Total lipids of the four varieties under investigation were found to range from 0.96 to 1.32 g/100 g dry wt, so they have a low lipid content. Decreases in lipid contents occur rapidly during germination. Crude fibres of the varieties tested ranged from 3.70 to 4.86 g. A substantial increase in crude fibre took place during germination.

Protein content of dry seeds varied from 24.1 to 27.6 g/100g dry wt. In lentil seedlings grown at 25 C in the dark, protein content decreased. This could be attributed to the fact that

protein synthesis had not yet started. The rate of decrease was high in the first 24 hours, thereafter it continued to decrease as germination proceeded but at a slower rate.

In lentils eighteen protein amino acids were identified: tryptophan, lysine, histidine, arginine, aspartic acid, glutamic acid, threonine, proline, glycine, alanine, cysteine, valine, methionine, leucine, isoleucine, serine, tyrosine and phenylalanine. The protein amino acids content varied from 80.8 to 83.5 g/100g protein, which was shown to decrease during germination.

The chemical score of protein ranged from 22-28, and the limiting amino acid was methionine. Chemical score (protein quality) decreased on germination, on average by 13 points.

Eight free amino acids were identified and were present in variable quantities: the predominant ones were arginine, glutamic acid and aspartic acid. During germination all free amino acids increased , with glutamic acid, aspartic acid and arginine increasing the most.

Lentil dry seeds are not a very good source of vitamin C, but they are a good source of the B vitamins, thiamine and riboflavin. Vitamin C ranged from 2.2 to 5.6 mg/100g dry wt, and at the end of the 5-day germination period, vitamin C increased 9 to 20-fold. Thiamine and riboflavin increased as well, but the increase after germination was not as high as that of vitamin C (0.6 to 0.8-fold and from 1.6 to 2.5-fold). Therefore while germinated seedlings are a better source of vitamin C, the seeds are a good source of thiamine and riboflavin.

During germination, the chemical and biochemical changes that occur in the seeds fall into two main categories:

(1) hydrolysis of certain compounds, and

(2) the synthesis of new compounds from the breakdown products formed (MAYER and POLJAKOFF-MAYBER, 1975).

As a result of germination at 25 C in the light, the rate of increase in the monosaccharides glucose and fructose and the disaccharide sucrose, was higher than in dark-grown seedlings (25 C). The decrease in the oligosaccharides, raffinose and stachyose was not as marked.

Total available carbohydrates decreased by about (18%), starches and dextrins also decreased (ranging from 18 to 29%). These results were unexpected since carbohydrates usually increase in light. The reason why this is not the case here is possibly due to the size of the leaves which are very small and therefore they can not photosynthesize fast enough for an accumulation of carbohydrates.

A rapid decrease in total lipids was observed, and they disappeared completely at the end of the 5 day-germination period.

On the other hand, crude fibres increased substantially. The protein content of seedlings grown at 25 C in the light decreased gradually; a 24% decrease of the original seed protein was observed on the fifth day after germination. In all seedlings grown in the light, the protein amino acids decreased. The decrease in essential amino acids, caused a reduction in protein quality as the chemical score was on the whole 13 points lower. Free amino acids increased in the range (x2.6 to x4.0) during germination at 25 C in the light, with glutamic acid and aspartic acid being the most abundant free amino acids.
The response of the three vitamins of lentils studied here and grown at 25 C in light was different to that grown in the dark at 25 C. Vitamin C increased significantly, but less so than in the dark; thiamine content was higher than that in the dark; riboflavin increased slightly, but more slowly than in dark-grown

seedlings.

In general terms, changes in the nutrients of seedlings grown in the light were characterised by either

(i) a gradual increase throughout the germination duration e.g. sugars and vitamins, or
(ii) a gradual decrease e.g. proteins.

Lentil seedlings grown in the light have a higher protein content than those grown in the dark. Although, the increase in vitamin C was not as high as in dark-grown seedlings, it was still found in higher amounts than in dry seeds. This is another advantage nutritionally for using light-grown lentil seedlings.

In lentil seedlings grown in the dark at the higher temperature (35 C), the monosaccharides (glucose and fructose) and the disaccharide (sucrose) increased rapidly and reached their maximum content either on the second or third day of germination, after which they decreased. The maximum content of fructose and glucose ranged from 0.96 to 1.89 g/100g and from 1.36 to 2.27 g/100g respectively, while maximum sucrose content varied from 1.67 to 2.85 g/100g. In terms of oligosaccharides, raffinose disappeared almost completely on the first day, while stachyose after three days of germination. Total available carbohydrates decreased as did starches and dextrins, while soluble sugars increased to a maximum and then decreased. Crude fibre increased substantially. Decreases in total lipids were more rapid than those of total carbohydrates and of proteins, and dissappeared in all varieties on the fourth day of germination.

In lentil seedlings grown at 35 C in the dark, the decrease in protein content was rapid during the first 24 h of germination, after which it decreased gradually. At the end of germination time about 45% of the protein was lost. At 35 C all protein amino acids decreased, as did protein quality. The protein chemical

score at the end of the germination period decreased by 14 points. The range of free amino acids increased from 3.7 to 5.8-fold. During germination of lentils at the higher temperature (35 C), vitamin C, thiamine and riboflavin showed similar responses: the trend was a rapid increase during the first 48 h or so, followed by a gradual decrease. Vitamin C at the end of the 5 day-germination period was still higher, thiamine was found in much lower amounts, and riboflavin was found to be just slightly lower than in dry seeds.

The higher temperature of 35 C exerts a considerable influence on the chemical and biochemical components studied in germinated lentils: in the early stages of germination there is either a rapid early increase followed by a decrease as in the case of mono- and di-saccharides, and vitamins or by a rapid decrease as in the case of proteins. Dark-grown lentil seedlings at 35 C were found to have lower protein quantity and quality (chemical score) compared to those at 25 C or those grown in the light at 25 C.

By germinating lentils in the dark at 25 C there is an increase in certain vitamins e.g. vitamin C, thiamine and riboflavin. Although these three vitamins increased in lentils germinating in the light at 25 C, their increases were not as high as those in the dark at 25 C. Lentils grown at 35 C in the dark suffered a higher nutritional loss.

Results from the present study have shown that germination at 25 C in the light has advantages in terms of protein, over germination in the dark at 25 C. On the other hand, seeds germinated at 35 C in the dark were not nutritionally as good as those in the other two cases. Therefore it is recommended to germinate lentil seeds at 25 C in the dark.

The four varieties of lentil seeds (AMR and LAG in particular) used in the present study were found to be good both in physical and in chemical terms. Therefore it would be advisable to

introduce these varieties to areas like Ethiopia and Yemen to replace existing varieties which have been found not to be as good in these respects (SOLH and ERSKINE, 1981).

Lentils subjectd to home processing (cooking)

Sucrose, raffinose and stachyose decreased after home processing (cooking), and losses in sucrose were greater than in stachyose. Sucrose retention ranged from 45 to 63%, raffinose retention varied from 36 to 46% and stachyose retention was found to range from 59 to 74%. Total available carbohydrates in AMR, LAG JOR and SML before cooking were 51.1, 49.2, 47.1 and 46.8 g/100g dry wt. and after cooking they were 41.9, 41.5, 38.2 and 36.5 g/100g respectively. Starches and dextrins retention ranged from 79 to 86%, and soluble sugars retention varied from 56 to 66%.

Crude fibre increased significantly after cooking, while total lipids appeared to decrease but this was found not to be significant. Proteins in AMR, LAG, JOR and SML before cooking were 24.1, 26.4, 25.3 and 27.5 g/100g dry weight respectively, and after cooking decreased to 21.9, 24.5, 24.6, and 26.2g/100g. The retention in total amino acids ranged from 88 to 92%. Protein quality decreased, as the chemical score decreased by 4-5 points, and free amino acids decreased to trace amounts.

Vitamin C retention was found to range from 33 to 45%. Thiamine retention was found to range from 44 to 54% and riboflavin retention varied from 59 to 65%.

Cooking, like any other processing, affects the stability or retention of nutrients, especially vitamins. It is not always possible to make generalisation about the effect of processing on vitamins, since a number of factors are important e.g. types of food, cooking time, temperature, exposure to air and protein

complexes in the food which may have a protective effect on vitamins (BENTERUD, 1977). Generalisation therefore is not possible in such cases. It is necessary then to cook each food under known conditions, and to determine the actual effect.

In an attempt to improve the cooking quality of lentils, they were soaked in various chemicals for a set time (p192). Ammonium carbonate followed by trisodium phosphate gave the best results in terms of decreasing the cooking time. In the present study, although the results obtained using different chemicals were not altogether successful, there was a hint that further work was required for lentils, since soaking certain other legumes in certain chemicals has proved very successful (ROCKLAND and METZLER, 1967; MEHROTRA, 1983).

Canning

Parameters for canning e.g. time and temperature for many foods are well established because the canning of such foods has been done for a long time now, while for other foods, including lentils, this has not been so. The difference is that the canning of these latter foods is more recent, and information on these is sadly lacking.

For canning lentil seeds they were processed at 245 F for 30 min (p.203). Total available carbohydrates before canning in AMR, LAG, JOR and SML were 51.5, 49.2, 47.1 and 46.8 g/100g dry wt, and after canning they were 45.0, 46.6, 43.5 and 41.8 g/100g respectively. After one year storage at 25 C they were found to be 40.5, 41.1, 39.7 and 38.1 g/100g respectively. Starch retention after canning ranged from 89 (SML) to 95%(LAG). Storage for twelve months at 25 C resulted in larger decreases. Soluble sugars retained after canning ranged from 76 to 84% and after one year storage ranged from 54 to 69%. Canning decreased sucrose, raffinose and stachyose. Storage of canned lentils at 25 and 35 C led to significant decreases in sucrose, raffinose and

stachyose. The monosaccharides (glucose and fructose) increased, which could be due to the hydrolysis of sucrose.

Crude fibre increased significantly as a result of canning and more increases occurred after one year storage at 25 C. Total lipids decreased slightly after canning and a significant decrease was observed after storage at 25 C.

Protein decreased slightly as a result of canning and more losses occurred after one year at 25 and at 35 C. Protein retention after 12 month-storage at 25 C ranged from 79 to 83%, at 35 C losses took place which were not highly significant. The effect of the storage temperatures of 25 and 35 C on protein was therefore very small. Canning decreased the total amino acids of lentils, and the decrease ranged from 70.8 to 73.4 g/100g. The amino acids most affected were: lysine, methionine, tryptophan, valine, and histidine. On storage for one year at 25 and 35 C further losses occurred. After one year storage, the two temperatures (25 and 35 C) have a neligible effect on total amino acids. Free amino acids retention after canning ranged from 58 (LAG) to 64% (SML). Storage for twelve months at 25 C resulted in further losses.

The three vitamins: vitamin C, thiamine and riboflavin

Vitamin C retention ranged from 57 (AMR) to 63% (LAG) after canning. After twelve months storage at 25 C, its retention ranged from 41 to 51%. At the end of 12 months storage at 35 C vitamin C retention was found to range from 25 to 35%. The rate of vitamin C loss at 35 C was therefore higher than at 25 C. Thiamine retention as a result of canning ranged from 68 to 75%. After twelve month storage at 25 C its retention ranged from 50 to 57% and at 35 C its retention varied from 44 to 49%. Riboflavin retention after canning ranged from 71 to 81%. After one year storage at 25 C, riboflavin retention ranged from 54 to 65% and at 35 C its retention varied from 47 to 56%. Losses in riboflavin

and thiamine as a function of storage time and temperature were found to be small compared to those of vitamin C.

Maximising nutrient retention for thermal processing has received a lot of attention recently, and for optimisation, each heating process and product must be considered individually (PRIESTLEY, 1979).

Storage temperature seems to be important as far as nutritional quality of canned lentils is concerned. Storage at 25 C appears to be more desirable than at 35 C. Higher storage temperature has a greater significant effect on vitamin C than on the other vitamins. So, although the advantages and benefits of food processing (canning) are numerous (p.199), it is important that certain precautions are taken during the storage of canned lentil seeds. It would be advantageous from a nutritional viewpoint to store them at the lower temperature (25 C) although, in certain countries the summer temperature can be as high as 35 C or even higher. It is advisable then not to store the cans at 35 C if at all possible.

Scanning Electron Microscopy

In the present study, the cells in the four varieties of lentils investigated ranged from 52 to 80 µm in diameter. The chief reserve is starch, present as granules ranging from 18.5 to 25.7 um in diameter. The cells and starch granules are smaller than those of other legumes e.g. beans (Phaseolus vulgaris) with cells about 160 µm in diameter and starch granules up to 50 µm in diameter (OPIK, 1966). Protein was observed as thin membranes surrounding the starch granules. During cooking starch granules changed shape, became rough and lose their birefrigence.

FINAL CONCLUSION

The world is faced with an increasing population problem and it is important therefore that enough food is available so that every one gets enough of the proper nutrients. FAO (1964) has issued the following guidelines:

1.In most underdeveloped regions it has been shown that protein intake is less than it was some 20 years ago.

2.The production of animal food e.g. meat, egg and fish is increasing slowly but is not keeping pace with the rise in world population.

3.Therefore it is essential to increase the supply of grains, especially those of legumes. This offers a partial solution to the problem of increasing the supply of world protein, and this increase can be achieved in a relatively short time.

Greater consumption can be brought about by increased availability of legumes and their introduction as new processed foods.

Lentils are nutritious and could play an important role in man's diet. In many countries like India the production of lentils in 1975 was nearly double that in 1966.

The canning of lentils has started recently in some countries e.g. U.S.A. which can supply these to the underdeveloped countries. This however costs money which the poorer countries can hardly afford. Further, commercial starch can be produced from lentil seeds and would afford a good revenue.

Lentils are considered to be the most easily digestible of grain legumes and are often served to hospital-patients for this reason.

A start has been made here to show the various nutrients that can be supplied by this commodity. Further research is required in an attempt to produce new lines in lentil seeds which would not only be bigger in size, larger in yield but would also have certain nutritional features and characters which would allow them to grow under extreme conditions. Finally humans should be encouraged to consume lentils in larger amounts to provide them with these essential nutrients and vitamins in a diet sadly lacking in them.

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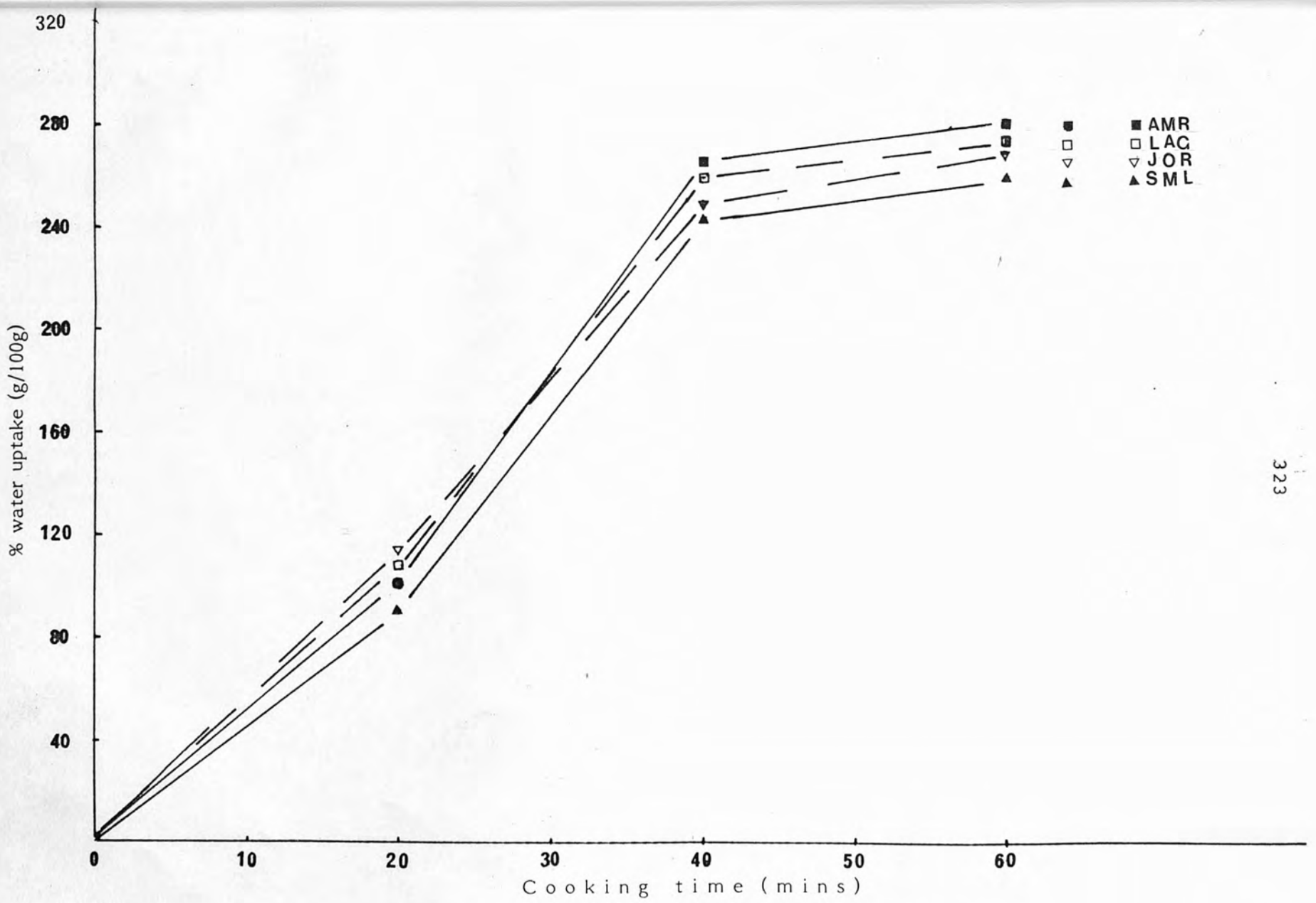
Appendix 1. For converting the difference in millilitres of 0.1N thiosulphate needed for the filtrate and the blank determination $(V_0 - V) \times 10 \times T$ to glucose

ml of O.IN thio $(V_0 - V) \times 10 \times T$	Glucose (mg)	Δ
1	2.4	
2	4.8	2.4
3	7.2	2.4
4	9.7	2.5
5	12.2	2.5
6	14.7	2.5
7	17.2	2.5
8	19.8	2.6
9	22.4	2.6
10	25.0	2.6
11	27.6	2.6
12	30.3	2.7
13	33.0	2.7
14	35.7	2.7
15	38.5	2.8
16	41.3	2.8
17	44.2	2.9
18	47.1	2.9
19	50.0	2.9
20	53.0	3.0
21	56.0	3.0
22	59.1	3.1
23	62.2	3.1

APPENDIX 2: Essential amino acids (EAA) of reference protein, (whole egg) (FAO, 1973; OSBORNE and VOOGT, 1978)

Lysine	Tryptophan	Isoleucine	Leucine	Valine	Methionine & cysteine	Threonine	Phenylalanine & tyrosine	Histidine	TOTAL EAA
6.6	1.7	5.8	8.9	7.4	5.7	5.0	9.3	2.2	52.6



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Appendix 3: Effect of cooking time on the % water uptake of lentils (Fig. 19)