LIPID PEROXIDATION AND AGEING IN SEEDS OF CABBAGE AND SOYA BEAN.

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Submitted in partial fulfilment of the
requirements of the degree of
Master of Science
in the
Department of Biology
University of Natal
Durban
1986

"Of seeds
some have more vitality than others
as to keeping."

Theophrastus (285 BC).

PREFACE

The work described in this thesis was carried out in the Department of Biology, University of Natal, Durban from January 1985 to December 1986 under the supervision of Mr M T Smith and Dr G K Campbell.

These studies represent the original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

ACKNOWLEDGEMENTS

I wish to thank my supervisors, Mr M T Smith and Dr G K Campbell, for their advice and assistance in the completion of this thesis, and Mr M Dhlamini for his help in the preparation of the graphic material.

I would also like to acknowledge the greatly appreciated use of the photocopying facilities of F G Knights and Son, (PTY) Ltd , as well Mayford Seed Company (PTY) Ltd for going out of their way in providing suitable seed material for this study.

This work was carried out with the financial support of the Council for Scientific and Industrial Research.

ABSTRACT

It has been suggested that lipid peroxidation is involved in the loss of seed vigour, although many attempts to examine the relationships between lipid peroxidation and seed vigour have proved equivocal. Studies were undertaken on seed lots of cabbage and soya bean to find evidence for peroxidation by the analysis of i) total and polar fatty acid levels; ii) lipid hydroperoxides; iii) volatile products produced on heating dry seeds; and iv) volatile products produced on imbibition.

The loss of polyunsaturated fatty acids (PUFAs) detected in the dry seeds was clearly related to germinability in both soya bean and cabbage seeds. Furthermore, an increase in hydroperoxides was observed in both seed types. Although the relationship of the level of hydroperoxides to germinability was less clear than for the decline in the level of PUFAs, these results suggested that the loss of PUFAs was possibly due to the peroxidation of the seed lipid, indirect evidence obtained from the heating of the seeds suggesting that hydroperoxide breakdown may be necessary in order that the changes in PUFAs become apparent.

In contrast to the poor relationship observed between germinability and hydroperoxide level, a marked relationship between hydroperoxide level and seed moisture content was observed in the cabbage seeds. This may be significant with regard to the observed relationship between storability and seed moisture content, although no such relationship was seen in the soya beans.

Certain volatile compounds derived from dry heated seeds were related to seed vigour in both seed types and evidence suggests that the lipid hydroperoxides were the source of these compounds.

Although the total volatiles counts evolved from imbibing cabbage seeds showed no quantitative relationship to seed vigour, one peak was noted which was clearly associated with the vigour of these seeds. The variability in the volatiles evolved from soya beans on imbibition, however, precluded the detection of any possible relationship between these and seed vigour. In both seed types, results suggest that the volatiles derived on imbibition were of a different source to those derived on heating.

A marked increase in the level of hydroperoxides was observed in whole cabbage seeds and soya bean axes of low vigour over the first hour of imbibition. This may suggest that an exacerbation of damage on imbibition was associated with low vigour seeds. In contrast to this, in the seeds of high vigour, the increase in hydroperoxide levels was markedly less or rapidly reduced, suggesting the possible activity of repair mechanisms.

Ferrous ions were shown to invigorate both seed types, particularly cabbage seeds. It is suggested that the invigorating effect of these compounds was due to the facilitaion of repair, including hydroperoxide breakdown and the quenching of any free radicals.

CONTENTS

INTRODUCTION

1.SEED AGEING.

Plant seeds constitute some of the longest lived assemblages of cells that do not undergo cell division. Despite the exceptional longevity exhibited, all seeds gradually lose vigour and ultimately viability. That seeds age has long been recognized. The underlying causes, however, remain to be determined.

Seeds can broadly be divided into two groups; recalcitrant and orthodox. Recalcitrant seeds are difficult to define, but can possibly be described as seeds which do not undergo dehydration on maturation. The loss of seed viability usually results if moisture content falls below a certain critical level. Orthodox seeds, on the other hand, upon the completion of maturation, enter into a period characterized by low moisture content and metabolic quiescence, with or without the imposition of dormancy. For further growth to proceed, the seeds must imbibe sufficient quantities of water to attain a moisture content conducive to germination. Depending on the environmental conditions, seeds may experience extended periods of time in the dry state. It is during this period of time, i.e. between maturation and germination, that seeds will age. All seeds, however, do not age at the same rate. Some can survive for very long periods of time, while others may not survive one growing season.

Although the phenomenon of ageing in general is well known, the underlying causes and mechanisms of ageing are still poorly understood. Two main schools of thought prevail (Kirkwood,1984). The one proposes that ageing is coded for by the genome, and primarily attempts to explain the observation that certain cell lines exhibit a marked increase in mortality after a

certain period of growth, while others are apparently 'immortal'. The other school of thought suggests that ageing is due to the stochastic accrual of deleterious changes within the cells that predispose it to death. Death follows when a critical level of damage occurs, resulting in an 'error catastrophe' (Holliday,1984).

Opinions vary as to which of these two theories apply, although it is probable that this depends very much on the specific organism under consideration - it is unlikely that all organisms age in the same way. There is also disagreement with regard to the origin of the changes that initiate the onset of ageing, some suggesting that it is the nucleus, while others favour a cytoplasmic origin (reviewed by Aufderheide,1984).

It is generally agreed that the loss of seed vigour is due to the gradual, random accumulation of damage by the seed tissue, leading to an error catastrophe (Osborne,1980). This process begins immediately following maturation, the rate of vigour loss being dependent on the rate and site of damage. Theoretically, longevity may be achieved in two ways. Firstly, the rate of the accumulation of damage may be reduced, delaying the attainment of the critical level of damage. Secondly, if the seeds show some form of dormancy, a high seed moisture content may be permitted without germination occuring. Under such conditions, damage may not only be prevented, but also repaired (Villiers,1974; Osborne,1982). In the absence of the above two mechanisms, the rate of vigour loss is thought to be determined largely by the conditions experienced by the seeds preceeding and following maturation. That is, the history of the seed during development and the temperature, moisture content, and the availability of oxygen in the gaseous environment during storage (Osborne,1980).

1.1.Pre-storage conditions that may effect seed vigour.

Pre-storage conditions that can markedly influence the vigour of the seed as well as its ability to withstand long periods of dry storage, will be discussed in the following sections.

1.1.a.GENETIC DIFFERENCES.

Some seeds appear to be genetically superior to others, in that they show a more vigorous growth and higher yields. This may even be apparent between seeds produced by the same parent plant at the same time (Bewley and Black,1982).

1.1.b.SPECIES DIFFERENCES.

Species differences can also have a marked effect on seed longevity. For example, Canna compacta seeds are reported to have survived c600 years and Nelumbo sp. for c237 years. Hard coated legumes such as Cassia, Albizzia and Mimosa are also very long lived, in marked contrast to other legumes such a soya beans (Osborne,1980).

1.1.c.DIFFERENCES IN MORPHOLOGY.

Differences in morphology can also influence vigour, larger seeds from a particular seed lot often being more vigorous than smaller ones. This may reflect the environmental conditions at the time of maturation, or be due to premature harvesting (Bewley and Black,1982).

1.1.d.MECHANICAL DAMAGE.

The means of harvesting the seed as well as post-harvest handling can also influence vigour due to the generation of mechanical damage such as bruising or fracturing of the seed tissue. This depends very much on the type of seed (Moore,1972), legumes being reported to be particularly susceptible to this kind of damage.

1.1.e.MICROFLORA.

Microflora, particularly fungi, can have a marked influence on seed vigour, particularly under humid storage conditons (Bewley and Black,1982). A causual relationship between infection and vigour has not, however, been convincingly demonstrated. That is, it is still not clear whether a certain degree of damage or ageing must have occured to allow for infection, or whether infection is independent of seed quality. Nevertheless, that infection by fungi can accelerate the loss of vigour is generally accepted (Roberts,1972).

1.2.Storage conditions and loss of vigour.

The conditions of storage, namely temperature and relative humidity (and hence seed moisture content), and to a lesser extent, the presence of oxygen, are the main factors thought to be responsible for the loss of seed vigour during dry storage (Bewley and Black,1982). It has been estimated that storage life may be doubled for each 1% reduction in seed moisture content or for every 5°C lowering of the temperature of storage (Harrington,1973).

This, however, only applies to a very narrow range of moisture contents and temperatures (Roberts and Ellis,1982). Although seed vigour will decline with increasing temperature and seed moisture content, the relationship is not linear. At moisture contents less than 4-5%, the seed is often damaged. The degree of damage depends on the seed type, and is thought to be due to the physical disruption of membranes at these moisture levels. Furthermore, at high moisture contents, the rate of the loss of vigour may be greatly reduced. It has been suggested that this may be due to a higher metabolic activity under these conditions, allowing for the repair of any damage (Villiers,1974).

Similarly, many seeds, particularly those of tropical origin, exhibit a sensitivity to temperatures lower than 10-12°C (Lyons,1973). This is thought to be due to the disorganisation of membranes, leading to the impairment of membrane related functions such as respiration and energy production.

The role of oxygen in the loss of seed vigour is equivocal. It appears to act synergistically with either temperature or moisture content (Ohlrogge and Kernan,1982), but does not seem to be of primary importance (Osborne,1980).

In dry storage, a number of cytological and physiological changes that have been observed to occur in aged seeds. These have been presented as possible causes of the loss of seed vigour and include a) damage to the genome, b) impairment of respiration, c) damage to protein and RNA synthesising machinery, d) loss of enzyme activity and e) loss of membrane integrity.

1.2.a.DNA INTEGRITY.

An increase in chromosomal aberrations was one of the first changes suggested to be involved in the loss of seed vigour (de Vries,1901). Navishin (1933) has reported that more than 80% of *Crepis tectorum* plants grown from old seeds showed chromosomal aberrations. More recently, Roberts *et al.*(1967) demonstrated a strong correlation between seed vigour and chromosome aberrations in rapidly aged peas, beans and barley. In their study, the percentage aberrations were closely related to the conditions of ageing. A similar result was been reported by Murata *et al.*(1980), also working on rapidly aged barley seeds.

The causes of these changes have not yet been clarified. The mutagenic effect of X-radiation led to the suggestion that normal background radiation may be responsible for the increase in aberrations (Roberts,

1983). However, Giles (1940) and Gundhardt et al.(1953) have both demonstrated that background radiation is insufficient to account for the mutation rates observed in the seeds they investigated. Furthermore, it is evident from work done by Roberts et al.(1967) and Murata et al.(1980) that the frequency of mutation is related to the physiological rather than the chronological age of the seed. This may suggest that it is the conditions of storage and not the duration of storage that is primarily responsible for the increase in damage to the DNA.

The accumulation of mutagenic metabolites has also been implicated as a cause of genetic damage (D'Amato,1954). Evidence for this has, however, proved equivocal. A number of workers have reported a mutagenic activity of extracts from aged seeds on fresh seeds (Floris and Anguillesi,1974), while others have observed no such deleterious effects (Roos,1982).

Another proposed cause of genetic damage is the action of DNases. DNA isolated from non-viable rye seeds is of low mean molecular weight and greatly fragmented (Osborne,1980). It has been suggested that this might be due to the activity of endodeoxyribonucleases. It is proposed that in fresh seeds, DNase activity is inhibited, but on ageing, this inhibition is lost, leading to DNA fragmentation. Alternatively, the impairment of DNA repair mechanisms might be responsible for the observed fragmentation, either directly due to loss of enzyme activity, or indirectly due to the failure of membrane integrity (Roberts and Ellis,1982). Loss of membrane integrity may also lead to the release of hydrolytic enzymes (Berjak and Villiers,1972).

Reduced oxygen species may also be responsible for chomosomal aberrations. Petry (1921) has shown that the presence of oxygen increased the levels of radiation damage in seeds. Furthermore, Lesko et al.(1980) has

demonstrated that reduced oxygen species, particularly the hydroxyl radical, was responsible for increasing single-stranded scissions in DNA *in vitro*. This may be significant with regard to the greater percentage of chromosomal aberrations observed in barley seeds aged under hyperbaric oxygen conditions (Roberts *et al.*, 1967).

There are a number of observations, however, which contradict the suggestion that damage to the genome is a major factor in the loss of seed vigour. Firstly, seeds appear to show an extremely large variation in the level of aberrations needed to result in a decline in vigour. For example, Roberts *et al.* (1967) has shown that bean seeds of 10% germinability had only a 12% level of aberrant genomes, while Harrison (1966) has reported that lettuce seeds with approximately 90% aberrant genomes had germinabilities of 50%. Secondly, poor correlations between chromosomal aberrations and germinability has been reported for X-radiated seeds (Abdul-Baki and Anderson,1972), while inactivation of the genome by gamma-irradiation did not impair radicle emergence in lettuce seeds (Haber and Luippold,1960). Furthermore, while treatments which resulted in the greatest degree of chromosomal aberrations in broad bean seeds also resulted in the least loss of germinability (Roberts *et al.*,1967), no increase in aberrant genomes was observed in rapidly aged lettuce seeds despite a fall in germinability from 100% to 10% (Harrison,1966). This may suggest that the increase in chromosomal aberrations may not be a cause, but rather a consequence of the loss of seed vigour.

1.2.b. RESPIRATION.

Opinions vary with regard to the changes in respiratory efficiency that occurs with declining vigour. Some workers (Kittock and Law,1968) have

reported a significant positive correlation between respiration and seedling vigour in wheat, while others (Anderson,1970) have reported no correlation between respiration as measured by oxygen uptake, and seed vigour, also in wheat. A negative correlation between respiratory quotient and vigour was, however, observed. Similarly, a good correlation has been observed between the degree of seed mechanical damage and respiratory quotient, but not oxygen uptake (Woodstock,1967). It has also been shown that the rates of respiration bore a better relationship to vigour during 2-6 hours (imbibition) and 20-30 hours (radicle emergence) after imbibition than during an intermediate period (Woodstock,1967). Furthermore, ageing can occur at different rates in different parts of the seed, and this may lead to a masking of changes in individual parts (Bewley and Black,1982).

Two patterns have, however, emerged i.e. an increase in respiratory quotient and a decline in oxygen uptake is often associated with seeds of low vigour. The reasons for this are not known, although Woodstock (1973) has suggested that this relationship may be indicative of a fundamental mechanism behind the loss of vigour viz. mitochondrial membrane integrity. This is supported by a number of observations. Abu-Shakra and Ching (1967) have reported a much higher P:O ratio from mitochondria isolated from fresh compared with aged soya beans. This may suggest that the mitochondria from aged seeds were endogenously uncoupled. Ching and Danielson (1972) have reported that aged lettuce seeds had lower ATP levels than fresh seeds, while the loss of mitochondrial structural integrity has been shown to be the first lesion to accompany the loss of vigour in rapidly aged maize (Zea mays) seeds (Berjak and Villiers,1972).

Loss of membrane integrity is not the only lesion

that could be responsible for the decline in respiratory activity. Impairment of associated enzymes may also be involved. Indeed, one of the most successfully applied tests for seed vigour to date, the tetrazolium test, is based upon the activity of dehydrogenases (Woodstock, 1973).

1.2.c.PROTEIN AND RNA SYNTHESIS.

Damage to long-lived mRNA has been suggested to be responsible for the loss of rye seed vigour. It has been proposed that protein synthesis immediately following imbibition is coded for by long-lived mRNAs and translated on 'old'ribosomes, or alternatively it is coded for by newly synthesized mRNA transcribed on imbibition (Osborne,1980). A number of workers agree that the decline in protein synthesising activity in aged seeds is due to the impaired processing of stored mRNA and an impaired synthesis of new mRNA (Smith and Bray,1982; Grilli et al.,1982). The reasons for this are not clear. PolyA-RNA isolated from non-viable rye seeds was still of high template activity, capable of coding for a number of discrete proteins. That these seeds were unable to synthesize protein may suggest that lesions to protein synthesis had occured elsewhere (Osborne,1980).

Ribosomes may also constitute a possible site of damage. It has been suggested that the loss of ribosomal integrity observed in aged rye seeds was due to the action of ribonucleases (Osborne,1980). The involvement of hydrolytic enzymes has also been implicated by Brockelhurst and Fraser (1980) and Grilli et al.(1982). This is supported the work of Ghosh and Chaudhuri (1984). These workers have shown that the loss of protein synthesising ability in aged rice was associated with an increase in RNAse activity. Therefore the release or activation of hydrolytic enzymes may be involved in the

above changes.

Damage to the enzymes associated with transcription and translation have also been implicated in the loss of protein synthesising capacity. It has been shown that the loss of activity of the transferase enzyme 1 closely parallels the loss of protein synthesising activity in seeds and embryos and closely reflects the extent of cellular damage (Bewley and Black,1982). Furthermore, Perl et al. (1978) has shown an increase in protease activity in rapidly aged sorghum and suggest that the loss of seed vigour was due to this protease activity.

Other factors that have been implicated include damage to the DNA template (Osborne,1982) and the depletion of ATP levels (Ching,1982). It has been suggested that the low rates of biosynthetic activity observed in aged rice seeds was due to a loss of the ability to synthesize and accumulate ATP rather than a loss of protein or RNA synthesis (Banerjee et al.,1980). This in turn may reflect a loss of membrane integrity (Roberts and Ellis,1982).

1.2.d.LOSS OF ENZYME ACTIVITY.

Apart from changes in enzymes of the protein synthesising machinery, a decline in the activity of other enzymes such as catalases, phosphatases and dehydrogenases has been shown to accompany the loss of seed vigour (MacLeod,1952). It appears that one of the manifestations of heterosis in maize is found in the superior biochemical activity of the NAD-linked mitochondrial enzymes, leading to more efficient electron transport and oxidative phosphorylation (Bewley and Black,1982). Furthermore, Perl et al. (1978) has demonstrated an increasing protease activity in rapidly aged sorghum. However, it is usually difficult to determine if the loss of enzyme activity is a cause or consequence of the loss of seed vigour. Furthermore, the

change in enzyme activity is often different under different ageing conditions. Grabe (1964) reported a high positive correlation between glutamic acid decarboxylase (GADA) activity and seed vigour in corn, while James (1968) has reported that GADA activity remained high even after the loss of viability in rapidly aged beans.

drop

1.2.e.MEMBRANE INTEGRITY. — get from literature.

A consistent observation in early ageing studies has been the increase in electrolyte leakage from less vigorous seeds (Ching and Schoolcraft,1968; Abdul-Baki and Anderson,1972). This is thought to be due to damage incured by the membranes during ageing, leading to the loss of semi-permeability. Furthermore, ultrastructural studies on rapidly aged maize showed that the loss of vigour observed was accompanied by an increase in cellular disorganization, and that practically all the degenerative changes in the ultrastructure could be the result of a general deterioration of the lipoprotein membranes of the cells (Berjak and Villiers,1972). The importance of the structural and functional integrity of cell membranes is self evident. This, together with the above mentioned observations, has led to the suggestion that changes in membrane integrity may be associated with the loss of seed vigour.

Damage to the plasma-membrane. The loss of plasma-membrane permeability has often been implicated in the increased leakage of solutes from aged seeds (Parish and Leopold,1977). Damage to the membrane constituents may lead to an impairment of normal membrane integrity, resulting in the loss of selective permeability. Simon (1974) has suggested that the initial leakiness of the seed during imbibition was due to the disorganisation of the membrane in the dry state. Alternatively, it has

been suggested that this leakiness may in fact be due to a reduced ability of the seed to utilize the reserves released on imbibition, leading to increased levels of these metabolites in the steep water (Bewley and Black,1882). However, Loomis and Smith (1980) have demonstrated a greater loss of K^+ and Cl^- from aged cabbage seeds. This may suggest that membrane permeability *per se* has been influenced. A further explanation has been put forward by Chabot and Leopold (1982). These workers suggest that the initial leakiness of the seeds may be due to the progress of cell expansion during imbibition that requires the "blebbing of large amounts of supplementary lipid, and this blebbing process may be associated with extensive solute leakage".

Damage to Mitochondrial Membranes. A number of workers have implicated damage to the mitochondrial membranes as a possible cause of the loss of seed vigour. Woodstock (1973) suggests that loss of mitochondrial membrane integrity may constitute the "fundamental mechanism" responsible for the loss of vigour in seeds. In their ultrasructural study of rapidly aged maize seeds, Berjak and Villiers (1972) noted that the onset of mitochondrial damage was one of the first symptoms to appear and suggest that it was this damage that was most critical to the loss of vigour in the seed investigated.

As has already been discussed, significant correlations between oxygen uptake, respiratory quotient and seed vigour have often been reported (Woodstock,1973). Furthermore, Woodstock and Taylorson (1981) and Gorecki *et al*.(1985) have demonstrated a significant correlation between seed vigour and the amount of ethanol and acetaldehyde produced from soya bean and pea seeds on imbibition. They suggest that this was possibly due to impaired mitochondrial function,

resulting in an imbalance between the glycolytic and mitochondrial respiratory payhways.

It has also been shown that mitochondria isolated from aged soya bean seeds take up 10%-40% more oxygen than those from fresh seeds, while the P:O ratios were 3.0 and 1.4 for the aged and unaged seeds respectively, suggesting that the mitochondria from aged seeds were endogenously uncoupled (Abu-Shakra and Ching,1967). In addition, a significant correlation between ATP content and seed vigour has been reported for lettuce seeds (Ching and Danielson,1972). Similarly, the ATP content of non-viable crimson clover (Trifolium incarnatum) seeds was less than 1% of that in the viable seeds (Ching,1973). All the above observations are consistent with the suggestion that mitochondrial membranes may be involved in the loss of seed vigour. However, the loss of membrane integrity is not the only factor that could cause the above changes.

2.LIPID PEROXIDATION.

2.1.The Free Radical Theory of Ageing.

A theory that is gaining increasing attention with regard to ageing in general is the free radical theory of ageing. This assumes that there is a single basic cause of ageing, modified by genetic and environmental factors, and postulates that free radical reactions are involved in ageing and related disorders (Harman,1981). One possible example of such free radicals is the superoxide radical. This can be produced directly by a number of enzymes, particularly peroxidases and dehydrogenases, in both plants and animals (Halliwell and Gutteridge,1985). Under normal circumstances, no toxicity results. This is thought to be due primarily to the action of superoxide dismutase (SOD), which is responsible for converting the superoxide into hydrogen

peroxide and oxygen (1).

$$2O_2^- + 2H^+ \text{------}\rightarrow H_2O_2 + O_2 \text{ ..... (1).}$$

The discovery of SOD therefore led to the proposal that the superoxide radical is a major factor in oxygen toxicity and that SOD is an essential defense against it. Toxicity will result if the production of the radical exceeds the ability of the cell to quench it or due to a failure or impairment of the defense mechanisms.

Another possible pathway for the onset of toxicity due to the production of the superoxide has been proposed. Under normal conditions, the hydrogen peroxide produced by SOD is broken down by the action of enzymes such as catalase and glutathione peroxidase. Under adverse conditions, however, the hydrogen peroxide may be broken down by a Haber-Weiss type reaction to yield the hydroxyl radical (2).

$$H_2O_2 + O_2^- \text{------}\rightarrow \cdot OH + OH^- + O_2 \text{ ..... (2).}$$

It is this radical that is thought to be the major cause of cellular damage *in vivo*. The second-order rate constants of the classical Haber-Weiss reaction are, however, virtually zero in aqueous solution, making its occurance *in vivo* very unlikely. This has led to the proposition that the reaction may be catalysed *in vivo* by traces of transition metals, particularly iron. It is envisaged that the superoxide reduces any traces of ferric iron present (3) which in turn reduces hydrogen peroxide by a Fenton type reaction (4) to produce the hydroxyl radical. The source of these iron complexes *in vivo* is not known, but may include low molecular weight iron complexes such as iron citrate and iron-ATP.

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2 \quad \ldots\ldots (3).$$

$$H_2O_2 + Fe^{2+} \longrightarrow \cdot OH + OH^- + Fe^{3+} \quad \ldots\ldots (4).$$

$$\text{Net: } O_2^- + H_2O_2 \longrightarrow O_2 + \cdot OH + OH^- \quad \ldots\ldots (5)$$

The hydroxyl radical is extremely reactive and can react with any organic molecule in its immediate surroundings. Furthermore, both hydrogen peroxide and the superoxide radical are much less reactive than the hydroxyl radical, and hence longer lived, and would thus increase the extent of damage by diffusing away from the site of production. Under normal circumstances, cells can maintain integrity by preventing the accumulation of the superoxide radical. This is achieved by the co-ordinated action of SOD, and peroxidases and catalases.

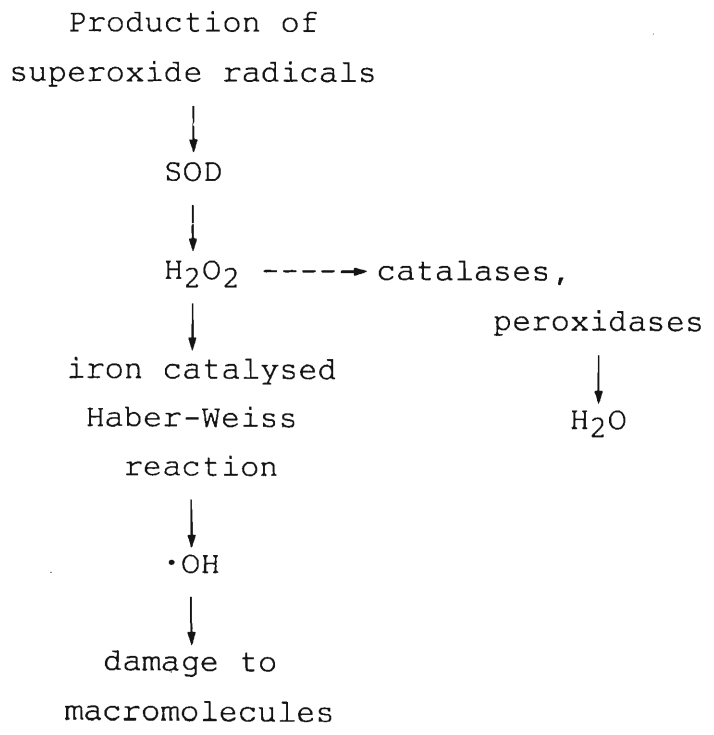


Figure 1.1. The superoxide theory of oxygen toxicity. (Halliwell and Gutteridge,1985).

Furthermore, natural antioxidants will scavange any free radicals produced. If this does not occur, iron-catalysed breakdown of H_2O_2 would produce the hydroxyl radical which would lead to an accumulating level of damage, resulting eventually in cell death (figure 1.1).

2.2.Lipid Peroxidation and membrane damage.

As mentioned above, the hydroxyl radical can react with all major organic molecules within the cell, including proteins, DNA, RNA and fatty acids of membranes. Although the free radical theory of ageing gives no indication as to which of these sites is the main site of damage, damage to cell membranes may be a major factor in the ageing process for a number of reasons. Firstly, polyunsaturated fatty acids are particularly susceptible to free radical attack (Halliwell and Gutteridge,1985). Secondly, free radical producing reactions, such as electron transport chains, are closely associated with membranes. Furthermore, the secondary products of lipid peroxidation, particularly alkoxy radicals and aldehydes, are potentially cytotoxic (Esterbauer,1980), while the changes to membrane properties that could result from peroxidation would lead to cellular dysfuction (Vladimirov et al.,1980).

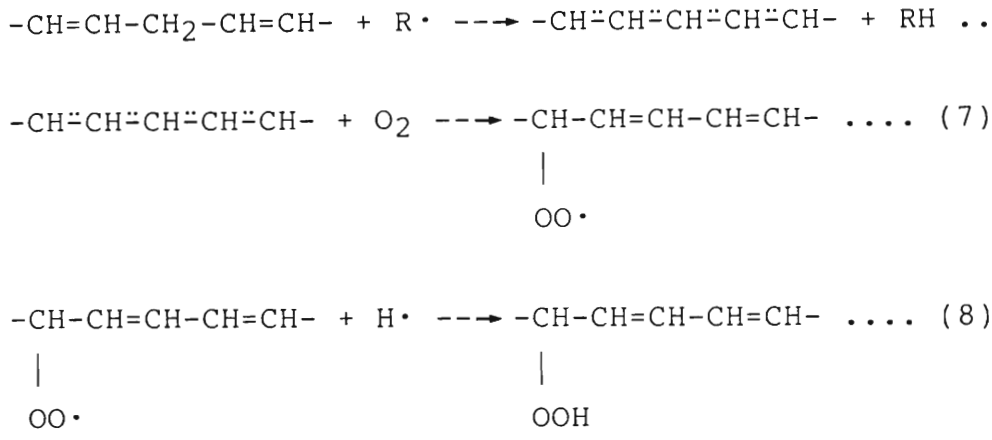
Free radical induced lipid peroxidation has been suggested to be the cause of membrane damage in seeds (Koostra and Harrington,1969). More recently, the effect of peroxidation on mitochondrial membranes in vitro has been investigated (Vladimirov et al.,1980). These workers have demonstrated that lipid peroxidation increases membrane rigidity and negative surface charge, leading to loss of respiratory control, an increase in membrane permeability to calcium and a loss of ATPase activity. These changes were attributed to the peroxidative loss of polyunsaturated fatty acids from the membrane. Furthermore, lipid peroxidation apparently

brought about an increase in membrane permeability to H^+ (or OH^-) ions and was responsible for the breakdown in membrane dielectric stability. The uncoupling of oxidative phosphorylation was also shown to be a direct result of lipid peroxidation and was suggested to be due to the above changes.

That lipid peroxidation can cause extensive damage to membranes is thus well established. If such changes were to occur *in vivo*, similar damage would possibly result.

2.3. Autoxidation.

In pure oils, the oxidation of polyunsaturated fatty acids is thought to proceed via a process of autoxidation. It has been proposed that lipid peroxidation is initiated by hydrogen abstraction by a free radical such as the superoxide or hydroxyl radicals (Frankel,1982). The presence of double bonds in unsaturated fatty acids weakens the C-H bonds adjacent to it, thus making polyunsaturated fatty acids particularly susceptible to free radical attack. This free radical attack results in the formation of a radical intermediate of very short life span (6) as it reacts rapidly with molecular oxygen to produce a peroxy radical (7). This radical in turn abstracts hydrogen from an adjacent molecule, usually another fatty acid, resulting in the formation of a lipid hydroperoxide and a further free radical, thus perpetuating the chain reaction (8). The hydroperoxide is relatively stable and will tend to accumulate. Under certain conditions, however, particularly at elevated temperature and in the presence of transition metals, it will be broken down to yield a number of secondary products, including alkoxy radicals, which will thus lead to chain branching (Vladimirov *et al.*,1980).



Autoxidation of Linoleate (after Halliwell and Gutteridge,1985).

In plants, three fatty acids usually predominate, namely oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Autoxidation of these oils usually produces a complex mixture of hydroperoxides, the relative proportion being dependent on which carbon the hydroperoxide is formed at and the conditions of oxidation (Frankel,1982).

Autoxidation of monolayers of these oils appears to be slightly different to that in bulk oils, as oxidation of linoleate and linolenate yields a much higher proportion of epoxy compounds (Logani and Davies,1979). The proportion of these epoxy compounds is also influenced by the conditions of oxidation, viz. temperature and the presence of a hydrogen donor. Furthermore, the inclusion of saturated fatty acids in the monolayer reduces both the rate of reaction and the amount of epoxides produced. The length of the saturated fatty acids has also been shown to influence the rate and products of oxidation, the longer chains being more effective in reducing the level of epoxides than the shorter ones (Mead,1980).

The inclusion of an antioxidant such as alpha-tocopherol, can further modify the reaction, usually

resulting in the development of a lag period before the onset of oxidation. It has been estimated that one molecule of alpha-tocopherol may protect up to 20 000 molecules of fatty acids, assuming the mobility of the antioxidant within the monolayer (Mead,1980).

That lipid peroxidation occurs *in vitro* is well established. Its occurrence and importance *in vivo* is still, however, under investigation. Evidence is nevertheless accumulating that would suggest that lipid peroxidation does occur *in vivo* (Halliwell and Gutteridge,1985).

Although the oxidation of membranes might be expected to be different to that of pure oils, the oxidation of erythrocytes *in vitro* has been shown to yield hydroperoxides as the major product (Wu *et al.*,1984). It therefore is probable that hydroperoxides may be the major product *in vivo*.

2.4.The fate of the hydroperoxide.

Hydroperoxides are apparently relatively benign. Vladimirov *et al.* (1980) has demonstrated that it was the breakdown products of the hydroperoxide and not the hydroperoxides themselves that were responsible for the change in permeability of artificial membranes (BLM). The non-enzymatic breakdown of hydroperoxides has been shown to yield a large number of products including short chain aldehydes and alkoxy radicals (Frankel,1982), both of which would be potentially damaging if produced *in vivo* (Esterbauer,1980). Not only are they implicated in changes in membrane fluidity, surface charge and permeability (Vladimirov *et al.*,1980), but they have been shown to react with polypeptides (Gardner ,1979) and may also be involved in polynucleotide damage (Inouye,1984).

Hydroperoxide breakdown is catalysed by the presence of reduced iron (Frankel,1982). *In vivo*, haeme

compounds such as cytochromes may provide such a source of reduced iron (Halliwell and Gutteridge,1985) and could therefore represent a potential source of cellular damage by catalysing lipid peroxidation (Bindoli et al.,1982) (figure 1.2.).

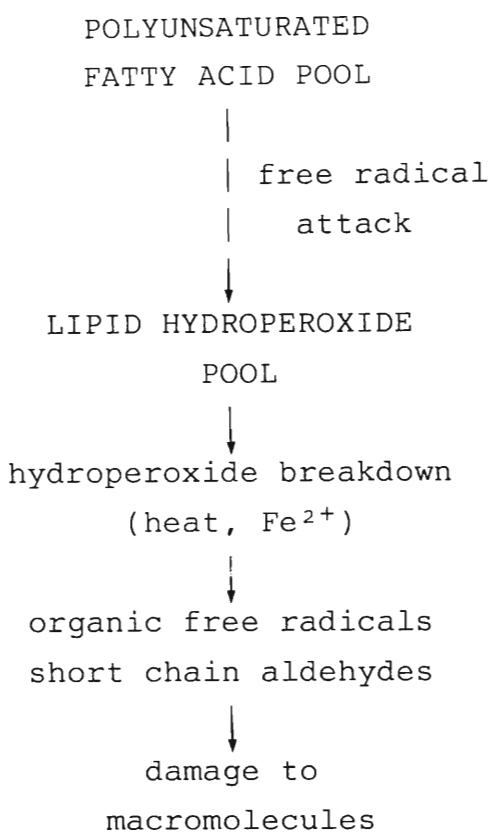


Figure 1.2 Free radical induced lipid peroxidation - the fate of the hydroperoxide.

2.5. Evidence for lipid peroxidation in seeds.

Although there is firm evidence for the occurrence of lipid peroxidation in seeds, a casual relationship between lipid peroxidation and seed vigour remains to be determined (Wilson and McDonald,1986a). There is some recent work, however, which suggests that lipid peroxidation may be associated with the loss of seed vigour. A decline in the levels of polyunsaturated fatty acids, particularly linoleate, in aged seeds has been

reported in peanut (Pearce and Abdel Samad,1980) and soya bean (Stewart and Bewley, 1980; Priestley and Leopold,1983). This was suggested to be due to lipid peroxidation. Furthermore, a number of workers have reported an increase in the products of lipid peroxidation with age. These include oxygenated fatty acids (Spencer et al.,1973) and malondialdehyde (MDA) (Radrupal and Basu,1982; Halder et al.,1983).

Buchvarov and Gantcheff (1984) have reported a greater level of free radicals in the axes of aged compared with unaged soya beans. That these free radicals may be responsible for the above changes is suggested by a number of observations. Firstly, pea seeds treated with antioxidants alpha-tocopherol and butylated hydroxytoluene show a significant resistance to vigour loss during rapid ageing (Gorecki and Harman, in press). Similarly, Radrupal and Basu (1979) have shown that the iodination of mungbean (Vigna radiata) seeds can reduce the rate of vigour loss under rapid ageing conditions. These workers suggest that this was due to the stabilising effect of the iodine on the double bonds of the unsaturated fatty acids, rendering them less susceptable to peroxidative attack.

The provision of a static electric charge during and after ageing markedly improved the vigour, and reduced the degree of electrolyte leakage and the percentage chromosomal abberations of rapidly aged maize seeds (Berjak,1978). It was proposed that the provision of electrons prevented extensive lipid peroxidation by quenching any free radicals produced.

Lipid peroxidation has been reported to alter the phase transition temperature of soya liposomes (Senaratna et al.,1985). Woodstock et al.(1984) has demonstrated that in high vigour soya bean seeds, the relationship between oxygen uptake and temperature is distinctly biphasic, while in low vigour seeds, no

transition was seen within the temperature range investigated. This may implicate the physical alteration of the membranes during ageing, leading to the impairment of respiration.

Finally, a lipid peroxidation model for seed ageing has recently been proposed (Wilson and McDonald,1986a). It is envisaged that storage subjects seed lipids to a slow consistant attack by oxygen, resulting in the formation of hydroperoxides. Prolonged storage, it is suggested, leads to the accumulation of these hydroperoxides, which on imbibition are broken down to yield a number of cytotoxic products, particularly aldehydes, and further free radicals. These are postulated to "inhibit respiration, protein synthesis, DNA synthesis and denature protein".

2.6.A tentative unifying hypothesis for seed ageing.

Of all the 'viability theories' proposed to date, the suggestion that membrane damage may be involved is the only one that provides evidence, albeit circumstantial, for an underlying cause of damage, namely lipid peroxidation. This, however, does not exclude or invalidate the other theories. On the contrary, it provides a hypothetical basis for a unifying hypothesis for seed ageing, that is, the free radical theory of ageing. Such a contention is supported by a number of observations. Not only are free radicals regarded as the initiators of lipid peroxidation, but they have also been implicated in damage to the DNA (Lesko et al.,1980). It is conceivable that free radicals may not only attack membranes and DNA, but also proteins and RNA. Harman (1981) has stated that the free radical theory of ageing assumes that there is a single basic cause of ageing, modified by genetic and environmental factors, and postulates that free radical reactions are involved in ageing. These reactions arise

from nonenzymatic reactions and from enzymatic reactions, particularly those of the major energy producing reactions of mitochondrial (and other) electron transport chains. In the light of the above discussion, this definition may be readily adapted to seed ageing. Furthermore, the polyunsaturated fatty acids of membranes might constitute the major site for the initiation and exacerbation of free radical damage, as suggested by Wilson and McDonald (1986a).

3.VOLATILE LIPID OXIDATION PRODUCTS AND SEED VIGOUR.

As has been already discussed, the primary products of lipid peroxidation are hydroperoxides. These can be broken down into a large variety of products, many of which are volatile. Numerous tests have been developed in order to monitor the extent of lipid peroxidation in animal and vegetable oils. This includes the measurement of thermally released pentane by gas chromatography. Scholz and Ptak (1966) have reported a good correlation between the production of pentane and the age of the oil sample. Similarly, Evans et al. (1969) has reported a linear relationship between the amount of pentane produced and the peroxide value.

A number of workers have investigated the volatile production from germinating seeds. Stotsky and Schenck (1976) have demonstrated a relationship between volatile production and the rate of seed germination. A greater volatile production has also been reported from aged pea and soya bean (Harman et al.,1980,1982). Furthermore, it was suggested that lipid peroxidation was the source of these volatiles. Similar relationships between the amount of volatile compounds, specifically ethanol and acetaldehyde, and seed vigour were obtained by Woodstock and Taylorson (1980) and Gorecki et al. (1985). Indeed, the level of aldehydes passively trapped from soya

beans during the first 24 hours of imbibition was highly correlated to field emergence (Wilson and McDonald, 1986b). However, these workers implicate mitochondrial dysfunction as the source of the volatiles.

Fielding and Goldsworthy (1982) have shown that volatile compounds were produced when dry wheat seeds were heated in sealed glass vials. Furthermore, this volatile production was related to seed vigour, aged seeds producing greater levels of volatiles. They suggest that lipid peroxidation was the source of these compounds.

Work in our laboratory has shown that volatiles evolved from dry and imbibing lettuce seeds were related to seed vigour. Further analysis of the volatiles evolved suggests that there is a marked relationship between the amount of hexanol produced and seed vigour (Smith, pers. comm.)[1].

4.SCOPE OF THE PRESENT INVESTIGATION.

Preliminary work in our laboratory (Blakeway,1985) has suggested that a possible relationship may exist between lipid peroxidation and seed vigour in a number of different cultivars of soya bean seeds. In view of the equivocal results from studies on the role of lipid peroxidation in seed ageing to date , particularly in soya beans (Priestley and Leopold, 1979, Stewart and Bewley,1980), it was decided to repeat the above work. In addition, a number of different cultivars of cabbage seeds were also investigated.

Evidence for lipid peroxidation in these two seed types was sought using a number of approaches.

1.The measurement of lipid hydroperoxides in the dry

[1]Mr M T Smith, Dept. Biology, Univ. Natal, Durban, S.A.

seeds in order to determine a) the extent of lipid peroxidation incurred by each seed lot and b) if any relationship existed between this and seed vigour.

2. The determination of the relative percentages of the fatty acids by GLC in order to establish a) if any changes had occured, particularly the polyunsaturated fatty acids, and b) if these changes bore any relationship to the extent of lipid peroxidation.

It was also attempted to monitor the changes in the levels of the lipid hydroperoxides during the first hour of imbibition in order to establish the fate of the hydroperoxides on imbibition. Furthermore, in the light of the above mentioned work on the volatiles derived from seeds, the analysis of volatile compounds produced from the seed a) on heating and b) during imbibition was undertaken using GLC in order to determine:

1. if any relationship existed between the evolution of volatiles and the extent of lipid peroxidation and

2. if any relationship existed between the evolution of volatiles and seed vigour.

Finally, the use of certain reducing agents as a means of chemically invigorating the seeds was investigated.

MATERIALS AND METHODS

1. **Materials.**

Seeds of cabbage (Brassica oleracea L) were obtained from Mayford Seed Company, Johannesburg and stored in sealed tins at laboratory temperatures until used. The soya bean seeds (Glycine max L) were obtained from the Summergrain Research Centre, Potchefstroom and stored in a cold room at 5°C. Tinoridine was obtained from the research laboratories, Yoshitomi Pharmaceutical Industries Ltd., Yoshitomi-cho, Chikujo-gun, Fukuoka-ken 871, Japan.

2. **Germination Tests.**

Cabbage. 100 seeds were germinated in 9cm Petri dishes on two sheets of Whatman No.1 filter paper and moistened initially with 5mℓ of water. Seeds were kept moist throughout the test period. Percentage germination (radicle emergence) was recorded after one week. Results were used to chose six lots of seeds with different germinability. Seeds from these six lots were then subjected to further germination tests. Seeds were germinated on paper towelling between two vertically orientated sheets of plastic to allow normal root/shoot development. Percentage radicle emergence and average radicle lengths were recorded.

Soya bean. Six lots were chosen for study based on the results of earlier investigations (Blakeway,1985). 100 seeds from each of these lots were germinated in 14cm Petri dishes lined with paper towelling. Initially, just

sufficient water to dampen the paper (50mℓ) was used. Further water was added throughout the experimental period. Percentage germination was recorded after 4 days.

3. **Moisture Content.**

1g of whole seeds of cabbage and soya were placed in aluminium foil trays and heated at 110°C overnight. Seeds were then reweighed, moisture content being determined by difference and expressed as a percentage of the wet weight of the seeds.

4. **Triphenyl-tetrazolium chloride (TZ) staining.**

Seeds were germinated for 24 hours as described in section 2. Seed coats were then removed from 5 seeds of each batch and the seeds submerged in a 1% solution of TZ for 1 hour. The staining pattern of the cotyledons and radicles of each batch was then recorded.

5. **Extraction of Lipid.**

a. Whole seeds.

Two different solvent systems were used for extracting lipid from the seeds. Initially, hexane/isopropanol (3/2,V/V) (Hara & Radin, 1978) was used, but for reasons of cost and safety methylene chloride/methanol (MM) (2/1,V/V) (Khor & Chan,1985) was subsequently used. In both cases the technique was the same. 5g of ground seed tissue was extracted with 20mℓ of solvent containing butylated hydroxytoluene (0.005% W/V) as antioxidant for 30 minutes with shaking. Following centrifugation, the supernatant was dried down at 45°C under nitrogen. Extracts were stored at 5°C

until used.

b. Axes.

20 axes of each seed lot were isolated and ground in a mortar and pestle in 10mℓ of methylene chloride/ methanol. After standing for 10 minutes, the extract was centrifuged at maximum speed in a bench-top centrifuge for 5 minutes at room temperature. The supernatant was then decanted and washed with a quarter volume of 1% NaCl solution (Folsch wash), recentrifuged and the lower phase taken to dryness under nitrogen at 45°C.

6. **Fractionation of Lipid.**

Lipid was fractionated by silicic acid column chromatography (Beutelmann & Kende,1977). The silicic acid was activated overnight at 100°C. 20 X 1cm Biorad econo-columns were filled with a silicic acid/ chloroform slurry. A sample of lipid dissolved in 1mℓ chloroform was placed onto the column and eluted with succesive 20mℓ volumes of chloroform, acetone and methanol, giving the neutral, glycolipid, and polar lipid fraction respectively. Eluates were dried down at 45°C under nitrogen and stored at 5°C.

7. **Determination of Hydroperoxide Level.**

In this study it was deemed desirable to have as sensitive a test as possible to allow the detection of low levels of hydroperoxides. For this reason a modification of the iron test described Stine et al. (1953) was used.

Reagents.

1.Benzene/methanol 7/3,V/V. Solvents were both

of analytical grade.

2.Ferrous chloride solution. 0.4g of barium chloride in 50mℓ of water was added to 0.5g ferrous sulphate in 50 mℓ water. 2mℓ of 10N HCl was added to facilitate precipitation. The solution was then cleared with a brief centrifugation (maximum speed for 5 minutes at room temperature).

3.Potassium thiocyanate solution. 15g KSCN was dissolved in 50mℓ water (30% solution).

The determination of hydroperoxide levels in dry seeds.

In this study, it was found that the ferrous chloride solution should preferably be added to the solvents before the lipid as the reverse resulted in a reduction in colour development. 20 microlitres ferrous chloride was added to 5mℓ of benzene/methanol and shaken. 20 microlitres of 100 microlitres of lipid in 0.5mℓ methylene chloride/methanol was then added to followed by 20 microlitres KSCN. Absorbance was read at 505nm against a blank of the reagents.

The determination of hydroperoxide levels on imbibition.

Whole seeds was imbibed on two sheets of filter paper moistened with 5mℓ of water. At ½ and 1 hour, 1g of seeds was submerged in hot (90°C) water for 30 seconds. Seeds were then processed as described above. The isolated axes of soya were imbibed on two sheets of filter paper moistened with 5mℓ water. At ½ and 1 hour, 20 axes were submerged in hot (90°C) water for 30 seconds and then the lipid

extracted as outlined above (section 5.b.). Peroxide values were then determined.

8. **Esterification of Fatty Acids.**

Methyl esters of the fatty acids were obtained using a modification of the organic base-catalyzed technique of Metcalfe and Wang (1981). An aliquot of lipid (100 microlitres) was dissolved in 1mℓ diethyl ether. 0.5mℓ of 1N tetramethylammonium hydroxide (TMAH) was added and the mixture shaken for 1 minute. 1mℓ of water was then added, the methyl esters partitioning into the upper ether phase. An aliquot of this was then analysed using gas-liquid chromatography.

9. **Gas-Liquid Chromatography.**

Fatty acid methyl esters.

A Pye Series 104 Gas Chromatograph fitted with a flame ionization detector (FID) and a 1.5m X 4mm I.D. glass column packed with 10% Silar 5CP on Chromosorb G was used. Nitrogen was used as the carrier gas at 30mℓ/min. For fatty acid analysis, injection temperature was 270°C, detector temperature, 300°C and column temperature was held isothermally at 200°C. Peak areas were integrated electronically and expressed as area percentages.

Volatile compounds.

For the analysis of volatile compounds from dry seeds, the above gas chromatograph was used. Injection and column temperature was changed to 100°C and carrier gas flow rate reduced to appproximately 10mℓ/min. For the analysis of volatile compounds from imbibing seeds, a Varian 3300 gas chromatograph fitted

with an FID and a 15m DB-1 megabore column was used. Injection and detector temperature were 250°C, column temperature was programed at 40°C for 5 minutes and then increased to 150°C at 10°/min. This was held for a further 10 minutes. Carrier gas was nitrogen at 3mℓ/min. Peak areas were expressed as area percentages. In the analysis of some of the data, total area counts were used and represented the mean of two injections.

10. **Analysis of Volatile Compounds.**

1.Heating of dry seeds.

5g of each batch of cabbage and soya seeds were sealed in 5mℓ serum vials and heated at 85°C for 22 h. 250 microlitres of the headspace of each vial was then analysed by GLC. Seeds were then processed as above for fatty acid and hydroperoxide determination.

2.Volatiles derived on imbibition.

Seeds were imbibed in Petri dishes for 20 hours as previously described (section 2). 2g of seeds were then sealed in a 5mℓ serum vial and heated at 85°C for 15min. 250 microlitres of headspace was then analysed by GLC.

0.2g of isolated axes were sealed in 5mℓ serum vials and moistened with 0.5mℓ of water. Axes were then allowed to imbibe water for 20 hours. Vials were then heated briefly (20 mins) and 100 microlitres of the headspace analysed by GLC.

11. **Seed invigoration with reducing agents and antioxidants.**

Seeds were germinated as described in section

2. Solutions of ferrous chloride, ferrous sulphate, potassium iodide, tinoridine and dimethylsulphoxide were used instead of water. The solution of ferrous chloride was made up fresh as described above (section 7).

12. **Statistical Methods.**

The significance of diffrerence was determined using Student's t-test where

$$tcalc = (\bar{x}_1 - \bar{x}_2)/((s_1^2 + s_2^2)/n)^{\frac{1}{2}}$$

for n-1 degrees of freedom, where $\bar{x}_1$ and $\bar{x}_2$ are the means of sample 1 and 2 respectively and s_1 and s_2 are the standard deviations of sample 1 and 2 respectively. The linear regressions (y on x) were calculated for the equation $y = mx + c$, where

$$c = (\sum y)(\sum x^2) - (\sum x)(\sum xy)/n\sum x^2 - (\sum x)^2$$

and

$$m = n\sum xy - (\sum x)(\sum y)/n\sum x^2 - (\sum x)^2.$$

The standard error for the regression was calculated as $SE = (\sum(y - yest)^2/n-2)^{\frac{1}{2}}$ where yest is the estimated value of y. The correlation coefficient of the fit of the line to the data was calculated as

$$r^2 = \sum(yest - \bar{y})^2/\sum(y - \bar{y})^2.$$

The significance of the correlation was determined using the t-test where

$$tcalc = r.(n-2)^{\frac{1}{2}}/(1-r^2)^{\frac{1}{2}}.$$

TABLE 1

The percentage radicle emergence of six cultivars of cabbage (*B. oleracea*) seeds. Standard deviation is given in parentheses.(n=4)

Cultivar.	Abbreviation	% G
Golden Acre	(G.A.)	100 (±0.6)
Glory of Enkhuizen	(G.E.)	98 (±1.7)
Cape Spitz	(C.S.)	82 (±1.7)
Savoy Perfection(A)	(S.P.A)	76 (±8.7)
Savoy Perfection(B)	(S.P.B)	73 (±8.0)
Savoy Perfection(C)	(S.P.C)	39 (±16.0)

TABLE 2

Percentage radicle emergence of six cultivars of soya bean (*G. max*) seeds. Standard deviation is given in parentheses.(n=4)

Cultivar.	Abbreviation	% G
Ibis	-	97 (±2.1)
Impala 4028	4028	95 (±8.6)
Impala 4023	4023	95 (±5.9)
Pioneer 5774	PNR	94 (±2.5)
Hartebeest	-	89 (±10.1)
Impala 4031	4031	82 (±22.1)

DISCUSSION OF RESULTS.

1.THE DETERMINATION OF SEED VIGOUR.

Germination tests were carried out on a number of cultivars (cvs) of soya and cabbage seeds. The results of these tests were then used to choose six seed lots of each seed type for the ageing studies. Germination percentages (percentage radicle emergence) for each cultivar are given in table 1 and 2 and the accumulative germination percentages for each lot are presented in figures 2.1 to 2.4. Average radicle lengths, moisture contents, and tetrazolium reducing ability of each of these seed lots was then also determined.

In the following discussion, extensive use is made of correlations and the ranking of the seeds according to one or other parameter without making express mention of the fact that the seed lots used in this study were all of different cultivars. That differences in cultivars, together with the presumed differences in environmental (preharvest), harvest and storage conditions could have influenced the results is therefore a recognized, but largely unknown parameter. The basic premise has therefore been that whatever technique was used to provide evidence for lipid peroxidation in seed ageing, results should show some measure of variability due to the above mentioned factors.

1.1. Radicle length.

In this study, the average radicle lengths of the cabbage seeds were less in the seeds giving low germination percentages. For example, cvs Golden Acre

Figure 2.1and 2.2 The germination curves of the six lots of cabbage seeds used in this study. (n=2)

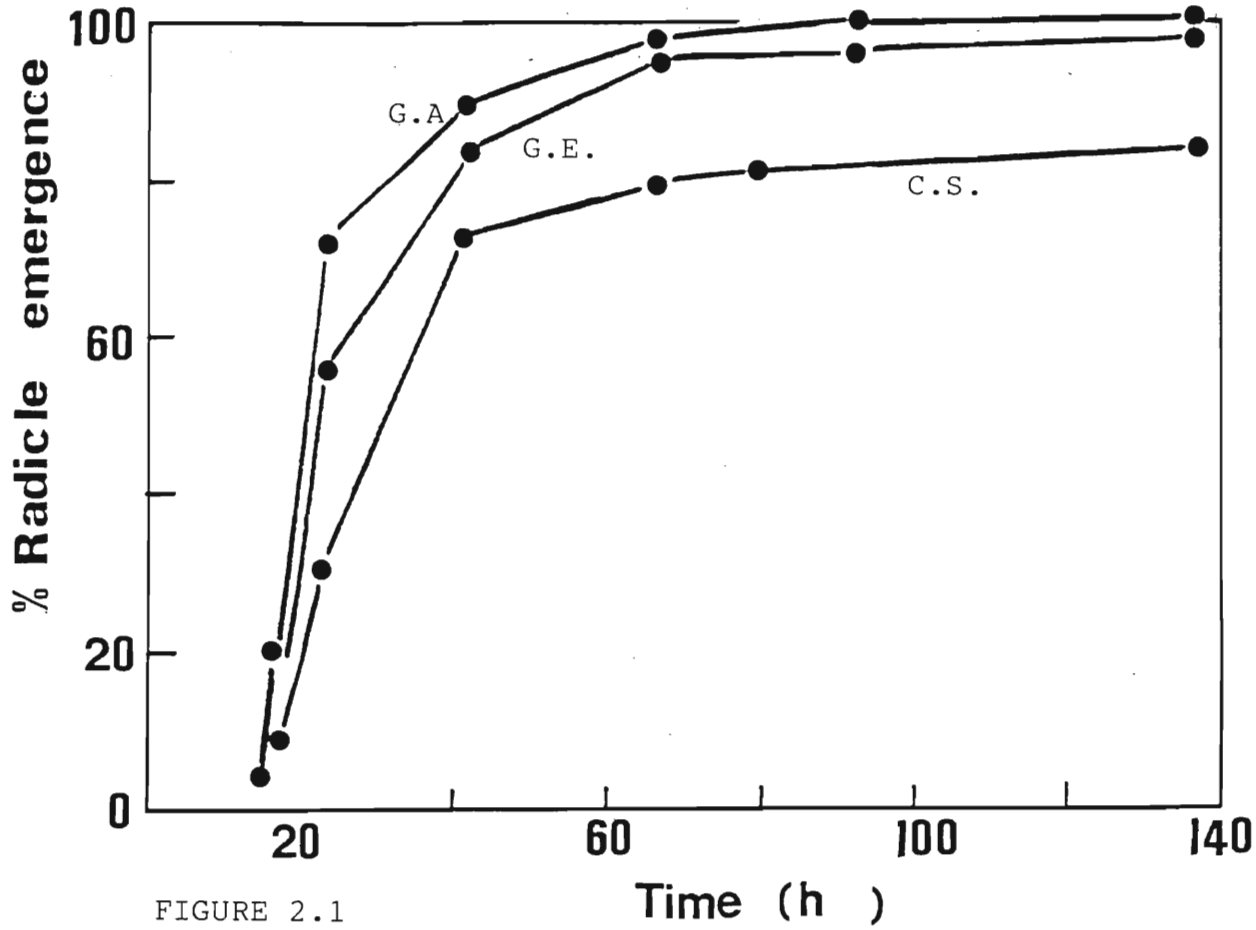


FIGURE 2.1

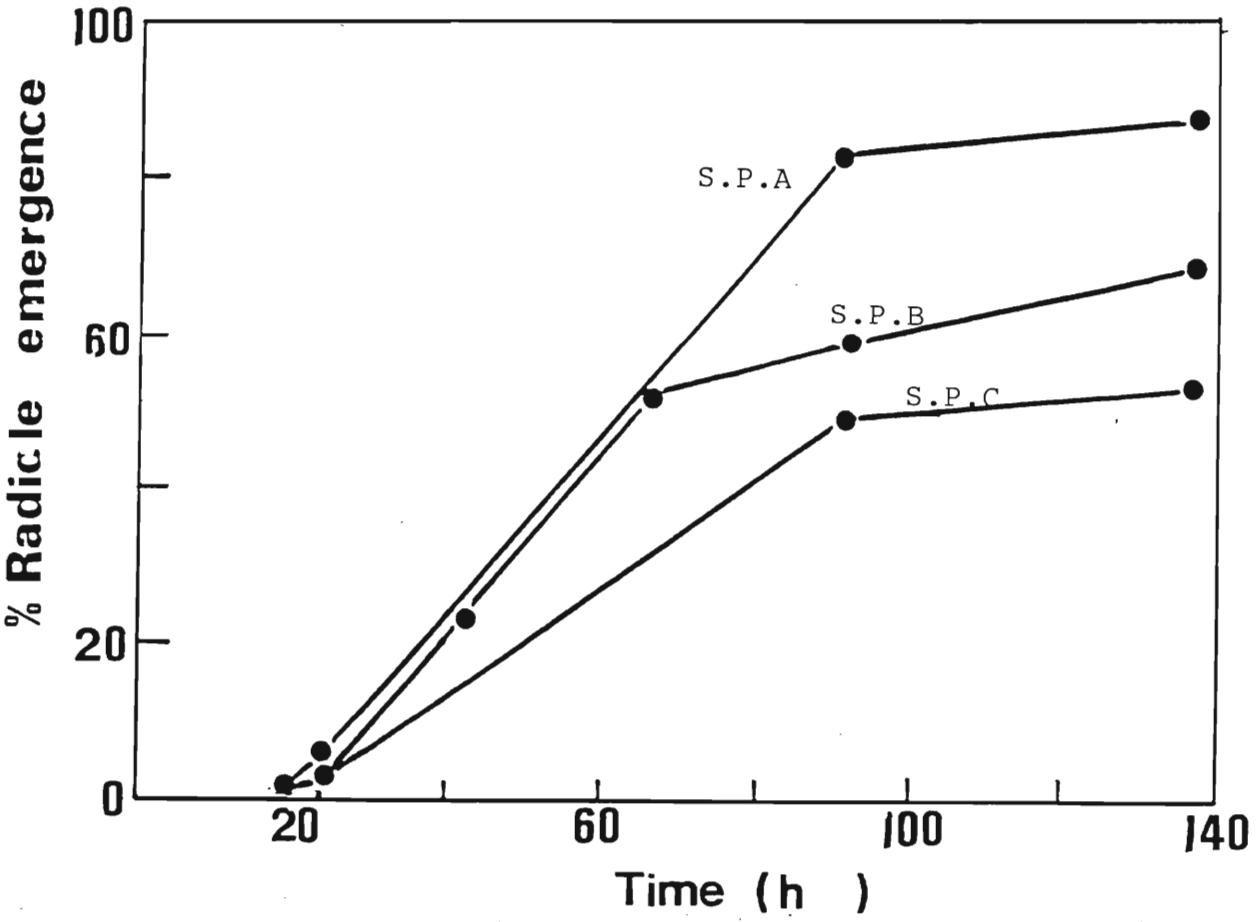


FIGURE 2.2

Figure 2.3 & 2.4 The germination curves of the six lots of soya bean seeds used in this study. (n=2)

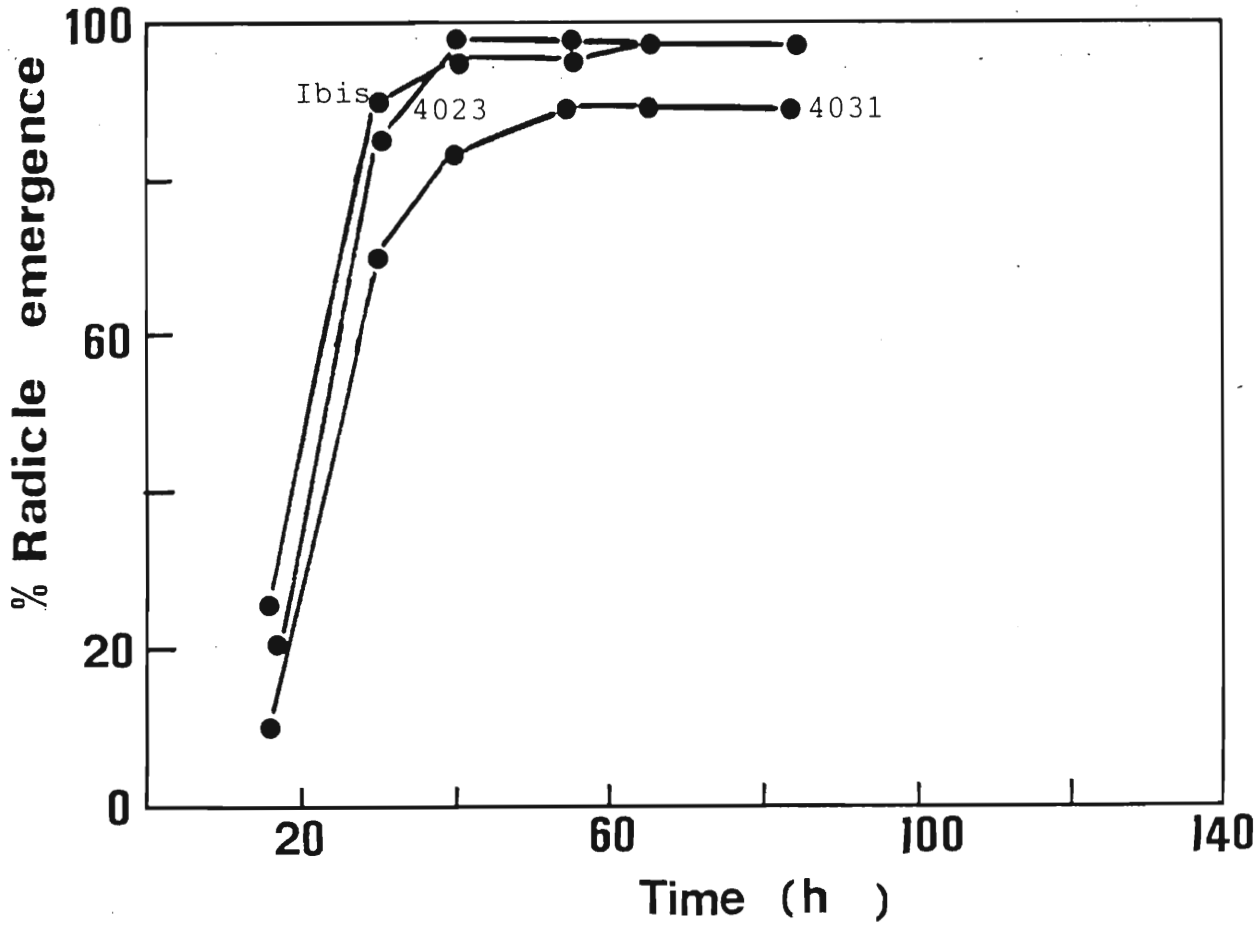


FIGURE 2.3

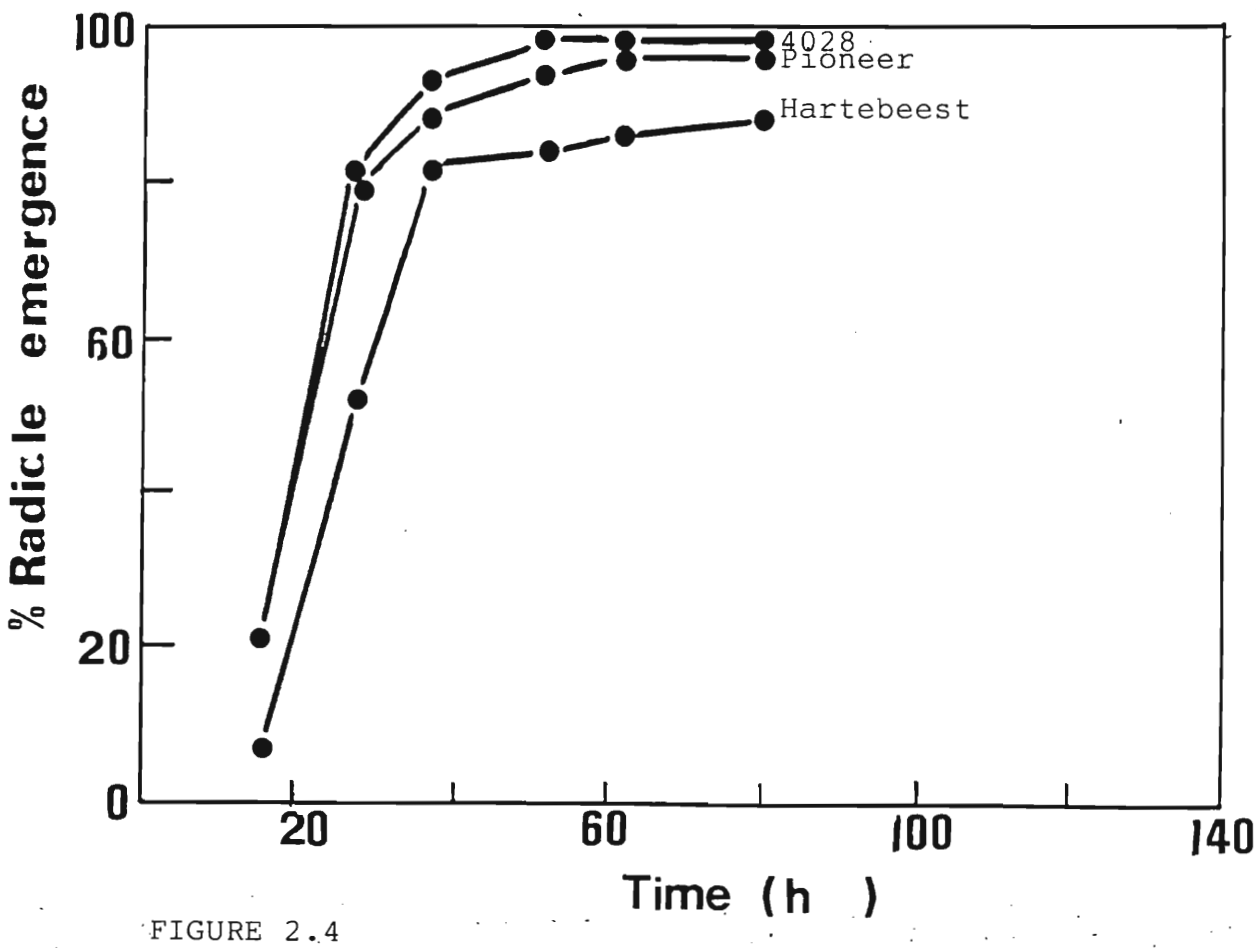


FIGURE 2.4

and Glory of Enkhuizen, which gave germination percentages of 100% and 98% respectively, had an average radicle length of 55mm and 65mm respectively, while Savoy Perfection, lot B, which gave 73% germination, and Savoy Perfection lot C, which gave a 39% germination, had average radicle lengths of 10 and 30mm respectively (figure 2.5). A similar decline in average radicle lengths as seed germinability declined was also seen in the soya beans (figure 2.6).

Woodstock and Tao (1981) have shown a marked decrease in the length of excised embryonic axes of soya beans after seven days growth following rapid ageing. A decline in average radicle lengths has also been reported by Priestley and Leopold (1983) in slowly aged soya beans. The reasons for this decline is unclear. It could reflect a delay in the time taken for radicle emergence to occur or an impairment of subsequent growth. That it involves damage to the axis itself is suggested by the work of Woodstock and Tao (1981). In addition to a reduction in average length, these workers also reported a greater degree of abnormal growth in seeds aged for longer periods of time. This clearly indicated that the mechanisms of growth were damaged in some way.

1.2.Seed Moisture Content.

Seed moisture content is a major factor in the loss of seed vigour during dry storage. Powell and Harman (1985) have shown a more rapid loss of germinability in seeds of higher moisture content subject to rapid ageing treatment. Despite the pivotal role of seed moisture content in seed ageing, however, very few workers have determined the moisture contents of the seeds under study when investigating the loss of seed vigour. Indeed, in many rapid ageing studies, seed moisture contents have been largely ignored, despite the fact

Figure 2.5 A regression (y on x) of the average radicle lengths and percentage radicle emergence of the six lots of cabbage seeds. The correlation coefficient (r) for the regression was 0.61. In this and all subsequent figures, **the broken lines indicate ±1 standard deviation.** (n=1)

Figure 2.6 A regression of the average radicle lengths and percentage radicle emergence of the six lots of soya bean seeds. (r=0.52). (n=2)

FIGURE 2.5

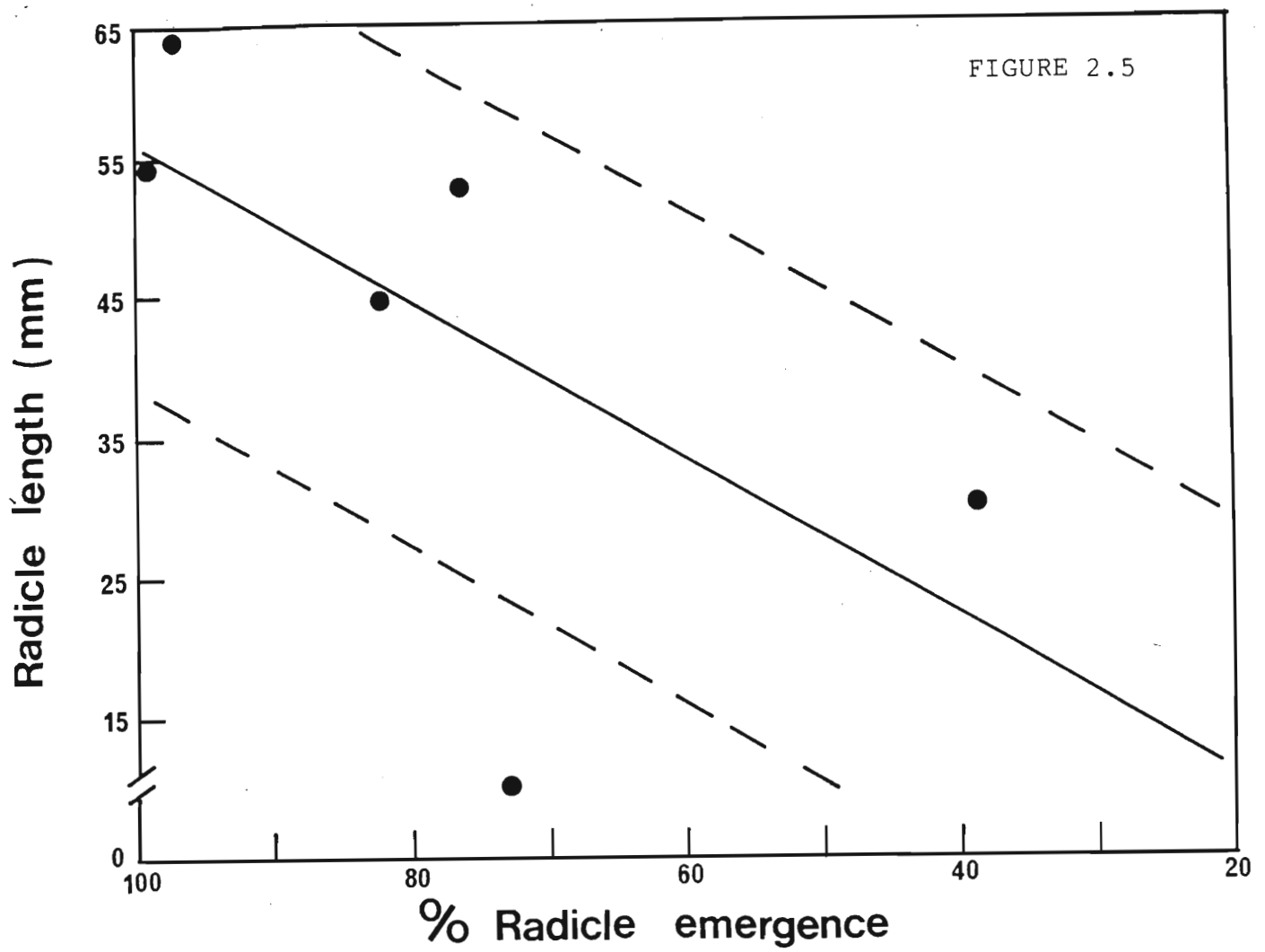
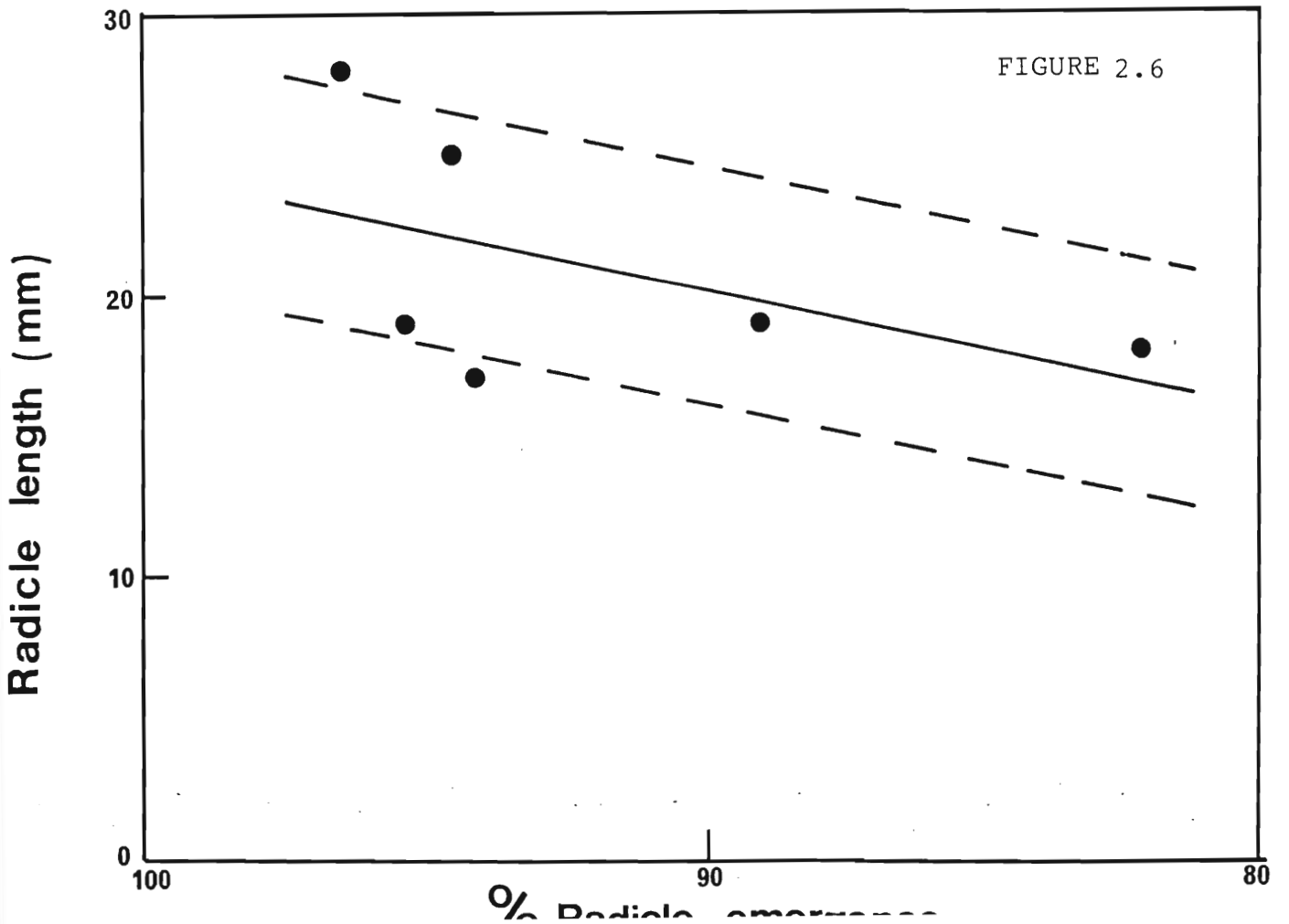


FIGURE 2.6



that it increases markedly during the treatment (Halder and Gupta,1982).

In this study, moisture contents of the cabbage seeds were about 6% on average, and those of soya bean, 8%. The correlations between moisture content and germinability were poor for both cabbage and soya, the correlation coefficients being 0.32 and 0.44 respectively (figure 2.7 & 2.8). Furthermore, moisture content was high in the cabbage seeds of low germinability while it was low in the soya beans of low germinability. It should, however, be borne in mind that the relationship determined is a product of the statistical treatment of the data and may be artificial rather than real. Nevertheless, in cabbage, a possible relationship between moisture content and germinability may have been present, the germination percentages decreasing as seed moisture content increased (figure 2.7). It has been estimated that a decline in moisture content of 1-2% will double seed storage life (Bewley and Black,1982). The relationship between seed moisture content and germinability (as opposed to storability) has not, however, been extensively investigated. In the present study, this relationship was poor in both seed types.

1.3.Tetrazolium staining.

A qualitative assessment of the tetrazolium reducing ability of all seed lots was carried out as described. In general, a loss of TZ reducing ability accompanied the loss of vigour in cabbage seeds. The radicle tip was a major site of this lesion. Figure2.9 summarizes the staining pattern observed. In the more vigorous seeds, the radicle tips were intensely stained, while the cotyledons were consistently well stained, particularly on the edges (figure 2.9a). In the less vigorous seeds, these two areas were consistently

Figure 2.7 A regression of the moisture content and percentage radicle emergence of the six lots of cabbage seeds. (r=0.32). (n=2)

Figure 2.8 A regression of the moisture content and percentage radicle emergence of the six lots of soya bean seeds. (r=0.44). (n=2)

FIGURE 2.7

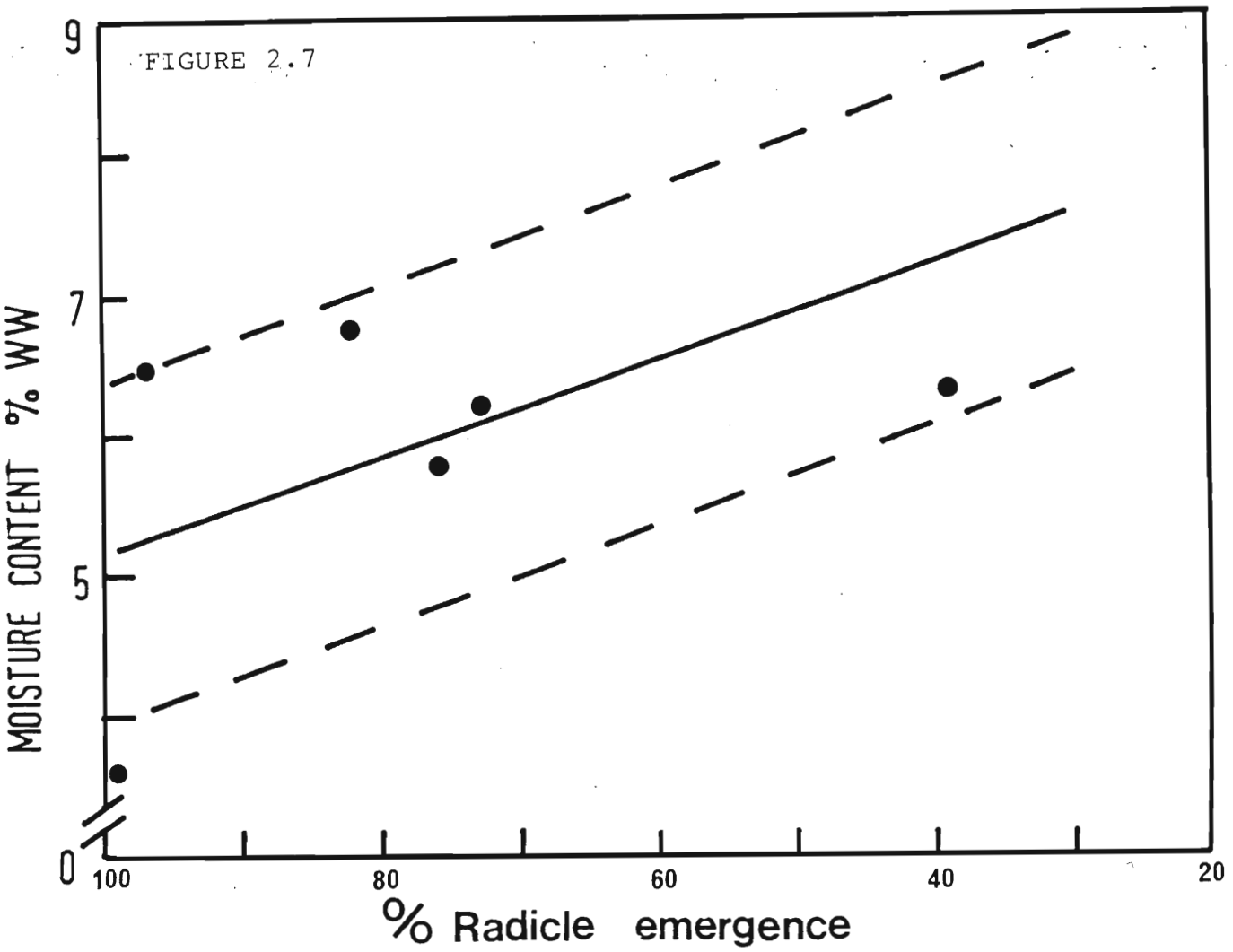


FIGURE 2.8

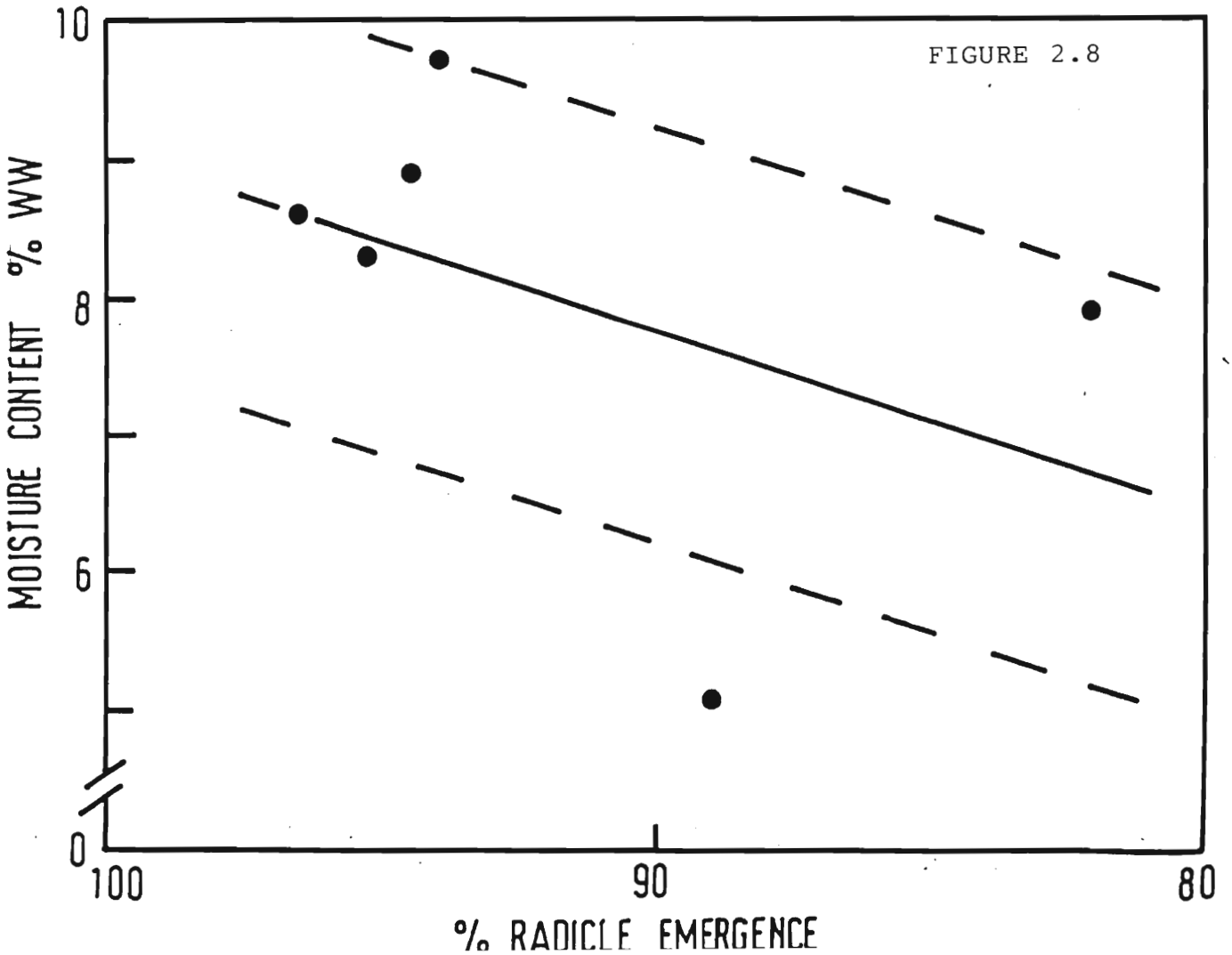


Figure 2.9 A schematic representation of the topographical staining pattern observed in the cabbage seeds.

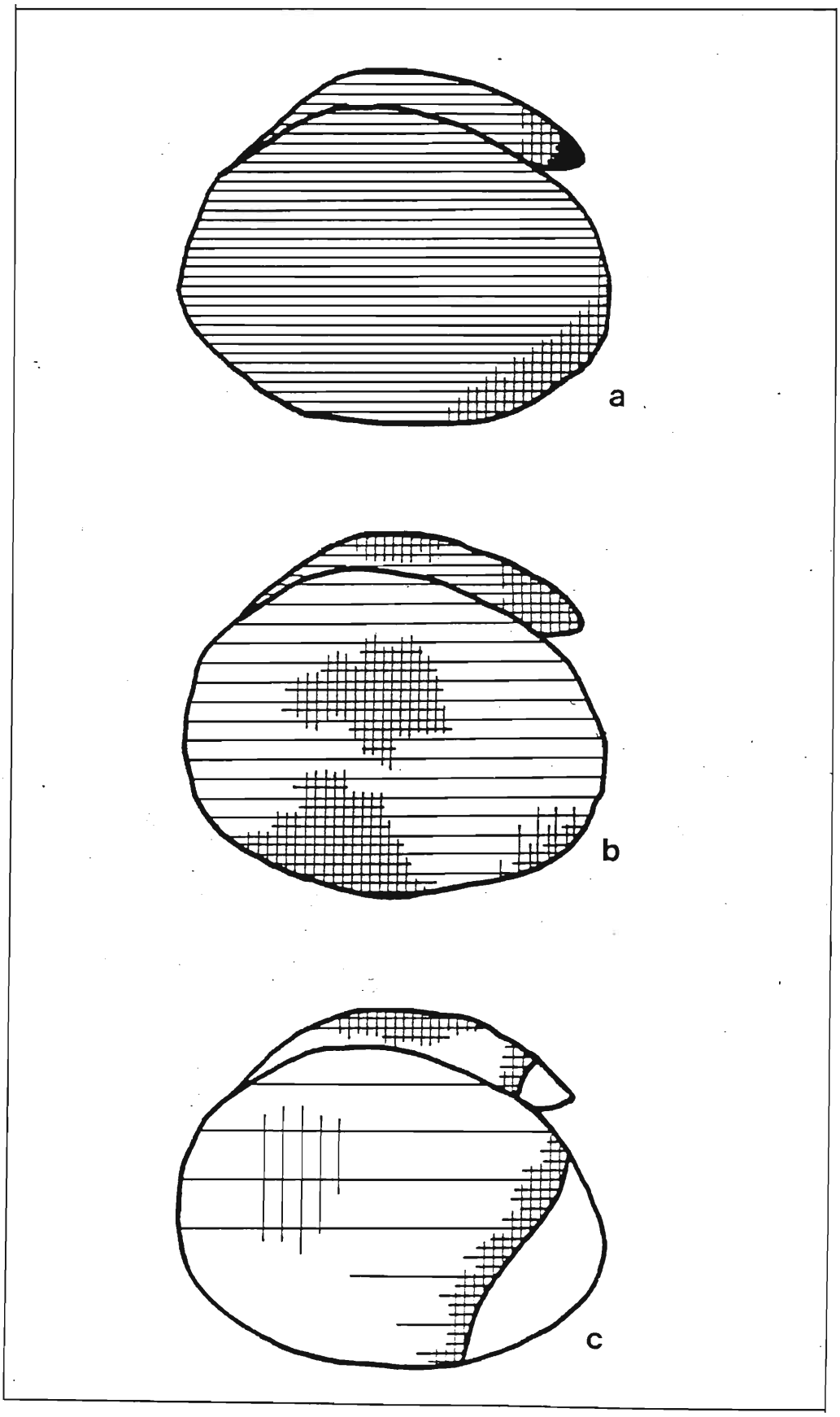


FIGURE 2.9

unstained (figure 2.9c).The staining pattern observed for each seed batch is briefly described in table 3.

No distinct pattern of tetrazolium staining was observed in soya beans. The staining pattern observed is described in table 4. Seed axes in general showed an equivalent TZ reducing ability, which in all seed lots was relatively high, particularly at the radicle tips. Cotyledonary staining varied greatly, both within and between seed lots. It was also observed that some cotyledons of seed lots of poor vigour (eg. 4031, table 4) stained very intensely. This was not, however, thought to represent the reducing ability of the seed per se. MacKay (1972) has stated that the presence of micro-organisms which can also reduce tetrazolium may prove misleading in the interpretation of this test. It is possible that the presence of micro-organisms in the lots of poor germinability was responsible for the intense staining observed.

2.THE ANALYSIS OF LIPID HYDROPEROXIDES

2.1. Hydroperoxide level of dry seeds.

Seed hydroperoxide level was determined as described. In cabbage, a possible relationship between the level of hydroperoxides and seed germinability was observed, the latter decreasing as peroxide value increased (figure 2.10). In soya bean, a similar relationship between seed germinability and hydroperoxide level was seen, viz. an increase in peroxide value as seed germinability declined (figure 2.11).

In cabbage, a marked relationship between peroxide value and moisture content was observed, seeds of low moisture contents giving low peroxide values, while those of high moisture contents had high peroxide values (figure 2.12). This was particularly marked in the

TABLE 3.

A summary of the tetrazolium staining pattern observed in cabbage (B. oleracea) seeds.

1.GOLDEN ACRE	
Radicles: Radicle tips were intensely stained. The rest of the radicle was in general uniformly, well stained.	**Cotyledons:** Uniformly, well stained, with slightly darker staining occuring on the cotyledonary edge opposite the radicle (figure 9.a.).
2.GLORY OF ENKHUIZEN	
Radicles: Staining of tips varied from completely unstained to intensely stained tips. Rest of the radicle was well stained, patchy.	**Cotyledons:** Unstained to slightly stained, patchy, mainly round the edge opposite the radicle.
3. CAPE SPITZ	
Radicles: Tips intensely stained. Rest unstained to well stained, patchy.	**Cotyledons:** Unstained to well stained, patchy. particularly round lower edges (figure 9.b.)
4.SAVOY PERFECTION LOT A	
Radicles: Tips unstained to intensely stained. Rest unstained to slightly stained, patchy.	**Cotyledons:**Poorly stained, uniform to well stained, patchy.
5.SAVOY PERFECTION LOT B	
Radicles: Tips unstained. Rest unstained to poorly stained, uniform.	**Cotyledons:** Unstained to poorly stained, patchy. Edge opposite radicle consistently unstained (figure 9.c.).
6.SAVOY PERFECTION LOT C	
Radicles: Tips unstained to poorly stained. Rest unstained to poorly stained, patchy.	**Cotyledons:** Unstained to poorly stained patchy. Edge opposite radicle consistently unstained.

TABLE 4.

A summary of the tetrazolium staining pattern observed in soya bean (G.max) seeds.

1.IBIS

Radicles: Tips intensely stained. Rest poorly stained, patchy. **Cotyledons:** Unstained to poorly stained.

2.IMPALA 4028.

Radicles: Tips well stained. Rest poorly stained, uniform. **Cotyledons:** Poorly stained, uniform.

3.IMPALA 4023.

Radicles: Tips well stained. Rest well to poorly stained, uniform. **Cotyledons:** Well stained, patchy.

4.PIONEER 5774.

Radicles: Tips well stained. Rest poorly stained, uniform. **Cotyledons:** Poorly stained to well stained patchy.

5.HARTEBEEST

Radicles: Tips intensely stained. Rest unstained to intensely stained, patchy. **Cotyledons:** Intensely stained, patchy.

6.IMPALA 4031.

Radicles: Tips well stained. Rest poorly stained, uniform to intensely stained patchy. **Cotyledons:** Poorly stained, uniform to intensely stained patchy. Edge opposite radicles often unstained.

Figure 2.10 A regression of the hydroperoxide level and percentage radicle emergence of the six lots of cabbage seeds. (r=0.35). (n=2)

Figure 2.11 A regression of the hydroperoxide level and percentage radicle emergence of the six lots of soya bean seeds. (r=0.39). (n=1)

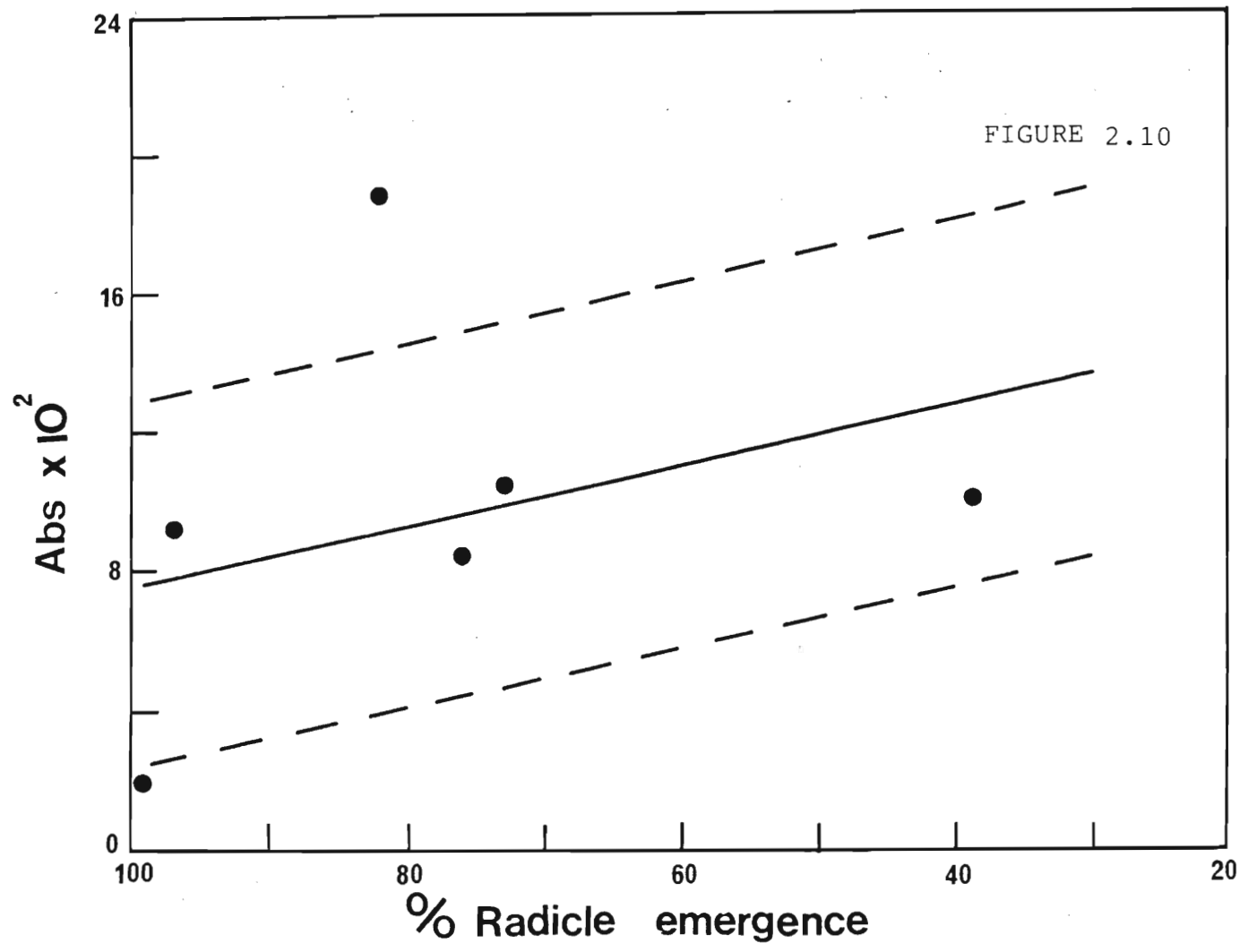


FIGURE 2.10

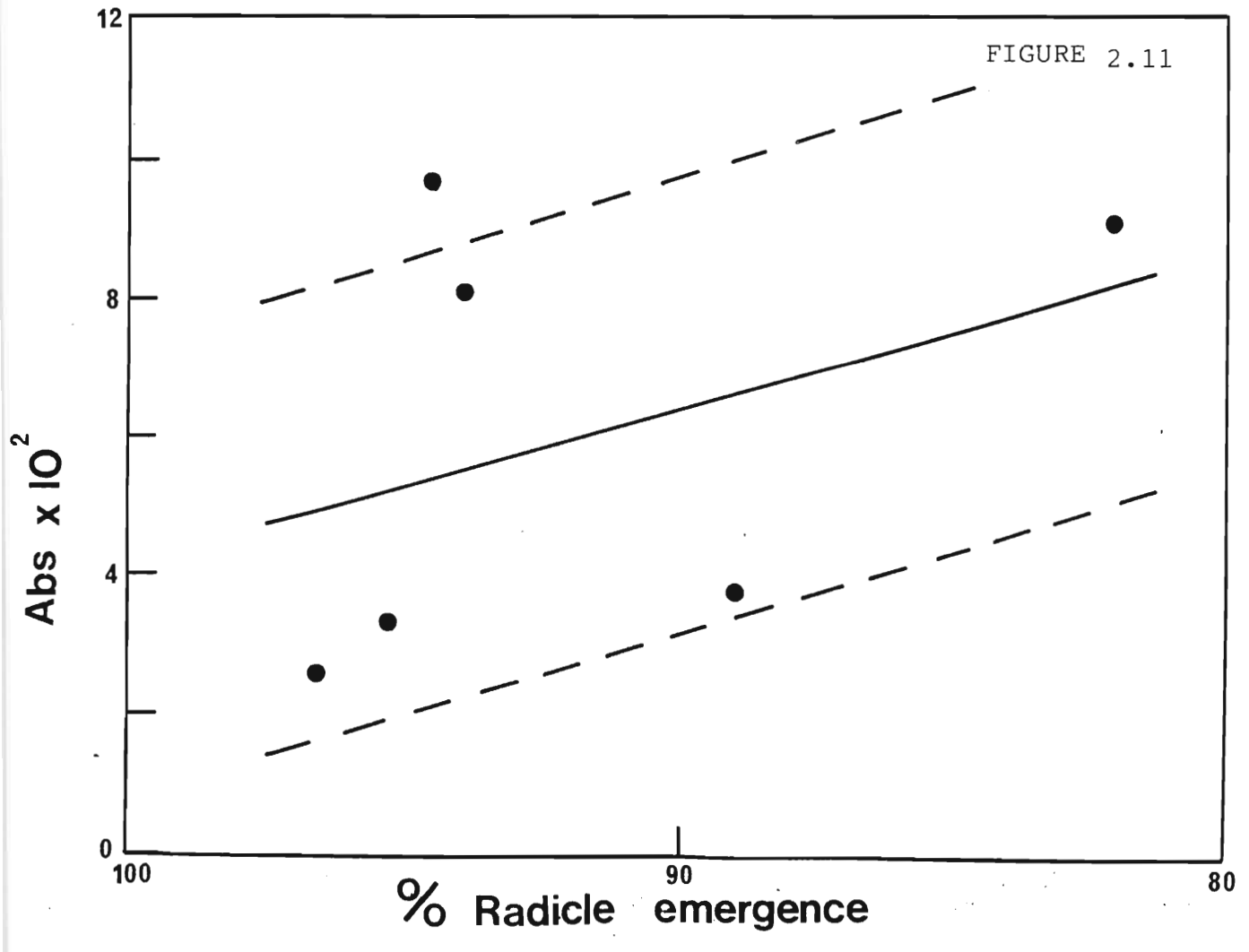


FIGURE 2.11

cv Cape Spitz. This seed lot had the highest moisture content (6.77%) and gave the highest peroxide value (0.188), while cv Golden Acre, which had a moisture content of 3.59%, gave a peroxide value of only 0.021. This may be significant with regard to the observed relationship between seed moisture content and storability, although no relationship between seed moisture content and peroxide value was observed in the soya beans (figure 2.13).

Surprisingly, very few workers have determined seed hydroperoxide levels. Pearce and Abdel Samad (1980) could detect no hydroperoxides in rapidly aged peanut seeds. On the other hand, Spencer et al. (1973) have demonstrated an increase in oxygenated fatty acids in seeds during storage. The vigour of the seeds was unfortunately not determined. Radrupal and Basu (1982) have however reported a highly significant correlation between lipid peroxidation as measured by the thiobarbituric acid test and seed germinability in seeds of wheat and mustard. That a relationship exists between hydroperoxide level and seed germinability is suggested by the results of this present study, although the correlation coefficients for both seed types were poor, being 0.35 and 0.39 for cabbage and soya respectively. Indeed, in cabbage the level of the hydroperoxides appeared to be more closely related to the seed moisture content. However, the inconsistencies in the relationship between hydroperoxide level and germinability may be due to the varietal differences and growth, harvest and storage conditions prior to purchase as this might be expected to lead to a certain degree of variability between seed lots.

2.2.Changes in the peroxide value of the total lipid extract after heating.

As the lipid hydroperoxide is thermally labile

Figure 2.13 The relationship between the hydroperoxide level and the seed moisture content of the soya beans. (n=2)

Figure 2.12 The relationship between the hydroperoxide level and moisture content of the seeds of cabbage. (n=6)

FIGURE 2.13

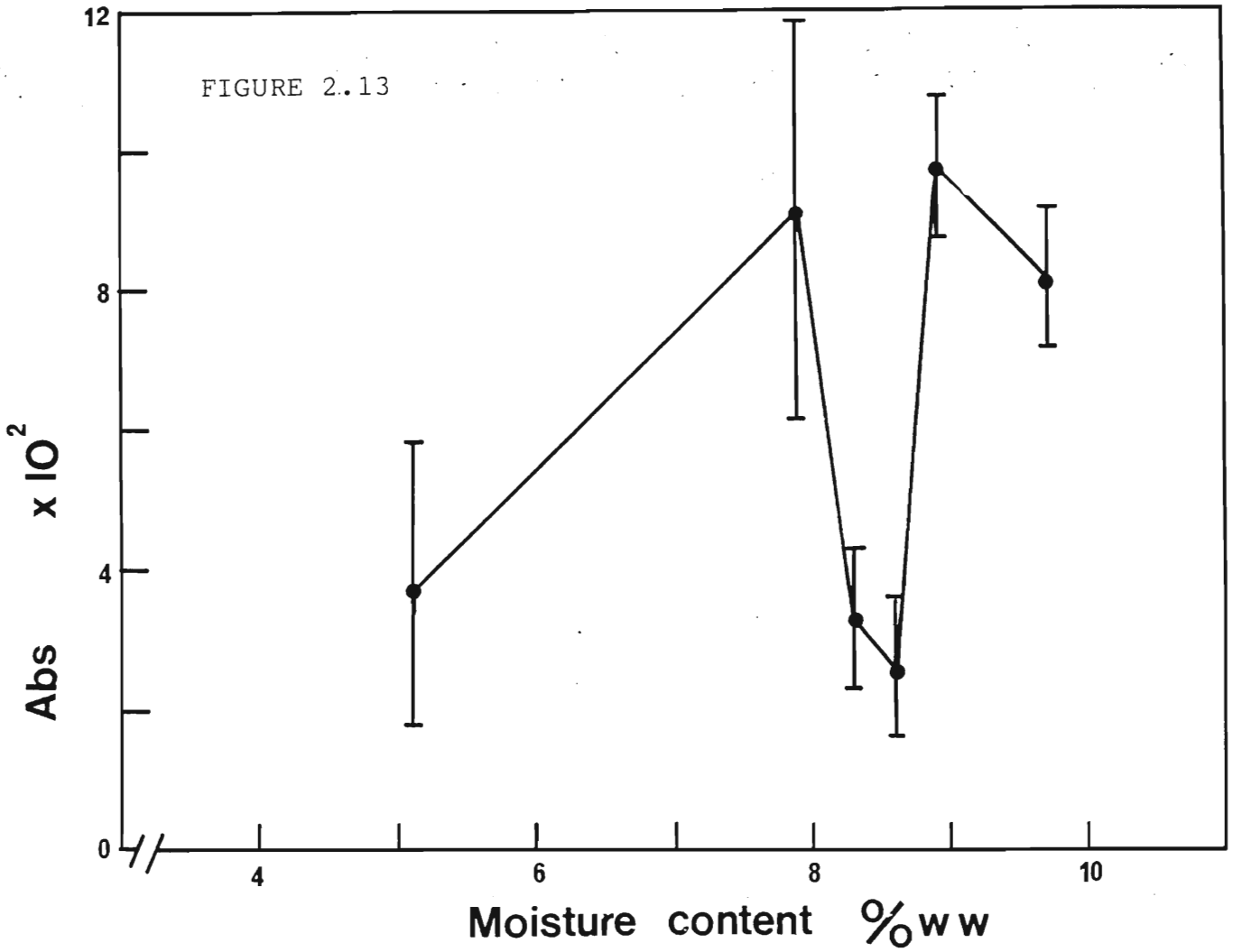


FIGURE 2.12

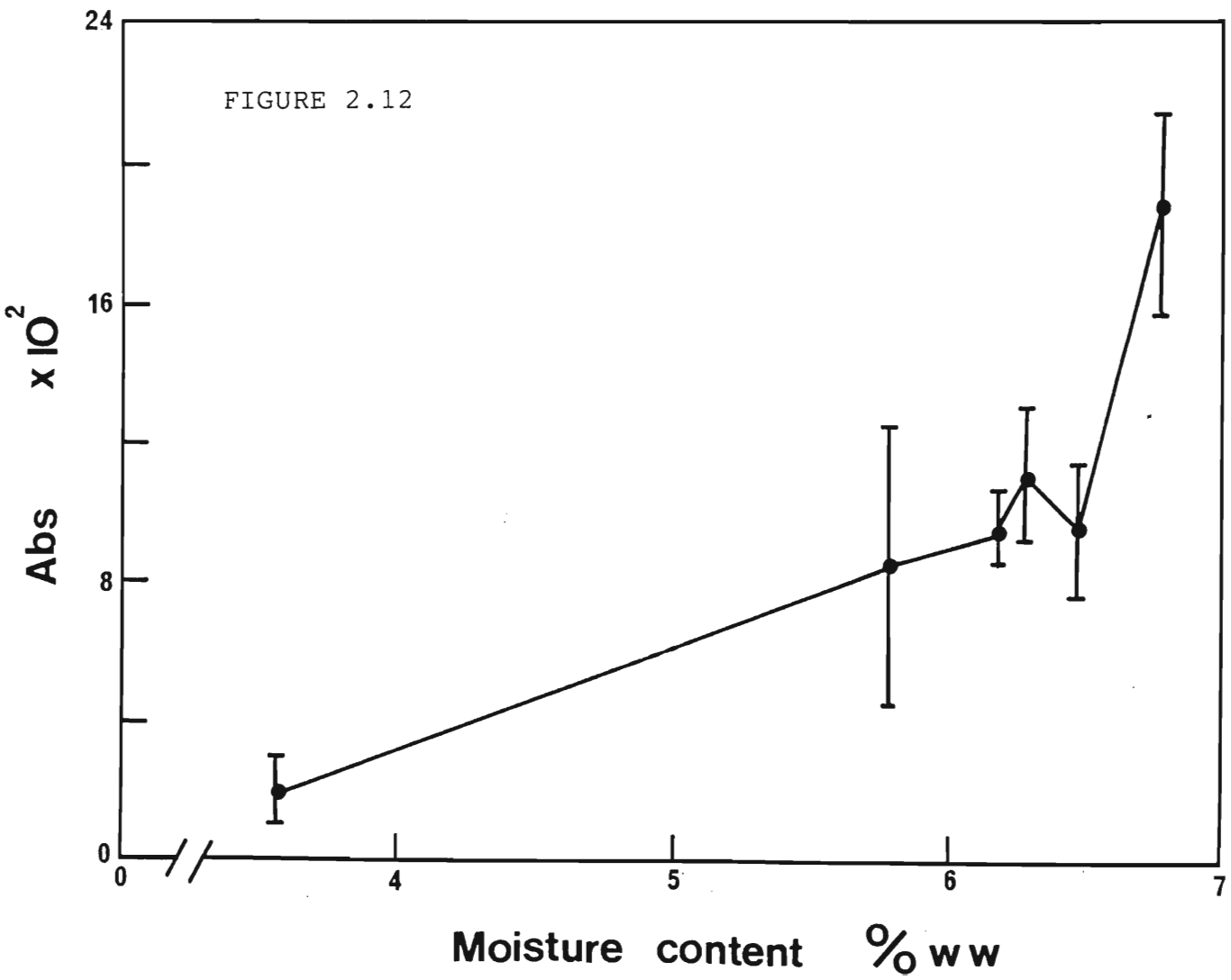


TABLE 5

Changes in the hydroperoxide level of cabbage (*B. oleracea*) seeds on heating (22hrs at 85°C). Standard deviation is given in parentheses. (n=3)

Cultivar.[1]	Unheated	Heated
Golden Acre	0.106 (±0.04)	0.103 (±0.02)
Glory of Enkhuizen	0.112 (±0.03)	0.052 (±0.03)
Cape Spitz	0.157 (±0.05)	0.140 (±0.04)
Savoy Perfection(A)	0.107 (±0.04)	0.078 (±0.03)
Savoy Perfection(B)	0.143 (±0.02)	0.041 (±0.02)
Savoy Perfection(C)	0.145 (±0.04)	0.082 (±0.02)

[1]seeds are ranked according to % germination

TABLE 6

Changes in the hydroperoxide level of soya bean (*G. max*) on heating (22hrs at 85°C). Standard deviation is given in parentheses. (n=4)

Cultivar.[1]	Unheated	Heated
Ibis	0.027 (±0.02)	0.021 (±0.01)
4028	0.022 (±0.02)	0.010 (±0.01)
4023	0.027 (±0.02)	0.018 (±0.01)
Pioneer	0.044 (±0.03)	0.022 (±0.01)
Hartebeest	0.052 (±0.03)	0.034 (±0.01)
4031	0.062 (±0.04)	0.014 (±0.01)

[1]seeds are ranked according to % germination

(Frankel,1982), a decline in the hydroperoxide value of the seeds might be expected to occur after heating. This was confirmed by examining the peroxide value of both soya and cabbage seeds after heating dry seeds for 24h at 85°C, a decline being observed in both cases (tables 5 and 6). If considered in isolation, the decline in the level of the hydroperoxides is not significant. However, this, together with the marked similarity between the volatiles evolved from whole seeds and pure oil (section 4.2), and the marked relationship between hydroperoxide value and volatile production (section 4.1), particularly in cabbage, suggests that the lipid hydroperoxides were the source of the volatile compounds evolved from dry seeds on heating.

2.3.Changes in the peroxide value on imbibition.

With regards to loss of seed vigour, it is not clear when the critical events occur; prior to or on imbibition. It may be argued that the accumulating levels of damage to the seed may be exacerbated on imbibition due to the increased moisture content. Any free radical species would be afforded greater mobility, which could thus increase the extent of damage, while hydroperoxide breakdown would be greatly facilitated, with the concomitant production of organic free radicals and other cytotoxic and mutagenic compounds. Furthermore, this increase in damage might be expected to be dependent on the level of damage suffered in dry storage, so that seeds having higher peroxide values would show a greater increase in damage.

In the seeds of both cabbage and soya bean, a change in hydroperoxide levels was observed during the first hour of imbibition. Cabbage seeds having a low initial peroxide levels showed a decline in hydroperoxide value on imbibition. With increasing initial hydroperoxide value, the decline in peroxide

value became progressively less, until in the cv. Savoy Perfection C, an increase in peroxide value was observed at ½ hour (figure 2.14).

A similar relationship between initial peroxide value and changes on imbibition were observed in the seeds of soya. In whole seeds, an increase on imbibition was generally observed, but the relationship to vigour was not apparent. For instance, cv Ibis with a 97% germination, showed the most marked increase in peroxide value, while the changes were much less for cv Impala 4031 which gave a 82% germination (figure 2.15). However, when peroxide values were determined for seed axes, a definite relationship was observed, seeds of high vigour showing a decline in peroxide value at ½ hour. Seeds of low vigour, on the other hand, showed an initial increase in the level of hydroperoxides, the increase being dependent on the initial peroxide value (figure 2.16).

The contrasting results obtained for whole seeds and isolated axes in soya provides further support for the suggestion that the analysis of whole seeds may mask changes in the respective parts (Stewart and Bewley,1980). More significantly, however, the widely fluctuating hydroperoxide levels observed in both whole seeds and axes on imbibition may indicate the possibility of increased peroxidative damage during the first hours of germination and may provide indirect support for the concept of repair on imbibition, the reduction of the levels of the hydroperoxides possibly being an important imbibition-related event.

3.THE ANALYSIS OF FATTY ACID-METHYL ESTERS.

3.1.Changes in the percentages of fatty acids.

The presence of double bonds in unsaturated fatty acids weakens the C-H bonds of the intermediate carbon,

Figure 2.14 The changes in the hydroperoxide levels that were observed to occur in four lots of cabbage seeds over the first hour of imbibition. (n=1)

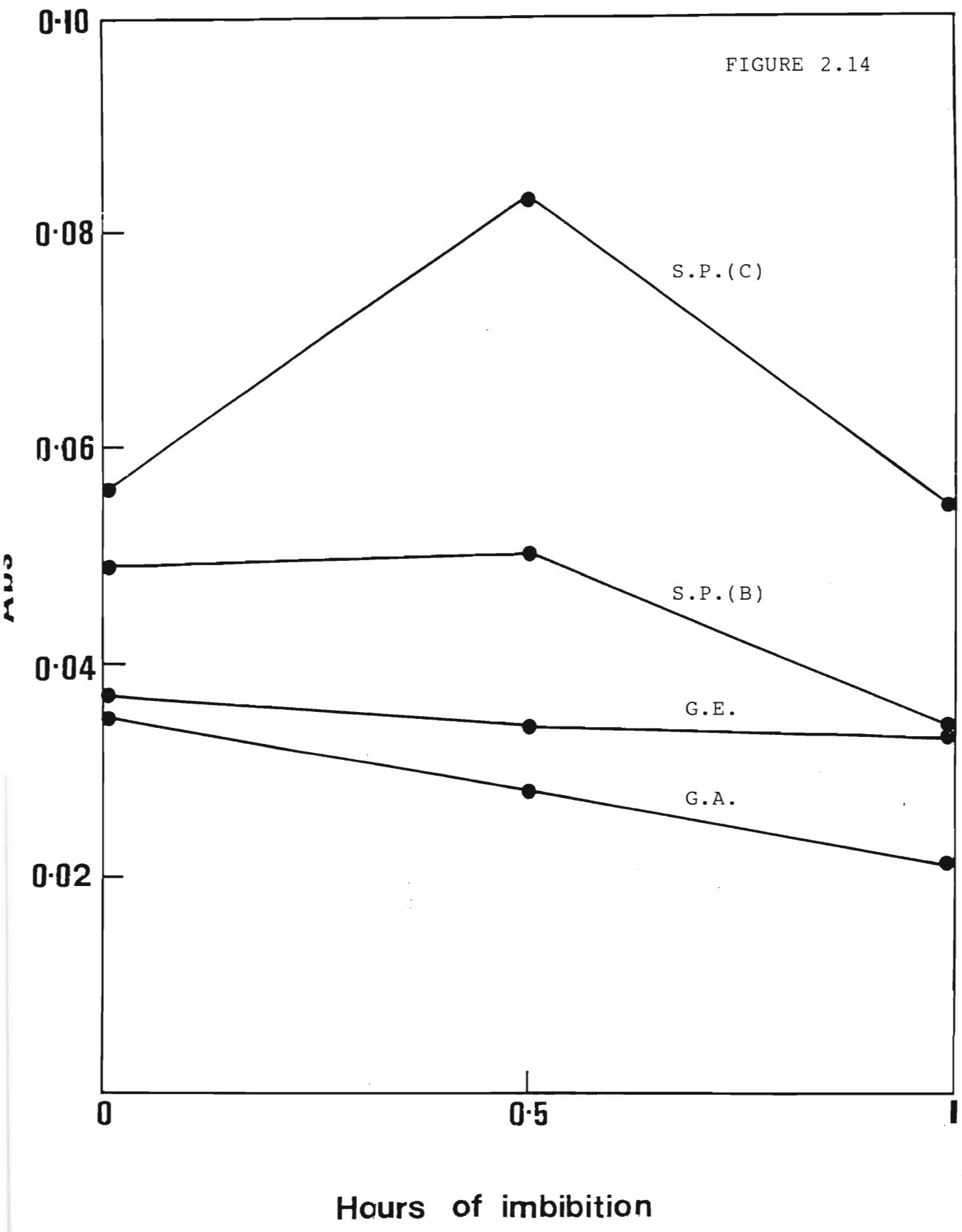


FIGURE 2.14
0·10
0·08
0·06
0·04
0·02
S.P.(C)
S.P.(B)
G.E.
G.A.
0
0·5
1
Hours of imbibition

Figure 2.15 The changes in the hydroperoxide levels that were observed to occur in four lots of soya bean seeds over the first hour of imbibition. (n=2)

FIGURE 2.15

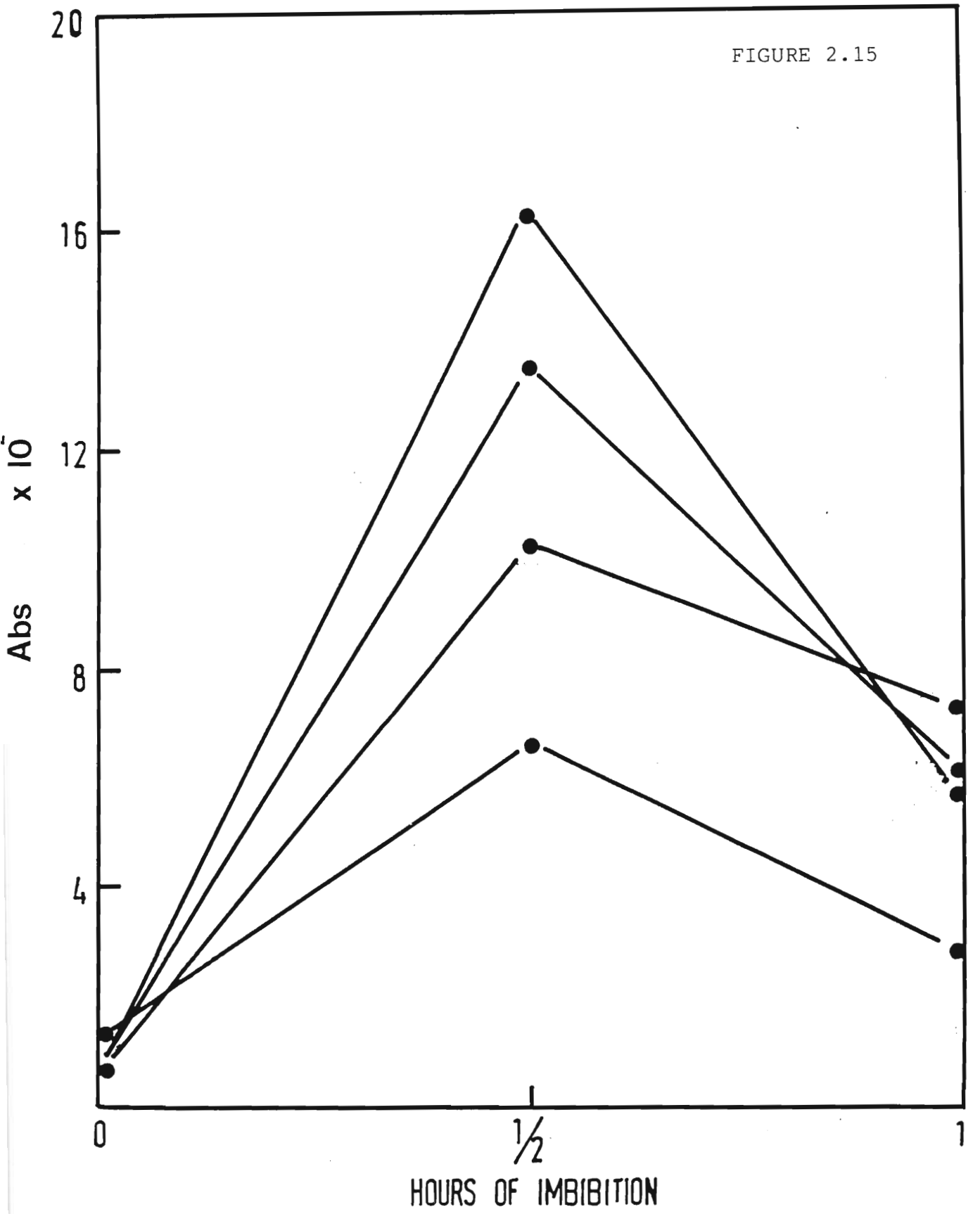


Figure 2.16 The changes in the hydroperoxide levels that were observed to occur in the axes isolated from four lots of soya bean seeds over the first hour of imbibition. (n=4)

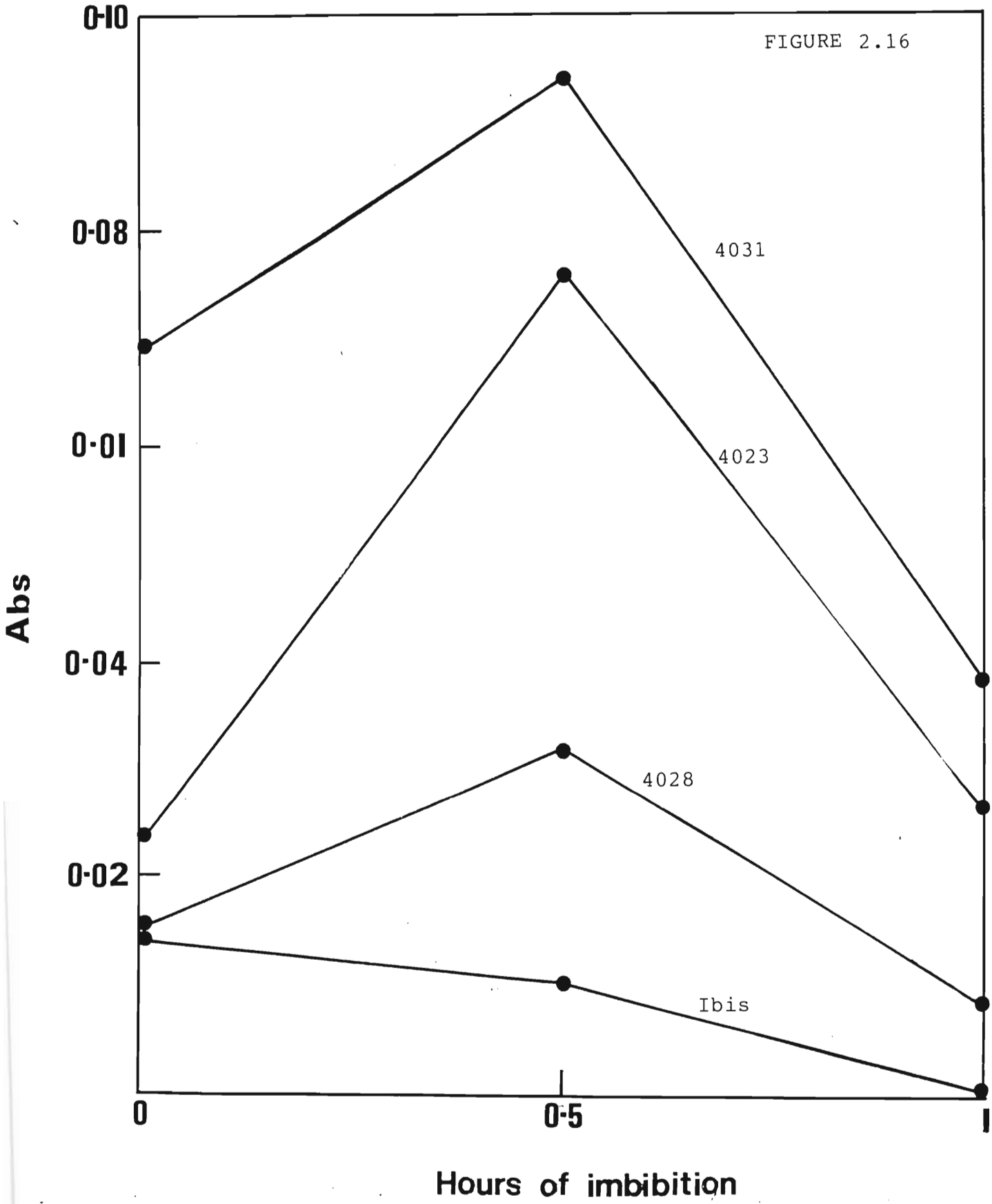


FIGURE 2.16
0·10
0·08
0·01
0·04
0·02
Abs
4031
4023
4028
Ibis
0
0·5
1
Hours of imbibition

making it more susceptible to peroxidative attack. Polyunsaturated fatty acids would therefore be preferentially oxidized, leading to their selective loss. This would lead to a change in the relative level of individual fatty acids. If the seeds have undergone peroxidative damage, as suggested by the increasing peroxide values in both cabbage and soya, changes in the relative percentages of the fatty acids, particularly the polyunsaturated fatty acids, might be expected to have occured.

Koostra and Harrington (1969) were probably the first to suggest that oxidation of membrane lipids during storage may be a cause of the increased electrolyte leakage from aged seeds. An increase in oxygenated fatty acids of seeds during storage has been reported by Spencer *et al*. (1973), although the relationship to seed vigour was not, however, determined. Priestley and Leopold (1979) reported no changes in the levels of the fatty acids in rapidly aged soya beans, concluding that oxidation of seed lipids was not involved in ageing. This contrasts with a later study by Stewart and Bewley (1980) where soya beans aged under similar conditions did show a decline in the relative percentages of the polyunsaturated fatty acids. They suggest that these changes could contribute to the loss of seed vigour. However, Flood and Sinclair (1981) have shown that the loss of germinability of scarified subterranean clover (*Trifolium subterraneum*) was accompanied by a significant decline in linoleic and linolenic acids. A further study by Priestley and Leopold (1983) demonstrated a decline in polyunsaturated fatty acids in slowly aged soya beans and these workers suggested that there may be fundamental differences between rapid and slow ageing and cautioned against direct comparison between the treatments.

The results of this present study showed a change

in the percentages of oleic (18:1), linoleic (18:2) and linolenic (18:3) acids in the total lipid extract as well as the polar lipid fraction of both the cabbage and soya bean seeds. Palmitic (16:0) and stearic (18:0) acid, although usually present, did not show any obvious change. In general, as seed vigour declined, the levels of linolenic and linoleic acids declined, while those of oleic acid increased. In cabbage, changes were more marked in the polar fraction, while in soya, the total lipid showed the greater changes.

In the total lipid extract of cabbage (figure 2.17), linolenic acid showed a slight decline from 22.5% in cv. Golden Acre to 17.6% in cv. Savoy Perfection lot C (figure 2.18). These two cultivars gave germination percentages of 100% and 39% respectively. Similarly, linoleic acid levels declined from 33.0% to 24.6%. The decline in the levels of both linolenic and linoleic acids was paralleled by a concomitant increase in the percentage of oleic acid from 20.7% to 30.0%.

Similar changes were observed in the polar lipid fraction (figure 2.19). The levels of linoleic acid decreased from 45.7% to 30.5%, while those of oleic acid increased from 20% to 36.5%. Linolenic acid showed an equivalent decline to that observed in the total extract.

Two unidentified peaks were present in the total extract of cabbage (figure 2.17) the larger of the two possibly representing erucic acid which is found in equivalent quantity in rape seeds (Swern,1964). Neither of these peaks showed any changes in relative percentage.

It was observed in this study that the level of oleic acid increased not only in relative percent (i.e. due to a loss of 18:2 & 3) but also in absolute amount i.e. in area counts (data not shown). Possible reasons for this will be considered later.

Figure 2.17 A representative gas chromatogram of a total lipid extract from cabbage seeds showing the fatty acid methyl esters detected. (16:0, palmitic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid). Two other major peaks were present (retention times 13.93 and 25.45 mins). The identity of these two peaks is unknown, although the larger of the two (25.45 mins) may be erucic acid.

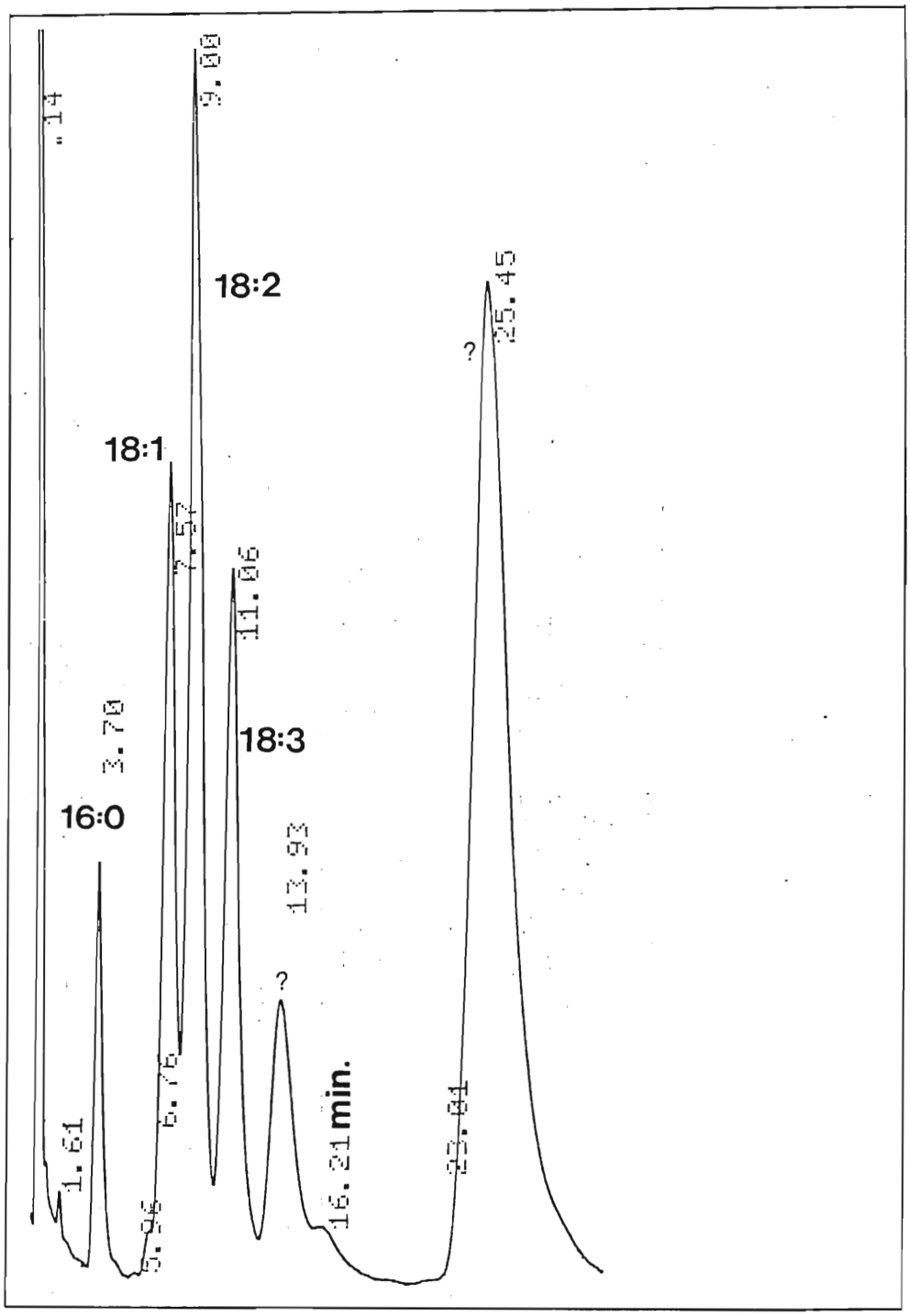

1.14
1.61
3.70
16:0
5.96
6.76
7.57
9.00
18:1
18:2
11.06
18:3
13.93
?
16.21 min.
23.04
?
25.45

FIGURE 2.17

Figure 2.18 The changes in the relative percentages of the fatty acid methyl esters of the total extract from cabbage seeds with declining seed vigour. Relative percentages have been corrected for erucic acid. (n=2)

Figure 2.19 The changes in the relative percentages of the fatty acid methyl esters of the polar fraction of cabbage seeds with declining seed vigour. (n=2)

FIGURE 2.18

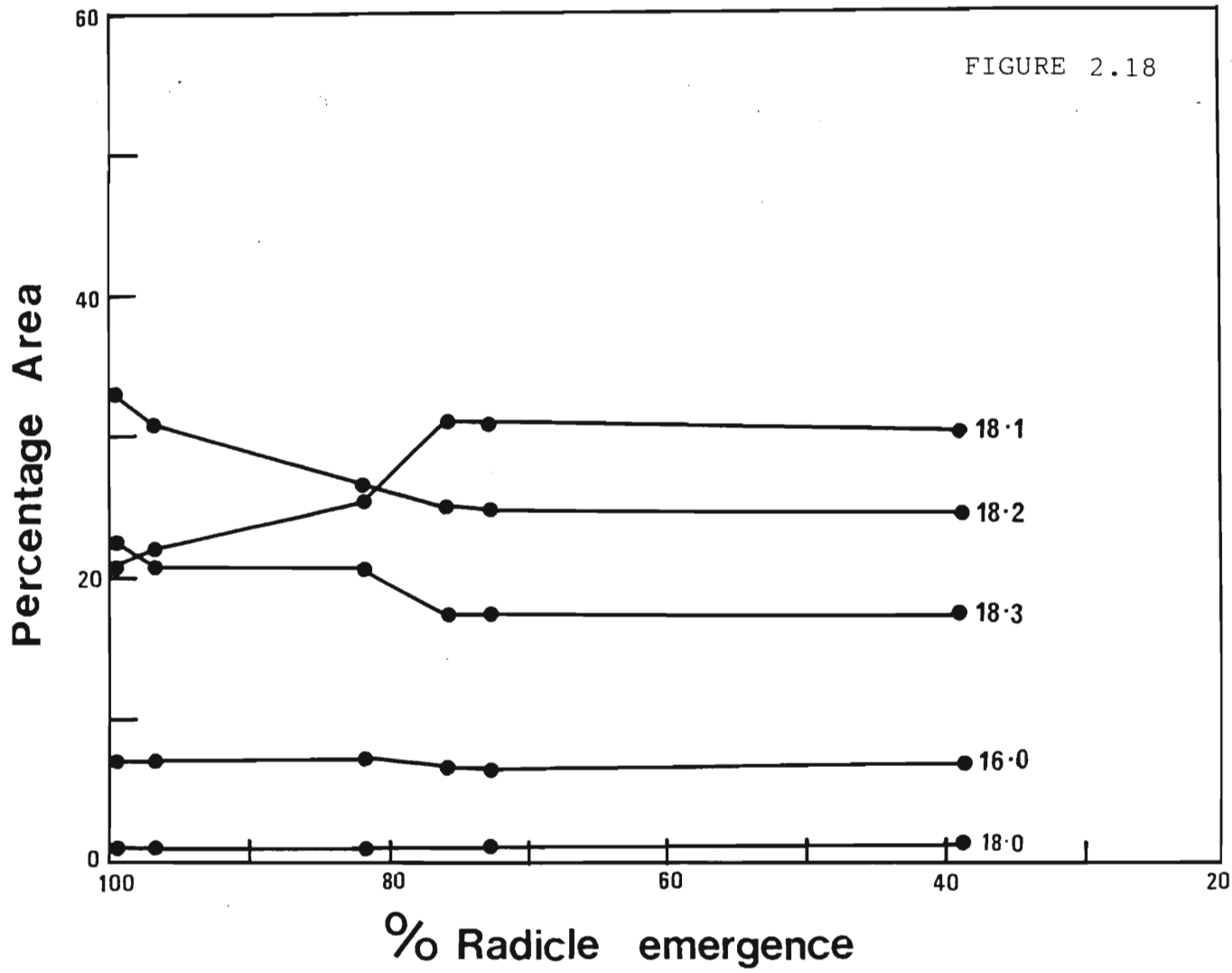
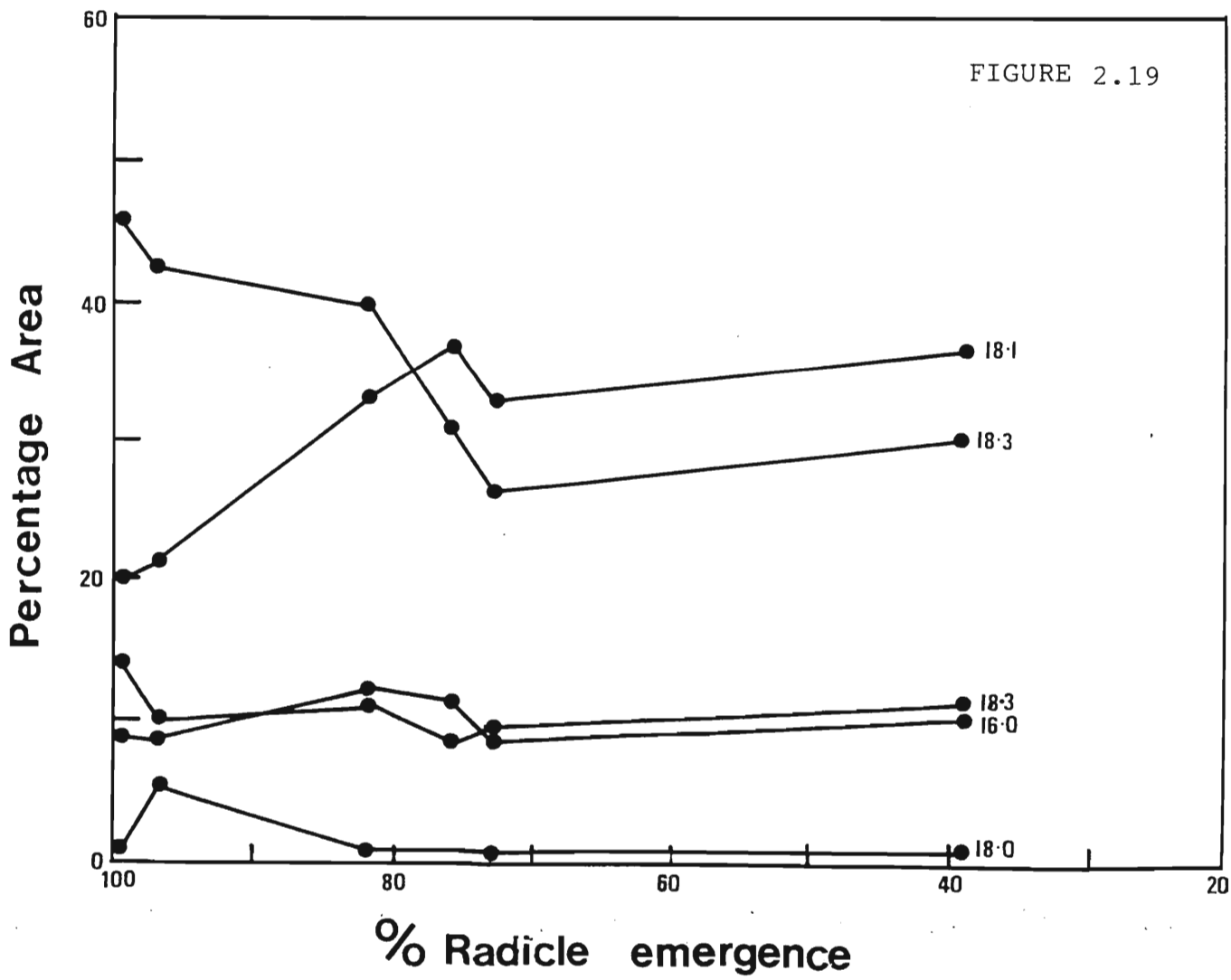


FIGURE 2.19



In the total extract of the soya beans (figure 2.20) linolenic acid levels declined from 9.2% in cv. Ibis (97% germination) to 4.9% in cv. Impala 4031 (82% germination). Similarly, linoleic (18:2) levels declined from 60.3% to 53.5%. The levels of oleic acid (18:1) rose from 18.0% to 28.2% (figure2.23). Similar changes were reported by Priestley and Leopold (1983) in the total fraction of slowly aged soya beans.

In the polar lipid fraction (table 21), only the levels of linolenic and possibly stearic acid (16:0) were seen to change; linolenic acid decreasing from 6.9% to 5.6% and stearic acid increasing from 2% to 2.5% (figure 2.22). In both cases the changes were extremely small, in marked contrast to the changes observed in the total extract. This is in agreeement with the results of Priestley and Leopold, who also demonstrated a smaller degree of change in the polar lipid fraction. Furthermore, the levels of linoleic acid showed only a very slight decline (0.2%) in their study. In this study the seeds were all of different cultivars, grown and harvested under different conditions. This could lead to a high degree of variability, as different seed lots might be expected to have different amounts of fatty acids initially i.e. before ageing, and this may mask any changes in the linoleic acid fraction. Nevertheless, the above results clearly indicate that the loss of polyunsaturated fatty acids has accompanied the loss of seed vigour in the seeds of both cabbage and soya.

3.2.Changes in fatty acid levels after heating.

Lipid hydroperoxides have been shown to be thermally labile, both in vitro (Frankel,1982) as well as in the ealier part of this present study (tables 5 and 6). It may therefore be expected that changes in the levels of the fatty acids might occur after heating.

In the total lipid extract of soya, the relative

Figure 2.20 & 2.21 A representative gas chromatogram of a total lipid extract (figure 20) and a polar lipid fraction (figure 21) from soya bean seeds showing the fatty acid methyl esters detected. (16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid).

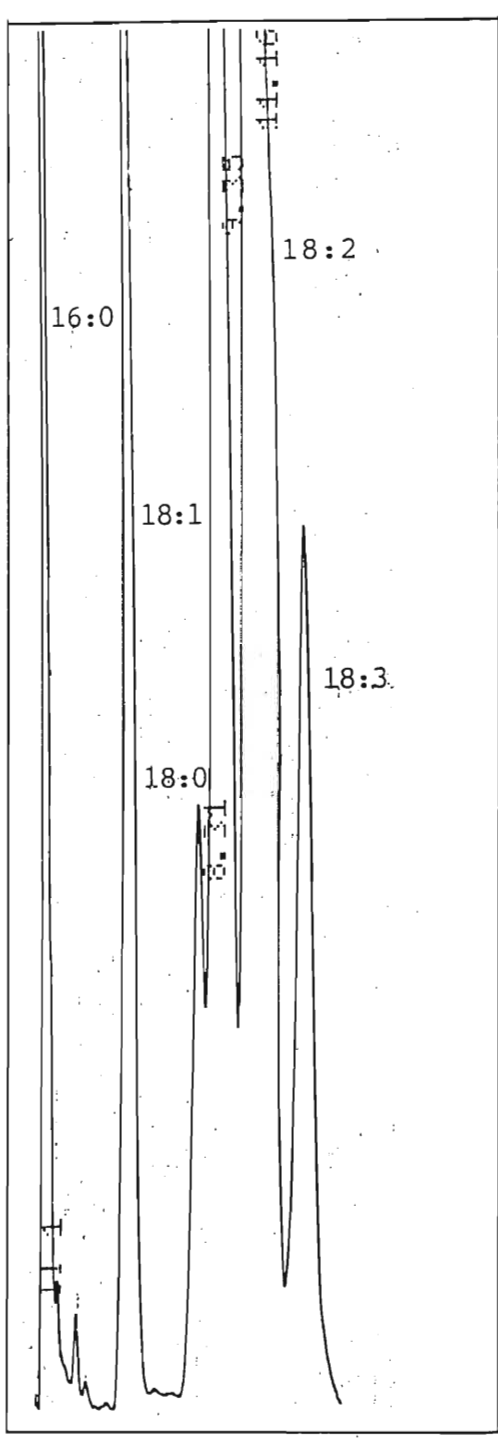


FIGURE 2.20

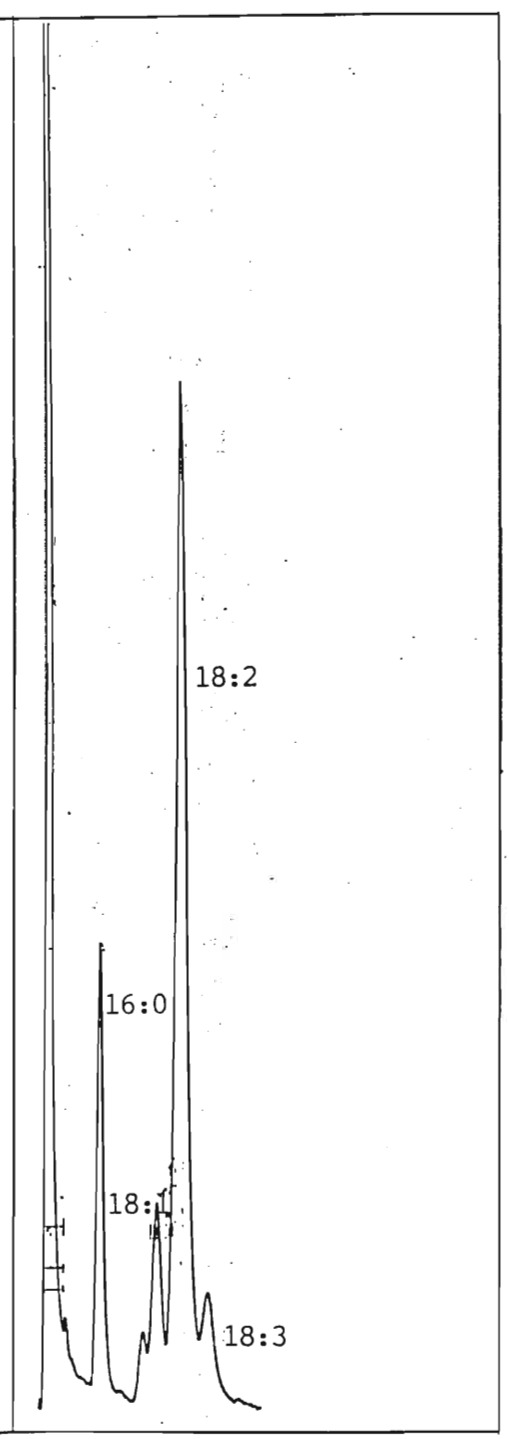


FIGURE 2.21

Figure 2.22 The changes in the relative percentages of the fatty acid methyl esters of the polar fraction from soya bean seeds with declining seed vigour. (n=2)

Figure 2.23 The changes in the relative percentages of the fatty acid methyl esters of the total extract of soya bean seeds with declining seed vigour. (n=2)

FIGURE 2.22

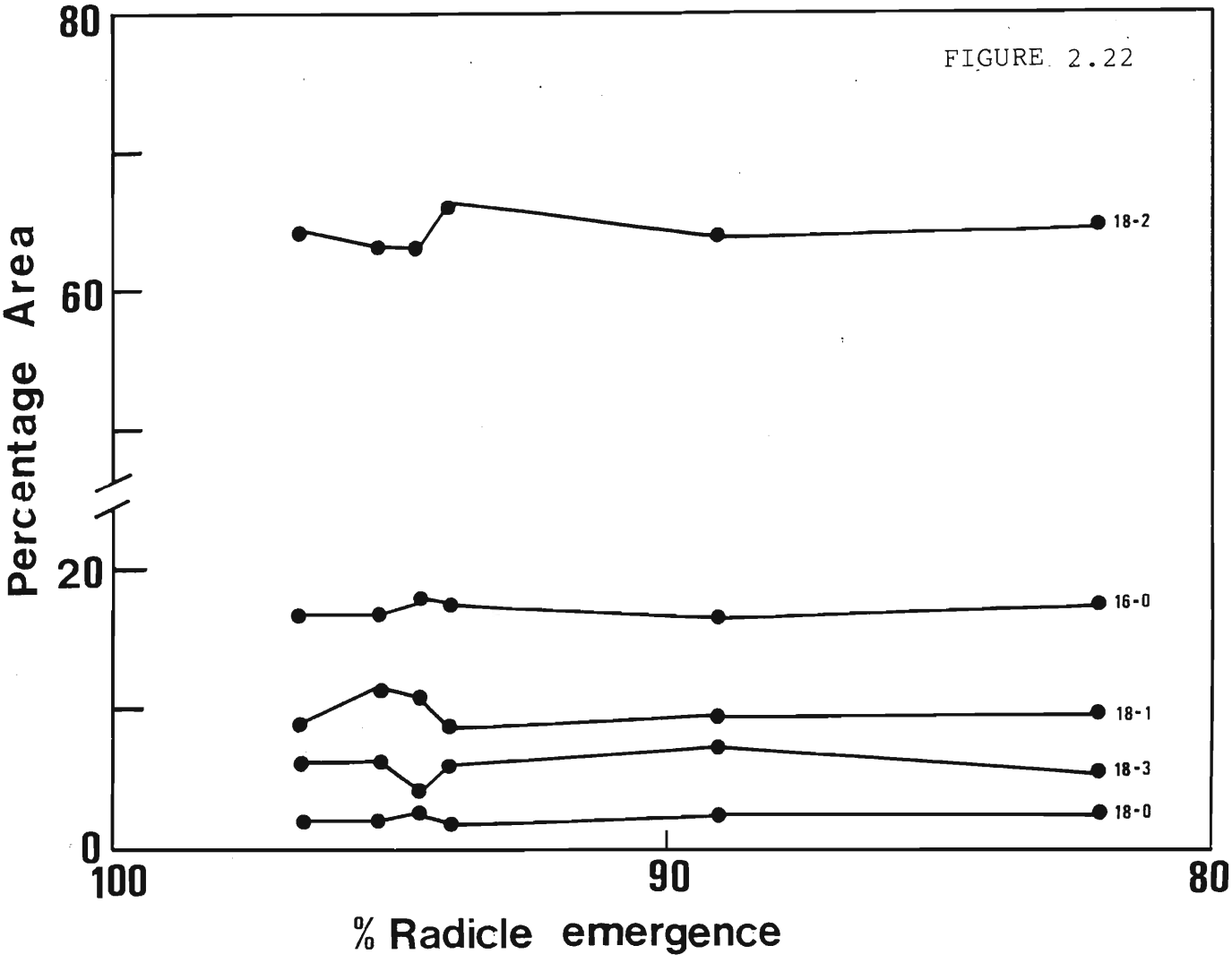


FIGURE 2.23

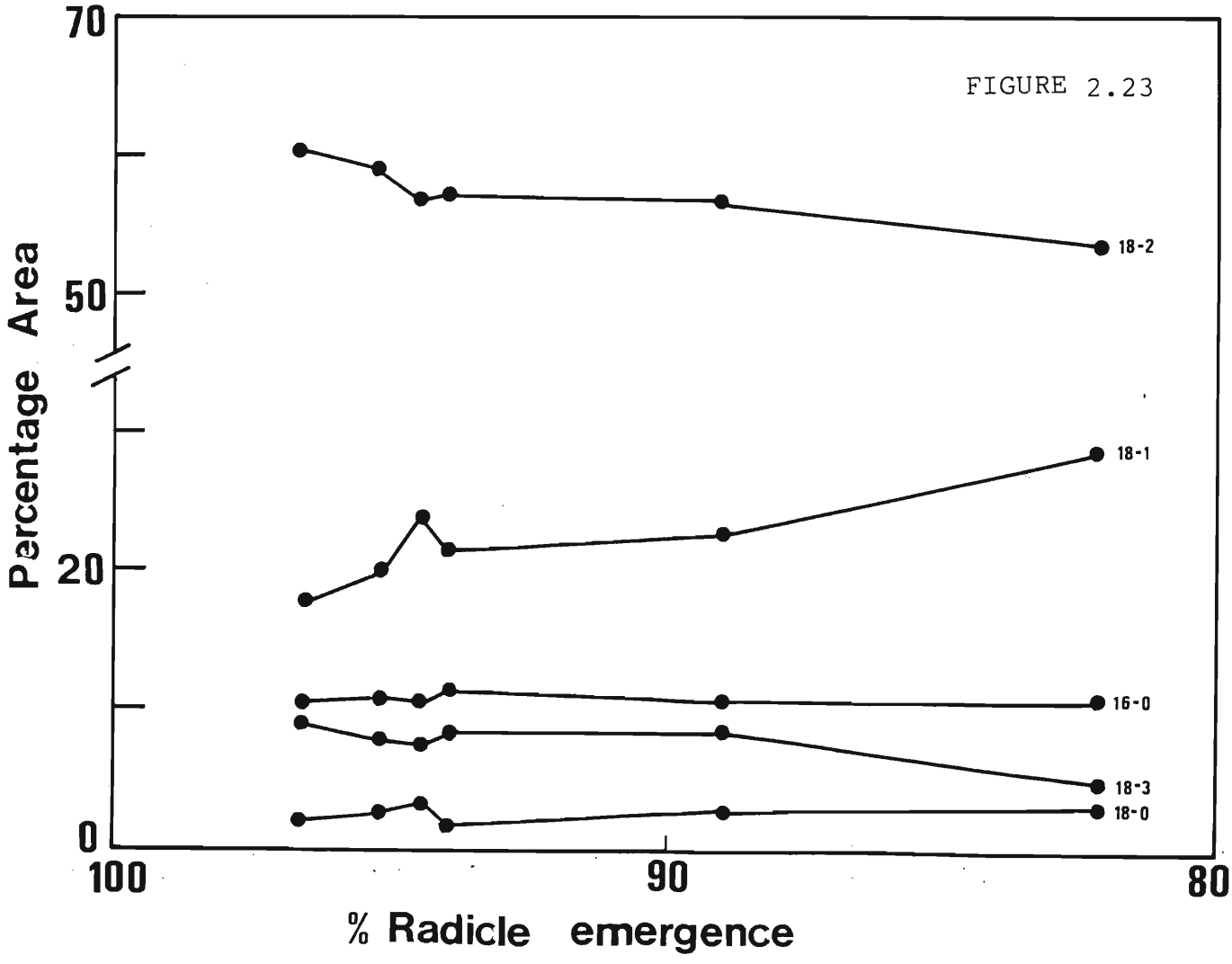


TABLE 7

Relative percentages of fatty acid methyl esters in the total lipid fraction of soya bean seeds before (A) and after (B) heating. Data represents the average of two analyses.

Cultivar.	16:0		18:1		18:2		18:3	
	A	B	A	B	A	B	A	B
Ibis	10.4	12.1	18.0	17.3	60.3	58.9	9.2	11.3
4028	10.9	12.9	19.8	15.7	59.0	56.2	7.8	11.8
4023	10.5	12.2	23.8	20.9	56.3	55.8	7.6	10.9
Pioneer	11.5	11.8	21.4	19.9	57.1	55.6	8.2	12.2
Hartebeest	10.3	12.1	22.4	21.1	56.1	54.8	8.5	11.2
4031	10.8	13.1	28.2	27.8	53.5	53.6	4.9	4.9

TABLE 8

Relative percentages of the fatty acid methyl esters of the total lipid fraction of cabbage seeds before (A) and after (B) heating. Data represents the average of two analyses.

Cultivar.	16:0		18:1		18:2		18:3	
	A	B	A	B	A	B	A	B
G.A.	6.7	6.8	20.7	20.7	33.0	30.7	22.5	23.1
G.E.	6.8	6.9	22.1	22.3	31.0	28.5	20.9	22.1
C.S.	7.1	7.0	25.4	25.9	26.3	24.3	20.6	22.1
S.P.(A)	6.5	6.9	31.1	31.7	25.1	23.5	17.6	17.2
S.P.(B)	6.4	6.9	30.6	31.0	24.9	23.0	17.7	18.0
S.P.(C)	6.7	6.7	30.0	30.3	24.6	22.5	17.6	18.3

percentages of linolenic and palmitic acid were slightly higher than those in the unheated seeds, while the levels of linoleic, oleic and stearic acid were in general less (table 7). A similar change was observed in the polar fraction, i.e. the levels of linolenic and palmitic acid were higher, and those of linoleic and oleic lower, than in the unheated seeds (data not shown). The levels of stearic acid were somewhat variable, although an increase in relative percent may have occured.

In the total lipid extract of cabbage, a slight decrease in the percentage of linoleic acid occured, while linolenic and oleic acid increased (table 8). The polar fraction showed no obvious change (data not shown). These changes are not statistically significant in either of the seed types. Nevertheless, the consistency of the changes would suggest that they had been due to the heating treatment and may indicate that lipid peroxidation was responsible for the changes in the fatty acids observed.

4.THE ANALYSIS OF VOLATILE COMPOUNDS.

4.1.Volatiles released on heating dry seeds.

The thermal decomposition of lipid hydroperoxides leads to the production of a large number of breakdown products, many of which are volatile. It may therefore be expected that volatile production from heated seeds may bear some relationship to seed vigour, as the higher the initial peroxide value of aged seeds, the greater the levels of volatiles that could be produced on heating. This is supported by the findings of Fielding and Goldsworthy (1982). These workers reported a relationship between volatile evolution and seed vigour in wheat. By heating seeds in sealed glass vials at 60°C

for 24 hours, a spectrum of volatile compounds was released which were readily analysed by GLC.

In both soya bean and cabbage, a quantitative increase in total volatile compounds released was observed with as seed vigour declined. Qualitatively, the spectrum of volatiles evolved was identical, except for peak 9 which was only observed in cabbage (figures 2.25 and 2.26).

Cabbage.

In general, peaks 1 to 6 showed an increase in area as seed vigour declined. Furthermore, these peaks were present in all of the seed samples (table 9). Two other large peaks were also detected, namely peaks 8 and 9. These did not show as clear a relationship with seed vigour as the preceeding peaks, and peak 9 was not detected in the sample from cv. Golden Acre.

Peak area percentages in all samples declined with declining seed vigour. This was probably due to the preponderance of peak 9. If peak area percentages were calculated with peaks 8 and 9 excluded, peak area percentages were observed to increase (table 9). The relationship between increase in peak area percentage and seed germinability was most marked in peaks 4 and 5 (figure2.27), which were observed to coelute (figure 2.25).

The areas i.e. the absolute amounts of peaks 4 & 5 showed a better relationship to peroxide value than to germinability (figure 2.28) and would be expected if hydroperoxides were the source of the volatiles. Area percentages on the other hand showed a better relationship to germinability (table 9). This may suggest that total peak production may be related to seed vigour, and may be significant if peaks are to be used on a predictive basis.

Figure 2.25 & 2.26 Gas chromatograms showing the volatiles derived from the seeds of cabbage and soya bean on heating. Peaks 4 and 5 in cabbage and 6 and 7 in soya coeluted. Peak 9 was found only in cabbage.

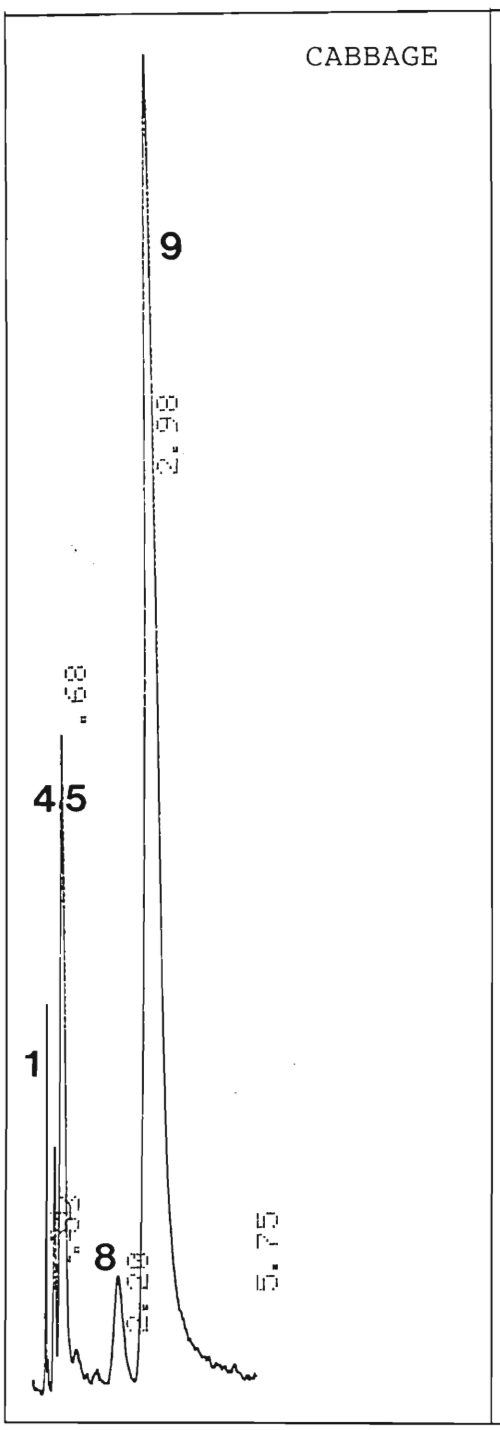


FIGURE 2.25

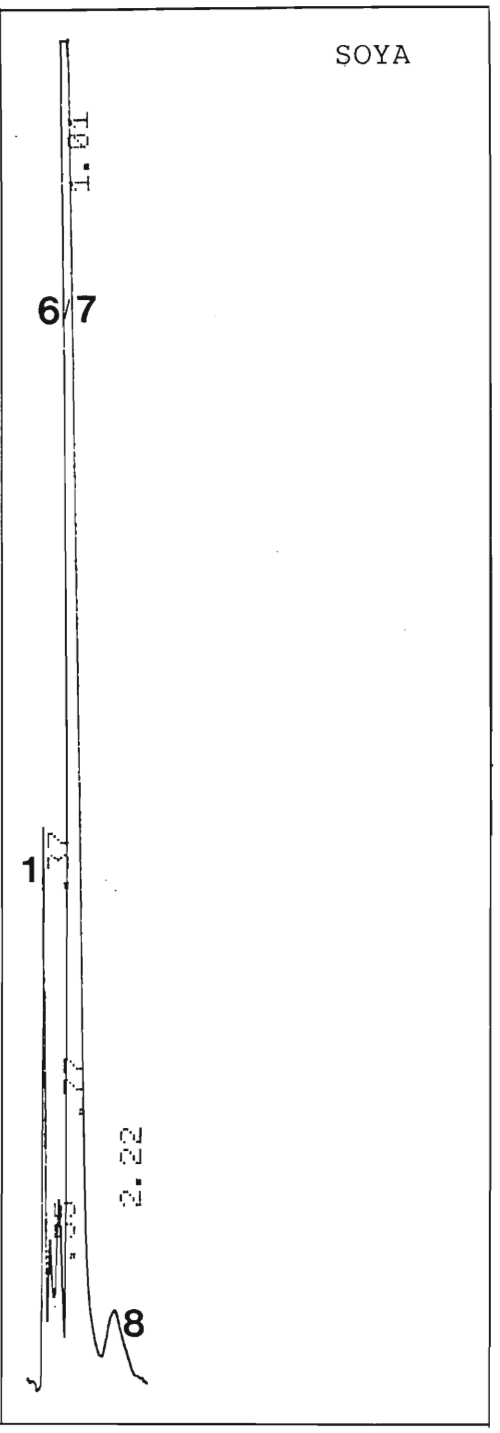


FIGURE 2.26

TABLE 9

Volatile compounds produced from cabbage (*B. oleracea*) seeds on heating (22hrs at 85°C). A, Area ($X10^{-3}$), B, Area Percent. Data represents the average of two analyses.

Peak.[1] Cultivar.	1.		3.		4&5[2]		9.	
	A	B	A	B	A	B[3]	A	B
G.A.	2.9	11.0	4.8	20.9	15.7	65.5	-	-
G.E.	5.0	1.7	12.3	4.2	44.4	71.8	278.1	76.0
C.S.	8.2	3.1	12.1	4.3	82.2	68.0	194.9	54.4
S.P.(A)	5.6	2.2	16.3	5.2	59.9	73.4	251.2	66.9
S.P.(B)	7.2	1.3	13.7	2.5	72.1	74.7	469.7	79.4
S.P.(C)	6.1	1.8	10.8	3.2	64.2	78.0	300.6	75.5

1, in order of elution, 2, peaks 4&5 coeluted, 3, calculated with peaks 8&9 excluded.

TABLE 10

Volatile compounds produced from soya bean (*G. max*) seeds on heating (22hrs at 85°C). A,Area ($X10^{-3}$), B,Area Percent. Data represents the average of two analyses.

Peak.[1] Cultivar.	1.		4&5[2].		6&7[2]		8.	
	A	B	A	B	A	B	A	B
Ibis	33.6	6.3	38.3	5.5	648.2	77.7	37.2	7.7
4028	37.3	6.6	54.7	9.1	578.0	77.9	40.1	5.1
4023	35.9	5.1	67.9	9.7	566.1	78.7	64.3	8.1
Pioneer	47.7	4.7	50.9	4.9	700.4	79.5	82.0	7.6
Hartebeest	42.9	4.7	83.6	10.4	703.8	78.9	63.6	5.6
4031	40.2	4.5	69.7	8.1	781.2	83.4	72.5	4.6

1, in order of elution, 2, peaks 4&5 and 6&7 coeluted.

Figure 2.27 A regression of the percentage area of peak 4&5 and percentage radicle emergence of the six lots of cabbage seeds. (r=0.87). The correlation was significant at the 5% level of confidence. (n=2)

Figure 2.29 A regression of the percentage area of peak 6&7 and percentage radicle emergence of the six lots of soya bean seeds. (r=0.91). The correlation was significant at the 5% confidence level. (n=2)

FIGURE 2.27

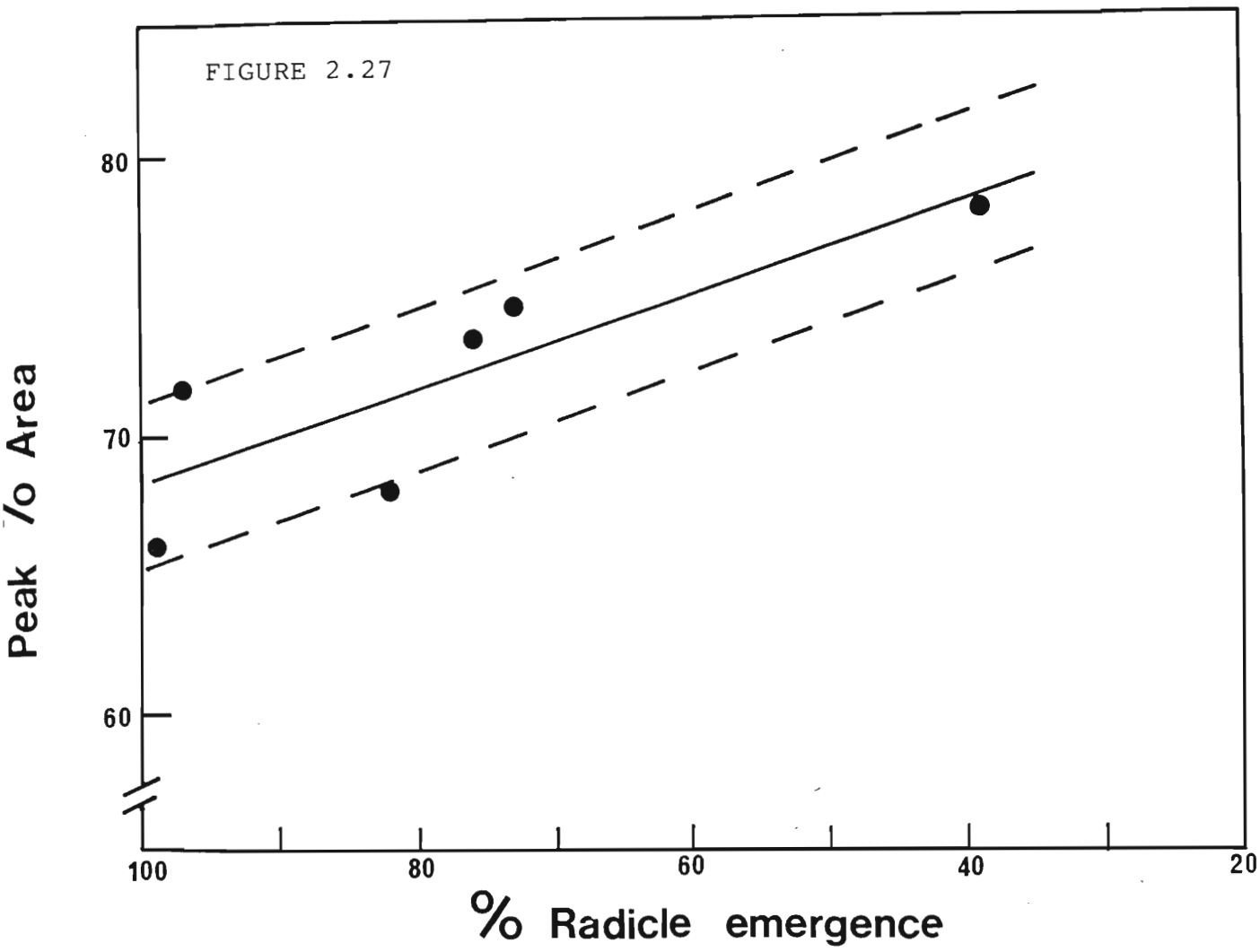


FIGURE 2.29

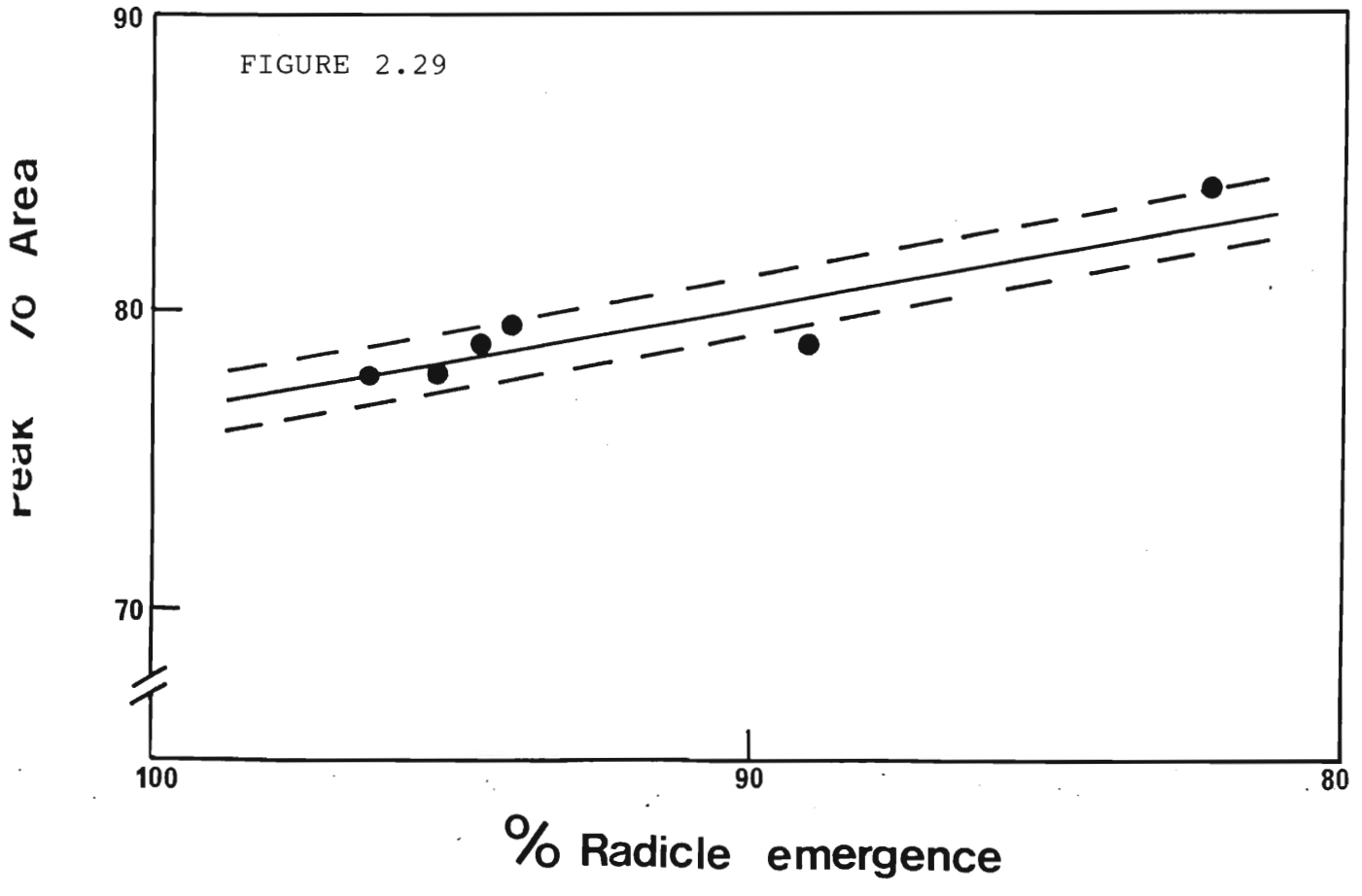


Figure 2.28 A regression of the hydroperoxide level and the area of peak 4&5 derived from the six lots of cabbage seeds. (r=0.87). The correlation was significant at the 5% level of confidence. (n=2)

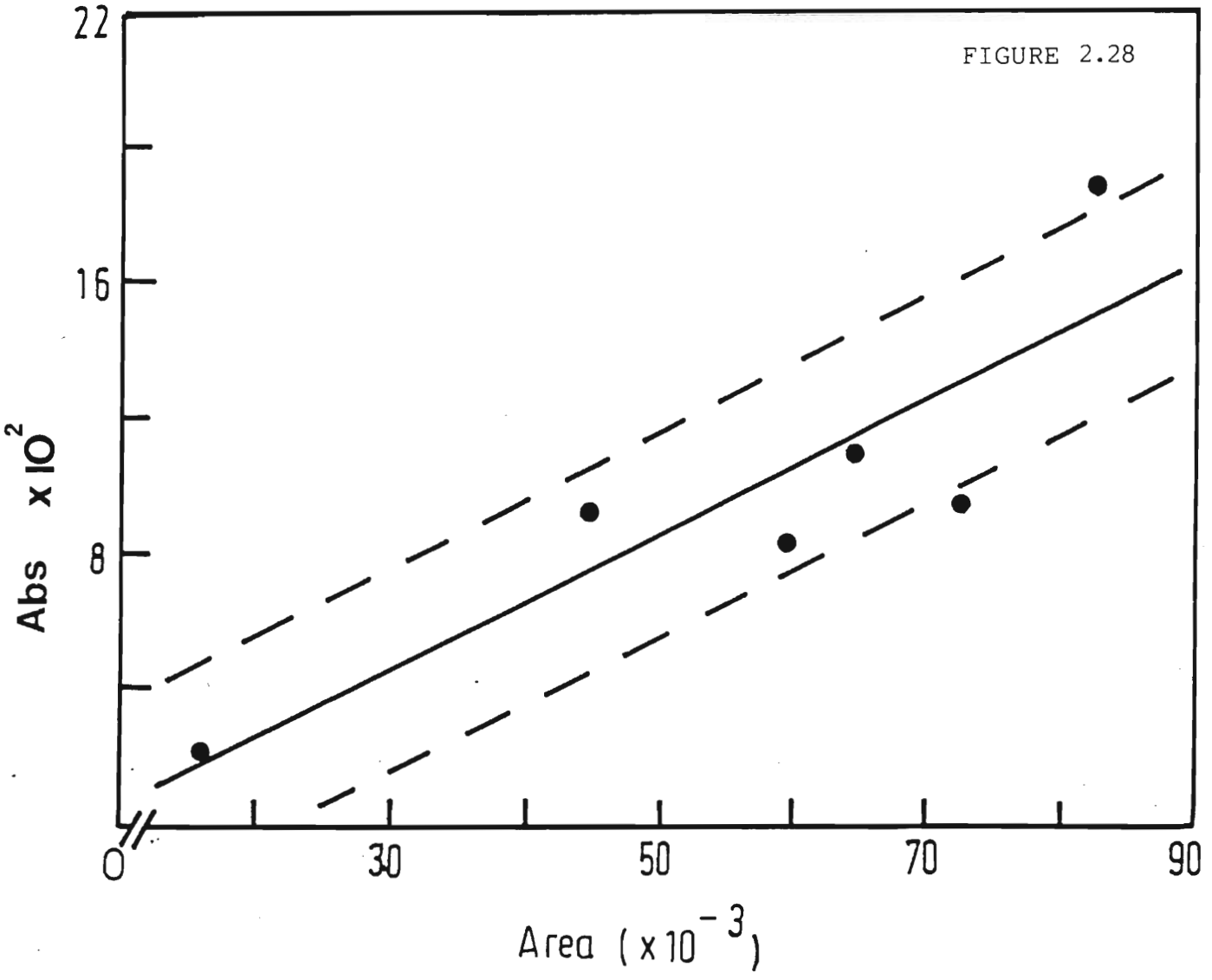


FIGURE 2.28

Soya bean.

The trends observed in soya were identical to those in cabbage, i.e. area counts of all peaks were observed to increase as seed vigour declined, particularly peaks 6 and 7 (figure 2.29) which were again seen to coelute (figure 2.26). Area percentages increased only in peaks 6 and 7 (table 10), the other peaks showing no distinct trend (peak 4/5) or declining (peaks 1 and 8). Peak 9 was consistently absent (figure 2.26).

The relationship between germinability and peroxide value was much more distinct in soya than in cabbage (section 1.) and hence the relationship between these and either areas or area percentages of peaks 6 & 7 was very similar, although a better relationship was still possibly present between area percentage and germinability (table 10). Moisture content, in contrast to cabbage, was apparently unrelated to the evolution of the volatile compounds.

It may be significant to note that the spectrum of volatiles derived from both soya and cabbage seeds was very similar to that reported by Fielding and Goldsworthy (1982) using similar chromatographic conditions. Furthermore, they stress the importance of a "peak 6". This bears a similarity to the peak 4/5 of cabbage and 6/7 of soya found in this study. In addition, the above workers state that it was this peak that was most clearly related to seed vigour, as was the case in this study. The above similarities, therefore, might suggest a common source and, if shown to be universal, will add further support to the suggestion of Fielding and Goldsworthy that this method may provide a very sensitive test for the routine determination of seed physiological age.

TABLE 11

Volatile compounds (percentage area) produced from soya bean (*G. max*) and cabbage (*B oleracea*) oils on heating (22hrs at 85°C). Data represents the mean of two analyses.

Soya Cultivar.	Peak[1] 6&7[2]	Cabbage Cultivar.	Peak[1] 4&5[2]
Ibis	68.8	Golden Acre	58.4
4028	66.9	Glory of Enkhuizen	66.8
4023	82.2	Cape Spitz	73.8
Pioneer	53.6	Savoy Perfection(A)	76.1
Hartebeest	75.6	Savoy Perfection(B)	60.1
4031	51.3	Savoy Perfection(C)	65.9

1, in order of elution. 2, peaks coeluted.

4.2.Volatile compounds evolved from total lipid extracts on heating.

In the analysis of the volatile compounds evolved from whole seeds, it is difficult to acertain with certainty the source of the compounds. If the lipid was the source, then the heating of the total lipid extract should give similar results.

In both cabbage and soya bean, no qualitative difference was observed between the volatile compounds evolved from dry seeds (figure 2.25 & 2.26) and those evolved from extracted oils (figures 2.30 and 2.31). A similar result has been reported by Fielding and Goldsworthy (1982) who showed that the volatile profile obtained from heated wheat germ oil was very similar to that obtained from heated wheat kernels. Peak 9, however, was not detected in any of the samples of the headspace from extracted cabbage oils. Furthermore, the quantitative relationship between the peaks derived from extracted oils and the vigour of the seeds was less marked than for those derived from the dry seeds, although a possible increase in the area percentage of peaks 4 and 5 in cabbage and 6 and 7 in soya bean was still observed (table 11). Nevertheless, the results clearly suggest that the volatiles were in all probability derived from the thermal decomposition of the lipid hydroperoxides.

4.3.The analysis of volatile compounds evolved during imbibition.

Harman et al. (1980) were probably the first to demonstrate a increased evolution of volatile carbonyl compounds in aged seeds on imbibition. These workers suggested that it was due to lipid peroxidaion. Subsequently, Woodstock and Taylorson (1981) have reported increased levels of ethanol and acetaldehyde in rapidly aged soya beans, although they suggest that

Figure 2.30 & 2.31 Gas chromatograms showing the volatiles derived from the extracted oils from seeds of cabbage and soya bean on heating.

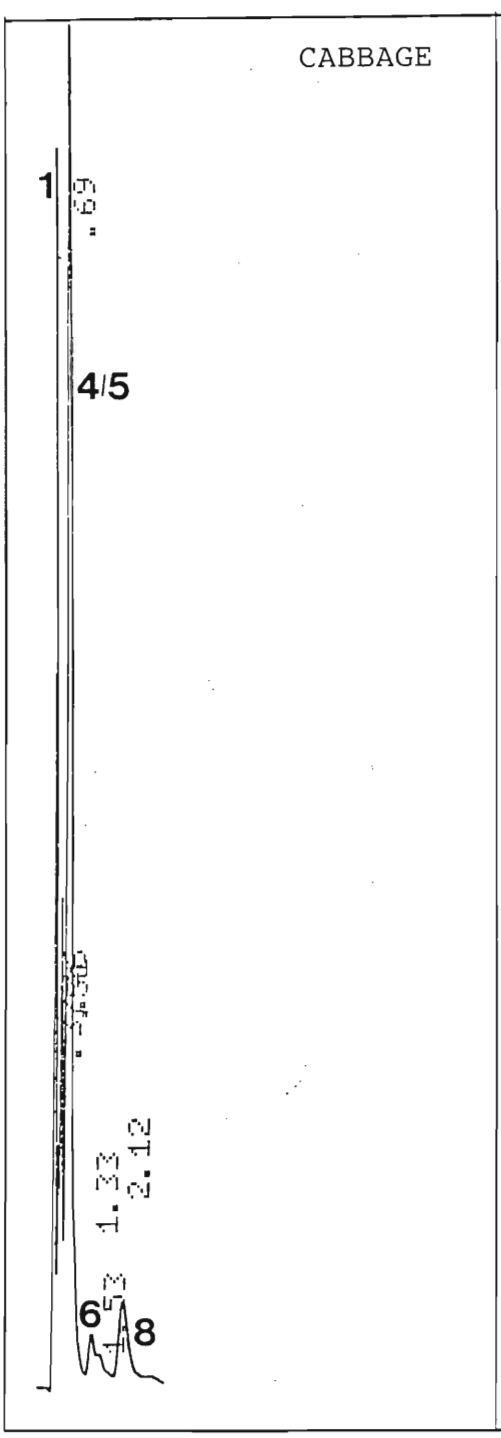


FIGURE 2.30

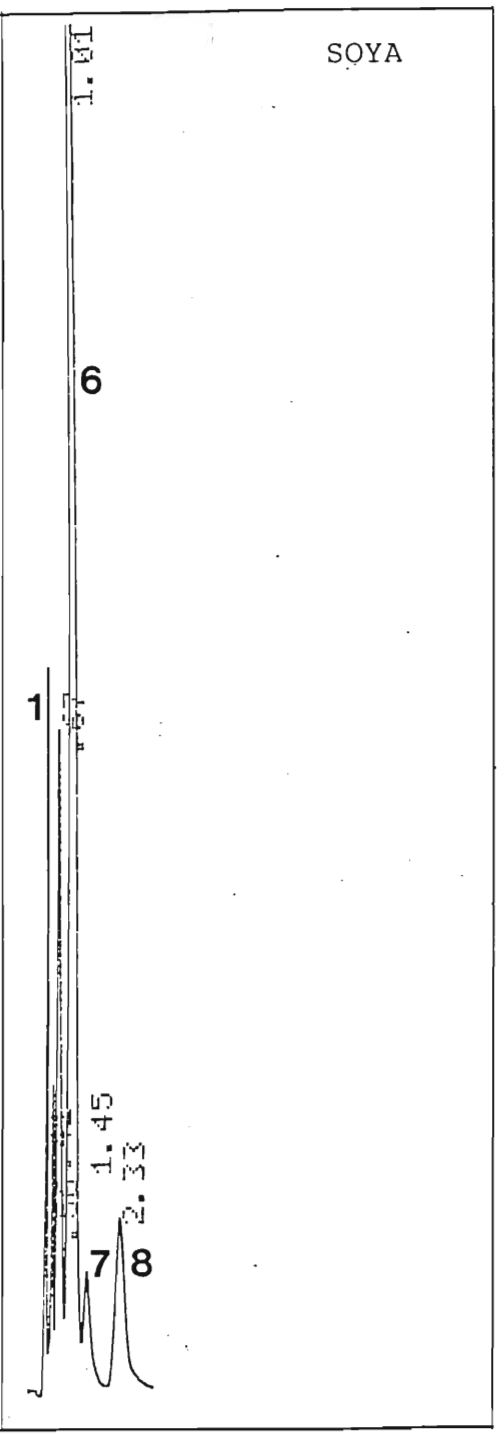


FIGURE 2.31

metabolic imbalance may be a probable source of the volatile compounds. Similar results have been reported by Gorecki et al.(1985) who showed an increase in the levels of ethanol and acetaldehyde with declining pea seed age. More recently, Wilson and McDonald (1986b) have demonstrated that the levels of volatile aldehydes produced during the first 24 hours of germination were highly correlated with field emergence in soya bean seeds.

The volatile compounds evolved from cabbage during the first 24 hours of imbibition are shown in figure 2.32. Qualitatively, all samples were identical, but varied somewhat quantitatively. For this reason, total volatile production during this period showed little relationship with seed vigour, in contrast to the results of the above workers. However, one peak was seen to bear a relationship to seed vigour (retention time 2.6 mins- figure 2.32 on an area and percentage area basis. This increased from undetectable levels in cv Golden acre (germination 100%) to 24 % of the total in cv Savoy Perfection C (germination 39%). The relationship between this peak and seed vigour was very marked (figure 2.33).

In contrast to cabbage, volatiles derived from whole seeds of soya (figure 2.34) showed considerable variation both quantitatively and qualitatively. No relationship between any peak and seed vigour could be found. Gorecki et al. (1985) have reported that infestation by micro-organisms led to highly variable volatile estimations. In order to eliminate this possibility in the present study, seeds were sterilized in 1% NaOCl for 5 minutes before being sealed into vials, and a shorter imbibition time before sampling was used. However, this treatment only slightly reduced the variability encountered. Nevertheless, a peak that eluted in the approximate position to the indicator peak in cabbage (retention time 2.46 mins - figure 2.34

Figure 2.32 Gas chromatograms showing the volatiles derived from seeds of cabbage after 24 hours of imbibition.

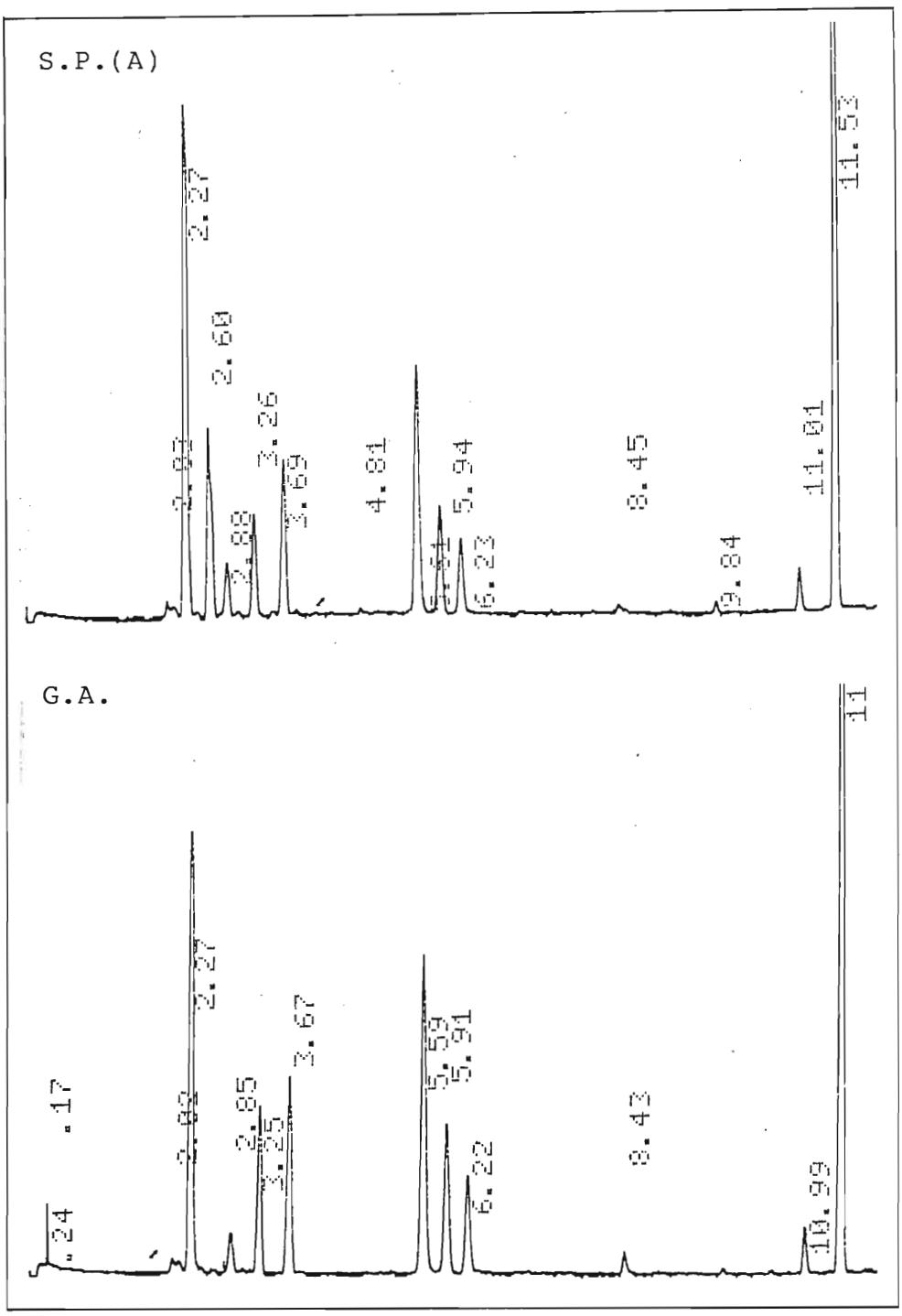

S.P.(A)
2.27
2.60
3.26
2.82
2.88
3.69
4.81
5.94
6.23
8.45
9.84
11.01
11.52
G.A.
.17
.24
2.27
2.82
2.85
2.95
3.67
5.53
5.91
6.22
8.43
10.99
11

FIGURE 2.32

Figure 2.33 The relationship between the percentage area and the area of the peak eluting at 2.60mins and the percentage radicle emergence of the six lots of cabbage seeds. (n=2)

FIGURE 2.33

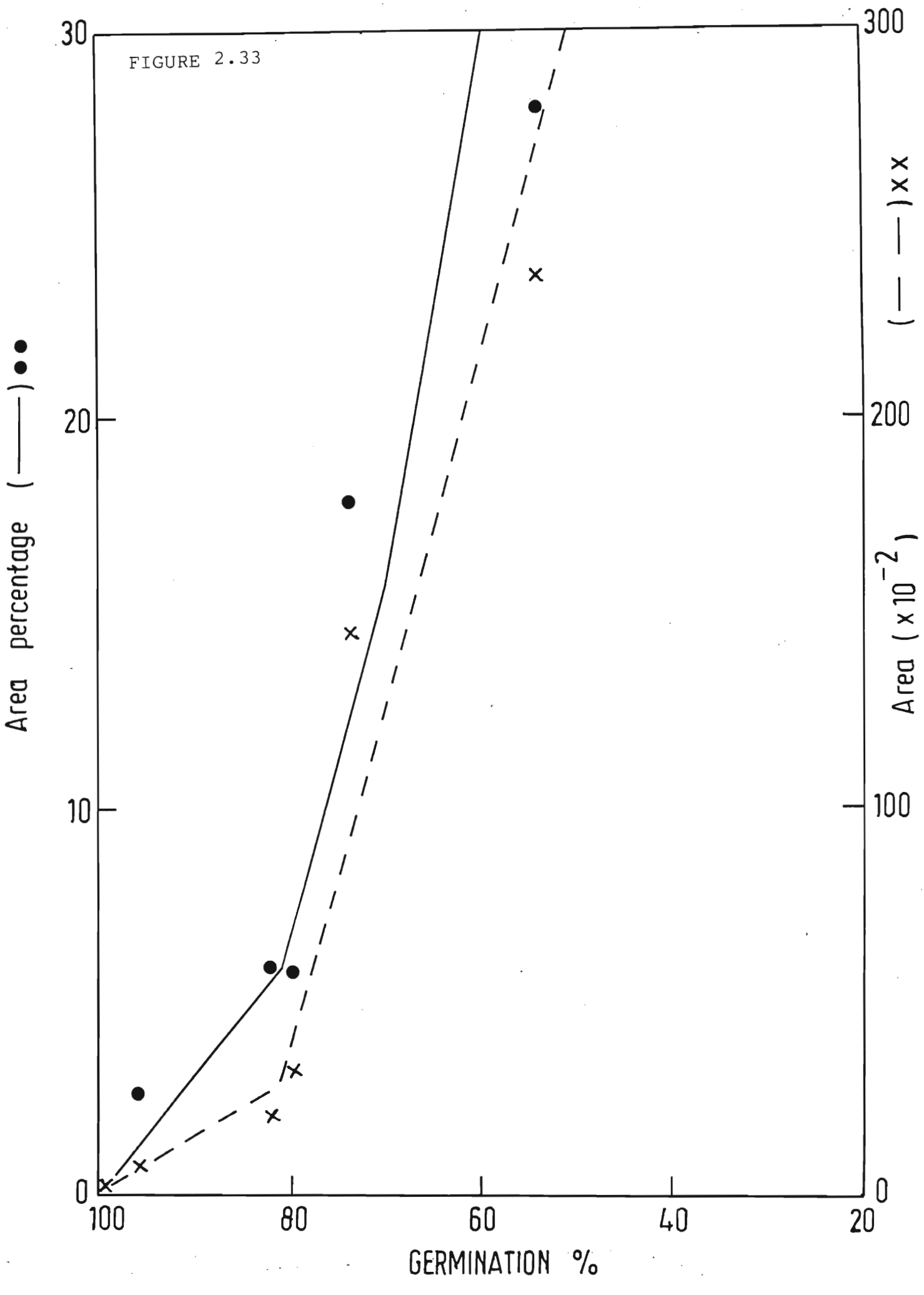

30
20
10
0
300
200
100
0
100
80
60
40
20
GERMINATION %
Area percentage (——) ●●
(— — —) x x
Area (x10⁻²)

Figure 2.34 Gas chromatograms showing the volatiles derived from seeds of soya bean after 24 hours of imbibition.

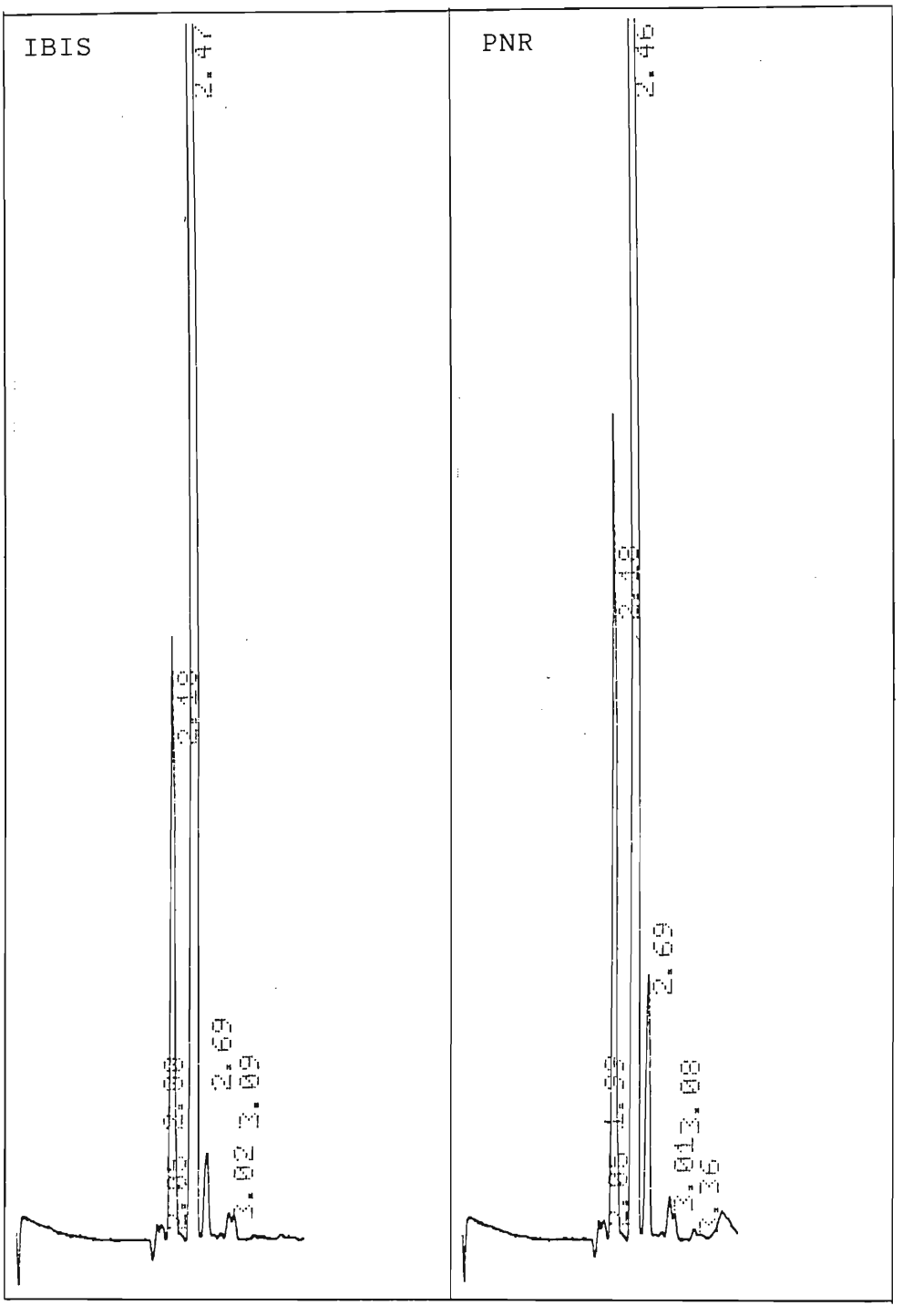


FIGURE 2.34

appeared to show some relationship to seed vigour (figure 2.35).

The use of isolated axes greatly reduced the variabilty encountered in the whole seeds. This may again highlight the need to distinguish between changes in individual seed parts as opposed to whole seeds. However, volatile production showed no relationship to seed vigour in either total counts or the relative percentage of the main peak (retention time 1.10-1.12 mins - figure 2.36 and 2.37).

5.SEED INVIGORATION BY CHEMICAL TREATMENT.

The idea of chemically treating seeds to improve germinability and storability is one of long standing. Kaloyereas et al. (1961) demonstrated that the pretreatment of onion and okra seeds with alpha-tocopherol or starch phosphate improved storability at ambient conditions. More recently, Radrupal and Basu (1979) have reported that iodination of mungbean seeds increased storability under rapid aging conditions. Similarly, Gorecki and Harman (in press) have shown an improved storability of pea seeds pretreated with alpha-tocopherol and butylated hydroxytoluene, also under rapid aging conditions. Water-soluble antioxidants, including ascorbate and glutathione, had little beneficial effect. Indeed, ascorbate actually accelerated the rate of vigour loss. This is probably due to the pro-oxidant behaviour of ascorbate at low concentrations (Halliwell and Gutteridge,1985). Most work using antioxidant treatment has been done on the influence of the treatment on storability rather than germinability. Nevertheless, Gorecki and Harman (in press) have stated that a slight improvement in seed germination and vigour was observed after treatment with sodium benzoate and glutathione.

Figure 2.37 The relationship between the percentage area of the peak eluting at 1.11-1.12mins (equivalent to that eluting at 2.46min - different chromatographic conditions were used) and percentage radical emergence of the six lots of soya bean seeds. (n=2)

Figure 2.35 A regression of the percentage area of the peak eluting at 2.46min and the percentage radicle emergence of the six lots of soya bean seeds. (r=0.57). (n=1)

FIGURE 2.37

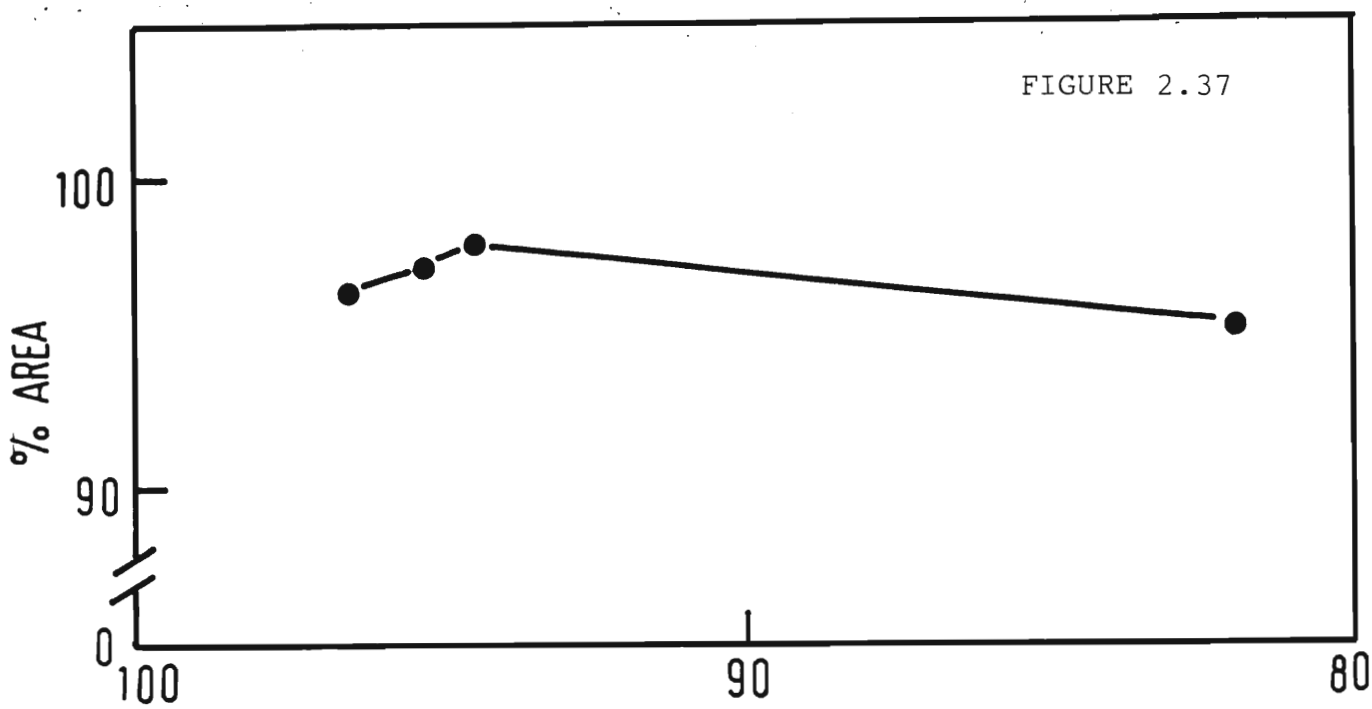


FIGURE 2.35

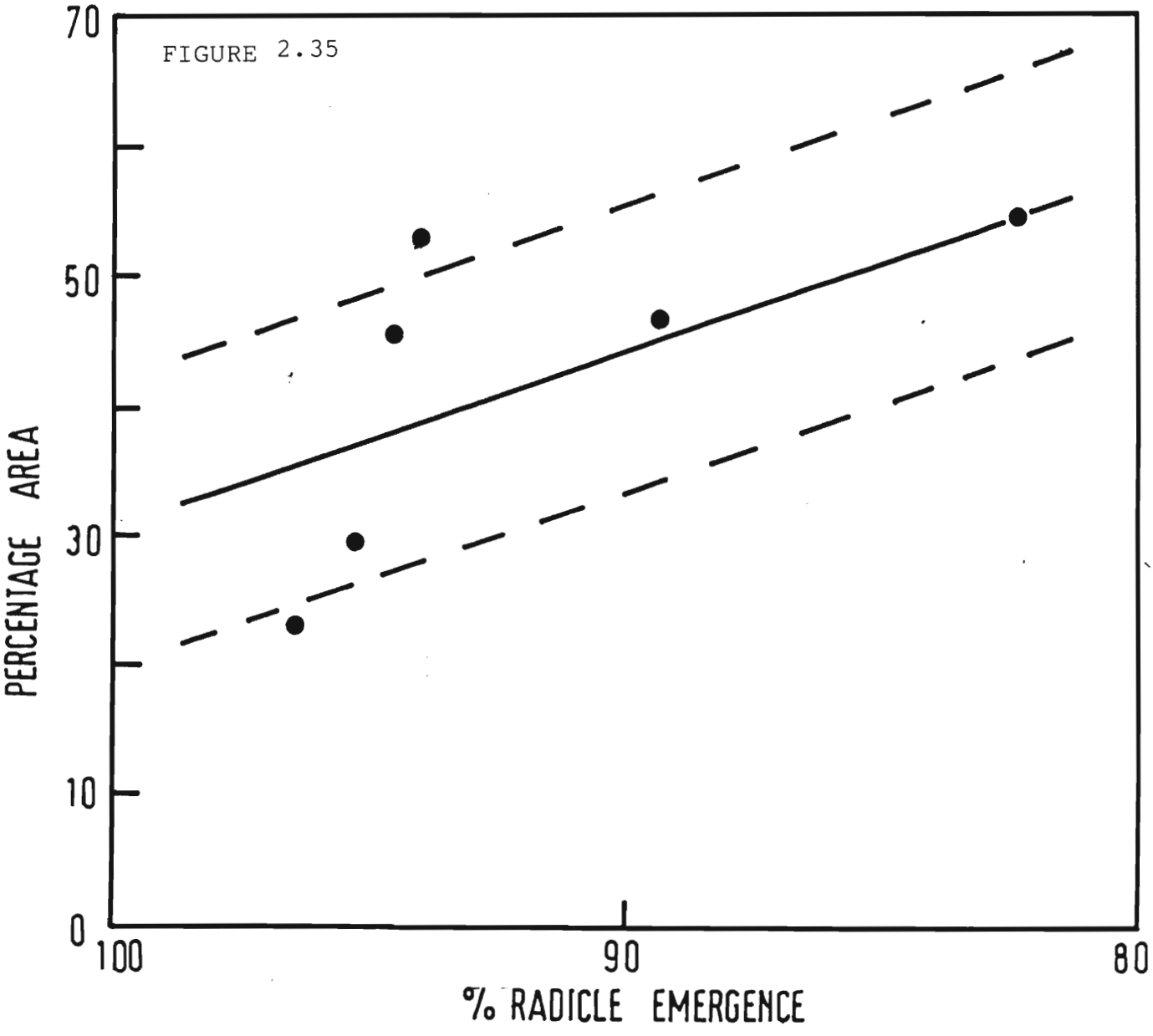


Figure 2.36 Gas chromatograms showing the volatiles derived from soya bean axes after 24 hours imbibition.

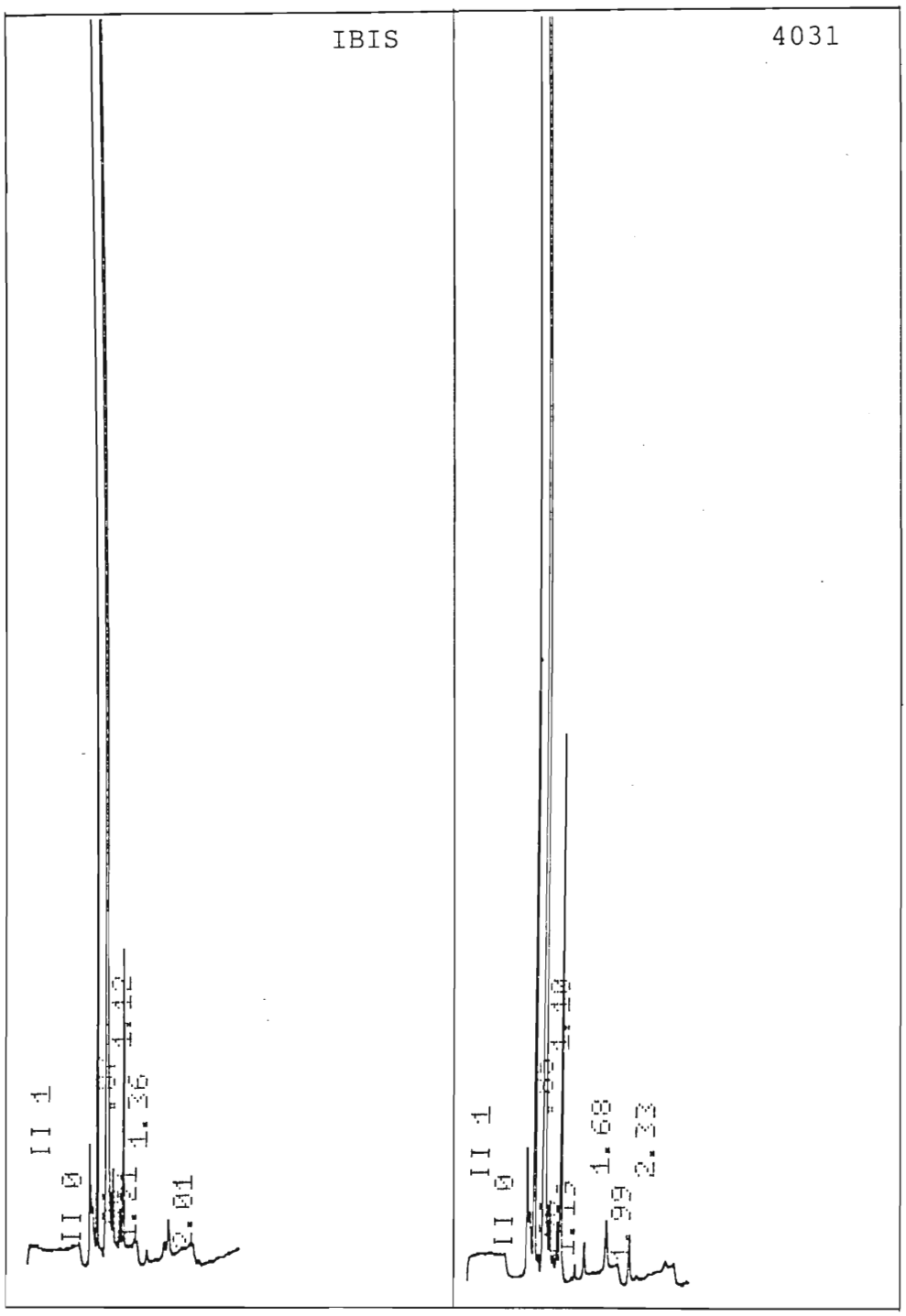


FIGURE 2.36

Berjak (1978) has shown that the provision of a static electric charge during and after rapid ageing markedly improved maize seed vigour over the controls. This improved vigour was reflected in the greater ultrastructural integrity of treated seeds. Furthermore, this treatment increased the rate of germination of seeds not subject to rapid ageing. This was suggested to be due to an inhibition of lipid peroxidation by providing a extra source of electrons, thus quenching any free radicals produced.

In the light of the above, it was decided to provide a chemical source of electrons, viz. simple reducing agents, to the seeds in order to determine if a similar invigorating effect could be achieved. Seeds of cabbage and soya bean were treated with solutions of ferrous chloride, potassium iodide and ferrous sulphate. The effect of antioxidants tinoridine, a water-soluble compound with alpha-tocopherol like activity (Yasuda et al.,1980) and dimethyl sulphoxide (DMSO), a hydroxyl radical scavenger, was also investigated.

When cabbage seeds (cv Savoy Perfection C) were treated with the above reducing agents, a marked invigoration of the seeds was observed (figure 2.38). The treatments improved both the rate of germination and the final percentage germination of the seeds. Both of the ferrous compounds showed a greater invigorating ability than potassium iodide. Furthermore, the invigorating effect increased with increasing concentration of these reducing agents. However, a reduction in concentration did not substantially reduce the invigorating effect of either of these compounds (figure 2.39).

Treatment with tinoridine, a compound reported to have 20 times the activity of alpha-tocopherol in inhibiting the onset of CCl_4 toxicity in rat liver (Yasuda et al.,1980), had only a slight invigorating effect (figure 2.38). This is in agreement with the work

Figure 2.38 The invigorating effect of antioxidants and reducing agents on cabbage seeds (cultivar Savoy Perfection C). (n=2)

Figure 2.39 The invigorating effect of different concentrations of reducing agents on the cabbage seeds. The invigorating effect was very similar for either 0.01M (———) and 0.005M (-----) solutions of the two compounds. (n=2)

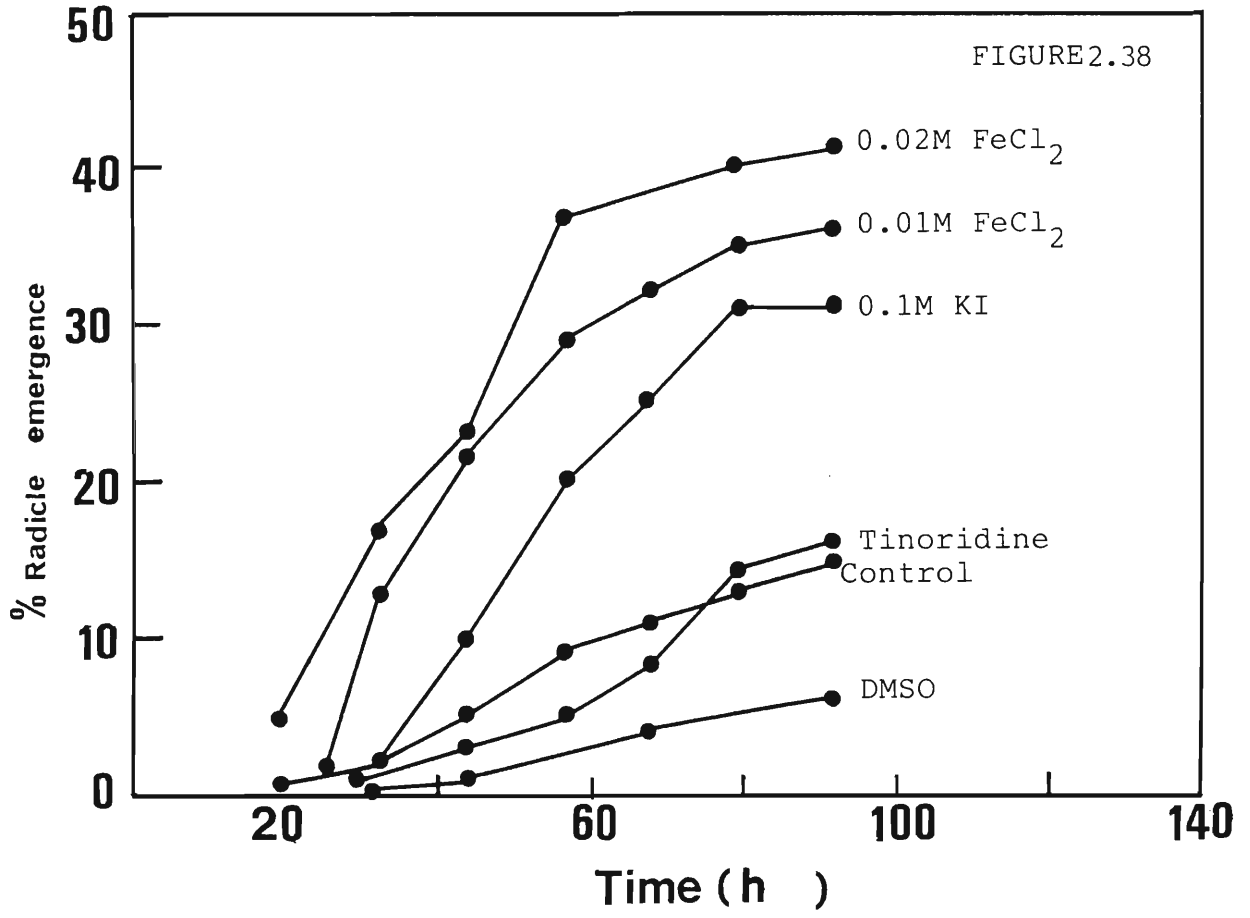


FIGURE2.38
0.02M FeCl2
0.01M FeCl2
0.1M KI
Tinoridine
Control
DMSO
% Radicle emergence
Time (h)
0
10
20
30
40
50
20
60
100
140

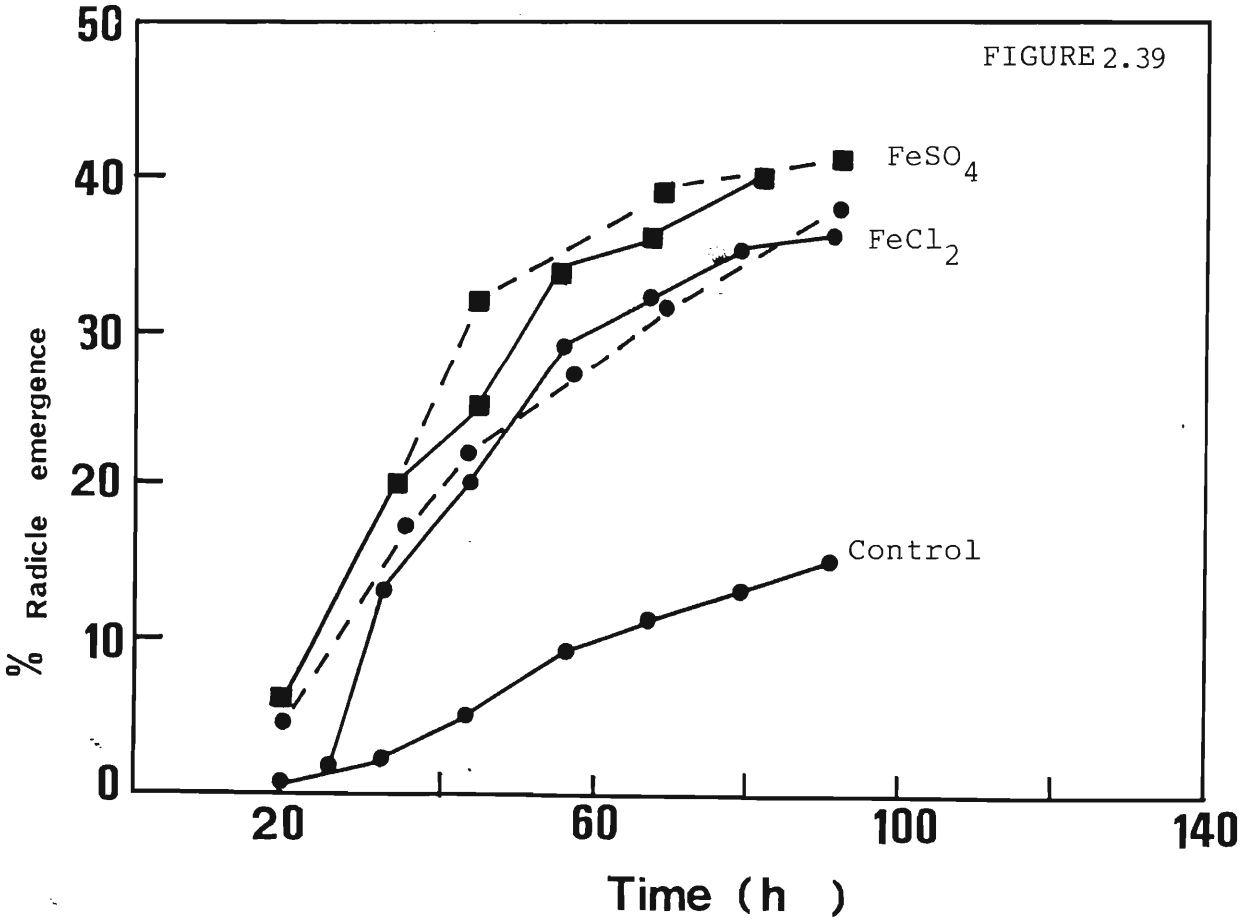


FIGURE 2.39
FeSO4
FeCl2
Control
% Radicle emergence
Time (h)
0
10
20
30
40
50
20
60
100
140

of Gorecki and Harman (in press) who reported no significant improvement in pea seed vigour when treated with alpha-tocopherol. Treatment of the seeds with DMSO resulted in a reduced rate of germination when compared to controls (figure 2.38). Although DMSO has been successfully used to inhibit lipid peroxidation *in vitro* (Halliwell and Gutteridge,1985), its strongly polar chemical properties may result in it being toxic *in vivo*.

The treatment of soya bean seeds (cv 4031) with a 0.02M solution of $FeCl_2$ reduced the rate of seed germination i.e. no invigorating effect was observed (figure 2.40). In contrast to this, treatment with 0.01M $FeSO_4$ consistantly improved the rate of germination, but not the final percentage germination (figure 2.41). The invigorating effect of this treatment was not significant at the 5% level. However treatment of the seeds with a saturated solution of tinoridine resulted in an equivalent improvement in the rate of germination to the seeds treated with 0.01M $FeSO_4$ (figure 2.40).

The above treatments with reducing agents were, however, toxic. The toxicity of ferrous chloride was apparently due to the chloride ion, as treatment with equimolar concentrations of ferrous sulphate resulted in a reduction in toxicity as well as an improved invigorating effect. Nevertheless, toxicity was still evident, resulting in a reduction in the rate of radicle extention, necrosis of the radicle tips and an inhibition of root hair development. Cotyledonary greening was also delayed. However, treatment of the seeds with 0.005M solutions of ferrous sulphate substantially reduced the toxicity, some seedlings showing normal development.

The hydroperoxide level of cabbage seeds treated with $FeCl_2$ was monitored over the first hour of imbibition. In both cases, a reduction in the

Figure 2.40 The invigorating effect of antioxidants and reducing agents on soya bean seeds (cultivar Impala 4031 Duiker). T, Tinoridine; C, control. (n=2)

Figure 2.41 The invigorating effect of a 0.01M solution of $FeSO_4$ on seeds of soya (4031). ■, control; ●, experiment. (n=6)

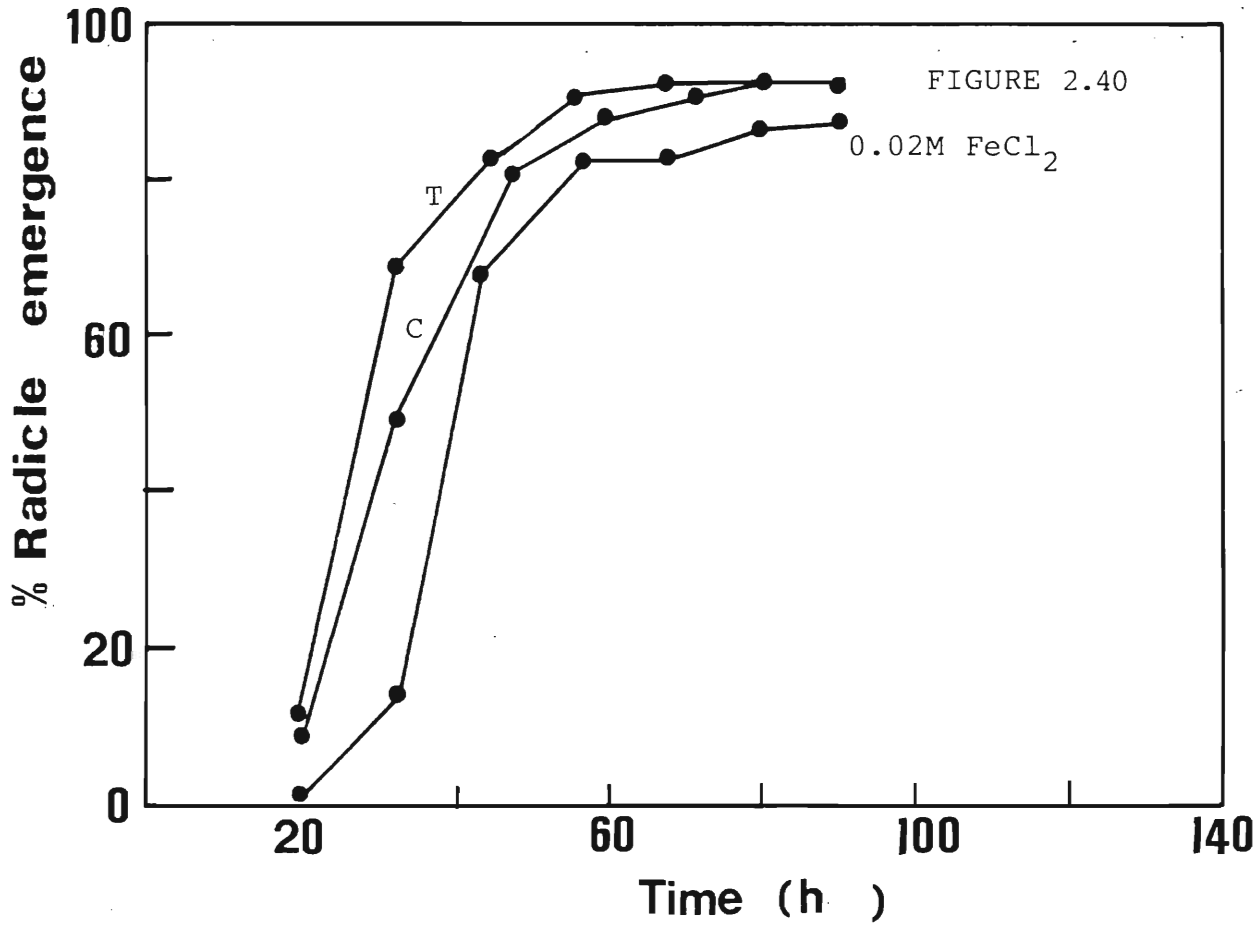


FIGURE 2.40

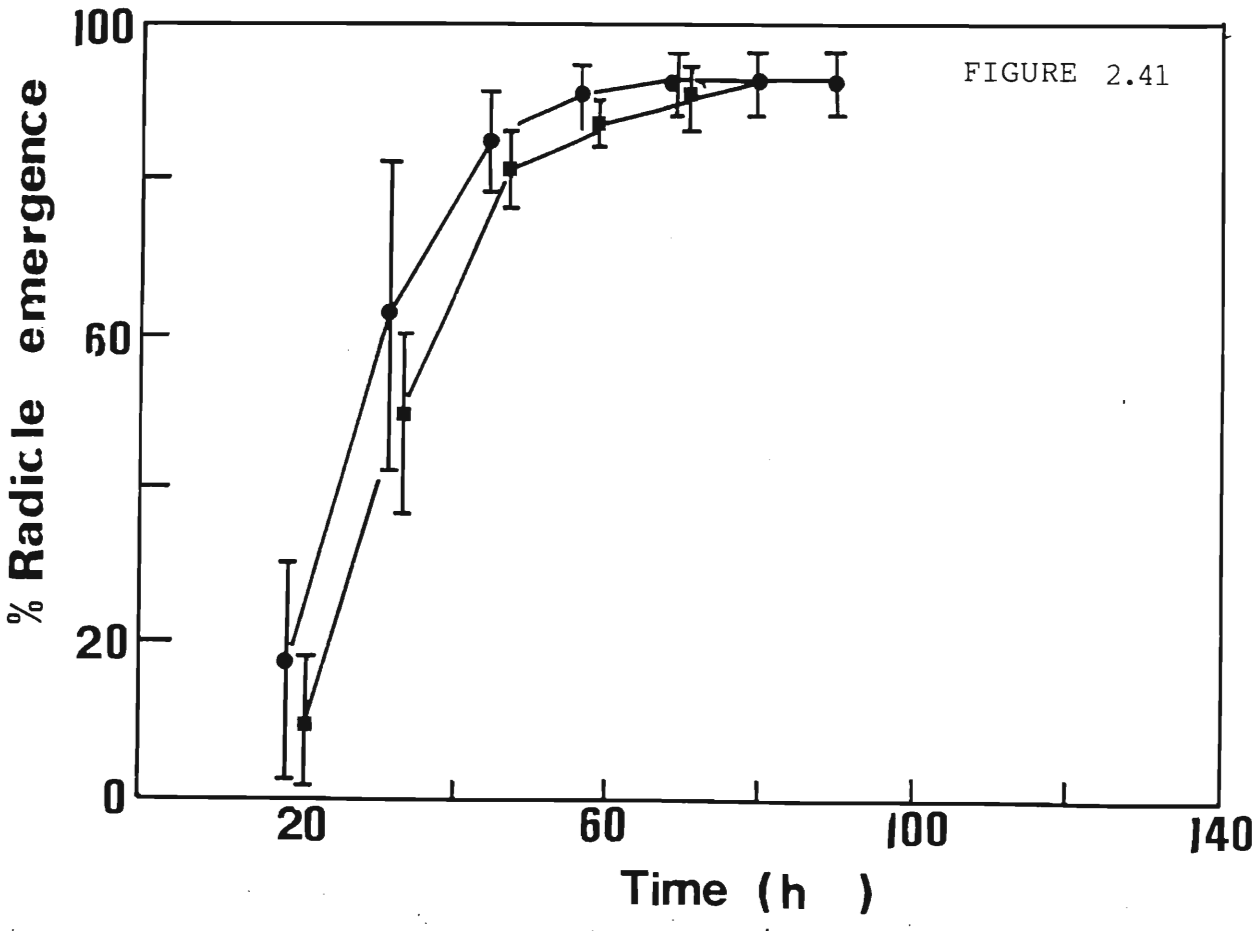
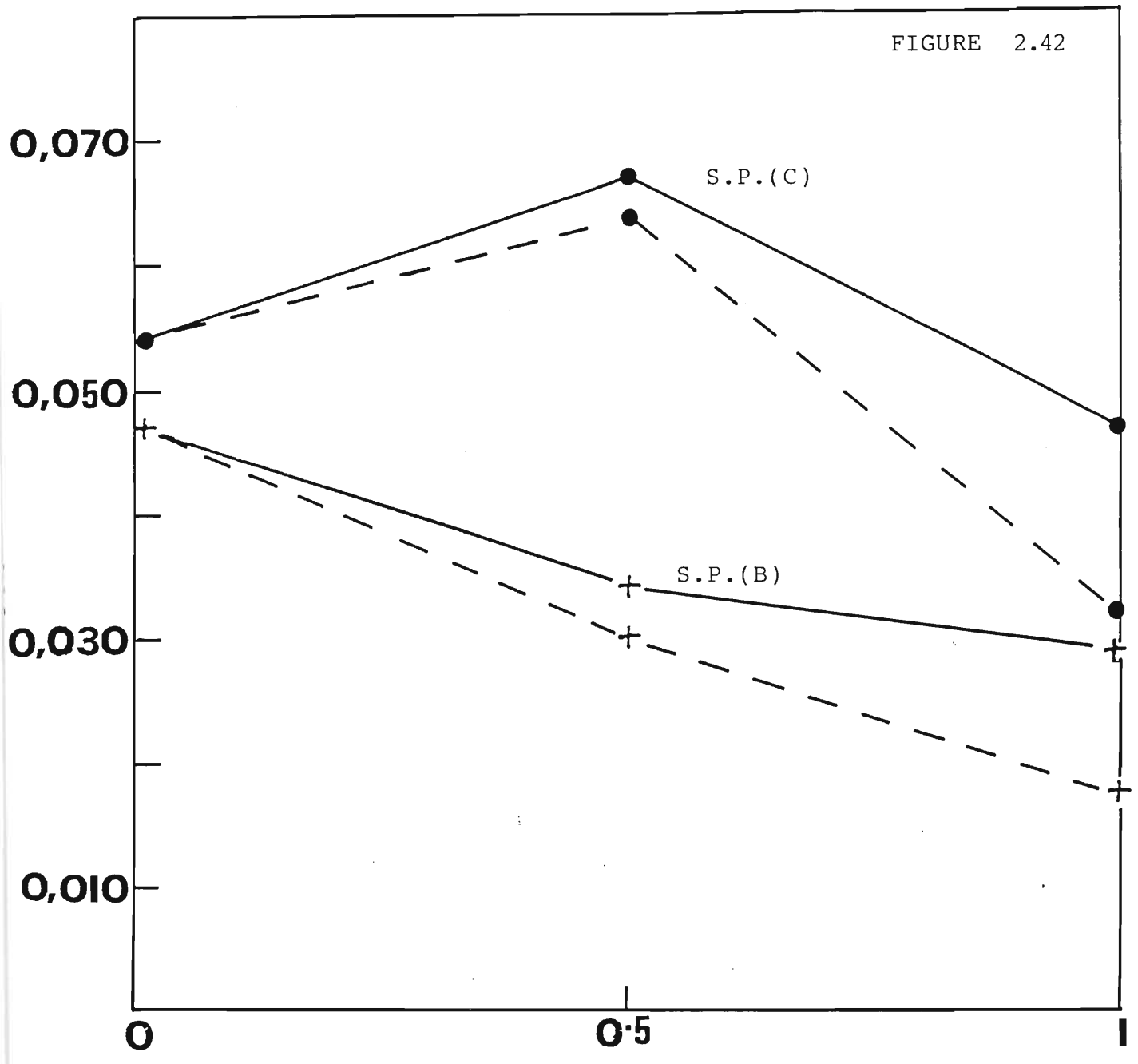


FIGURE 2.41

hydroperoxide level was observed in the treated seeds (figure 2. 42). As the results were not treated statistically, it was not possible to determine if they were significant or not, although it may be possible to speculate that one of the effects of the treatments was an impairment of the formation of the hydroperoxides, the facilitation of the breakdown of these compounds, or both. This may be paticularly pertinant in the light of the observed fluctuations of hydroperoxide levels on imbibition, and may indicate that the reduction of the level of hydroperoxides may be an important imbibition-related event. This may be achieved in two ways. The ferrous iron may act as an antioxidant, quenching any free radicals present or produced on imbibition, and thus retarding lipid peroxidation. Alternatively, the iron compounds may catalyse the breakdown of the hydroperoxides by a Fenton type reaction, thus limiting the rate of accumulation of these compounds. This latter reaction would not be expected to be beneficial per se, as it results in the formation of free radical products. However, if this occured in conjunction with the antioxidant activity, the observed invigorating effect of these treatments may be explained. The antioxidants used in this study showed only a very slight invigorating effect. DMSO was apparently toxic, while tinoridine was only obviously beneficial in soya beans. This may suggest that either the membranes were only slightly permeable to tinoridine, or that the beneficial effect of the reducing agents was due to activity other than that suggested above i.e. they were not acting as antioxidants.

Figure 2.42 The effect of 0.01M $FeCl_2$ on the changes in the hydroperoxide levels during the first hour of imbibition in two lots of cabbage seeds. In both cases, the treated seeds (------) showed a reduced level of hydroperoxides. (n=2)

FIGURE 2.42



GENERAL DISCUSSION

The bulk of the evidence presented to date for a role of lipid peroxidation in seed ageing is largely circumstantial and a causual relationship remains to be determined. Despite the significant period of time that the mechanisms of seed ageing have been under investigation, very little progress has been made in the elucidation of the causual cellular and biochemical changes of seed ageing. This is possibly due to the basic complexity of the ageing process as well as the limited amount of work done on the subject. However, a large portion of the blame may be due to the fact that "different workers have studied different facets of deterioration, have used different techniques on different species or cultivars of seeds which have been aged in different ways to different extents, and whose viability response varies from zero germination, through reduced seedling vigour, to no apparent visible change" (Bewley & Black,1982). Making comparisons between such data is very difficult, if not impossible in many cases.

1.Lipid peroxidation in seeds.

Koostra and Harrington (1969) were probably the first to suggest that oxidative damage to the membrane fatty acids may be responsible for a loss of membrane permeability leading to the greater degree of electrolyte leakage observed in aged seeds. Increased oxidation of seed lipid accompanying ageing has been reported by Spencer *et al.* (1973) working on *Crepis thomsonii*, *C. vescaria* and *Cichorium intybus* seeds. They demonstrated an increase in epoxy and conjugated hydroxy acids, due primarily to the oxidation of linoleic acid, a 10% loss being observed in all seeds. Furthermore, the rate of oxidation was substantially higher in seeds kept at room temperature (22-25°C) than

those stored at 5°C. The vigour of the seeds was not, however, determined.

Radrupal and Basu (1982), working on wheat (T. aestivum) and mustard (Brassica juncea), have reported a highly significant correlation between germinability, lipid peroxidation and electrolyte leakage under all conditions of ageing. From the above, it would seem clear that lipid peroxidation does occur in seeds during storage, and furthermore, a relationship between lipid peroxidation and seed vigour may exist. This is supported by the results from the present study.

2.Rapid ageing.

Koostra and Harrington (1969), in their work on cucumber (Cucumis sativus) seeds applied the technique of accelerated ageing, thus providing a series of seed lots of different vigour in a very short period of time. This was achieved by storing the seeds under conditions of high relative humidity (100%) and high temperature (40°C). At the time, they cautioned against direct comparison with slow ageing as they felt that changes induced under the above mentioned conditions might not be the same as those in slowly aged seeds. However, subsequent to this, the technique has been widely applied, while very little attention has been paid to the warning initially given. Furthermore, recent work on the subject has provided evidence which suggests that changes induced by rapid ageing may not be the same as those in slow ageing (Priestley and Leopold,1983). Indeed, Wilson and McDonald (1986a) identify the use of 'accelerated ageing' as one of two "major deficiencies" in many of the studies to date on seed ageing.

Priestley and Leopold (1979) have reported that soya beans rapidly aged at 40°C and 100% relative humidity (r.h.) showed no decline in the level of polyunsaturated fatty acids during ageing, suggesting

that oxidation of seed lipids was unrelated to the loss of the seed vigour.

Stewart and Bewley (1980) rapidly aged soya bean seeds at 45°C and 100% r.h. Controls were kept at 45°C under desiccated conditions. The seeds kept at high temperature and high r.h. were all dead within 2 days, while the germination % of the controls remained unaltered over the 7 day experimental period. Under the former conditions, lipid peroxidation as measured by an increase in electrolyte leakage, an increase in the levels of malondialdehyde (MDA) - a breakdown product of lipid hydroperoxides, and a decline in the relative percentages of the polyunsaturated fatty acids (PUFAs) linoleic and linolenic acid was observed to occur. However, these changes only occured after loss of viability. Thus, it appears that lipid peroxidation was not responsible for the loss of viability observed in these seeds.

Superoxide dismutase (SOD) activity during the first 5 hrs of imbibition was also investigated. In the controls, SOD activity peaked at 3½h , while the aged seeds showed a complete absence of SOD activity during the first 5h of imbibition. Although a loss of SOD activity alone could be implicated as the cause of viability loss, as suggested by the authors, it may also reflect a more general loss of enzyme activity within the seeds. Therefore, the loss of viabilty observed in this study was possibly due to the combined action of both high temperature and high r.h., and may be associated with a general loss of enzyme activity.

Pearce and Abdel Samad (1980) have investigated the changes associated with the loss of vigour in both slowly and rapidly aged peanut (Arachis hypogea) seeds. The slowly aged seeds (stored at 5°C) showed a definite correlation between the loss of vigour, the increase in electrolyte leakage and the decline in relative percent

of PUFAs observed. Their failure to detect hydroperoxides is surprising, although their technique of boiling the lipid samples prior to analysis is perhaps questionable. In the rapidly aged seeds an equivalent loss of PUFAs, a greater decline in germination percentage (15% as opposed to 39%) and a greater increase in electrical conductivity (about 850 $\mu S/cm^2$ as opposed to 353 $\mu S/cm^2$) was observed. These workers concluded that the loss of seed vigour in both slowly and rapidly aged seeds appeared to be due to a loss of membrane integrity, but that the involvement of lipid peroxidation in the changes was uncertain. Rather, the authors implicate the action of enzyme(s). The larger changes observed in percentage germination and electrolyte leakage in the rapidly aged seeds despite an equivalent loss of PUFAs in both the rapidly and slowly aged seeds may be significant in this context, as this might suggest that loss of PUFAs was not the major lesion in the rapidly aged seeds.

Halder and Gupta (1982) have investigated the mechanisms of deterioration of rapidly aged sunflower (Helianthus annuus) seeds. Unfortunately, a variety of ageing conditions were used, making comparison very difficult. Nevertheless, their results are in broad agreement with those of Stewart and Bewley (1980). Lipid peroxidation appeared to have accompanied the loss of seed vigour as evidenced by an increase in MDA levels and electrolyte leakage. However, both these changes show a poor correlation with the decline in vigour.

A marked relationship between the loss of phospholipids, particularly phosphatidylcholine (PC), and loss of vigour was observed. It is interesting to note that the loss of phospholipid appears, at least initially, to be unrelated to increase in MDA levels i.e. to lipid peroxidation. On the other hand, there was a marked loss of catalase activity after 30 days of

ageing. This correlates very well with the increase in MDA levels and the increase in electrolyte leakage, particularly in the pre-imbibed seeds. This might reflect a general loss of enzyme activity at 30 days. If this includes the enzymes associated with the control of lipid peroxidation, as is suggested by the decline in catalase activity, oxidation of membranes may be facilitated, leading to the increase in the levels of MDA and electrolyte leakage. This is supported by the suggestion that the reduced level of sugar leaching under high r.h. might be a function of reduced hydrolysing enzyme activity. Thus it appears again that the loss of vigour observed in these seeds under rapid ageing conditions may be due to changes in enzyme activity. The loss in dehydrogenase activity reported may also be significant in this regard.

. Therefore, in nearly all of the above studies, changes in enzyme activity have been implicated in the loss of vigour observed under rapid ageing conditions. That such changes occur is clearly demonstrated by the work of Perl et al (1978). They reported a poor correlation between electrolyte leakage and vigour in rapidly aged sorghum, suggesting that membrane damage was not associated with the ageing observed. Enzyme activities, however, showed distinct temporal patterns. Amylase, glutamate-pyruvate-transaminase, RNAse and glutamate decarboxylase activity all increased initially, correlating with seed invigoration, followed by a rapid decline. Acid phosphatase, dehydrogenase (tetrazolium reducing activity) and peroxidase activity declined throughout the ageing period. However, protease activity increased throughout the ageing period. The authors suggest that the loss of enzyme activity, and possibly the loss of vigour, observed was associated with the increased protease activity.

Thus it appears that there may be a fundamental

difference between slow and rapid ageing based on changes in enzyme activity. Wilson and McDonald (1986a) state that the mechanisms of rapid ageing are "different physiologically" from slow ageing. They suggest that the conditions associated with rapid ageing treatments may facilitate "the enzymatic activation of toxic chemicals" (possibly oxygenated fatty acids) leading to cellular damage and eventually death. However, in the light of the above discussion, these changes may alternatively be due to the increased activity of hydrolytic enzymes. The high relative humidities used in the rapid ageing treatment may raise seed moisture contents to a point where certain germination-related processes may be permitted to occur, including those of repair (Ward and Powell,1983) and hydrolysis (Perl et al.,1978). However, partial hydration may lead to an imbalance between such processes, resulting in the breakdown of molecules without their replacement.

3.Changes in phospholipids during rapid ageing.

A consistent observation under rapid ageing conditions has been the loss of phospholipid, particularly PC. Petruzzelli and Taranto (1984) have investigated the changes in phospholipids in slowly and rapidly aged wheat (Triticum durum) embryos. They report that the total extractable phospholipid declined more in the rapidly aged embryos, with a concomitant increase in phosphatidic acid (PA). This was primarily due to a decline in PC. Furthermore, in the slowly aged seeds, the shifts in the major phospholipid classes were small in comparison to those in rapidly aged seeds, while the marked loss of phospholipid observed occurs in the absence of a decline in the germinability of the rapidly aged seeds. They state that lipid peroxidation did not seem to have occured under the ageing conditions used in their study, as no phospholipid remained at the origin

of the TLC plates. Rather, they suggest that the conditions of rapid ageing may facilitate or accelerate the action of hydrolytic enzymes. Similar changes in phospholipids were obtained by Francis and Coolbear (1984) working on rapidly aged tomato (Lycopersicon esculentum) seeds. They reported a direct relationship between loss of viability due to controlled deterioration and amount of phospholipid present in the tomato seeds. Again, this was due mainly to a loss of PC.

Powell and Harman (1985) have reported a significant loss in total phospholipid (primarily PC) in rapidly aged pea seeds. Their data are difficult to analyse, but suggest that different changes are taking place at different rates under the different storage conditions. Lipid peroxidation did not appear to be involved in the loss of vigour observed in the two high humidity treatments. These workers suggest that lipid hydrolysis was responsible for the changes observed. Furthermore, they mention that a balance may exist within seeds between the processes of deterioration and repair, and that the outcome of this balance depends very much on the conditions of storage.

In erythrocytes, similar losses have been associated with lipid peroxidation, ageing or both. Lubin et al. (1972) have reported an increased lipid turnover in vitamin E deficient erythrocytes incubated in H_2O_2. Furthermore, they associate this with increased phospholipase activity. Shukla and Hanahan (1982) have demonstrated an increased phospholipase A2 activity in aged erythrocytes. In both cases, phosphatidylethanolamine (PE) is preferentially hydrolysed. Lubin et al. (1972) point out that PE has a higher percentage of unsaturated fatty acids, and is therefore more susceptible to peroxidative attack. It may be that PC is selectively hydrolysed in seeds for similar reasons,

although loss of phospholipid is not always associated with increased levels of peroxidation. However, less vigorous seeds have been shown to be more susceptible to rapid ageing treatment (Powell & Matthews,1981), and this may be a reflection of a greater substrate level for hydrolytic enzyme activity due to previous oxidative damage during storage. The high relative humidities and hence seed moisture contents used in rapid ageing may therefore facilitate the activity or release of hydrolytic enzymes which are inhibited or impaired under dry storage conditions.

It appears, therefore, that the conditions of rapid ageing may bring about an incomplete or retarded germination, and may lead to some form of differential enzymatic activity, as suggested by Powell and Harman (1985), and hence deleterious changes within the cells.

4.The loss of unsaturated fatty acids.

As already discussed, evidence to date on the loss of unsaturated fatty acids during ageing is equivocal (Priestley and Leopold, 1979; Stewart and Bewley,1980). Recent work, however, seems to suggest that loss of PUFAs is associated with loss of vigour in slowly aged seeds (Priestley and Leopold, 1983).

In the present study, a decline in PUFAs was clearly associated with the loss of vigour in both soya bean and cabbage seeds. In soya, heat treatment suggested that lipid peroxidation may have been the cause of this loss i.e. a decline in hydroperoxide value in all seed batches after heating was associated with a characteristic change in the levels of the fatty acids. Although a similar change in peroxide value was observed in the cabbage seeds, only slight changes in the relative percentages of the fatty acids were observed after heating.

It is generally held that the loss of PUFAs is due

to peroxidative damage to the lipids. Hydroperoxide levels were, however, only poorly related to the decline in PUFAs and to seed vigour in this study. For example, in cabbage, cultivar Cape Spitz was of high germinability despite the highest recorded hydroperoxide level. Furthermore, this high peroxide value was not reflected in the changes in PUFA levels. This may suggest that the proposed relationship between lipid peroxidation, PUFA levels and seed vigour may not be as close or direct as possibly expected.

However, this cultivar did show a marked decline in the relative percentage of linoleate (8%) and an increase in palmitate (12%) in the polar fraction after heating, suggesting that relatively large shifts in the relative levels of the individual fatty acids had occured during the treatment. This may be a reflection of the high peroxide value of this seed lot.

It may also be possible that hydroperoxide breakdown is necessary for the changes in PUFAs to result. This may not have occured to a large extent in this cultivar during storage. However, this seed had the highest moisture content, and hydroperoxide breakdown might be expected to be facilitated under such conditions. Nevertheless, it may be that this lot had only recently undergone an elevation in moisture content and thus only recently undergone peroxidative damage.

A consistent observation in both the cabbage and the soya beans was an increase in the absolute amount of oleic acid. It may be that the oxygenated fatty acids if not immediately cleaved from the glycerol backbone, would become esterified during preparation. These might show slightly different elution characteristics to the parent compound due to the changes in chemical structure following oxidation, while esterification and high temperature GLC analysis might result in additional chemical changes, also influencing elution times. Morris

et al. (1960) has reported the alteration of long chain esters during gas chromatography. Unsaturated hydroxy and hydroperoxy compounds were altered during GLC to give conjugated unsaturated esters. Furthermore, these unsaturated hydroperoxides were altered to more highly unsaturated derivatives. Methyl oleate and methyl linoleate were converted to conjugated dienoates and conjugated trienoates respectively. That esterification of oxygenated fatty acids had occured is suggested by the changes in fatty acid levels after heating in soya bean and cabbage. It is therefore possible that the increase in the level of oleate may be due to the presence of oxygenated forms of PUFAs fatty acids co-eluting with it. Indeed, oleate was observed to decline in all batches after heating in the total lipid extract from soya bean seeds, which would not perhaps be expected of such a relatively stable unsaturate. In the light of this, the analysis of changes in fatty acid levels in future work may be more properly analysed using the greater resolving power of capillary columns. Special preparation of the oxygenated forms may also be employed to achieve seperation of coeluting compounds.

5.Changes in hydroperoxide levels.

Despite the significant amount of work done on seeds in order to determine the occurrence of lipid peroxidation, very few workers have attempted to measure the level of hydroperoxides, while those who have, have reported that hydroperoxides could not be detected (Pearce and Abdel Samad,1980) or yielded equivocal results (Powell and Harman,1985).

Wilson and McDonald (1986a) suggest that by measuring the amount of oxygenated fatty acids, the physiological age of the seed can be determined. This was not, however, observed to apply in this study. Although an increase in peroxide value with declining

vigour was observed in both seed types, the correlations between peroxide value and germinability were poor. Furthermore, in cabbage, the extent of peroxidation showed a better relationship with moisture content. This may be due to the higher seed moisture content permitting a higher level of peroxidative attack, possibly by affording greater mobility to any free radical products of lipid peroxidation or by facilitating the formation of free radical species. Data obtained from the measurement of hydroperoxides should possibly be treated with caution. Halliwell and Gutteridge (1985) have pointed out that the level of the hydroperoxide will be dependent on the rate of production and the rate of breakdown. There is no reason why this should not be the case in dry seeds. Thus hydroperoxides should not be seen to only accumulate. Hydroperoxide breakdown could result in a seed batch having a relatively low peroxide value, but having suffered a significant extent of damage. Furthermore, hydroperoxide breakdown may exacerbate damage due to the production of cytotoxic secondary products such as aldehydes. This may explain the apparent discrepancy in results for the cabbage seeds investigated in this study, the peroxide value showing a better relationship to moisture contents than germinability.

6.The initiation of lipid peroxidation in seeds.

It has been suggested that the peroxidation of seed lipids proceeds by the process of autoxidation in the presence of oxygen (Wilson and McDonald,1986a). It is also possible, however, that lipid peroxidation may be initiated by the activity of certain enzymes.

It has been postulated that in hydrated systems, the hydroxyl radical may represent the primary radical species responsible for hydrogen abstraction (Halliwell and Gutteridge,1985). This in turn is thought to be due

to the production of the superoxide radical (see Introduction: Section 2). In animals, electron transport chains are thought to be the major source of the superoxide radical. Two possible sources exist, cytochromes and the coenzyme Q. In mitochondria isolated from animal tissues, the NADH-coenzyme Q reductase complex and the reduced forms of coenzyme Q itself (semiquinone), appears to be the main site of 'leakage' of the superoxide radical. Mitochondria isolated from plant cells have also been shown to produce the superoxide, probably by a similar mechanism. (The cytochrome P450 may also constitute an important source of the radical) (Halliwell and Gutteridge,1985).

However, the above observations have been made on hydrated animal and plant cells. The extent of metabolism in dry seeds is uncertain. Nevertheless, evidence does exist for metabolic activity in dry dormant oats and wheat (Bewley and Black,1982). It is therefore possible that a low level of metabolism does occur in dry seeds.

The physical state of membranes at very low moisture content has been subject to some investigation. The liquid-crystalline state is apparently necessary for functional integrity (Singer and Nicholson,1972). Below a certain moisture level, however, membranes undergo a phase change to a gel state and associated enzymes, e.g. mitochondrial enzymes, might be 'displaced'.

It may be possible, therefore, that under dry storage conditions, metabolic intermediates, including the superoxide, may be 'leaked' into the cytoplasm. This may initiate lipid peroxidation itself, or at higher moisture contents, particularly in the presence of haeme compounds (cytochromes), it may lead to hydroxyl radical formation which could also result in the onset of lipid peroxidation.

Another possible source of lipid peroxidation in

dry seeds is lipoxygenase(LOX) activity. Lipoxygenase is responsible for the oxidation of the cis,cis-1,4-pentadiene system of unsaturated fatty acids, particularly linoleic and linolenic acid (Galliard and Chan,1980). Its activity involves the formation of free radical species (1). Furthermore, the associated co-oxidation reaction (2) can generate further free radicals by abstracting a hydrogen from an adjacent molecule (Halliwell and Gutteridge,1985).

$$L\text{-}H \xrightarrow{LOX} L\cdot \xrightarrow{O_2} LOO\cdot \xrightarrow{LOX} LOOH \quad \ldots\ldots (1).$$

$$LOO\cdot + XH \longrightarrow LOOH + X\cdot \quad \ldots\ldots (2).$$

Furthermore, lipoxygenase is apparently closely associated with mitochondria. Siedow and Girvin (1980) reported that they had great difficulty in obtaining mitochondria free from lipoxygenase activity, while Haydar and Hadziyev (1973) and Dupont (1971) have reported lipoxygenase activity that is endogenous to mitochondria isolated from pea seeds and cauliflower respectively. This, however, may also be due to the activity of the alternate respiratory pathway (Dupont et al.,1982). Nevertheless, lipoxygenase has been shown to be active at very low moisture contents (Mangold,1981). This might suggest that a low constant activity of lipoxygenase in dry seeds may be a potential source of free radicals, leading to lipid peroxidation.

6.Volatile compounds and seed vigour.

The measurement of volatile secondary breakdown products as an index of lipid peroxidation has been used in the analysis of oils (Scholtz and Ptak,1966). This technique has also been applied to animal systems (Dillard and Tappel,1979), while in plants, Stotsky and

Schenck (1976) have reported the evolution of volatile aldehydes from imbibing seeds. That this is related to seed vigour was suggested by the work of Harman et al. (1980) who have reported a greater volatile production from less vigorous seeds. This is supported by recent work on imbibing soya bean and pea seeds (Woodstock and Taylerson, 1980; Gorecki et al., 1984; Wilson and McDonald,1986b) all of whom have reported a relationship between volatile aldehydes and seed vigour from imbibing seeds.

Stotsky and Schenck (1976) reported no detectable volatile evolution from dry seeds. Other workers (Heydanek and McGorrin,1981) however, have detected volatile compounds from dry oats, including hexanal. Ross (pers. comm.)[2] has shown that the thermal decomposition of linoleate was accompanied by an increase in volatile secondary products, particularly hexanal. Recently, Fielding and Goldsworthy (1982) have demonstrated a possible relationship between volatile compounds evolved from heated dry wheat and seed age. This is supported by recent work by Smith (pers. comm.)[1] who has shown a possible correlation between the amount of hexanol evolved and seed vigour in dry-heated lettuce seeds.

The possible use of such volatile compounds as an index of seed vigour has been suggested by a number of workers (Fielding and Goldsworthy,1982; Wilson and McDonald, 1986b). Two possible sources of these volatile compounds have been proposed; breakdown of oxygenated products of lipid peroxidation or metabolic imbalance due to damage to the mitochondria (Wilson and McDonald, 1986a).

Lipid peroxidation as a source of these volatile compounds in seeds was probably first suggested by

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[2]Mrs G. Ross, Dept. Applied Cemistry, Univ. Natal.

Harman et al. (1980). As already mentioned, hydroperoxides can be broken down both enzymatically and nonenzymatically. Nonenzymatic mechanisms have recently been reviewed by Frankel (1982). Hydroperoxides decompose readily, both thermally and in the presence of transition metals, giving rise to a large number of predominately carbonyl, hydroxyl and hydrocarbon products.

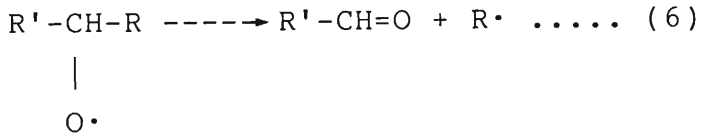
The alkoxy radical is considered the primary intermediate in hydroperoxide breakdown. This is thought to be produced in three main ways; homolytic cleavage of a hydroperoxide (3), interaction of two peroxy radicals (4) and homolytic cleavage of a peroxide (5).

$$ROOH \longrightarrow RO\cdot + \cdot OH \quad \ldots\ldots (3)$$

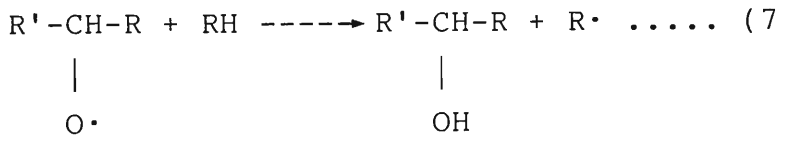
$$ROO\cdot + ROO\cdot \longrightarrow 2RO\cdot + O_2 \quad \ldots\ldots (4)$$

$$ROOR \longrightarrow RO\cdot + RO\cdot \quad \ldots\ldots (5)$$

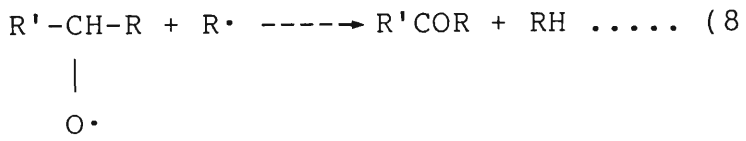
The secondary alkoxy radical may then cleave to form aldehydes, a major product of hydroperoxide breakdown (6). The alkoxy radical may also react with the substrate (7), with the corresponding alkyl radical (8) or with another alkoxy radical (9). Reactions 6 and 7 will perpetuate the chain reaction, as they produce further free radicals, while reactions 8 and 9 will terminate it by forming stable products. Such breakdown yields a large number of products, particularly aldehydes (6) and hydrocarbons (10), many of which are volatile.



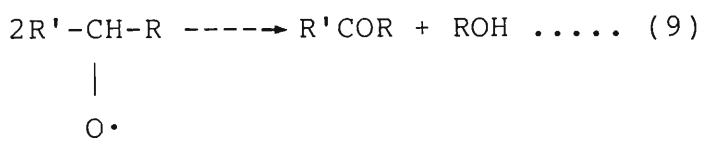
$$R'\text{-}CH(O\cdot)\text{-}R \longrightarrow R'\text{-}CH{=}O + R\cdot \quad \ldots\ldots (6)$$



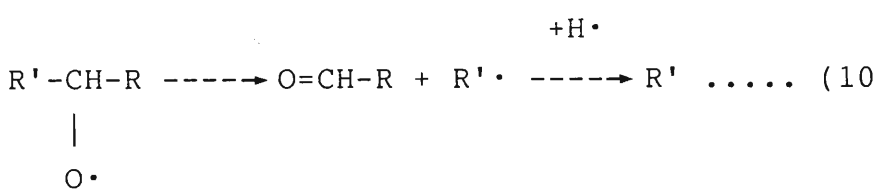
$$R'\text{-}CH(O\cdot)\text{-}R + RH \longrightarrow R'\text{-}CH(OH)\text{-}R + R\cdot \quad \ldots\ldots (7)$$



$$R'\text{-}CH(O\cdot)\text{-}R + R\cdot \longrightarrow R'COR + RH \quad \ldots\ldots (8)$$



$$2R'\text{-}CH(O\cdot)\text{-}R \longrightarrow R'COR + ROH \quad \ldots\ldots (9)$$



$$R'\text{-}CH(O\cdot)\text{-}R \longrightarrow O{=}CH\text{-}R + R'\cdot \xrightarrow{+H\cdot} R' \quad \ldots\ldots (10)$$

(R'- Hydrocarbon end of fatty acid)

Enzymatic breakdown has been shown to give rise to similar breakdown products. Lipoxygenase and concomitant hydrolase activity has been suggested to be responsible for the burst of volatile aldehyde and carbonyl compounds during the wound response in plant tissues (Halliwell and Gutteridge,1985). Vick and Zimmerman (1976) have reported 'hydroperoxide lyase' activity in watermelon (Citrullus lanatus L.) seedlings. Similar activity has been reported in cucumber and alfafa

(Medicago sativa L.) seedlings (Sekiya et al.,1979). Enzymatic breakdown, however, would probably only be of significance in imbibed, germinating seeds, volatiles derived from dry seeds been due predominately to nonenzymatic breakdown. This is suggested by the observation that volatiles derived from dry and imbibing oats were compositionally different (Heydanek and McGorrin,1981). A similar result was obtained with regard to the volatile spectra derived from dry and imbibing cabbage and soya bean seeds in this present study.

The significance of haeme compounds in hydroperoxide breakdown should possibly be considered. An antibody raised against the microsomal flavoprotein NADH-cytochrome P450 reductase inhibits peroxidation by 90% (Halliwell and Gutteridge,1985). Similarly, Bindoli et al. (1982) has shown that the cytochrome P450 inhibitor, SKF52A, will stongly inhibit cumene hydroperoxide induced mitochondrial lipid peroxidation. They suggest that cytochrome P450 is responsible for the breakdown of cumene hydroperoxide by a Fenton type reaction, yielding primarily alkoxy radicals. Such compounds, therefore, might provide potential sites for hydroperoxide breakdown, and in so doing would greatly increase the level of cytotoxic radicals within the cell. In this regard, it is interesting to note that reducing molecular species such as NADH and NADPH can stimulate damage by maintaining a high level of reduced haeme compounds within the cell (Lötscher et al.,1980; Maridonneau et al.,1982).

In the present study, a possible relationship between volatile evolution and seed vigour was observed in both soya bean and cabbage seeds. In dry cabbage seeds, a marked relationship between initial peroxide level and volatile evolution was observed. This together with the observation that oils extracted from the seeds

produced virtually identical volatile spectra and that heating resulted in a general decline in the hydroperoxide level in both seed types, might suggest that volatile production from dry seeds was due to the thermal decomposition of the hydroperoxides.

Volatiles derived from imbibing cabbage seeds, in contrast to dry seeds, did not show an obvious relationship between the total quantity produced and seed vigour. One peak, however, was definitely related to seed vigour, increasing from undetectable levels in the most vigorous seeds to 28% of the total count in the least vigorous seeds. In contrast to volatiles derived from dry seeds, this peak did not appear to be related to the initial hydroperoxide level. This may suggest that the source of these volatiles was not the same as for those from dry seeds. Similar results were obtained by Heydanek and McGorrin (1981), who demonstrated a compositional difference between volatiles derived from dry and imbibing oats. Soya beans showed no obvious relationship to vigour whether volatiles were derived from whole seeds or isolated axes.

As mentioned above, the source of these volatiles appears to be different to those derived from dry seeds. Woodstock and Taylorson (1981) have implicated an imbalance between the tricarboxylic and glycolytic activities, suggesting that this may be more pronounced in aged seeds. Gorecki et al. (1985) also implicate mitochondrial dysfunction as the source of the volatile aldehydes they detected in pea seeds, although they sugggest the operation of an alternate respiratory pathway in the absence of oxidative phosphorylation. It appears, therefore, that volatile compounds produced during imbibition reflect general seed metabolism, and for this reason might provide a better indication of seed vigour and field performance than those derived from dry seeds. Nevertheless, in this study it was the

latter technique that was able to distinguish between soya bean seeds of very similar vigour. Furthermore, only one peak in cabbage showed any relationship to seed vigour in volatiles derived on imbibition, while most, if not all the peaks derived on heating showed a relationship to vigour. However, the peak detected in imbibing cabbage seeds did show a better relationship to seed vigour than any of those derived on heating.

It therefore appears that in this study, the measurement of volatiles derived on heating was the better of the two techniques. One possible downfall of this technique, however, is that it appears to measure the extent of lipid peroxidation, but this may give very little information as to the general vigour of the seed, as suggested by the results obtained in this study. It is suggested that the use of both techniques in conjunction may provide a more comprehensive index of seed vigour, as this will provide information on peroxidative damage and general seed vigour. It is important, however, that the difference between the two techniques be recognized at the outset, as it is clear from this and other work that the two techniques are measuring volatiles derived from different sources.

7.Changes in the peroxide value during imbibition.

Although it has been shown that the rate of loss of vigour increases with increasing moisture content, a certain level is reached after which a marked reduction in the rate of damage occurs (Wilson and McDonald,1986a). It has been shown that thermodormant lettuce seeds kept fully imbibibed showed an extended viability and greater chromosomal stability than dry seeds when subject to rapid ageing. It was suggested that the imbibed state permits the activity of repair processes, thus preventing the accumulation of damage. In dry seeds, on the other hand, such repair mechanisms

are impaired, leading to an increasing level of damage (Villiers,1974).

Berjak and Villiers (1972) have shown a higher rate of protein and RNA synthesis during the first 48 hours of imbibition in rapidly aged as opposed to unaged maize seeds. They suggest that the higher activity was due to repair processes. Osborne (1982) has recently reviewed the accumulating evidence for DNA repair mechanisms in seeds. Wilson and McDonald (1986a) in their review of seed ageing have stated that "evidence suggests that imbibing seeds undergo extensive repair and macromolecular turnover".

This has led to the suggestion that the greater lag period before germination in less vigorous seeds may be indicative of repair processes, and that damage must be repaired before germination can proceed. The efficacy of hydration-dehydration treatments has also been suggested to be due to the activity of repair processes. However, Marcus et al. (1966) have shown that in slowly aged wheat, protein synthesis is inhibited during imbibition. Furthermore, a number of workers have expressed doubt as to the significance of repair processes in hydration-dehydration treatments. Bewley and Black (1982) suggest that on hydration, the early metabolic processes of germination are initiated, and that these events are arrested on subsequent dehydration, but not reversed. The phenomenon of the invigoration of aged seeds was not, however, addressed. Wilson and McDonald (1986a) state that the fact that seed responses to hydration can be detected after only a few hours of imbibition (even 15 minutes in wheat seeds and in the absence of oxygen) "casts some doubt on the cellular repair hypothesis". They suggest that on imbibition, "toxic products" are released or produced, resulting eventually in cellular death. The ameliorating effect of hydration-dehydration treatments is attributed by them to a controlled

destruction of these toxic products, possibly oxygenated fatty acids, and not to the repair of damage.

A number of observations would suggest that imbibition is associated with an increase in cellular damage, this increase being greater in less vigorous seeds. Gorecki et al. (1985) have shown that the burst in volatile acetaldehyde and ethanol production was greater in the less vigorous seeds. Furthermore, the subsequent decline in volatile production with imbibition in the more vigorous seeds preceeded that in the less vigorous seeds. In the present study, imbibition of vigorous seeds was associated with a decline in hydroperoxide value. However, with increasing initial peroxide value, the peroxide value on imbibition either declined to a smaller degree or even markedly increased. Thus it appears that from results of this study that increasing seed age (or rather peroxide value) was associated with an increase in hydroperoxide production on imbibition. A possible explanation for this phenomenon could be that the greater moisture content on imbibition might facilitate the mobility of any cytotoxic compounds that have accumulated during storage or that are produced on imbibition, including organic free radicals and aldehydes. If damage does not exceed the ability of the tissue to repair it, repair processes involving enzymes such as peroxidases and 'peroxygenases' might rapidly break down any oxygenated fatty acids present. However, if damage should exceed such repair ability, hydroperoxides may be broken down by other pathways, such as Fenton type reactions in association with haeme compounds, particularly cytochromes. This may lead to the production of further free radicals, thus resulting in a 'burst' of further lipid peroxidation. Furthermore, this increased level of hydroperoxides will result in a greater level of toxic secondary products particularly aldehydes, thus greatly

exacerbating the extent of damage (Wilson and McDonald, 1986a).

8.The invigorating effect of reducing compounds.

The pretreatment of seeds with antioxidants in order to improve storage ability and germination response has met with limited success. Kaloyereas et al. (1961) has shown that the viability of onion (Allium cepa L.) and okra (Abelmoschus esculentus L.) seeds could be extended by pretreatment with alpha-tocopherol and starch phosphate. More recently, Gorecki and Harman (in press) have shown that peas treated with alpha-tocopherol and butylated hydroxytoluene were more resistant to rapid ageing than untreated controls. Similarly, Radrupal and Basu (1979) have shown that iodinated mungbean (Vigna radiata L.) seeds showed a resistance to vigour loss, also under rapid ageing treatments. They attributed this effect to the stabilising effect of iodine on the unsaturated fatty acids.

If free radical species are associated with the onset of damage on imbibition, then the provision of reducing agents might provide a source of electrons, thus quenching any free radicals present (Berjak,1978). Initially, ferrous chloride and potassium iodide were chosen. Seeds treated with these two compounds showed a marked improvement in rate of germination and final percentage germination. However, seeds treated with a compound, tinoridine, with reported antioxidant activity of 20 times that of alpha-tocopherol (Yasuda et al.,1980) had very little beneficial effect. Furthermore, dimethyl sulphoxide, (DMSO) a hydroxyl radical scavenger, was observed to be toxic to the seeds on imbibition. (DMSO is, however, known to permeabilize membranes). This might suggest that it was the reducing ability of the compounds that was beneficial, and that

they were not acting primarily as antioxidants.

The hydroperoxide levels of two batches of cabbage seeds treated with ferrous chloride were monitored over the first hour of imbibition. In both cases, the level of hydroperoxides was lower than that in the controls. It therefore seems that one of the effects of the treatment was a reduction of the lipid hydroperoxide, presumably by a Fenton-type reaction. It is possible therefore that the combined antioxidant activity and reducing ability of the reducing agents may facilitate the breakdown of oxygenated fatty acids and quench any free radicals present or produced, thus enhancing the processes of repair.

9.The lipid peroxidation model of seed ageing.

In the introduction, a unifying hypothesis for seed ageing was presented. This was based upon the free radical theory of ageing. It should be noted, however, that the free radical theory of ageing has been developed for hydrated systems and therefore may not be directly applicable to dry seeds.

Wilson and McDonald (1986a) have put forward a lipid peroxidation model for seed ageing in which they suggest that during dry storage, direct autocatalytic attack of unsaturated fatty acids by atmospheric oxygen leads to the accumulation of oxygenated fatty acids. Enzymatic, and to a lesser extent nonenzymatic breakdown of these oxygenated fatty acids on imbibition is suggested to lead to the production of free radicals and toxic secondary products which "inhibit respiration, protein synthesis, DNA synthesis and denature protein".

The above authors consider time to be a major parameter in their model. However, earlier unpublished work by the present writer has shown that the extent of lipid peroxidation as measured by peroxide value in a number of batches of lettuce, cabbage, radish (Raphanus

sativus) and carrot (Daucus carota) seeds showed no obvious relationship to chronological age. On the other hand, a relationship between seed germinabiliy and peroxide value was observed.

Wilson and McDonald (1986a) have also proposed that autoxidation of seed lipids during storage leads to the accumulation of hydroperoxides and that by measuring the level of the hydroperoxides, the physiological age of the seeds can be determined. However, this proposal may not be correct since cabbage seed peroxide levels were only poorly correlated to germinability.

Thus it would appear that the lipid hydroperoxide level is dependent not only on the chronological age of the seed, but also upon the rate of formation and the rate of breakdown. This in turn will be dependent largely upon the storage conditions the seed has been subject to, particularly those of temperature and relative humidity. For example, seed moisture content, which is dependent upon the relative humidity of the environment, may markedly influence the rate of lipid peroxidation, as was suggested by the results of this study.

As autoxidation is inhibited at higher moisture contents, enzyme activity may be implicated as a possible alternative cause of peroxidation at higher moisture levels (Wilson and McDonald, 1986a). They have suggested that lipoxygenase activity might provide an alternative mechanism for lipid oxidation at higher moisture levels. Furthermore, the observation that the mitochondria are the first organelles to show signs of damage in rapidly aged maize (Berjak and Villiers,1972) may support a role of enzymes, particularly those of the electron transport chain, in the initiation of damage. Such a proposal has been recently made by Berjak, Dini and Gevers (1983). However, this may also be a reflection of the relatively high proportion of PUFAs

found in the inner membrane (Moreau et al.,1974), thus making them more susceptible to autoxidation.

While Wilson and McDonald (1986a) state that the level of hydroperoxides accumulated during storage will represent "damage that would be manifest on imbibition", on the basis of results reported in this thesis, the present writer would like to suggest that it may not be the level of hydroperoxide per se that will determine seed vigour, but rather the extent of breakdown during storage or the ability of the seed to remove the hydroperoxides without causing damage on imbibition.

Wilson and McDonald (1986a) favour an enzymatic breakdown of the hydroperoxide on imbibition, mentioning the possible alternative involvement of haeme compounds such as cytochromes. However, it is suggested that the latter pathway of hydroperoxide breakdown may be more significant, particularly with regard to the production of toxic products such as aldehydes and organic free radicals.

In summary, it is suggested that, depending upon the conditions of storage, the lipids of the seed membranes may be subject to direct attack by atmospheric oxygen. Alternatively, at higher seed moisture contents, the activity of enzymes such as lipoxygenases and those associated with electron transport chains, particularly mitochondrial electron transport chains, may 'leak' toxic intermediates. Free radicals produced from either source are proposed to cause oxidative damage to membrane lipids in particular, but also other macromolecules in general, leading to an accumulating level of damage. If on imbibition, the level of damage exceeds the ability of the cell to repair it, pathological changes would ensue, including primarily changes in membrane integrity, but also the impairment of respiration, protein and DNA synthesis, damage to the genome and the denaturation of macromolecules such as

proteins and RNA. Indeed, the loss of membrane integrity will directly influence many of these processes. The loss of membrane integrity is proposed to be the central pathological event associated with the reduction of seed vigour, particularly on imbibition. The involvement of haeme compounds in the breakdown of the hydroperoxides on imbibition may be of particular significance, as this would greatly exacerbate damage due to the production of further cytotoxic compounds (figure 1). Enzymes such as lyases may also be involved in the breakdown of the hydroperoxides on imbibition.

10. The use of volatile analysis as a vigour test.

A significant correlation between volatile production from dry seeds and seed vigour was observed in both the soya beans and cabbage seeds investigated in this study. A number of workers have suggested the use of these volatiles on a predictive basis. Fielding and Goldsworthy (1982) have stated that the measurement of volatiles derived from heating dry seeds may provide a very sensitive indication of their physiological age and potential vigour. Similarly, Wilson and McDonald (1986a) have stated that the passive trapping of aldehydes released on imbibition may be useful as a rapid, simple and highly predictive vigour test for soybean seeds. These workers reported a correlation of -0.97 between aldehydes passively trapped over the first 24 hours of germination and field emergence. This contrasts with the lack of success in the present study to find a relationship between volatiles derived on imbibition and germinability, particularly in the seeds of soya. Some modification of the chromatographic technique used in the present study to make headspace sampling more comparable with that of Wilson and McDonald (1986b) might yield more satisfactory results. Nevertheless, a further investigation of the predictive capability of

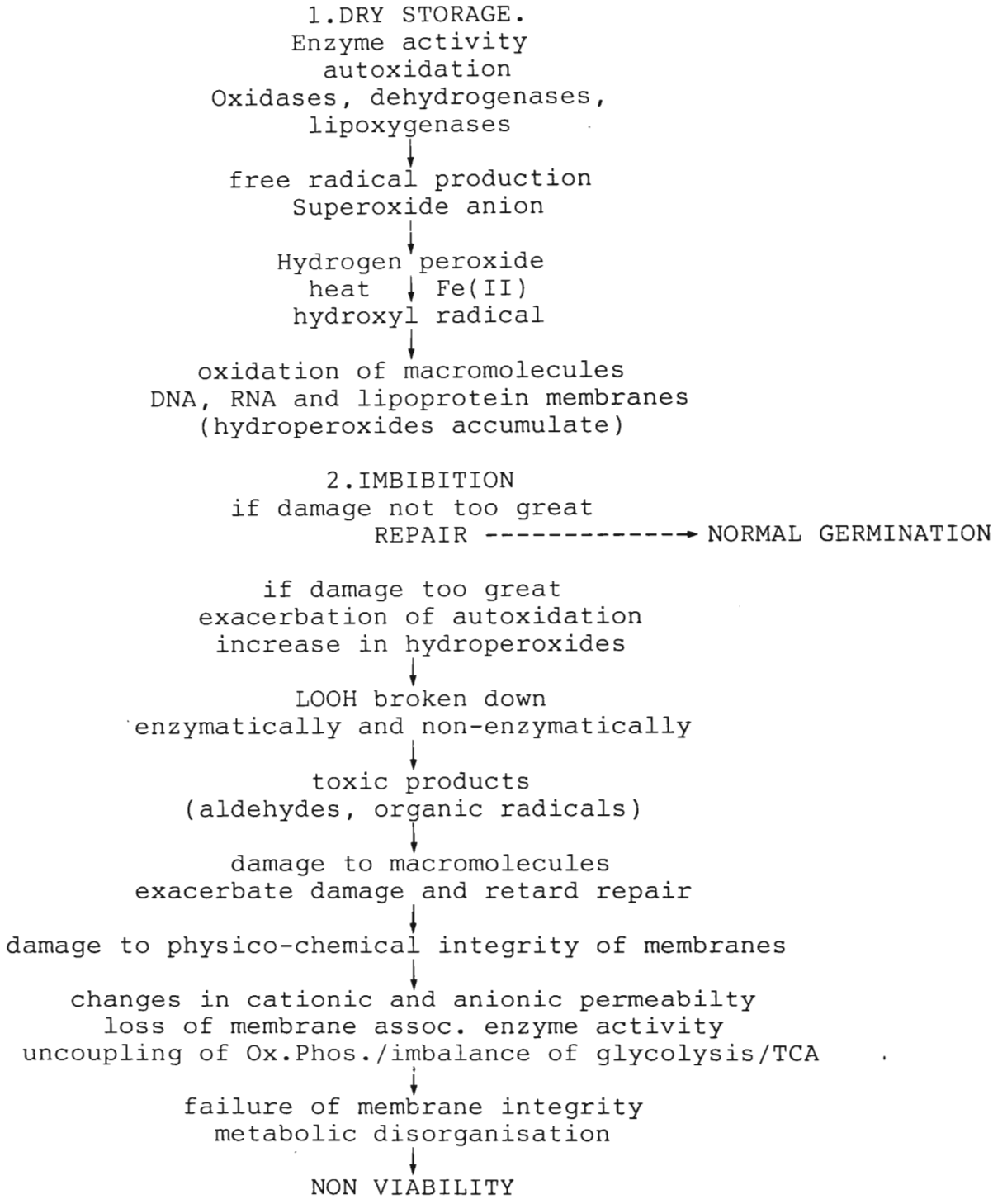


Figure 1. A two phase scheme for free radical mediated ageing in dry seeds.

the volatiles derived both from dry and imbibing seeds might be expedient and may provide a valuable tool in the routine analysis of seed vigour, or at least serve as an index of lipid peroxidation.

CONCLUSION.

In conclusion, it appears the that loss of PUFAs that had occured in both seed types was associated with the loss of seed vigour and was possibly due to lipid peroxidation. Furthermore, volatile compounds evolved on heating were clearly related to seed vigour, particularly seed hydroperoxide levels. Volatiles evolved on imbibition yielded equivocal results, but did show some relationship to seed vigour in cabbage seeds. The above results therefore provide further support for a role of lipid peroxidation in seed ageing.

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