THE EFFECT OF ROASTING, IRRADIATION AND STORAGE ON THE MAIN NUTRIENTS OF THREE VARIETIES OF ALMONDS

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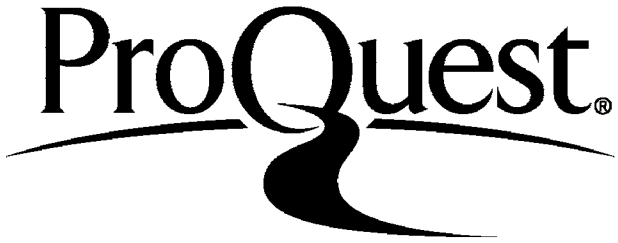

TO THE MEMORY OF MY BELOVED

FATHER WHO IS ALWAYS IN

MY THOUGHTS

ABSTRACT

The effects of processing (irradiation and heat treatment) and storage on the main nutrients (carbohydrates, proteins and lipids) of almonds (Prunus amygdalus Batsch) have been investigated.

Two varieties of Iranian almonds: P. amygdalus var. hard shelled, and P. amygdalus var. fragile, and one American variety, P. amygdalus var. semi-hard have been used in the present study. Variations between the nutrient composition of the different varieties were observed, and the range was between 93 and 132 mg/g for carbohydrates; 170 and 180 mg/g for proteins and 400 and 500 mg/g for lipids.

The almonds were then subjected to the following three processes:

1. A sample of shelled almonds was subjected to 2 Mrad irradiation using ^{60}Co. Changes followed similar trends in the three almond varieties after irradiation. There were no significant losses in total available carbohydrates, total proteins and total lipids, whereas increases were observed in free amino acids, free sugars and free fatty acids. Storage of the irradiated almonds at 22°C for one year did not bring about any changes in total available carbohydrates, total protein and total lipids. However, there were changes in the individual free sugar fractions, while no changes were observed in free amino acids and in free fatty acids.

2. Samples of shelled almonds were pre-soaked in 17% salt solution for 4 hours with slow agitation. After removing them from the solution, the salted nuts were transferred to a rotary drum and dried at 70°C for one hour, until the moisture taken up during the soaking treatment was removed.

The temperature was then raised to 120°C within 30 minutes and roasted for 20 further minutes. Losses occurred in both carbohydrates and proteins, while the total lipid content remained unchanged after the above processing. Decreases were noted in free amino acids and in free sugars, but there were increases in free fatty acids. Storage at 22°C for one year resulted in no further losses of carbohydrates and proteins. Free amino acids increased, indicating some protein hydrolysis. A further increase in free fatty acids was observed.

3. A sample of shelled almonds was first roasted as described under (2) and then irradiated as described under (1); this being referred to as combined processing (dual treatment). The same three nutrients followed very similar trends to those found in roasted samples.

So, on the whole, irradiation at 2 Mrad seems to preserve the nutrients of almonds much better than does roasting, especially during storage for one year.

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ABBREVIATIONS USED IN THE TEXT

Digalactosyl diglycerides	DGDG
Diglycerides	DG
Free Fatty acids	FFA
Free sterols	FS
Hydrocarbon	HC
Lyso-phosphatidyl choline	LPC
Monoglycerides	MG
Phosphatidic acid	PA
Phosphatidyl choline	PC
Phosphatidyl ethanolamine	PE
Phosphatidyl inositol	PI
Relative humidity	RH
Sitosterol	SI
Sterol glycoside	SG
Sterol esters	SE
Sulpholipid	SL
Triglyceride	TG
Trimethylsilyl	TMS
Total available carbohydrates	TACHO
Trace	T

For other abbreviations see page 53.

For petroleum ether read light petroleum.

GENERAL INTRODUCTION

Almonds are one of the most popular nuts in the world. Botanically they are the stones of drupes, enclosing the edible seed. They are borne by Prunus amygdalus Batsch (family Rosaceae), a small tree closely related to the peach. It appears to be native to the eastern part of the Mediterranean region, from where it has spread westwards to Europe. The first European cultivators of almonds were the Greeks and Romans. Today they are cultivated in all temperate regions, but they do not grow in the tropics. The almond tree was introduced into America (California), Australia and South Africa from Europe.

The six countries accounting for nearly all of the world's commercial production of sweet almonds are the United States, Spain, Italy, Iran, Portugal and Morocco (Table 1).

Two of the varieties used in this study originated from Iran, while one of the varieties used was Californian. More than twenty different varieties of almonds exist in Iran, spreading from the northern Elburz mountains to the southern Bandar Abas and then moving eastwards (Fig. 1).

The earliest almond trees in the United States were those grown from seeds introduced from Mexico and Spain, but these crops failed. Trees were then imported from Europe in the 1940's but these did not thrive in the Eastern parts of the States and eventually were established in California which is now the only important almond-producing state in that country. Almond orchards in California provide a living for more than 6,000 growers and their families. In fact, almonds are one of the fastest expanding tree crops in California, expanding nearly three fold in fifteen years.

Fig. 1 : Map of IRAN and its neighbouring countries.


TURKEY
U. S. S. R.
U. S. S. R.
Mt Ararat
CASPIAN SEA
Tabriz
Lake Rezaiyeh
Rasht
ELBURZ MOUNTAINS
Mashad
Qasvin
Mt. Damavand
Tehran
ZAGROS MOUNTAINS
DASHT-E KAVIR
SYRIA
Hamadan
Qom
IRAN
AFGHANISTAN
Baghdad
Isfahan
R. Zayandeh
IRAQ
R. Tigris
R. Euphrates
Yazd
DASHT-E LUT
R. Karun
Ahwaz
Kerman
Abadan
Persepolis
Shiraz
FARS MOUNTAINS
Kharg Is.
Bam
ARABIA
Bandar Abbas
PAKISTAN
PERSIAN GULF
Char Bahar
GULF OF OMAN
A.F.L.
IRAN
Extent of Persian Empire 500 BC
N
Kilometres
0
100
200
Oilfield Areas

Table 1: COMMERCIAL ALMOND PRODUCTION

IN SELECTED COUNTRIES

1972 - 1976

Shelled basis

(WOODROOF 1979)

REGION AND COUNTRY	(1000 metric tons)				
	1972	1973	1974	1975	1976
Iran	9.0	8.1	8.5	8.3	8.0
Italy	15.0	8.0	14.0	20.0	25.0
Morocco	4.8	5.5	3.0	3.0	5.0
Portugal	5.5	8.0	5.0	3.5	6.0
Spain	50.0	37.0	55.0	43.5	65.0
United States	64.4	67.7	98.7	84.0	127.0
TOTAL	148.7	134.3	185.2	162.3	236.0

Minor producers include Turkey, Cyprus, Greece, Algeria, Afghanistan and Tunisia. Although the major almond producers mainly use the nuts for domestic purposes, a large proportion is also exported.

Table 2: ALMOND EXPORTS FROM SELECTED COUNTRIES 1971-1975

(Shelled basis, WOODROOF, 1979)

COUNTRY	1971-72	1972-73	1973-74	1974-75	1975-76
Iran	5.4	5.6	5.5	2.5	1.8
Italy	13.2	9.6	3.3	2.2	8.0
Morocco	1.5	3.6	4.3	1.6	0.8
Portugal	5.2	4.5	6.6	3.5	3.0
Spain	19.0	33.0	19.0	20.0	25.0
United States	35.7	26.2	32.3	45.0	46.0
TOTAL	80.0	82.5	71.0	74.8	86.1

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Almonds are exported as either 'unselected' or 'selected'. Unselected almonds are simply those that have not been as carefully culled, graded or sized as selected almonds. The admixture with other cultivars or types may be greater in that there may be more bitters, doubles (twins), broken, splits and pieces as well as damaged kernels and other defects.

There are two varieties of almonds, the sweet almond (Prunus amygdalus var. dulcis) and the bitter almond (Prunus amygdalus var. amara). Bitter almonds are used for the expression of almond oil and volatile substances, because they are cheaper than the sweet (edible) almonds, and for this reason they are often mixed with the sweet variety. According to most authorities, (BROUK, 1975) the sweet almond is never bitter when ripe. Sweet or edible almonds may vary from soft to hard shells.

Morphologically the almond fruit has the three distinctive parts of a drupe. The exocarp is more or less pubescent; the mesocarp (hull) is fleshy but becomes dry and leathery at maturity as it loses moisture, and the endocarp (shell) varies from being very hard to very thin and soft.

The seed contains a full size embryo surrounded by seed coats. Fruits develop in two stages, differing from other stone fruits in that there is no second period of enlargement preceding ripening (BROOKS, 1943); rather, the mesocarp dehisces and opens to expose the nut.

The most ideal climatic conditions for production exist where there is a Mediterranean-type climate which has a rainy, relatively mild winter combined with a warm, rainless spring and summer.

One undesirable feature of almonds results from the fact that almond flower contains two ovules; one or both may develop into a seed. If both develop, two kernels are produced that are usually misshapen and therefore, difficulty arises in screening and sizing. The tendency to produce only one single well-formed kernel is a highly desirable characteristic.

One advantage of almonds over other nuts is that they come in different sizes: the bigger ones are used for snack purposes, while the smaller, misshapen ones are used ground as ingredients in the making of cakes and sweets etc.

Entire almonds (i.e. almond kernels) are eaten either peeled or with the testa, a thin, brown skin which peels off easily when the seed is soaked or boiled. Almonds consumed as dessert nuts may be salted and roasted after removal of testa, or they may be added to chocolate. Ground almonds with or without testa are also often used for special doughs or cream. Almonds

added in small quantities to cakes, pastries, savoury sauces and meats should be considered as flavouring or decorative material rather than as nuts per se (BROUK, 1975). When the shelled nuts, which contain about 50% oil, are dried coarsely, ground and cold pressed, about 70% of the oil is removed. The oil is low enough in free fatty acids to be refined as a high-grade saleable product after a bleaching agent has been used (WOODROOF, 1979). Recently, another use for almonds has been found and almond butter is catching on fast in the U.S.A. as a butter rich in unsaturated fatty acids. Almond butter is a versatile, new ingredient, which may be used specially in the baking, candy and ice cream industries as well as in other food processing fields (AXER, 1984).

In general, because the ingredient is both a source of flavour and of nutritional components of natural origin, almonds are very widely used.

The increased use of almonds on the market has been due to effective merchandising and the development of new uses. Marketing has been aided by increased buying power of the consuming public all over the world. The results of these factors has been spectacular increases in per acre yield, expansion to new areas, and an increase in total production. The prospects are that almond growing will continue to intensify in various parts of the world (KESTER and ASAY, 1975). It is thought then that this crop is an economic asset to a producing country.

There is a lack of information on the quantity of almonds that are lost due to post harvest diseases.

Seeds should be kept in a viable condition until they are used or processed because rapid deterioration begins at the time of death of the seeds.

Seeds stored in bulk are not in a natural environment and deterioration can be very rapid when conditions of storage are adverse (BARTON, 1941).

With the increased use of mechanical harvesters, there has been a great tendency to harvest seeds while they are still moist. These seeds are ready prey for insects and fungi.

Many species of fungi, generally referred to as storage fungi, present superficially on the seeds at the time of harvest, pose a distinct hazard because their spores germinate and the fungi grow on stored seeds. There is no doubt that the nutritional quality of seeds in storage are adversely affected by the proliferation of fungi in seeds and the production of toxic fungus metabolites. These substances include aflatoxins produced by Aspergillus flavus and A. parasiticus. These two fungi are capable of producing aflatoxins on a wide range of crops and processed foods.

In fact, the presence of aflatoxins is a warning that other higher-toxic fungi metabolites may also be present, possibly in substantial concentrations.

The growth and proliferation of insects, such as the Naval orangeworm (Peramyelois transitella Walk) and the peach twig-bore (Ansaria lineatella Zella) in the case of almonds is particularly destructive, not only causing loss of seeds, but also in the build up of their secretions that give foul tastes and odours.

On the whole, losses in stored seeds in the United States (comprising the major cereal and oil seeds only) was estimated to be in excess of ten million tons per year. The latter reflects the gravity of the situation in terms of introducing effective measures to prevent these large losses. Countries

like Spain, Iran and the western states of North America, where almonds are grown, have highly favourable conditions for grain-storage insetcs and microbial spoilage.

In the spring of 1972 a consignment of Iranian nuts entering the U.S.A. was checked for the first time for aflatoxins and was found to be heavily contaminated. This resulted in strict control of nuts imported to the States, particularly pistachio nuts, of which, in the following months, about thirty-six consignments were either detained or sent back.

Storage conditions in the Mediterranean countries are far from perfect. In Iran, for example, in order to prevent damage during storage, fumigation with methyl bromide, sulphur dioxide or carbon dioxide gas has been suggested but this needs to be repeated every few months, especially in warehouses without adequate ventilation.

Radiation of food material shortly after harvesting or prior to storage may contribute much towards reducing these losses, and hence increasing the world's food resources. This is therefore a subject of universal interest. In recent years the potential use of irradiation for the control of insect infestation and microorganism damage in stored grains has become well recognised (ISMAIL et al., 1976).

Another potential advantage of food irradiation is the possibility that, in some cases, the use of chemicals, especially the persistent pesticides derived from chlorinated hydrocarbons, would diminish. For example, the use of lindane for controlling insect infestation in wheat grain could be eliminated completely by applying low-dosage irradiation treatment. Some of the application and dose rates of irradiation used commercially are shown in Table 3.

Table 3: MAIN FOOD APPLICATION UNDER INVESTIGATION AND DOSE LEVELS REQUIRED

APPLICATION	CAUSE OF SPOILAGE	DOSE (Mrad)
Sterilisation of meats (room temperature storage)	Bacteria e.g. Clostridium botulinum	4-5
Sterilisation of special food ingredient e.g. spices	Bacteria vegetative and spore forming	1-3
Elimination of food poisoning organisms	Salmonella	0.5 - 1.0
Disinfectation of grain	Insect	0.02
Disinfectation of dried fruits	Insect	0.1

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REPORTED FROM THE MEETING ON THE WHOLESOMENESS OF IRRADIATED FOODS (1962)

To date, a systematic study of the effects of gamma irradiation on the major nutrients of almond nuts has not been undertaken and the object of the present study was to investigate the effect of 2 Mrad irradiation which is high enough to produce almonds free from any possible contamination due to bacteria, insect and fungi.

With regard to the utilisation of almonds, it is also important to keep in mind consumer acceptance. A common method of enhancing the flavour and acceptability of almonds is to roast them after soaking in salt solution.

In connection with this, it was thought useful to study whether commercial roasting, in fact, produced losses in the nutritional value of almond nuts.

Furthermore, it is now a well-established fact that some micro-organisms may survive low-dosage irradiation treatment (PARISI and ANTOINE, 1974). FARKAS (1977) has stated that it was generally recognised that one of the most promising means of increasing the effectiveness of irradiation in the control of spoilage of foods without adversely effecting normal organoleptic qualities was to combine it with heat processing. Therefore, a combined irradiation treatment with roasting and its effect on nutrients was also considered.

The present study therefore deals with aspects of 2 Mrad gamma irradiation, roasting and combined treatment on various nutrients in three cultivars of Prunus amygdalus. These will be discussed as follows:

Chapter I: Significance and use of food processing methods

Chapter II: Preliminary investigation of raw and processed almonds

Chapter III: Effect of irradiation and storage on carbohydrates

Chapter IV: Effect of salting-roasting, dual treatment and storage on carbohydrates.

Chapter V: Effect of irradiation and storage on proteins.

Chapter VI: Effect of salting-roasting, dual treatment and storage on proteins

Chapter VII: Effect of irradiation and storage on lipids

Chapter VIII: Effect of salting-roasting, dual treatment and storage on lipids

Chapter IX: General Conclusions

CHAPTER I

SIGNIFICANCE AND USE OF FOOD PROCESSING METHODS

The term 'processing' covers an enormous field of widely different treatments carried out for a diversity of purposes. The impact of industrial processing is increasing not only in width but also in depth (BERK, 1970).

Food processing has a number of objectives which include the improvement of nutritional value, improvement of sensory acceptability, improvement of convenience for the consumer and increasing the shelf-life of a product. One of the prime concerns is that of preservation. With the high concentration of urban population, an adequate amount of food cannot be supplied in the great variety encountered at present without preservation techniques.

Before considering in detail some of the food processing techniques used, certain principles need to be borne in mind.

1) Some losses are inevitable. Processing is carried out for a variety of purposes including improvement of palatability, texture, eating properties and destruction of toxins. These involve application of heat and water both of which may cause losses in nutrients.

2) Manufacturing processing sometimes involves partial or complete cooking, so factory losses simply replace those that would inevitably take place at home.

3) Some losses may occur during the transportation of food and by instantly processing food prior to transportation, losses may be avoided.

4) The availability of certain nutrients may be increased simply by destroying a toxic substance present.

5) Finally, there is not always a choice between fresh and processed food as crops may be seasonal. It is often a choice between processed food and no food at all.

Interest in the nutrient contents of foods has significantly increased recently, partially due to the strict labelling requirements of processed foods by the U.S. Food and Drug Administration (F.D.A.) (NESHEIM, 1974).

Foods are subjected to a vast number of processes. In the course of the present study, special emphasis will be placed on the following methods: irradiation, roasting,and combination of roasting and irradiation (dual treatment)

IRRADIATION

Introduction

One of the main aims in the application of ionizing radiation to food is to control microbiological spoilage. The objectives of such treatment of perishable food are either to delay the onset of spoilage by substantially decreasing the number of spoilage organisms or to destroy them to such an extent that the product will be indefinitely stable microbiologically. Another possible use is an indirect one, namely the radiation decontamination of dry ingredients (e.g. spices) to be used in the manufacture of food products. This effectively reduces the microbial count of food into which these ingredients are incorporated. Almonds are often used as ingredients in the production of a variety of food.

It has been known for many years that preservation of foods by irradiation offers considerable promise, although meaningful research did not start until the 1940's (PROCTOR et al., 1943). It is one of the truly peaceful uses of atomic energy and has aroused great interest in many countries (FAO, 1965). The first federal agency interested in food irradiation was the Atomic Energy Commission (AEC) which started a programme in 1950 and the U.S. Army started theirs in 1953. Considerable research has been carried out on the technology of the process and on the suitability of irradiated foods for human consumption. The results have shown that a number of applications of ionizing radiation for the preservation of foods is technically feasible and is also highly successful.

Since irradiated foods may become the subject of widespread commercial interest in the future · Food Agricultural Organization (FAO), International Atomic Energy Agency (IAEA) and World Health Organization (WHO) have created committees to make recommendations which may influence legislation in various countries on the control of production and use of irradiated foods, thereby facilitating international acceptance of the process.

Over fifty-five countries are known to have research programs in progress on one or other aspects of food irradiation (GORESLINE, 1973), therefore it has become desirable to encourage a more positive attitude towards national legislation to deal with particular controls required for irradiated food (POTTER, 1972).

Radiation and radiation sources

Only certain types of ionizing radiation possess properties suitable for the treatment of foods. These are electromagnetic radiation in the form of gamma or X-rays, and beams of electrons or negative beta particles within a certain energy range. Electromagnetic radiation of very short wavelength as in the case of gamma rays is generally emitted by the nuclei of radioactive substances during decay. X-rays are similar in nature but are produced by machines. Both gamma and X-rays are highly penetrating, with the effective depth depending on their energy.

As the other particles such as α -particles or neutrons induce radioactivity, they are not employed in food irradiation. For the purpose of the present study, gamma irradiation produced from a cobalt ^{60} source was used

for irradiating almonds. ^{60}Co induces ratioactivity by isomeric transition which is 10^{10} times weaker than the natural radioactivity of foods. There is, therefore, no danger regarding induced radioactivity in the use of such sources.

In any given application, the amount of radiation is controlled by the rate of energy output of the source. This is done by controlling the physical relationship (mainly distance) between the source and target material, and by controlling the length of treatment. The amount of energy absorbed is termed the 'dose' and has been usually measured in rad.

Application of irradiation

Nutrients contained in food are linked both by covalent and ionic bonding and because covalent bond energies are less than the energy of ionizing of an outer electron, all types of ionizing radiation break covalent bonds and bring about chemical changes (SOMMER and FORTLAGE, 1966).

When X-rays hit water molecules or nutrients within the food, primary electrons or activated molecules may arise. These may consequently kill bacteria and viruses or even inactivate certain enzymes. The radiation inactivation of enzymes in foodstuff is of considerable technical importance as it is necessary to prevent enzymatic spoilage as well as microbial spoilage in preserving food. For a wide variety of enzymes, inactivation is not serious at doses of up to 1 Mrad and as expected, this varies from enzyme to enzyme (DESROSIER and ROSENSTOCK, 1960).

For the purpose of preventing spoilage and for extending shelf-life, gamma irradiation now does what thermal processing (canning) has achieved

for years.

The use of radiation offers certain advantages over other methods for the purpose of insect disinfestation. Many insect species known to attack food products have become resistant to certain chemical insecticides and residues of some chemicals have been found to be poisonous to other forms of life. For this and other reasons, the Food and Drug Administration (FDA) has become increasingly concerned with the hazard to public health which comes from chemical contamination of air and water supplies. Low dose radiation (700 Krad) is mostly adequate to disinfect a product without altering it chemically and does not leave a toxic residue. Elimination of insect damage in a number of food items continue to be the subject of studies by Scientists working with food irradiation. It has been confirmed in laboratories of various countries that irradiation disinfestation is a highly practical proposition to control insects in grains, flour, peanuts, walnuts and dried fruits (ANON, 1973).

In general for sterilisation or radiation processing of foods, a dose of 2 to 5 Mrad is used (Table 3).

As regards the extent of processing and its effect upon food constituents, radiation sterilisation is comparable to canning or to the thermal processing of food. Because the public has accepted thermally processed food for many years now, it is relevant to compare the relative destructive effect on the nutritive values of irradiated and of heat-processed foods.

Irradiation may substitute for a number of alternative traditional methods of food preservation and could cope with a number of special problems which cannot be solved by the more traditional procedures.

Although this may be a valuable supplement to food preservation, it may never completely replace them. It is still a comparatively new technique and should not be misused in the early stages of its development. As with other processing techniques, irradiation can lead to certain biochemical and physical changes in treated food. The present study sets out to identify and investigate some of these changes. If irradiation of certain products is to be accepted with confidence by the public, the products must be shown to be wholesome when consumed in quantity over a long period of time. For this reason it was also decided to consider the effect of storage on irradiated foods.

Implication of irradiation to almonds

Recent concern over aflatoxins in California tree nut crops has led to a large number of studies on the incidence of aflatoxin. It has been shown that the occurence of aflatoxins, especially in almonds, is associated with the previous insect damage of kernels, so conditions after harvest should be controlled to prevent the growth of moulds.

LORENZ (1975) has stated that little thought has been given to the possibility of using ionizing radiation to control storage fungi, especially those of the Aspergillus groups as compared to the control of bacteria. As demonstrated by YEN et al (1956) and MOHYUDDIN and SKOROPAD (1970) fungi can be eliminated from stored nuts and grain by means of gamma irradiation.

It must be borne in mind that harmful fungi have to be destroyed as soon after harvesting as possible in order to secure the optimum effect of the irradiation treatment. This is in line with the principle of 'prevention

is better than cure' because once the concentration of aflatoxin builds up in the nuts, removal is difficult. This is supported by studies on the resistance of aflatoxin to chemical and biological changes after gamma irradiation (7, 15 and 30 Mrad) carried out by MIYAKI et al., (1967), who have found that aflatoxin was biologically quite resistant to irradiation at those doses.

Advantages offered to other food material by the irradiation treatment may also be applicable to almonds. In the case of maize, the microbial actions of radiation were shown to improve the quality of starch preparation immensely, thus improving hygienic characteristics of food prepared with starch.

The same has been shown for meat products prepared with irradiated spices, where the meat could be heated at a lower temperature and yet would keep longer than those contaminated with the usually high bacterial content of non-treated spices (ANON, 1973).

As more and more research is being carried out on this unique technology for food preservation, undoubtedly the product quality and the nutritional value of food will be improved. Some of the areas which require further exploration are the stability of nutrients during long storage periods following gamma-irradiation.

It has recently been recognised worldwide that despite all difficulties, the number of irradiated food items authorised with or without restriction have grown from 8 to 19 and the number of countries accepting one or more irradiated products for human consumption have increased from

three to eleven. This number will no doubt be increased further in the future.

Irradiation of three different varieties of almond nuts

Irradiation treatment was carried out by Irradiation Product Ltd (IPL), Swindon, Wiltshire.

Two samples of almonds (200g each) were placed in polythene bags and put aside as a control: one immediately after processing and the other as a control for stored products.

Ten more samples of 40 g each were placed in separate polythene bags and sealed. These were then irradiated by a dose of 2 Mrad for fungal and bacterial control (URBAIN, 1978).

Samples for immediate analysis were held in a cold room at 10°C, these included one control plus five treated samples. The rest were to be used for storage and therefore kept in an incubator at 22°C.

The irradiation conditions were as follows:

Type:	γ rays
Source:	^{60}Co
Temperature:	20°C
Gaseous condition:	air
Dose rate:	1.66 Mrad (time of 1h 12 min 17 sec given)

The reason for irradiating in the presence of air was that moulds are aerobes and therefore irradiating the food in the presence of oxygen increases the kill while treatment in its absence has reduced killing effects (DESROSIER and ROSENSTOCK, 1960).

ROASTING

Introduction

Roasting and salting is the largest outlet for tree nuts, because they are eaten mostly in this form. However, in the case of almonds a large proportion also goes into confectionery. Nuts are roasted either dry or in oil: dry roasting is by radiant heat. Before roasting, the nuts should be graded for size, colour and imperfections and then separated from shells and other foreign materials (Fig 2).

There are now possibly fewer nut salters in number but they produce larger amounts due to mechanization. Because of larger than average profits and relatively low initial outlay, the industry is dominated by regional companies. To compensate for high labour and material costs, nut salters have turned to automation and quantity production.

Roasting as compared to the raw state alters and substantially improves the flavour, texture, colour and appearance of these nuts. In the case of most nuts the flavour changes from a 'green raw' taste to the roasted flavour which is more enjoyable. The textural changes that nuts undergo after roasting are almost as important as flavour changes. The raw nuts are soft and pliable to a certain extent and may be termed 'soggy.' In contrast, the roasted nuts are crisp which is highly desirable to the consumers.

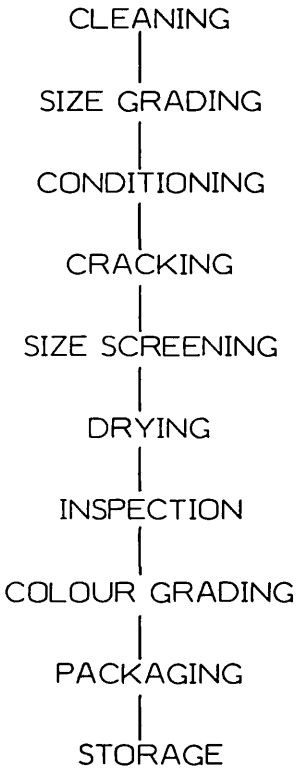


FIG 2: TWELVE STEPS IN PROCESSING SHELLED NUTS

Woodroof (1979)

Deep fat roasting

Unshelled nuts are mostly roasted by immersion in heated oil. The term 'deep fat roasting' is the processing of nuts by complete submersion in edible fat heated to about 121°C to 176°C. The nuts are held in a basket or on a belt submerged in hot fat, which is in turn held in an open top holding tank. The nut may be in a stationary position or conveyed through the heated fat until roasted. They are removed from the fat, cooled, salted and dressed with oil (SCOFIED, 1963). Nuts roasted by deep-fat roasting are more uniform in colour, flavour and texture than those that are dry roasted.

Dry Roasting

The dry roasting principle is that of heating the nut in a vessel until it reaches a certain temperature at which point desirable changes occur and bring about an improvement in texture, colour and flavour. In general, as the internal temperature of the nuts increases, the processing changes occur more rapidly and become more complex. Many different vessels have been used for this purpose, from a simple saucepan to the most sophisticated forced air circulation rotary drum dryer.

Dry roasting can be carried out on a batch of nuts or continuously in which salting, drying, roasting, cooling and packing of the nuts can be done in sequence (WOODROOF, 1979).

Implication of roasting to almonds

When considering a roasting process applicable to any food, certain factors need to be kept in mind. Drastic changes in the physical properties of the cell must be avoided. When a dry food material is put into water

the walls absorb water and soften and owing to their natural elasticity they tend to expand. High temperature heating could disrupt cell walls thereby increasing the chance of nutrient leaching. On the other hand, the roasting temperature must not be so low as to allow the growth of bacteria.

Protein constituents are affected by heating but quality may be maintained under certain conditions.

A roasted food containing 3-6% water and in equilibrium with 10-30% relative humidity is unstable and hygroscopic. During storage, flavour losses may occur together with a slow increase in toughness. Another problem is that rancidity is often increased at low water content especially so in the case of almonds which are rich in lipids.

In general, it can be said that roasting may be used not only to improve the flavour of almonds but also as a means by which spoilage and pathogenic microorganisms are destroyed, together with an effect on inactivating enzymes.

Salting and Roasting of three varieties of almond nuts

Three hundred grams of almond nuts of each variety were used. Each sample was salted by soaking in 17% salt (NaCl) solution (W/V) for four hours with slow agitation. After removing them from the solution, the salted nuts were transferred to a rotary drum and dried out at 70°C for one hour until the moisture taken up during the soaking treatment was removed. The temperature was then raised to 120°C within thirty minutes, and roasted for twenty minutes further (BLOCH and BREKKE, 1960; WOODROOF, 1979). After roasting the samples were cooled at room temperature and put into

polythene bags.

Almonds for immediate analysis were held in the cold room at a temperature of 10°C while those for storage studies were kept in an incubator at 22°C. at 60 - 70%RH.

HEAT-IRRADIATION (DUAL) TREATMENT

Introduction

It is now a well-known fact that micro-organisms that survive radiation treatment are more demanding as to the environmental conditions (temperature, nutrients and inhibitors) than untreated ones (ROWLEY et al, 1974; PARISI and ANTOINE, 1974). Therefore attempts to combine the irradiation treatment with other processing agents are of the utmost importance in enhancing the microbiological effectiveness, hence improving the quality of the product.

One of the most promising means of increasing the effectiveness of irradiation in the control of spoilage of foods without adversely affecting normal organoleptic qualities is to combine it with heat.

The effect of heat radiation treatment has been investigated by Indian workers with fungal spores suspended in saline (PADWAL-DESAI et al, 1973; PADWAL-DESAI, 1974). They have studied the effect of heat irradiation on sensitization of Aspergillus flavus (toxigenic) and Aspergillus flavus oryzae (non-toxigenic). Heating followed by irradiation caused maximum sensitization in both strains as compared to heating after irradiation or with simultaneous heating and irradiation.

The combination of mild heat treatment and subsequent irradiation has been investigated in Canada (HOANG and JULIEN, 1975) for the preservation of apple juice. Organoleptic tests have shown that juice heated at

70°C for eight seconds and irradiated at 350 Krad had an excellent score after four weeks storage at 30°C.

In ground nuts a combination of heat (65°C) plus irradiation (50 Krad) was found to inactivate toxigenic fungi like Aspergillus flavus (PADWAL-DESAI, 1974). The irradiated nuts were shelf-stable for several months when vacuum sealing or packaging under nitrogen was employed. Though the finding is quite encouraging, the use of this procedure for fungal control in ground nuts requires presealing of the commodity in suitable packs so as to prevent recontamination of the product after treatment. This may limit the use of this process.

A combined treatment using mild heat (twenty minutes at 60°C) and a low radiation dose (50 Krad) resulted in a synergistic anti-fungal effect in sliced, packaged bread and at the same time delayed the staling of the bread (KISS et al, 1974).

Similarly, pre-packaged chapaties (unleavened Indian bread) remained mould-free and shelf-stable up to two and a half months after a thermal-radiation (50 Krad) followed by dry heat (65°C for thirty minutes) (PADWAL-DESAI et al, 1973.) The latter have shown that radiation treatment may also precede heating which is contrary to the procedure followed by a number of other authors.

Inoculated pack studies using spores of Aspergillus sp. isolated from naturally infected bread and chapaties confirmed the efficiency of the combined treatment (SREENIVASAN, 1973).

In certain Indian experiments the refrigerated storage life of fish fillets packed in polyethylene pouches was considerably lengthened by a combined treatment of steam cooking for three to five minutes plus 100 Krad gamma irradiation (GHADI et al, 1976).

It is worth noting that thermo-radiation has proved quite effective in inactivating viruses in an environment that was highly protective when heat and irradiation were applied separately (WARD, 1977).

However, to the author's knowledge, the effect of the thermo-radiation process on the content of various nutrients of foods has not yet been carried out.

From the observations made so far, one may assume that an appropriate combination of heat and water activity could effectively reduce the radiation dose necessary for food preservation. However, more confirmatory tests on food would have to be made before extrapolating this data to different food products. In addition, the nutritional changes and eating quality of such products would also need critical evaluation.

One may conclude that, despite the progress made in this field, the available reports on combination treatments are still fragmentary and in many cases related to model systems. Therefore it is imperative that research and development in the field of combined treatments should be carried out in a more systematic manner.

In the present study, a thorough investigation has been made with regards to the nutritional changes occuring in the almonds when subjected to the dual treatment.

Dual treatment of three varieties of amond nuts

The three varieties of almonds were first subjected to the salting and roasting treatment as described on page 44 . These samples were then irradiated (2 Mrad), using the procedures described on page 40 . Together this constituted the dual treatment of roasting and irradiation.

The reason why the radiation dose and temperature used in the dual processing procedure were kept the same as those used in the single treatments was in order to establish a direct comparison between these results.

However, on a commercial basis, these conditions would be changed in order to reduce the cost of the whole process. This is because ordinarily, if irradiation was combined with heat processing, a lower radiation dose would be necessary to complete the sterilisation of food with regards to the microorganisms present.

CHAPTER II

PRELIMINARY INVESTIGATION OF THREE VARIETIES OF RAW AND PROCESSED ALMONDS

Introduction

Preliminary data of raw nuts and those under various processing conditions before and after storage was obtained. In this regard the weight and moisture content of the almonds were established.

The different samples used are given below together with the process under investigation. The abbreviation given will denote the stated samples and processes throughout the rest of the study. A suffix 'S' is added to the abbreviations for the processes to denote one year storage.

In this study two cultivars of Iranian almond nuts have been used. They were provided by the Institute of Modification and Preparation of seeds and seedlings, Tehran, Iran. Care has been taken to choose a representative sample of these two cultivars whenever required. Almonds of Peerless type grown in California were purchased from the local Supermarket.

Samples

Fragile (Monagha) Iranian (abb., F)

Semi-hard shell(Peerless)Californian (abb., S)

Hard-shell (Sangi) Iranian (abb., H)

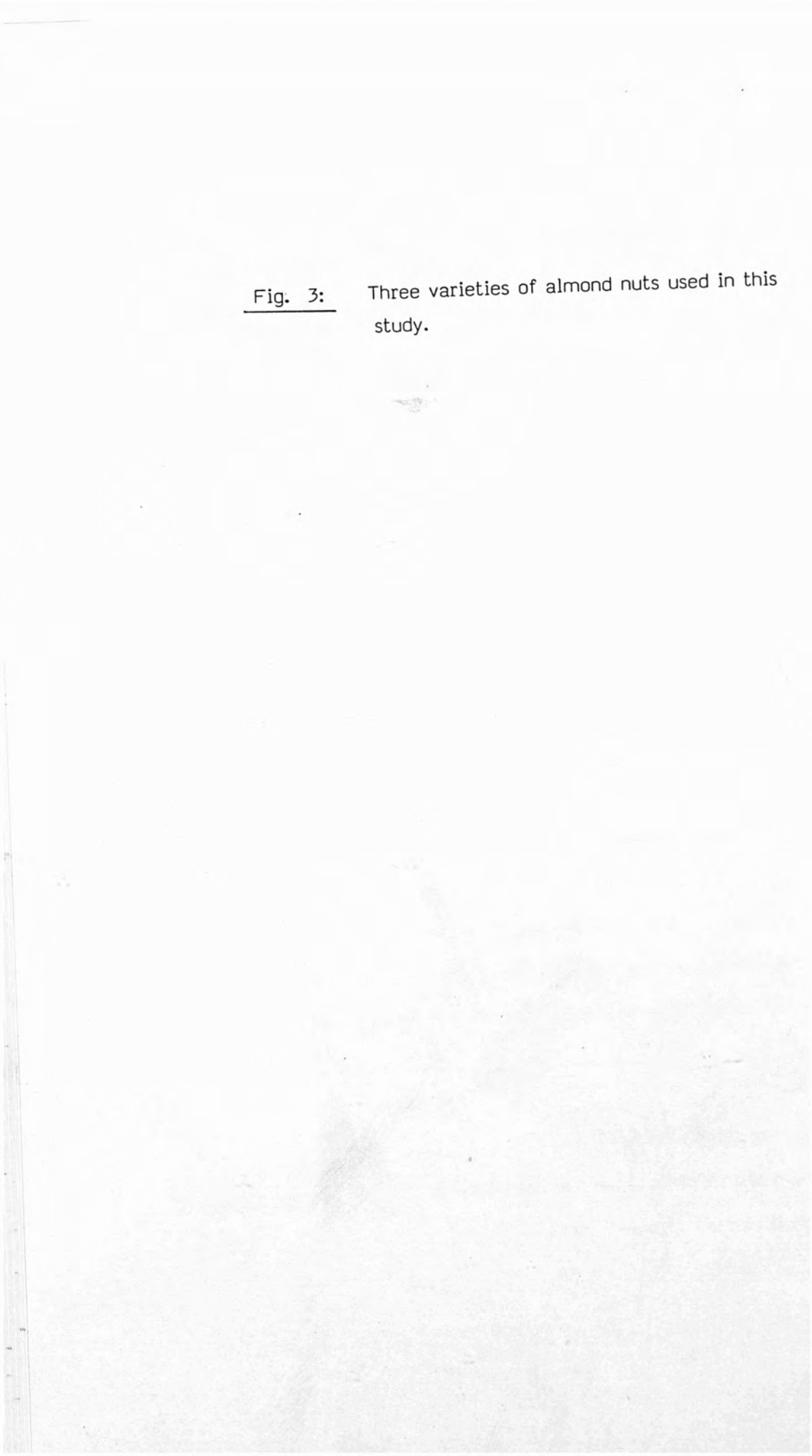
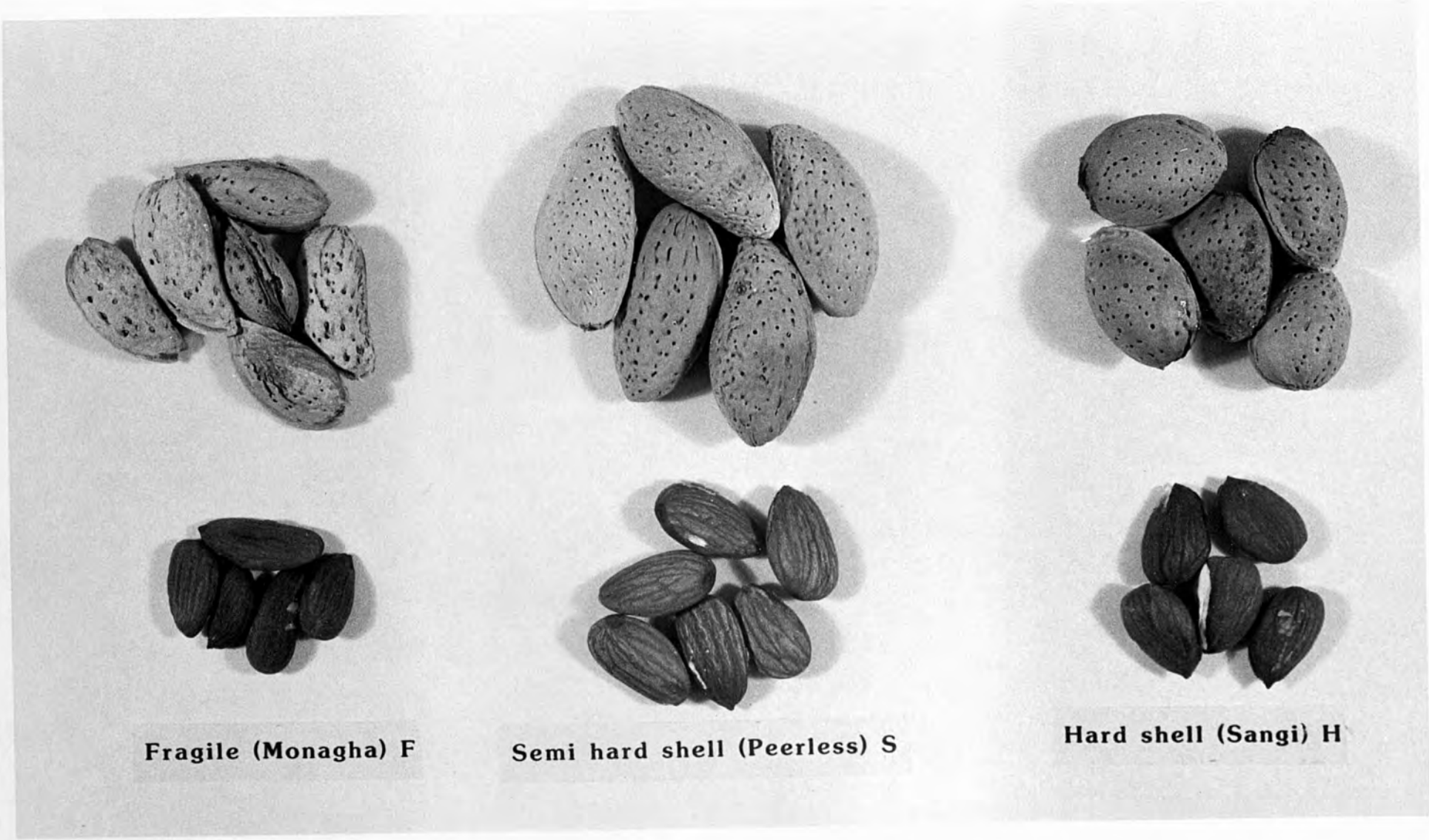


Fig. 3: Three varieties of almond nuts used in this study.



Fragile (Monagha) F

Semi hard shell (Peerless) S

Hard shell (Sangi) H

Processes

Irradiation	I
Salting and Roasting	R
Dual Treatment (Roasting/Irradiation)	RI
Unprocessed samples (controls)	C

MATERIAL AND METHODS

Preparation of sample

The samples were spread out on a clean, dry surface and all foreign matter was removed by hand.

(a) Determination of shell and kernels as a percentage of whole nut

Procedure

One hundred gram approximately of almond nuts were weighed and all the nuts were shelled. The shells and the kernels were kept separately and weighed. The percentage of shells and kernels with respect to whole nuts were calculated directly for all three varieties.

Calculation

Shell and kernels as percentage of whole nuts were calculated using the following formula:

$$\% \text{ shell} = \frac{\text{weight of shell}}{\text{weight of whole nuts}} \times 100$$

$$\% \text{ kernels} = \frac{\text{weight of kernels}}{\text{weight of whole nuts}} \times 100$$

A.O.C.S.

official method (Ab-2-49)

(b) Determination of moisture and volatile matter

This method determines the moisture and any other material which is volatile under the condition of the test.

Procedure

A known weight of kernels (approximately 10 g) was weighed in a tared moisture dish and left in a drying oven at 70° ± 3°C for eight hours. The dish was then removed from the oven and was cooled to room temperature in a desiccator and weighed again. This process was repeated until constant weight was obtained.

Calculation

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

$$\text{The moisture \% in kernels} = \frac{\text{loss in weight of kernels}}{\text{Wt. of kernels}} \times 100$$

(c) Determination of weight of hundred nuts

Procedure

The samples of almond nuts were shelled and re-examined. Any foreign matter was removed before they were used for analysis. The kernels were weighed accurately (app. 500g) and the number of individual nuts were counted. The weight of one hundred kernels for all three varieties were calculated using the following formula:

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

Determination of % of bitter almonds in sweet almonds

The bitter taste of almonds is due to the presence of amygdalins. These compounds are hydrolysed in the presence of water to produce hydrocyanic acid (HCN) which is then detected using a special paper called 'Picrosedeh'. This experiment is based on the principal that HCN in

alkaline media produces a red colour with picric acid. According to the Institute of Standards and Industrial Research of Iran (ISIRI), the percentage of bitter almonds in a sample of sweet almonds should not be higher than 1.5% (ISIRI, 1972).

Procedure

One gram of picric acid was dissolved in 100 ml of distilled water by heating at 50°C. Ten grams of sodium carbonate ($Na_2 CO_3$, 10 H_2O) was reacted with this mixture to produce sodium picrate. Part of the carbonate remains in the free form but it was ensured that the solution, although yellow, remained clear of any precipitate. Filter paper (Whatman No. 1) was immersed in the above solution and then dried before cutting into 1 cm wide strips. These were kept in a dark coloured bottle ready for the next part of the experiment. picrosedeh paper

One hundred almonds were placed separately in one hundred test tubes to which 20 ml of distilled water was added. One strip of picrosedeh paper was suspended in each test tube which was then firmly closed. After placing in a water bath (70°C for 30 mins) the tubes were removed and dried. If the colour of the picrosedeh paper changed to red, this indicated the presence of amygdalin, hence detecting bitter almonds.

In the present study the colour of the picrosedeh paper stayed as before and did not change to red hence showing the absence of bitter almonds in the three varieties under study.

RESULTS AND DISCUSSION

Table 4 gives shells and kernels as a percentage of whole nuts, the weight of 100 kernels and the total weight of 100 nuts.

The whole nut weight for each variety was significantly different, ranging from 191.8 for F to 525.5 g for S. This tells us that the nuts for the semi-hard shelled Californian variety are the largest, while the fragile-shelled Iranian variety are the smallest in comparison.

However, it was found that the greater % of shell (almost 75% of nut) in both S and H was mainly responsible for the higher weights, while for F only 55% of the nut was made up of shell. Despite this, the kernel of S was more than double the size of H. Furthermore, the weight of 100 kernels for F was 18g heavier than for H, although the weight of the whole nut for H was higher.

It seems that there is no direct relationship between variety, the weight of the kernels and the weight of the whole nut in the three varieties studied.

Table 5 shows the moisture contentsfor the different varieties of almond kernels before and after processing and after one year storage. It was observed that the moisture contentsof the three varieties were not significantly different from one another and ranged from 5.9 to 6.8%.

Irradiation treatment itself did not cause any change in the moisture content. One year storage of irradiated almonds resulted in a small but significant increase. The same general trend was observed for all three

Table 4: Shell and kernels as percentage of whole nuts, the weight of 100 kernels and the total weight of 100 whole nuts of three varieties of almonds.

	Shell (%)	Kernel (%)	Dry wt. of 100 kernels (g)	Total dry wt. of 100 nuts (g)
F	55	45	86.3±4.1	191.8±5.1
S	72.5	27.5	144.5±2.5	525.5±3.5
H	74.6	25.4	68.2±6.2	268.5± 6.9

Table 5: Moisture contents before processing, after processing and after one year storage of three varieties of almond nuts.

	F	S	H
	Percentage moisture (g/100g ± SE)		
C	6.2 ± 0.5	6.8 ± 0.4	5.9 ± 0.6
C-S	8.4 ± 0.6	8.7 ± 0.6	8.3 ± 0.5
I	5.4 ± 0.4	6.1 ± 0.3	5.7 ± 0.3
I-S	7.3 ± 0.5	7.3 ± 0.5	6.6 ± 0.4
R	4.1 ± 0.3	4.2 ± 0.2	3.9 ± 0.2
R-S	6.5 ± 0.5	6.7 ± 0.6	5.5 ± 0.3
RI	4.4 ± 0.4	4.1 ± 0.2	4.2 ± 0.3
RI-S	6.8 ± 0.6	5.6 ± 0.3	5.3 ± 0.3

varieties.

Both roasting (R) and the dual treatment (RI) brought about a significant decrease in moisture content as compared to controls. No doubt this was directly as a result of the heat applied during roasting.

After one year storage the original moisture content was, however, once again brought back to the original level recorded for controls. This would have been due to reabsorption of moisture from the atmosphere. This may be a significant factor in determining chemical changes occurring in the food during storage. Further reference to this will be made in relation to changes in nutrient components after processing and storage in this study.

CHAPTER III

EFFECT OF GAMMA IRRADIATION AND STORAGE ON CARBOHYDRATES

INTRODUCTION

Carbohydrates form one of the most important sources of dietary energy, and as such have a vital role to play in the food industry. These materials are very widely distributed in nature. Modern food technology utilises the numerous characteristic properties of carbohydrates, which can vary from inducing sweetness to gel formation (GREENWOOD and MUNRO, 1979).

The carbohydrates found in food may be divided into two main fractions:

(1) Total available carbohydrates. These include polysaccharides such as starch, sugars (glucose, fructose and sucrose). The available carbohydrates are digested and absorbed by man (McCANE and LAWRENCE, 1929).

(2) Unavailable carbohydrates. This group is mainly composed of celluloses, hemicelluloses and pectin. These are not digested by endogenous secretions of the human digestive tract and are collectively termed dietary fibre (TROWELL, 1972).

The classification of the carbohydrates in foods can be illustrated schematically as follows:

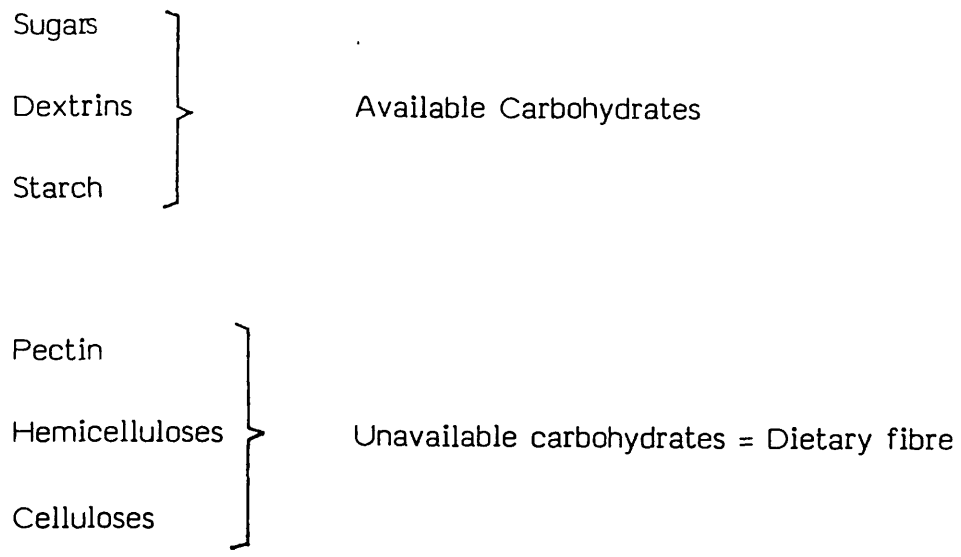


FIG. 4: CLASSIFICATION OF CARBOHYDRATES IN FOODS

The main effects of irradiation on carbohydrates are those of conversion of one form to another, hydrolysis and oxidative degradation (JOSEPHSON et al., 1975). Examples of interconversions include glucose to arabinose (MORI et al., 1965) and sorbitol to D-glucose (WOLFROM et al, 1955). In the case of hydrolysis, sucrose was found to yield fructose and glucose (SCHUBERT, 1974) while raffinose produces melli-biose, D-glucose and D-galactose (MAEDA and MURAKAMI, 1968).

Irradiation of sugar in the pure state leads to marked degradation processes and the formation of radiolytic products such as hydrogen, carbon monoxide, aldehydes, ketones, acids and other related carbohydrates. However, up to 100 Krad irradiation, the concentration of these radiolytic products may be considered negligible (DAUPHIN and ST. LEBE, 1977).

Moreover, the physical and chemical changes produced in the sugar by such an irradiation dose are very slight and less extensive than those following heat treatment.

The changes occurring in carbohydrates are brought about either through a release of ionizing energy upon the target molecule (direct effect) or through the action of secondary intermediates such as free radicals (FAUST and MASSEY, 1966). The deposition of ionizing radiation energy in a medium leads directly to the formation of ions and excited molecules which become distributed throughout the medium. Subsequently, reactions with other compounds in the system occur, leading to the indirect formation of secondary free radicals (TAUB and KAPRIELIAN, 1977).

The extent to which these changes take place depends upon the nature and physical state of the system. For water, which is present in almost all foodstuff, radiolysis leads to the formation of OH° (hydroxyl radicals) $\bar{e}$ (solvated electron), H° (hydrogen atom), H_2 and H_2O_2 (SPINKS and WOOD, 1976).

The influence of water on the radiolysis of pure sugar is complex. When sugars are irradiated in the solid state, water exerts a protective effect either by energy transfer via H-bond or by reaction of water with the radicals of irradiated sugars resulting in the re-formation of the initial product. The situation differs on irradiating sugars in solutions. In addition to the direct action of irradiation on the sugar and on water, there is the indirect effect of radicals derived from water on the sugar with the action of OH° radicals whose concentration is greater than that of other radicals.

These conclusions appear plausible although the radiolysis of sugar in a mixture or in food stuff has received little attention. The reaction particles can undergo reactions with common constituents in food, such as

free amino acids, lipids and carbohydrates. Further chemical studies are therefore required on the radiolysis of sugar in mixtures or in food material. However, progressive changes in the smaller molecules found upon the destruction of macromolecules depend on factors such as dose (BRASCH et al., 1952), water content, (EHRENBERG et al., 1957) and physical form of samples. Although irradiation may cause changes in the physical and chemical properties of foods high in carbohydrates, these have been shown not to be of any nutritional significance (READ, et al., 1961). These authors have found that the availability of carbohydrates of eight foods sterilised by a dose of 5.58 Mrad did not have any effect on rats.

Irradiation may also cause physical changes in sugar, affecting melting points, refractive index, optical rotation and colour. These changes are minimal for commercial doses of irradiation and are usually only of academic interest (DAUPHIN and ST. LEBE, 1977). Effects produced by irradiation are continued during storage (KRAYBILL, 1982). This is of course dependent upon the presence of water and temperature.

The purpose of this part of the study was to identify the carbohydrates found in almond kernels and to investigate the effect of irradiation and subsequent storage on carbohydrates.

MATERIALS AND METHODS

Estimation of total available carbohydrates: (Clegg anthrone method, OSBORNE and VOOGT, 1978).

This method may be used to estimate the amount of starch and dextrins together with soluble sugars (total available carbohydrates). The samples are digested with perchloric acid. Hydrolysed starches together with soluble sugars are determined colorimetrically by anthrone whose depth of colour is proportional to the amount of carbohydrate present.

The anthrone-sugar complex forms a green colour which has a maximum absorption at 630nm.

Reagents

1. Perchloric acid 52%. 250 ml of perchloric acid (sp. gr. 1.70) was added to 100 ml of water and was kept cold before use.

2. Sulphuric acid: 750 ml of sulphuric acid (sp. gr. 1.84) was added to 330 ml of water and kept cold before use.

3. Anthrone reagent: Sufficient 0.1% anthrone was made up in the above sulphuric acid for each day's requirements. This solution had to be made fresh, daily.

4. Glucose standard solution: 100 mg glucose was dissolved in 1 litre of distilled water.

Extraction

Two grams of almond kernels were macerated in a mortar with 10 ml

of water and then transferred into a 100ml measuring cylinder. Thirteen ml of 52% perchloric acid was added, and the mixture was stirred frequently with a glass rod for 20 minutes, and then diluted to 100ml. The mixture was passed through Whatman No 542 filter paper and made up to 250ml.

Sample and Standard Preparation

Ten ml of the above filtrate was diluted to 100ml with water and 1ml of this solution was placed in a test tube. One ml of diluted glucose standard solution (1ml = 0.1 mg glucose) and 1ml of sample were placed in separate test tubes. Five ml of freshly prepared anthrone (0.1% reagent) were added rapidly to each test tube which was stoppered and the contents mixed thoroughly. The tubes were then placed in a boiling water bath for exactly 12 minutes and cooled to room temperature. A green colour was formed with the anthrone reagent which was stable for at least 2 hours. The coloured solutions were transferred to glass cu vettes and the absorbance of the sample and standard were read (using a Unicam SP-800 spectrophotometer) against a blank solution containing 1ml distilled water plus 5ml anthrone reagent.

Calculation

Total available carbohydrates were calculated as a percentage using the formula:

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

Where w = weight (g) of sample

a = absorbance of diluted glucose sample

b = absorbance of diluted sample

QUALITATIVE AND QUANTITATIVE ESTIMATION OF NEUTRAL (SOLUBLE) SUGARS BY GAS-LIQUID CHROMATOGRAPHY

Preparation of the sample for analysis

The first stage in the analysis of free sugars depends primarily on whether the sugars are in solution or whether they need to be extracted from the foodstuff. The main requirement in the treatment of these samples is the removal of substances likely to interfere with subsequent measurements. Protein will interfere with sugar determination and it is desirable to remove the bulk of the protein before attempting analysis. Care is necessary in the choice of deproteinising agents. SOMOGYI (1945) made an extensive study of deproteinisation reagents in connection with the measurement of sugars. He came to the conlcusion that a zinc hydroxide precipitate formed in the reaction between zinc sulphate and sodium hydroxide provided a satisfactory method for deproteinisation, and gave a clear neutral filtrate. Frequently barium hydroxide is used instead of sodium hydroxide (DELENTE and LADENBURG, 1972).

All simple sugars are soluble in water, and most sugars have a relatively high degree of solubility. Values between 30% and 80% are quite common. The simple sugars are also fairly soluble in alcohol, but as molecular weight increases, solubility in alcohol falls off (BIRCH et al,1972).

So in the extraction of carbohydrates two points have to be observed; the procedure should be completely exhaustive and prevent any artefacts from being produced..

Different techniques with aqueous alcohol have been employed for extracting sugars, among them, ethanol, methanol and isopropanol have been used extensively (AOAC, 1975; FRIEDMANN et al., 1967; SOUTHGATE, 1969). A general precaution which has to be adhered to is that the extracting medium must be neutral and remain neutral during the extraction. Significant amounts of organic acids may be present in the sample and these will bring about a partial hydrolysis of polysaccharides. To prevent this from happening, solid calcium carbonate is often added to the extraction medium (SOUTHGATE, 1976).

Sugars are crystalline polyhydroxy compounds which are strongly hydrogen bonded, hence they have high melting points(200°- 300°C) (BIRCH, 1973). They are not volatile components but can be simply converted to volatile derivatives such as trimethylsilyl ethers by reacting with trimethylsilyl chloride (TMSCl) which are readily analysed by gas chromatography.

The use of gas chromatography technique has been widespread (SWEELEY and WALKER, 1964; BISHOP, 1964; CLAMP et al, 1971; HOLLINGAN, 1971). The most commonly used derivatives are the aditol acetates (SAWARDEKER et al, 1965), trimethylsilylethers (TMS) and methyl ethers. Alditol acetates are less suitable when fructose is to be determined in the presence of glucose (this is because on reducing fructose, it yields a mixture of mannitol and glucitol and as glucitol is also derived from glucose, direct quantitation is impossible.)

Acetate and methyl ethers may be used for monosaccharides but at the temperature needed for higher molecular weight sugars (such as di and tri

saccharides) thermal degradation effects occur: anhydro-derivatives may be formed or sugar ring inversion may occur. The TMS ethers (Fig 5) have the advantage of combined stability and volatility which allows the separation of di and tri-saccharides.

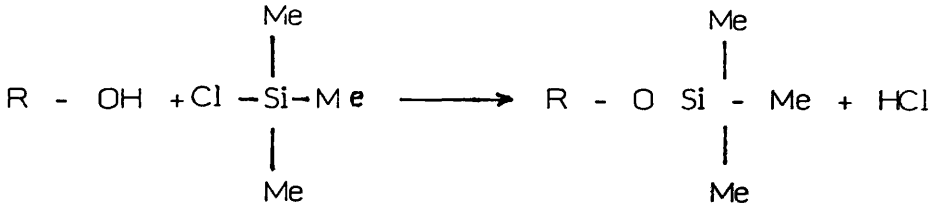


FIG 5: SIMPLIFIED SCHEME OF THE FORMATION OF TRIMETHYL SILYLDERIVATIVE OF SUGARS

Pyridine is universally accepted as the preferred solvent (BIRCH, 1973). The formation of TMS derivatives in pyridine occurs very rapidly at ambient temperature (SWEELEY et al, 1963).

Extraction

Ten grams of almond kernels were ground in a mortar and the lipid which could otherwise interfere in subsequent measurements, was removed. This was done by extracting with 150ml petroleum ether (B.P. 40-60°C) and the ethereal layer discarded. Free sugars were extracted by the methods used by DELENTE and LADENBURG (1972) with slight modifications.

Five grams of defatted and dried almond kernels were homogenised with a mixture of(methanol: chloroform (1:1,V/V)): water (1:2,V/V). To

this was added 0.25 g of solid anhydrous calcium carbonate. After standing overnight at 4°C, the homogenate was agitated for 15 minutes with a magnetic stirrer. Approximately 5ml (accurately measured) of suspension was centrifuged for f minutes at 20,000 r.p.m. To purify the sample, 1ml of the supernatant was added slowly with agitation to 2ml of 1.8% barium hydroxide solution, followed by 2ml of a 2% zinc sulphate solution. After standing for five minutes, the sample was centrifuged for a further 5 minutes, at 20,000 r.p.m. to give a clear extract.

Silylation

Silylation was carried out as follows: 1ml of the extract was lyophilised and 1ml of tri-sil-Z reagent (Pierce Chemical Co.) was added to it. This was heated at 60°C for 20 minutes. The sample was then ready for GLC analysis.

Gas liquid chromatograph

A Pye (series 104) chromatograph equipped with a hydrogen flame ionisation detector, nitrogen carrier and full scale drift per hour of 10^{-12} was used. Gas column (1.5m long and 2mm in diameter) was packed with 3% OV-17 (phenylmethylsilicone) on gas chrom Q, 100-120 mesh, supplied by Camlab, England. Gas chromatograph was operated with H_2 pressure of 12 p.s.i., air pressure of 10 p.s.i. and nitrogen flow of 50ml min^{-1}.

Two temperature conditions were investigated:

(a) temperature programmed from 150°C to 320°C at 8°C min^{-1} (AMAN, 1979)

(b) temperature programmed from 220°C to 270°C at 4°C min^{-1}.

(b) showed excellent results, hence this was used throughout the analysis (KLINE et al, 1970) (Fig. 6).

The chart speed was 25.4 cm h^{-1}, and with an attenuation of 5×10^{-5}.

Peak Identification

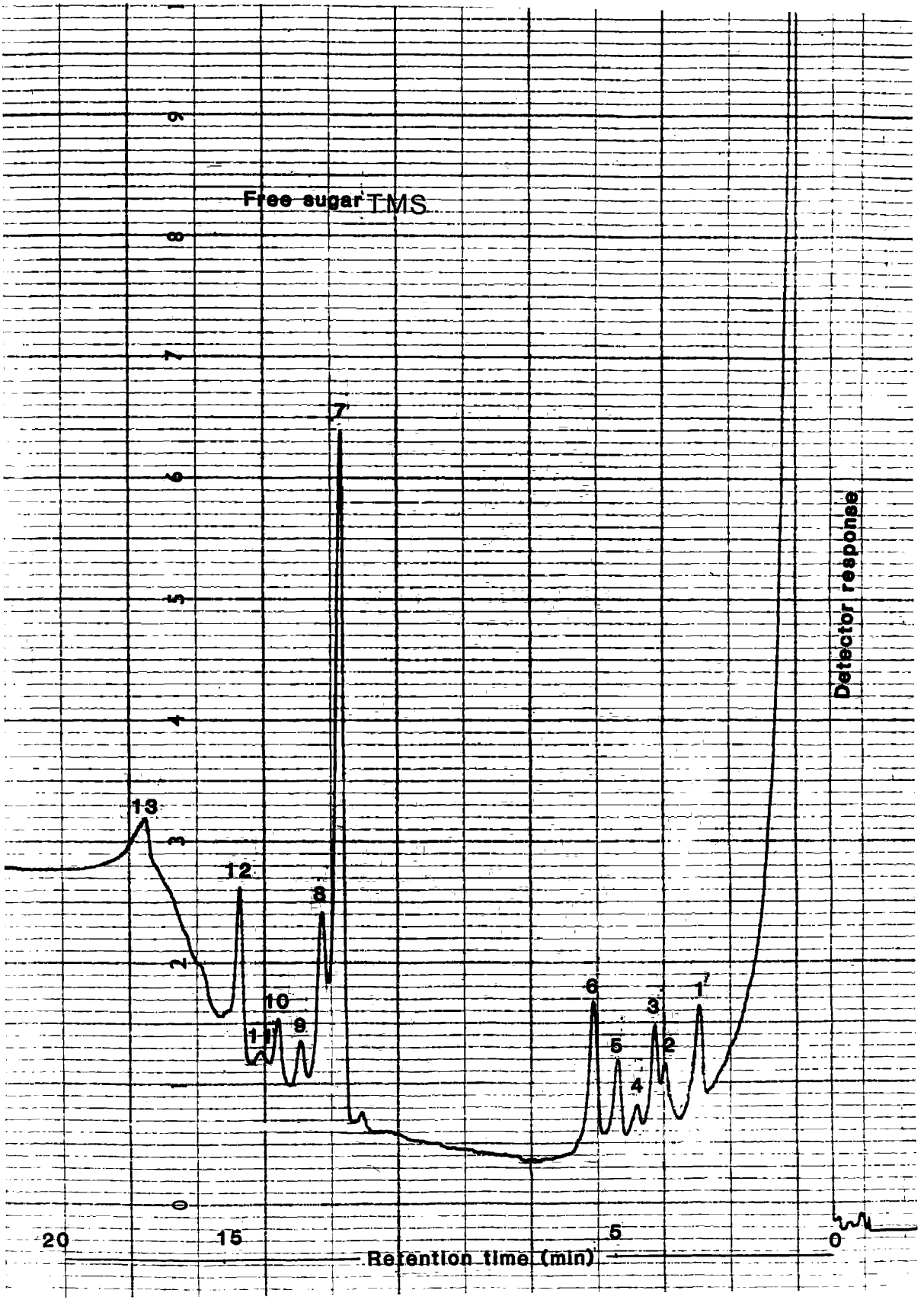
All the different sugar peaks were identified both by comparing retention times with those of pure sugar standards, and by co-chromatography where peak coincidence and height increase were observed.

It is well-known that retention times of volatile sugar derivatives increase with increasing molecular weights.

Fig. 6: Gas-liquid chromatography of free sugar methyl ethers on a 150 cm 3% OV-17 column with temperature programmed from 220°C to 270°C at 4°C min^{-1}

Peaks were identified as follows:

1.	Fructose
2.	Galactose
3, 5	Glucose
4.	Sorbitol
6.	Inositol
7.	Sucrose
8.	Maltose
9.	Isomaltose
10.	Cellobiose
11.	Mellibiose
12.	Raffinose
13.	Stachyose


Free sugar TMS
Detector response
Retention time (min)
20
15
5
0
1'
2
3
4
5
6
7'
8
9
10
11
12
13

STARCH AND TOTAL LOW MOLECULAR WEIGHT SUGARS

(LUFF-SCHOORL METHOD)

Principle:

Free sugars are extracted from the food product using hot ethanol. After hydrolysis in aqueous solution with hydrochloric acid the sugar can then be determined titrimetrically as glucose. Starches were isolated from the residue of the hot ethanolic extraction after treatment with potassium hydroxide solution. On subsequent hydrolysis the starch was converted to glucose which was then determined titrimetrically.

Reagents

1. Sodium hydroxide solution (150g/500ml)
2. Hydrochloric acid solution (IN)
3. Ethanolic potassium hydroxide solution. 25g of potassium hydroxide was dissolved in 400ml of 95% (V/V) ethanol and diluted with ethanol to 500ml.
4. Deproteinising solution I. 26.5g of potassium ferrocyanide (K_4 Fe $(CN)_6 . 3H_2$ O) in water and diluted to 250ml.
5. Deproteinising solution II. 43.8g of zinc acetate [$Zn(CH_3COO)_2 . 2H_2O$] was dissolved in water. Six ml of glacial acetic acid was added and the mixture was further diluted with water to 200ml.
6. Copper reagent. 37.5g of anhydrous sodium carbonate (Na_2 CO_3), 24.5g of sodium hydrogen carbonate (NaH CO_3), 35g of trisodium citrate dihydrate (Na_3 C_6 H_5 O_7, $2H_2O$), and 12.5g of powder copper sulphate pentahydrate (Cu $So_4 \cdot 5H_2O$) were weighed and the

salts were mixed in a beaker and dissolved in about 400ml of cold water, stirring continuously. The mixture was made up to 500ml with water (if after standing for one day turbidity or precipitate had formed, the solution should be decanted or filtered). The pH after 1 + 49 dilution with freshly boiled water should be 10.0 ± 0.1

7. Bromothymol blue solution; 2g in 200ml of 95% ethanol (V/V).

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

9. Sodium thiosulphate standard solution (O.IN). 12.5g of sodium thiosulphate ($Na_2S_2O_3$) and 0.1g of sodium carbonate (Na_2CO_3, 10 H_2O) were dissolved in 500ml of freshly boiled and subsequently cooled water. The solution was allowed to stand for one day before standardising.

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

11. Potassium iodide solution, 5g of potassium iodide was dissolved in water and diluted to 50ml. The solution was stored in a dark bottle.

12. Hydrochloric acid solution, 6N. 100ml concentrated hydrochloric acid (sp. gr. 1.18) was diluted with 85ml of water.

13. Potassium thiocyanate solution. 10g of potassium thiocyanate (KCNS) was dissolved in water and diluted to 50ml.

Procedure:

Starch Determination

Twenty five grams of sample was weighed into a 250ml centrifuge bottle, to which 150ml of 80% ethanol was added and the bottle covered with a watch glass. After heating on a steam bath, with occasional stirring for 1 hour, it was centrifuged for 10 minutes at 3000 r.p.m. The supernatant was decanted. The residue containing starch was washed with 50 ml of 80% ethanol and centrifuged for 10 minutes at 3000 r.p.m. The supernatant solution was again decanted, the residue was transferred to a 500ml beaker and 300 ml hot ethanolic potassium hydroxide was added, stirring continuously with a glass rod. The beaker was covered with a watch glass and heated on the boiling water bath for 1 hour with occasional stirring. The solution was decanted through a Whatman No 542 filter paper and the starch residue was washed directly with hot ethanol 80% (V/V) on the filter paper and stirred by means of a rubber-tipped glass rod. The filter was kept moist while the precipitate was loosened from the paper. A hole was pierced in the filter paper and the starch was washed through it into a 250ml beaker. A hundred ml of hot IN hydrochloric acid solution was added and the beaker was again covered with a watch glass and placed in a boiling water bath for 2½ hours, and stirred occasionally with a glass rod. The solution was cooled and neutralised to about pH 6.5 by dropwise addition of sodium hydroxide solution (pH checked using pH meter). The mixture was transferred to a 200ml graduated flask using distilled water to which 3ml of deproteinising solution I was added, and after mixing for 20 seconds, 3ml of deproteinising solution II was added, and the total diluted to 200 ml. After mixing again and filtering through a fluted paper the solution was

ready for titrimetric determination. The filtrate was made alkaline to bromothymol blue by adding 1-2 drops of the sodium hydroxide. A 25 ml aliquot was then pipetted into a 250ml conical flask to which 25ml of the copper reagent and several anti bumping granules were added. The flask was connected to a condenser and stood on a wire gauge. The contents of the flask were brought to the boil in about 2 minutes and then allowed to boil for exactly 10 minutes. The flask was cooled quickly to room temperature; 5ml of potassium iodide solution was added followed carefully but quickly by 20ml of 6N hydrochloric acid. After the addition of 10ml of thiocyanate solution the liberated iodine was titrated with standard solution thiosulphate. When the solution became pale yellow 1ml of starch indicator was added and the titration continued until the blue colour disappeared. A blank determination was carried out using 25ml of water instead of 25ml of filtrate.

Calculation

The amount (ml) of O.IN thiosulphate solution was calculated as follows:

$$(Vo - V) \times T \times 10$$

Vo = volume (ml) of standard thiosulphate solution needed for the blank

V = volume (ml) of standard thiosulphate solution needed for the determination

T = Normality of the standard thiosulphate solution

The corresponding amount of glucose was found in the conversion table (Appendix 1).

Let: this amount (mg) = g

Then: the starch content (%) = $\frac{g \times 800 \times 0.9}{W}$

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

In a preliminary experiment the Luff-Schoorl method of estimating starch was used. The quantitative values obtained for the latter were not significantly different (at the 5% level) from those obtained by the subtraction of values for total free sugars from total available carbohydrates. Values for starch plus dextrins in the present study were therefore obtained by subtraction.

Quantification:

All experiments were repeated four times. The results wherever possible are expressed as $\bar{X} \pm sd$, where $\bar{X}$ is the mean of four replicates and sd is the standard deviation. In this case sd is the same as standard error of the mean ($\frac{2sd}{\sqrt{n}}$, n is the number of samples) with 95% confidence limit. In cases where the SE has been omitted, the SE was never greater than 10% of the mean value. All % retention given in the tables represent increases or decreases compared to processed samples.

RESULTS AND DISCUSSION

Total available carbohydrates of almonds:

Almonds were found to contain 101.6, 132.4 and 93.6 mg g^{-1} dry weight total available carbohydrates in varieties F, S and H respectively (table 6), these being 10.2, 13.2 and 9.4% dry weight. However, a value quoted for almonds by OSBORNE and VOOGT (1978) was 5% on a dry weight basis. WOODROOF (1979) found almonds to contain 4.5% free sugar and stated that starch was absent.

In the present study, a blue colour was noted on adding iodine solution to the concentrated sugar extract residue, indicating the presence of starch. The low values reported for total available carbohydrates in the literature may have been due to the incomplete or low recovery of carbohydrates during extraction.

On comparing the three varieties, there was no significant difference in total available carbohydrates between F and H, but both of these were significantly different from that of S. This suggests that even between varieties, the content of total available carbohydrates varies considerably. This observation may also support the finding that total available carbohydrate values reported in this study are appreciably higher than those reported by OSBORNE and VOOGT (1978), and therefore depend on the variety of almonds used. It should be stressed at this point that varying values for total available carbohydrates which have been reported in the literature may possibly be due to the different techniques used by the different authors.

Table 6: The effect of 2 Mrad irradiation on total available carbohydrates, starch and free sugars of almond nuts.

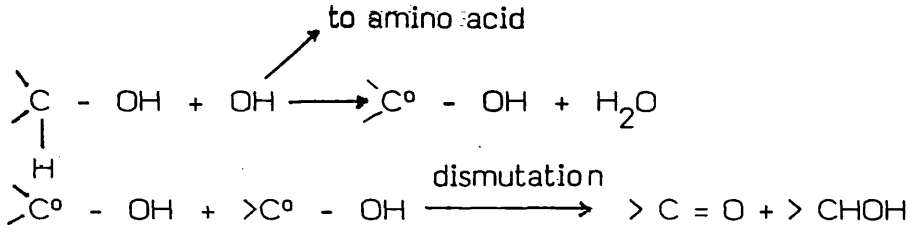
		Free sugars (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg g^{-1} ± SE					
F	C	67.2 ± 4.5	34.4 ± 2.2	101.6 ± 6.2	116.8	58.1	96.9
	I	78.5 ± 3.3	20.0 ± 2.1	98.5 ± 4.9			
S	C	73.5 ± 4.5	58.9 ± 4.6	132.4 ± 8.5	112.6	77.2	96.9
	I	82.8 ± 3.6	45.5 ± 3.7	128.3 ± 6.9			
H	C	49.5 ± 3.9	44.1 ± 3.9	93.6 ± 4.3	120.8	66.9	95.4
	I	59.8 ± 5.3	29.5 ± 2.0	89.3 ± 4.5			

Effect of irradiation on total available carbohydrates

It was found that the total available carbohydrate content of the three varieties F, S and H after irradiation (table 6) was 98.5, 128.3 and 89.3 mg g^{-1}dry weight respectively. This corresponds to 9.9, 12.8 and 8.9% dry weight of almonds respectively and was not significantly different from that recorded for controls. It may therefore be concluded that irradiation has no significant effect on total available carbohydrate content of the three varieties of almonds used.

Many substances including amino acids and proteins provide protection against irradiation of carbohydrates (PHILLIPS, 1972). Protein or amino acids appear to diminish the radiolysis of sugar by their respective action on OH° radicals, without modifying qualitatively the degradation process. Consequently, the radiolytic effects are usually less for mixture than for individual components. In particular, the protective effect of protein and amino acids on the radiolysis of sugar has already been noted by HILL (1964) and MORITA et al (1969).

The OH radicals have been shown to break the C - H bond as follows:



DAUPHIN and SAINT-LEBE (1977)

The presence of cysteine (10^{-3}M) in a solution of glucose (10^{-2}M) completely inhibited the formation of carbonyl products from the sugar.

If methionine was used instead, the amount of carbonyl products found was about 16% of the quantities obtained when a solution of glucose alone is irradiated at the same concentration (DIEHL et al., 1978).

Similarly, the presence of amino acids protects trehalose solution particularly from radiolytic degradation; cysteine being the most protective compound (MORITA et al., 1969). This observation can be explained by the high rate of reaction between cysteine and OH radicals compared to that of sugars and by the transfer of hydrogen from cysteine to the glucosidic radicals.

Although there is no overall change in carbohydrates in the present study, this does not necessarily imply that individual constituents may not have been affected. The next stage of the study was to determine the changes, if any, occurring in individual sugars.

Starch, Dextrins and free sugars of almonds:

SHALLENBERGER and BIRCH (1975) gave the following definition for oligosaccharides and polysaccharides. When two or ten monosaccharide units are combined glycosidically, the molecule is termed an oligosaccharide. If ten or more monosaccharides are joined, the compound is a polysaccharide. Although about 40 oligosaccharides are found free in nature, only a few are abundant in foods, namely sucrose, lactose, maltose raffinose and stachyose.

The most abundant oligosaccharides are composed of some combination of the monosaccharides, D-glucose, D-galactose and D-fructose.

Table 7: The effect of 2 Mrad irradiation on individual free sugars of almond nuts.

Free sugars *	F		S		H	
	C	I	C	I	C	I
	mg/g dry wt.					
Fructose	4.8	7.2	5.7	8.3	4.2	7.4
Galactose	2.7	4.2	3.6	4.9	2.1	3.5
Glucose	8.7	14.1	8.1	13.8	5.7	9.9
Sorbitol	1.8	4.5	2.1	4.5	0.9	2.7
Inositol	4.5	6.2	5.1	6.9	3.6	5.2
Sucrose	26.7	21.4	32.4	25.0	21.6	15.8
Maltose	7.8	11.6	5.4	9.1	4.5	8.1
Cellobiose	1.2	3.1	1.2	2.0	1.2	2.6
Mellibiose	0.3	0.6	0.3	1.4	0.3	1.1
Raffinose	6.9	4.7	8.1	6.0	4.2	3.2
Stachyose	1.8	0.9	1.5	0.9	1.2	0.3

* Determined by GLC as on page 70.

In the present study, sugars, which were readily separated by GLC and which had four or less monosaccharide units per molecule, were regarded as free sugars. The free sugars generally include glucose, sucrose, raffinose and stachyose, while the polysaccharide group consists of starch and dextrins.

Table 6 gives the content of starch and total free sugars, while table 7 gives individual free sugars.

For varieties F, S and H the starch content was found to be 34.4, 58.9 and 44.1 mg/g respectively, corresponding to 33.8, 44.5 and 47.1% of total available carbohydrates. This shows that free sugars made up a higher proportion of total available carbohydrates and any changes brought about by irradiation would most likely affect these.

The composition of the polysaccharide group including starch was confirmed qualitatively by the iodine test and quantitatively by the LUFF-SCHOORL method. Any further assessment would depend on any changes reflected in the free sugar fraction after the almond had been processed.

Among the free sugars, eleven individual constituents were identified. These were fructose, galactose, glucose, sorbitol, inositol, sucrose, maltose, cellobiose, mellibiose, raffinose and stachyose (table 7). The brief composition of each of these free sugars is given in Fig 7.

FIG 7

Sucrose = glucose β (1-2) fructose

Maltose = glucose α (1-4) glucose

Cellobiose = glucose β (1-4) glucose

Mellibiose = galactose (1-6) α glucose

Raffinose = galactose (1-6) α glucose β(1-2) fructose

Stachyose = galactose (1-6) α galactose (1-6) α glucose β (1-2) fructose

The presence of the rare sugar alcohol, sorbitol, in plants of the genus Rosaceae was confirmed by STRAIN (1937).

Total free sugar contents for varieties F, S and H were 67.2, 73.5 and 49.5 mg g^{-1} respectively (table 6), representing 6.7, 7.4 and 5.0% of the dry weight of almonds. It was surprising to note that the sugar profile found in this study was different from that of almond samples of CALIXTO et al (1981). They carried out quantitative analyses of sugars on the crude extracts by colorimetric, volumetric and gravimetric methods and did not obtain any reducing sugars, as only sucrose was identified definitely in their almond samples. One explanation for this could have been that the analytical techniques they used were not as sensitive as the GLC procedure used in the present study. On the other hand, SEQUEIRA and LEW (1970) did identify fructose, glucose, sorbitol and inositol, while VIDAL-VALVERDE et al (1979) reported the presence of only sucrose, raffinose and stachyose, but not glucose and fructose. Again, these differences may have been due to the techniques and varieties used. However, it is the first time to the author's knowledge that the following sugars: galactose, maltose, cellobiose and mellibiose have been reported as being present in almonds. This shows the high sensitivity of GLC in identifying sugars.

However, the percentage of free sugars in the samples of CALIXTO et al (1981) was reported to be 5.5% which was comparable to the 5 to

7.4% found in the almonds used in the present study.

Sucrose was the major, individual free sugar in all three varieties of almonds, making up 40 - 44% of total free sugars. Other sugars found in large amounts were: glucose (11-13% of free sugars), maltose (7-12%), and raffinose (8-11%).

Effect of irradiation on starch and free sugars

The content of starch after irradiation was found to be 20.0, 45.5 and 29.5 mg g^{-1} in varieties F, S and H respectively (table 6). This corresponds to a retention of 58.8, 77.2 and 66.9% respectively when compared to controls.

These values indicate a significant loss in starch and dextrins in all three varieties of almonds. Although changes in starch were significant, these losses were not reflected in total available carbohydrate content, implying that there must have been increases in the free sugar fraction

Values found after irradiation for total free sugars were 78.5, 82.8 and 59.8 mg g^{-1} for F, S and H respectively (table 6). Retention values were calculated as 116.8, 112.6 and 120.8% and these correspond to significant increases in total free sugars. This supports the fact that irradiating almonds had led to a decrease in starch content with a corresponding increase in free sugars, without any net loss in total available carbohydrates.

This was not altogether unexpected. Polysaccharides such as cellulose and starch are depolymerized by irradiation as is sucrose. This is supported

by the results of LAWTON et al (1951) who found that wood cellulose irradiated at 6.5 to 7.5 Mrad had a greatly increased level of water soluble solids and reducing sugars. Furthermore, it is possible to degrade cellulose to digestible sugars by irradiation of up to 100 Mrad (WOLFROM et al, 1954).

A further point to note was that the percentage increase in free sugars in the three varieties ranged form 12.7% in variety S to 21% in variety H. To obtain a clearer picture of the direct effect of irradiation, a close analysis of the results with regards to individual sugar constituents was made (table 7).

The first main feature was that individual sugars followed similar trends in all three varieties, hence any interpretation on one variety may be generalised and would similarly be applicable to any other sample.

Total monosaccharide content increased significantly as did each individual monosaccharide (table 7). Conversely, the oligosaccharides decreased, except for maltose, which increased in all three varieties. The fact that glucose and maltose increased suggests that possibly starch hydrolysis had occurred. It was therefore assumed that the hydrolysis of polysaccharides led to an increase in maltose and glucose, thereby supporting the previous observation that starch was originally present in almond kernels. Similar results have been reported by DESCHREIDER (1960) who observed an increase in the reducing power of wheat flour after irradiation.

The maltose values of irradiated wheat are increased by irradiation with dosages higher than 0.5 Mrad (LINKO and MILNER, 1960). This is

attributed to depolymerization of polysaccharides due to increased susceptibility of starch to enzymes.

Irradiation of polysaccharides that leads to depolymerization and a reduction in viscosity is of interest to the food industry, as a result of technological changes occurring. It is thought, however, that the final break-down of starch by irradiation is not similar to acid hydrolysis of starch. In the former, splitting may occur within the pyranose structure of the glucose molecule (KOROTCHENKO, et al, 1973; KERTESZ et al, 1959), while in the latter starch is simply hydrolysed with the eventual production of glucose molecule. The ultimate products of starch breakdown by irradiation include glucose, maltose, maltotriose, gluconic acid and other lower molecular weight organic acids (PHILLIPS and LOFROTH, 1966; KOROTCHENKO et al, 1973; BERGER and SAINT-LEBE, 1973).

Irradiation produces random breakage in the α (1-4) bond rather than a systematic rupture beginning with the non-reducing ends of the anhydro-glucose chains (BOURNE et al., 1956). Moreover, increases in reducing sugars are detectable at doses above 0.1 Mrad (KERTESZ et al, 1959).

The increase in sorbitol in the present study supports the suggestion that irradiation has resulted in the formation of free H radicals which have then reduced glucose and fructose molecules to form sorbitol, possibly according to the following:

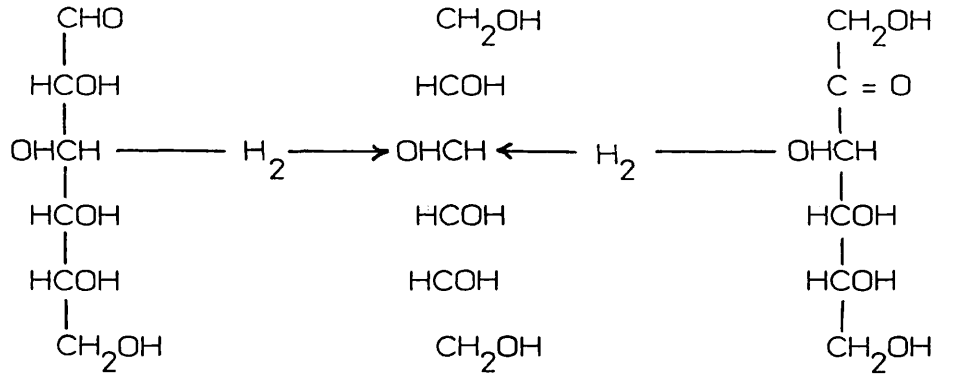
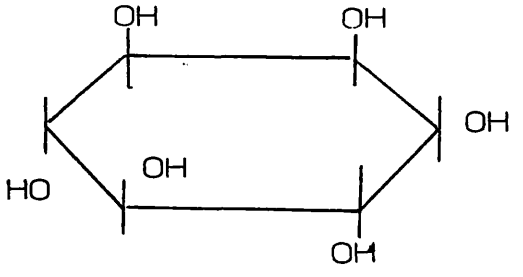


FIG. 8: CONVERSION OF GLUCOSE AND FRUCTOSE TO SORBITOL BY REDUCTION

(SHALLENBERGER and BIRCH, 1975)

Inositol, often referred to as a sugar derivative, is widely distributed in plant material and is biologically important in lipid synthesis. Inositol is actually the hexahydroxy derivative of cyclohexane and while it is not the direct product of hexose reduction, it is usually regarded as a hexose derivative in view of the similarity in structure. The most abundant stereoisomer of inositol is myo-inositol (BERK, 1976).



MYO-INOSITOL

Since other sugars cannot be converted to myo-inositol, it appears that the observed increase in this constituent is due to the breakdown of lipids rather than that of carbohydrates. Therefore, this will be further discussed in the lipid chapter (p. 268)

The net increase in fructose, galactose and glucose is comparable to losses observed in sucrose, raffinose and stachyose, assuming that part of the glucose is produced from maltose. From these results, it may be suggested that the breakdown products of sucrose, raffinose and stachyose include fructose, galactose and glucose (Fig 7).

The presence of higher saccharides of the raffinose series was not confirmed in the present study.

In general, it was found that irradiation treatment had no effect on total available carbohydrates. However, the oligosaccharides, sucrose, raffinose and stachyose underwent substantial hydrolyses to yield their component monosaccharides, such as glucose, fructose and galactose, which were all observed to increase. Certain reactions may have occurred leading to the conversion of glucose and fructose to sorbitol while the inositol may have been released from lipid hydrolysis.

EFFECT OF STORAGE ON CARBOHYDRATES OF IRRADIATED ALMONDS

Total available carbohydrates:

Immediately after irradiating the almond samples, they were stored in plastic bags at a temperature of 22°C. In order to determine the effect of storage on carbohydrates of irradiated almonds, comparisons were made between results taken immediately after processing and those after one year storage. Results for total available carbohydrates, free sugars and starch are shown in table 8.

Total available carbohydrates of varieties F, S and H after storage were 101.3, 124.9 and 85.9 mg g^{-1} respectively. These results correspond to retention values of 102.8, 97.3 and 96.2% which were not significantly different from those of samples analysed immediately after irradiation. It can therefore be assumed that storing irradiated almonds for one year did not affect the total available carbohydrates.

The same, however, cannot be said for the carbohydrates of control almonds which were stored for the same period at 22°C (table 9). Retention in the latter for F, S and H was found to be 87.2, 84.7 and 83.7% respectively. This suggests that storage of untreated almonds for one year brings about a significant loss in carbohydrates. Therefore, storing irradiated almonds for one year at 22°C offers an advantage over non-irradiated ones stored under the same conditions in terms of maintaining higher levels of total available carbohydrates.

Table 8: The effect of one year storage on total available carbohydrates, starch and total free sugars of irradiated almond nuts.

		Free sugars (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg/g ± SE					
F	I	78.5 ± 3.3	20.0 ± 2.1	98.5 ± 4.9	103.6	100.0	102.8
	I-S	81.3 ± 4.2	20.0 ± 1.2	101.3 ± 5.3			
S	I	82.8 ± 3.6	45.5 ± 3.7	128.3 ± 6.9	103.0	86.8	97.3
	I-S	85.4 ± 4.2	39.5 ± 2.3	124.9 ± 7.5			
H	I	59.8 ± 5.3	29.5 ± 2.0	89.3 ± 4.5	102.0	84.4	96.2
	I-S	61.0 ± 3.9	24.9 ± 2.7	85.9 ± 4.7			

Table 9: The effect of one year storage on total available carbohydrates, starch and total free sugars of control almond nuts.

		Free sugars (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg/g ± SE					
F	C	67.2 ± 4.5	34.4 ± 2.2	101.6 ± 6.2	86.9	87.8	87.2
	C-S	58.4 ± 3.2	30.2 ± 2.3	88.6 ± 5.6			
S	C	73.5 ± 4.5	58.9 ± 4.6	132.4 ± 8.5	83.3	86.4	84.7
	C-S	61.2 ± 5.1	50.9 ± 4.8	112.1 ± 9.1			
H	C	49.5 ± 3.9	44.1 ± 3.9	93.6 ± 4.3	82.2	85.3	83.7
	C-S	40.7 ± 3.2	37.6 ± 2.8	78.3 ± 5.5			

Starch and free sugars

From the results in table 8, it is obvious that starch and free sugars of irradiated almonds do not change significantly after one year storage.

Although total available carbohydrates, starch and total free sugars of irradiated-stored almonds were not affected, there were changes among the consituents of the free sugar fractions. A more detailed picture of these changes may be observed from table 10. As the three varieties of irradiated-stored almonds showed similar trends, the results may therefore be generalised in this case.

The first point to emerge from table 10 is the significant increase in fructose, galactose and glucose. The question then arose as to the source of this increase. From Fig. 7 it can be assumed that the hydrolysis of sucrose, raffinose and stachyose would lead to an increase in fructose, galactose and glucose, which was indeed found to be the case. Sucrose together with both raffinose and stachyose of almonds decreased significantly, the cumulative decrease ranging from 7.6 mg g^{-1} in H to 8.2 mg g^{-1} in F, while monosaccharide increases ranged from 6.5 mg g^{-1} in H to 8 mg g^{-1} in F.

Sugars of the raffinose family cannot be digested very well by the human tract, therefore, their breakdown during storage is beneficial in terms of increasing digestibility of carbohydrates in almonds.

The second point was that the cellobiose content increased significantly. This implied that cellulose degradation continued during storage.

Table 10: The effect of one year storage on individual free sugars of irradiated almond nuts.

Free sugars	F		S		H	
	I	I-S	I	I-S	I	I-S
	mg/g dry wt.					
Fructose	7.2	9.1	8.3	10.2	7.4	9.7
Galactose	4.2	6.3	4.9	6.9	3.5	5.5
Glucose	14.1	18.1	13.8	17.2	9.9	12.1
Sorbitol	4.5	5.5	4.5	4.9	2.7	3.2
Inositol	6.2	7.5	6.9	6.5	5.2	5.5
Sucrose	21.4	16.2	25.0	20.0	15.8	10.8
Maltose	11.6	10.9	9.1	10.1	8.1	7.9
Cellobiose	3.1	4.0	2.0	4.1	2.6	3.7
Mellibiose	0.6	1.1	1.4	1.6	1.1	1.7
Raffinose	4.7	2.6	6.0	3.9	3.2	0.9
Stachyose	0.9	0.0	0.9	0.0	0.3	0.0

When control-stored almonds were compared to controls, (table 9), the starch content was not significantly changed while free sugars decreased significantly: their retention values ranging from 82.2 to 86.9.

On closer observation, it was found that individual free sugars of control-stored almonds (table 11) followed a different pattern to those observed in irradiated-stored almonds.

A significant decrease in sucrose, raffinose and stachyose should have led in theory to an increase in monosaccharides. This was not the case as fructose, galactose and glucose actually decreased in amounts. A possible explanation for this may be that the loss in these monosaccharides may have been due to the occurrence of the Maillard reaction (p. 100).

The Maillard reaction therefore seems to be prominent in control-stored almonds, while this was not the case for the irradiated-stored almonds.

In summary, it appears that the irradiation treatment somehow provides a means by which either the Maillard reaction is inhibited or else the monosaccharides are protected to some extent from browning. The absence of this protection was observed in controls, where there was a decrease in monosaccharides.

Table 11: The effect of one year storage on individual free sugars of control almond nuts.

Free sugars	F		S		H	
	C	C-S	C	C-S	C	C-S
	mg/g dry wt.					
Fructose	4.8	3.5	5.7	4.3	4.2	3.2
Galactose	2.7	2.1	3.6	1.8	2.1	1.3
Glucose	8.7	6.8	8.1	5.8	5.7	3.6
Sorbitol	1.8	2.4	2.1	2.8	0.9	1.6
Inositol	4.5	5.7	5.1	6.5	3.6	4.7
Sucrose	26.7	21.3	32.4	26.2	21.6	16.4
Maltose	7.8	8.7	5.4	6.3	4.5	5.2
Cellobiose	1.2	1.4	1.2	1.5	1.2	1.3
Mellibiose	0.3	T	0.3	T	0.3	T
Raffinose	6.9	5.1	8.1	5.4	4.2	3.1
Stachyose	1.8	1.4	1.5	0.6	1.2	0.3

CHAPTER IV

EFFECT OF SALTING-ROASTING, DUAL TREATMENT AND STORAGE ON CARBOHYDRATES

INTRODUCTION

Compared with labile nutrients such as vitamins, carbohydrates are generally regarded as being less vulnerable to heat processing (BENDER, 1978). Heat processing leads to hydrolysis of polysaccharides producing simple sugars. The rate of reaction depends upon time and temperature of processing. SALEM (1975) has found that during the baking of broad beans (120°C for 15 mins) starch content decreased, being first converted into dextrins then into reducing sugars.

The effect of heat on mono- and oligo- saccharides is various. These effects include caramelization, pyrolysis and interactions with other food components.

When reaction is caused by heat in the absence of nitrogenous compounds, they are described as "Caramelization" reactions. When they occur in the presence of nitrogenous compounds, they are called 'Browning' or 'Maillard' reactions (HODGE, 1953).

Caramel was originally made by burning sugar and its formation was said to result from loss of water from the sugar molecule to give 'anhydro sugar', perhaps followed by further unknown reactions to give brown-coloured degradation and polymerisation products (GREENWOOD and MUNRO, 1979).

Pyrolysis implies severe heating of sugar molecule, which again results in brown colouration; carbon-carbon linkages probably being broken (HOUMINER, 1973).

Many foods are subjected to the so-called "non-enzymic" browning reaction, either during processing or upon storage of the finished products. The reactions are accompanied by flavour development, texture alteration and the initiation of yellow, red-brown and black colouration. Food nutritive value is invariably altered. Colour and flavour in many food is generated by inducing browning reactions. On the other hand in processed food (dehydrated or concentrated) naturally browning reactions occur, when colour and flavour of the product is altered. Such reactions may also occur during the storage of food even at relatively low temperatures.

The French chemist Maillard was the first to study the condensation of sugars with amino acids. He reported in 1912 that when a mixture of amino acids and sugars was heated, brown substances were formed. Since then, Maillard reactions have been considered as the major cause of non-enzymic browning in food. The course of non-enzymic browning as a consequence of the Maillard reaction may be as in Fig. 9 (BERK, 1976).

(a) Sugar reacts with the primary and secondary amines to form glycosylamines. The reaction is reversible and the role of water is important, for at low moisture content there is substantial formation of these compounds. Therefore, non-enzymic browning is believed to be a significant pathway for browning in dried and concentrated foods. Similar condensation reactions occur with free amino groups of peptides and proteins.

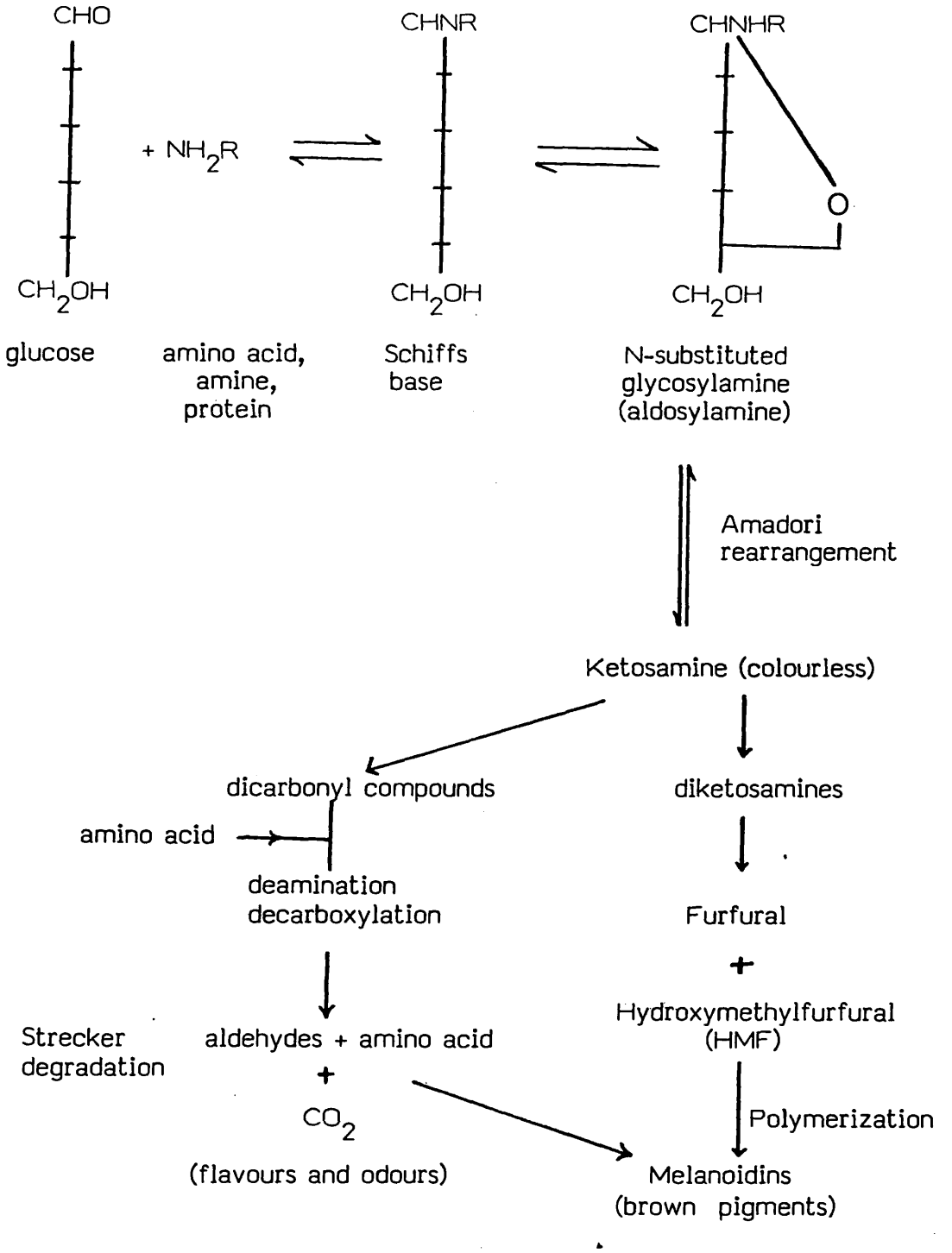


FIG 9: GENERAL SCHEME OF NON-ENZYMIC (MAILLARD) REACTIONS

(b) Amino acids involved in the reaction serve the dual purpose of amino source and acid catalyst and ketose-amines are formed immediately. When aldose reacts with amino compounds the reaction mixture is soon found to contain ketose-derivatives. Conversely aldose derivatives are formed when the starting material is a ketose. This isomerization is known as the Amadori rearrangement. It is reversible and the products are still colourless.

(c) Condensation of the ketose-amines with an additional molecule of aldose gives a diketose-amine product. As the reaction between amino acids and sugars progresses, dark products known as 'Melanoidins' are gradually formed.

(d) In the presence of certain dicarbonylic compounds, amino acids are known to undergo decarboxylation. This reaction known as Strecker degradation, results in the simultaneous deamination and decarboxylation of certain amino acids to form aldehydes.

(e) Polymerization into brown pigments.

Maillard reactions and Strecker degradation have usually been considered the most important flavour producing reactions in such heat processes (BERK, 1976).

The purpose of this part of the study was to investigate the effect of salting-roasting and the subsequent storage of samples on carbohydrates of almond nuts.

Throughout the study, unless otherwise mentioned, roasting will always refer to salting followed by roasting.

RESULTS AND DISCUSSION

Total available carbohydrates:

Total available carbohydrate content of roasted almonds in F, S and H is 82.6, 108.4 and 71.9 mg g^{-1} respectively (table 12). These values correspond to a retention of 81.3, 81.9 and 76.8% with respect to controls. These losses range from 19.0 mg g^{-1} to 24 mg g^{-1} and were shown to be significant. It is concluded that by roasting almonds a significant loss in total available carbohydrates was observed.

To determine the fraction responsible for this overall loss, both starch and free sugars were investigated in detail.

Starch and free sugars:

It may be seen from table 12 that the main effect of heat was on the free sugar fraction. In fact, significant losses in free sugars ranged from 12.2 mg g^{-1}(24.6%) in H to 16.4 mg g^{-1}(22.3%) in S.

Starch content in all three varieties of almonds also decreased significantly. These decreases ranged from 6.3 mg g^{-1} in variety F to 9.5 mg g^{-1} in H, corresponding to 18.3 and 21.5% respectively.

These losses were higher than those found by SALEM (1975) who showed a decrease of 1.5% in free sugars of broad beans. He also mentioned that baking broad beans resulted in a 2.5% loss in starch content. He went on to state that the action of heat in the presence of water caused a decrease in starch content due to its hydrolysis to dextrins, then to reducing sugars.

Table 12: The effect of heat on the total available carbohydrates, starch and the free sugars of almond nuts.

		Free sugar (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg g^{-1} ± SE					
F	C	67.2 ± 4.5	34.4 ± 2.2	101.6 ± 6.2	81.1	81.7	81.3
	R	54.5 ± 4.1	28.1 ± 2.5	82.6 ± 5.5			
S	C	73.5 ± 4.5	58.9 ± 4.6	132.4 ± 8.5	77.7	87.1	81.9
	R	57.1 ± 5.1	51.3 ± 2.7	108.4 ± 9.2			
H	C	49.5 ± 3.9	44.1 ± 3.9	93.6 ± 4.3	75.3	78.5	76.8
	R	37.3 ± 3.3	34.6 ± 2.2	71.9 ± 4.9			

Some loss of free sugars may have been inevitable, in that during four hours of soaking prior to roasting a certain amount of sugar may well have been extracted in the water. This would be due to the interaction between the protein molecule of the seed coat and the salt in the soaking solution. A more detailed explanation is given in chapter 6 (effect of salting, roasting and storage on protein).

The softening of the seed-coat (as a result of bonds within the protein constituents being weakened) could have thus allowed the leaching of water-soluble constituents from the nuts. Alternatively, water could have been imbibed by the almonds as observed by the increase in moisture content from 6% to 16% after soaking, thereby providing a suitable environment for a number of reactions, either enzymic or non-enzymic, to take place.

During roasting, the temperature of the nuts was adequate to bring about the hydrolysis of the starch to dextrins and dextrins to free sugars. BENDER (1978) has stated that in roasting or drying of food material, once the latter dried, its temperature will then rapidly rise to that of the heating surface. It might be at this stage that starches are hydrolysed to produce free sugars and at the same time some of the free sugars may react with proteins and amino acids to produce brown pigments through the Maillard reactions.

A closer examination of individual free sugars obtained after salting and roasting (table 13) shows the following:

(1) All the free sugars are seen to decrease significantly

Table 13: The effect of salting and roasting on individual free sugars of almond nuts.

Free sugars	F		S		H	
	C	R	C	R	C	R
	mg/g dry wt.					
Fructose	4.8	3.5	5.7	3.9	4.2	2.9
Galactose	2.7	1.8	3.6	2.0	2.1	1.1
Glucose	8.7	5.9	8.1	5.2	5.7	2.6
Sorbitol	1.8	1.7	2.1	1.8	0.9	0.3
Inositol	4.5	4.3	5.1	5.5	3.6	3.7
Sucrose	26.7	20.8	32.4	24.6	21.6	15.7
Maltose	7.8	9.9	5.4	7.5	4.5	7.7
Cellobiose	1.2	0.8	1.2	0.9	1.2	0.5
Mellibiose	0.3	T	0.3	T	0.3	T
Raffinose	6.9	5.1	8.1	5.0	4.2	2.4
Stachyose	1.8	0.7	1.5	0.7	1.2	0.4

except for maltose which actually increased. The increase in maltose reflects starch and dextrin hydrolysis.

(2) Oligosaccharides in general are most affected in that their total weight decreased by an average of 9.7 mg g^{-1} while the monosaccharides decreased by 5.6 mg g^{-1}.

A proposed pathway for the sequence of events which may have occurred is as follows:

As previously mentioned, soaking in salt solution may have weakened the cell membranes and increased enzymic or non-enzymic activity within the cells. The presence of water would have provided a medium for hydrolysis reactions to occur. In the present study it appears that in addition to starch, free sugars were also hydrolysed. At this stage an increase in monosaccharides would have been expected. The results (table13) show a net decrease in monosaccharides. Therefore at the same time part of free sugar fraction would have been released into the soaking water, and on subsequent heating, some of the free sugars would then be available to be used up by combining with proteins and amino acids (Maillard reaction) to produce brown pigments. At the same time, decreases in the amino acid contents were observed during salting and roasting and this will be discussed later in chapter 6.

To account for the substantial loss in free sugars, two reasons have been suggested. Firstly, by leaching into the soak water and secondly, via the Maillard reactions. The fact that the latter may have occurred in

almonds containing about 4% moisture after roasting is in direct contrast to reports (LEA and HANNON, 1949; WILLIAMS, 1976) that the Maillard reactions in foods are inhibited at very low moisture content. However, it is possible that these reactions may have occurred during the initial stages of heating (just after soaking) when moisture levels were as high as 17% in the nuts. Subsequently, as the moisture level fell with time, the reaction may have been inhibited.

Different sugars are considered to react at different rates in the Maillard reaction. BERK (1976) has stated that, in general, aldoses are more reactive than ketoses, monosaccharides are more reactive than disaccharides; therefore, it would be expected that if Maillard reaction had occurred, then glucose would have been used up to ten times (LEWIS and LEA, 1959) more rapidly than fructose. SHALLENBERGER and BIRCH (1975) have also mentioned that galactose is even more reactive than glucose, but this was found not to be the case in the present study.

Oligosaccharides are generally inert in terms of reacting with protein, therefore constituents such as sucrose, raffinose and stachyose probably undergo hydrolysis into their constituent monomers prior to entering the Maillard reaction.

In the present study, not a great deal of variation between the losses of the above sugars were noted. However, it should be mentioned that the differences in reactivity found by SHALLENBERGER and BIRCH (1975) and LEWIS AND LEW (1959) were found in model systems, hence it is most likely that in a complex system such as food, a different situation exists.

EFFECT OF STORAGE ON CARBOHYDRATES OF ROASTED ALMONDS

Total available carbohydrates:

After salting and roasting the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on carbohydrates of salted and roasted almonds, comparisons were made between results taken immediately after processing and those after one year storage.

Table 14 shows the total available carbohydrate content of F, S and H after one year storage, which were 82.9, 109.1 and 76.2 mg g^{-1} respectively. These results were found not to be significant when compared to unstored roasted samples.

The total available carbohydrates remaining in roasted almonds after one year storage were not significantly different from those of control-stored almonds (table 9). However, there were no actual storage losses for roasted samples as compared to control-stored ones. It therefore appears that roasted almonds have been stabilized to some degree by the heat treatment.

Starch and Free sugars

The amount of starch in F, S and H were found not to have changed significantly after storage (table 14). This suggests that there was no further breakdown of starch during subsequent storage after the heat treatment. The initial breakdown of starch in roasted almonds may have been due to enzymic action but these enzymes were probably destroyed

Table 14: The effect of one year storage on total available carbohydrates, starch and total free sugars of salted-roasted almond nuts.

		Free sugar (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg/g ± SE					
F	R	54.5 ± 4.1	28.1 ± 2.5	82.6 ± 5.5	98.3	104.2	100.4
	R-S	53.6 ± 3.8	29.3 ± 1.2	82.9 ± 4.6			
S	R	57.1 ± 5.1	51.3 ± 2.7	108.4 ± 9.2	104.0	96.9	100.6
	R-S	59.4 ± 3.1	49.7 ± 3.8	109.1 ± 6.7			
H	R	37.3 ± 3.3	34.6 ± 2.2	71.9 ± 4.9	99.5	113.0	105.9
	R-S	37.1 ± 2.5	39.1 ± 3.6	76.2 ± 3.1			

by the heat treatment. This could explain why starch was retained so well after storage.

From table 14 it was also observed that total free sugars were not significantly changed after storage. A more detailed account of individual free sugars is given in table 15. It was apparent that all varieties of almonds behaved similarly in terms of changes in individual sugars, therefore it was possible to generalise the results.

Only a few component sugars increased noticeably, for instance: sorbitol, inositol and cellobiose. An increase in sorbitol implied that environmental conditions produced in the almond cell during storage were such as to promote its formation from glucose and fructose. For this reaction to occur free hydrogen radicals must be present in the cells. These can be the by-products formed during lipid autoxidation. Lipid breakdown would also provide an explanation for the increase in free inositol, the latter being a hydrolytic product of phosphatidyl-inositol.

Increases in cellobiose reflected a substantial cellulose dègradation during storage. The results in this study have included both α—and β—glucose under the general heading of glucose, but GLC profiles show that the β-glucose peaks increased significantly. This evidently supports cellulose breakdown, the latter being a polymer of β-glucose.

The absence of a build-up of cellobiose in control samples during storage suggests that cellulose breakdown in roasted stored almonds was a result of the initial heat treatment.

Table 15: The effect of one year storage on individual free sugars of salted-roasted almond nuts.

Free sugars	F		S		H	
	R	R-S	R	R-S	R	R-S
			mg/g dry wt.			
Fructose	3.5	3.0	3.9	3.5	2.9	2.4
Galactose	1.8	1.6	2.0	1.8	1.1	1.0
Glucose	5.9	5.1	5.2	4.9	2.6	2.2
Sorbitol	1.7	2.6	1.8	2.7	0.3	1.2
Inositol	4.3	5.6	5.5	7.1	3.7	4.8
Sucrose	20.8	20.3	24.6	23.9	15.7	15.1
Maltose	9.9	8.6	7.5	7.9	7.7	6.7
Cellobiose	0.8	1.8	0.9	1.9	0.5	1.4
Mellibiose	T	T	T	T	T	T
Raffinose	5.1	4.5	5.0	4.8	2.4	2.1
Stachyose	0.7	0.5	0.7	0.9	0.4	0.2

There was no evidence for the occurrence of further browning reactions. It may be assumed that some degree of stabilization, as found in the case of starch, was given to the sugars, thereby limiting or inhibiting the Maillard reaction. The low moisture content of roasted almonds may itself have acted as the stabilizing factor.

EFFECT OF COMBINED PROCESSING ON CARBOHYDRATES

Total available carbohydrates:

The total available carbohydrate content of F, S and H after the combined treatment of roasting and irradiation are given in table 16: 79.3, 104.9 and 68.2 mg g^{-1} respectively. These results signified losses of 22.3 mg g^{-1} for F to 27.5 mg g^{-1} for S or 20.8% to 27.1% of the total available carbohydrates.

These results are very similar to those found previously for roasted samples, where loss of total available carbohydrates ranged from 19.0 mg g^{-1} to 24 mg g^{-1}. This implies that in the combined treatment of roasting and irradiation, it is the heat treatment that appears to have the more pronounced effect in decreasing carbohydrates in almonds. This supports previous observations that irradiation does not significantly affect total available carbohydrates.

Starch and free sugars:

Starch and free sugars decrease in all three varieties of almonds following heat and irradiation treatment (table 16). The decrease in starch ranged from 8.3 mg g^{-1} in F to 13.2 mg g^{-1} in H, while that of free sugars ranged from 14.0 in F to 17.9 in S. These trends followed those found previously for roasted almonds where the decrease in starch was 6.3 in variety F to 9.5 mg g^{-1} in variety H, and the decrease in free sugars was from 12.2 mg g^{-1} in variety H to 16.4 mg g^{-1} in variety S. Once more it is assumed that the changes brought about in the carbo-

Table 16: The effect of roasting and irradiation on total available carbohydrates, starch and total free sugars of almond nuts.

		Free sugar (FS)	Starch (ST)	TACHO	% Retention FS	ST	TACHO
		mg/g^{-1} ± SE					
F	C	67.2 ± 4.5	34.4 ± 2.2	101.6 ± 6.2	79.2	75.9	78.0
	RI	53.2 ± 3.7	26.1 ± 2.4	79.3 ± 5.3			
S	C	73.5 ± 4.5	58.9 ± 4.6	132.4 ± 8.5	75.6	83.7	79.2
	RI	55.6 ± 3.3	49.3 ± 3.1	104.9 ± 7.7			
H	C	49.5 ± 3.9	44.1 ± 3.9	93.6 ± 4.3	75.3	70.1	72.9
	RI	37.3 ± 4.2	30.9 ± 2.2	68.2 ± 4.8			

-hydrates of roasted-irradiated treated almonds have resulted from the roasting with very little contribution made by the irradiation treatment. Further support for this assumption is offered by the results of table 17, showing changes in individual free sugars after the combined treatment. It may be observed that while maltose increased (indicating starch breakdown) all other individual sugars decreased significantly, except for inositol which also increased.

As suggested earlier, it is proposed that the loss of free sugars resulted from their extraction into the soaking salt solution prior to heat treatment, and the occurence of the Maillard reaction. The evidence points to the fact that the combined treatment affects carbohydrates in a very similar way to that of roasting, and at the same time reducing or masking any changes brought about by irradiation alone.

Table 17: The effect of roasting and irradiation on individual free sugars of almond nuts.

Free sugars	F		S		H	
	C	RI	C	RI	C	RI
	mg/g dry wt.					
Fructose	4.8	3.2	5.7	4.5	4.2	2.7
Galactose	2.7	1.4	3.6	2.5	2.1	1.4
Glucose	8.7	5.9	8.1	5.9	5.7	2.9
Sorbitol	1.8	0.8	2.1	1.5	0.9	0.5
Inositol	4.5	5.9	5.1	6.4	3.6	4.6
Sucrose	26.7	19.9	32.4	23.1	21.6	15.6
Maltose	7.8	9.7	5.4	6.8	4.5	5.9
Cellobiose	1.2	0.8	1.2	0.6	1.2	0.7
Mellibiose	0.3	T	0.3	T	0.3	T
Raffinose	6.9	4.9	8.1	3.9	4.2	2.5
Stachyose	1.8	0.7	1.5	0.4	1.2	0.5

EFFECT OF STORAGE ON CARBOHYDRATES OF DUAL TREATED ALMONDS

Total available carbohydrates:

After salting, roasting and irradiating the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on carbohydrates of dual treated almonds, comparisons were made between results taken immediately after processing and those after one year storage.

The total available carbohydrate content of F, S and H were found to be 83.2, 107.6 and 73.4 mg g^{-1} in roasted-irradiated-stored almonds (table 18) These results were not significantly different from those obtained before storage.

An interesting point is that total available carbohydrates remaining in roasted-irradiated-stored almonds were significantly lower than those found in irradiated-stored samples (table 8) but not significantly different from those of roasted-stored almonds (table 14). It follows that during storage, stabilisation of carbohydrate in roasted-irradiated and roasted-almonds may have taken place.

The carbohydrate content of roasted-irradiated-stored almonds does not differ significantly from that of control-stored samples, which, however, is significantly lower than that of non-stored control samples. At the same time, there is no significant difference when the carbohydrate content of RI-S samples are compared with RI. In this case, storage has no effect on the carbohydrate content after the dual treatment.

Table 18: The effect of one year storage on total available carbohydrates, starch and total free sugars of roasted-irradiated almond nuts.

		Free sugar (FS)	Starch (ST)	TACHO	% Retention FS	% Retention ST	% Retention TACHO
		mg g^{-1} ± SE					
F	RI	53.2 ± 3.7	26.1 ± 2.4	79.3 ± 5.3	101.1	112.6	104.9
	RI-S	53.8 ± 3.3	29.4 ± 2.7	83.2 ± 6.5			
S	RI	55.6 ± 3.3	49.3 ± 3.1	104.9 ± 7.7	105.0	99.8	102.6
	RI-S	58.4 ± 4.3	49.2 ± 2.9	107.6 ± 6.2			
H	RI	37.3 ± 4.2	30.9 ± 2.2	68.2 ± 4.8	106.7	108.7	107.6
	RI-S	39.8 ± 2.2	33.6 ± 2.4	73.4 ± 4.5			

In order to gain a clearer insight into the changes occurring during the storage of almonds, it was thought worthwhile considering further the starch and free sugar fractions of roasted-irradiated-stored almonds.

Starch and free sugars:

Following the combined processing, there was no further hydrolysis of starch in F, S and H for roasted irradiated samples after one year storage (table 18). This observation was very similar to that made for the starch content of roasted-stored and irradiated-stored almonds.

There was no change in the total free sugar content of F, H and S for roasted-irradiated stored samples (table 18). Again, this was similar to that found for roasted-stored almonds and different from that of irradiated-stored almonds.

A detailed examination of table 19 shows that only two notable changes were observed in the content of individual free sugars in roasted-irradiated stored almonds. These were that inositol and cellobiose content were both found to increase. These increases suggested the breakdown of larger molecules, i.e. inositol from phosphatidyl inositol (among lipids) and cellobiose (among the unavailable carbohydrates).

The breakdown of cellulose was to be expected, especially as the same occurred for both roasted-stored and irradiated-stored almonds. Moreover, the change in cellobiose on a percentage basis was similar for both roasted-irradiated and for roasted only after storage. Both of these were, however, higher than those for irradiated-stored almonds. This was most likely due to the effect of heat.

Table 19: The effect of one year storage on individual free sugars of roasted-irradiated almond nuts.

Free sugars	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	mg/g dry wt.					
Fructose	3.2	3.1	4.5	4.3	2.7	2.2
Galactose	1.4	1.5	2.5	2.2	1.4	1.2
Glucose	5.9	6.1	5.9	5.5	2.9	3.0
Sorbitol	0.8	0.9	1.5	1.2	0.5	0.6
Inositol	5.9	7.3	6.4	8.1	4.6	6.2
Sucrose	19.9	20.1	23.1	23.9	15.6	16.9
Maltose	9.7	8.2	6.8	6.7	5.9	5.1
Cellobiose	0.8	1.8	0.6	1.5	0.7	1.6
Mellibiose	T	T	T	T	T	T
Raffinose	4.9	4.5	3.9	4.5	2.5	2.7
Stachyose	0.7	0.3	0.4	0.5	0.5	0.3

Another interesting point to emerge was that the sorbitol content remained unchanged. However, sorbitol had been observed to increase in roasted-stored almonds. Therefore, at the cellular level, conditions within these cells (roasted-irradiated) were probably such as not to allow reactions involving the reduction of glucose to sorbitol to occur.

In contrast, hydrolyses of oligosaccharides were observed in irradiated-stored almonds, so that some degree of hydrolysing of oligosaccharides would be expected in roasted-irradiated-stored almonds. This was not the case, thus suggesting that the heat processing part of the combined treatment may have aided in stabilising oligosaccharides, so that no further hydrolysis occurred during storage.

In general, it appears that the dual treatment of roasting and irradiation has aided in preserving free sugar and starch content in almonds during storage.

SUMMARY

Control

In this summary, the three different almond varieties will not be discussed individually, but a general account is presented for the purpose of brevity and clarity.

Total available carbohydrate content of almonds was found to range from 93.6 to 132.4 mg g^{-1}. This was higher than that cited in the literature. A possible reason for this was that while other authors had failed to report the presence of starch in almonds, in the present study, starch was found to be present, therefore increasing total available carbohydrate content on a quantitative basis.

Starch content was found to range from 34.4 to 58.9 mg g^{-1}, while total free sugar content ranged from 49.5 to 73.5 mg g^{-1}. Among the free sugars, eleven individual sugars were identified. These were fructose, glucose, galactose, sorbitol, inositol, sucrose, maltose, cellobiose, mellibiose, raffinose and stachyose.

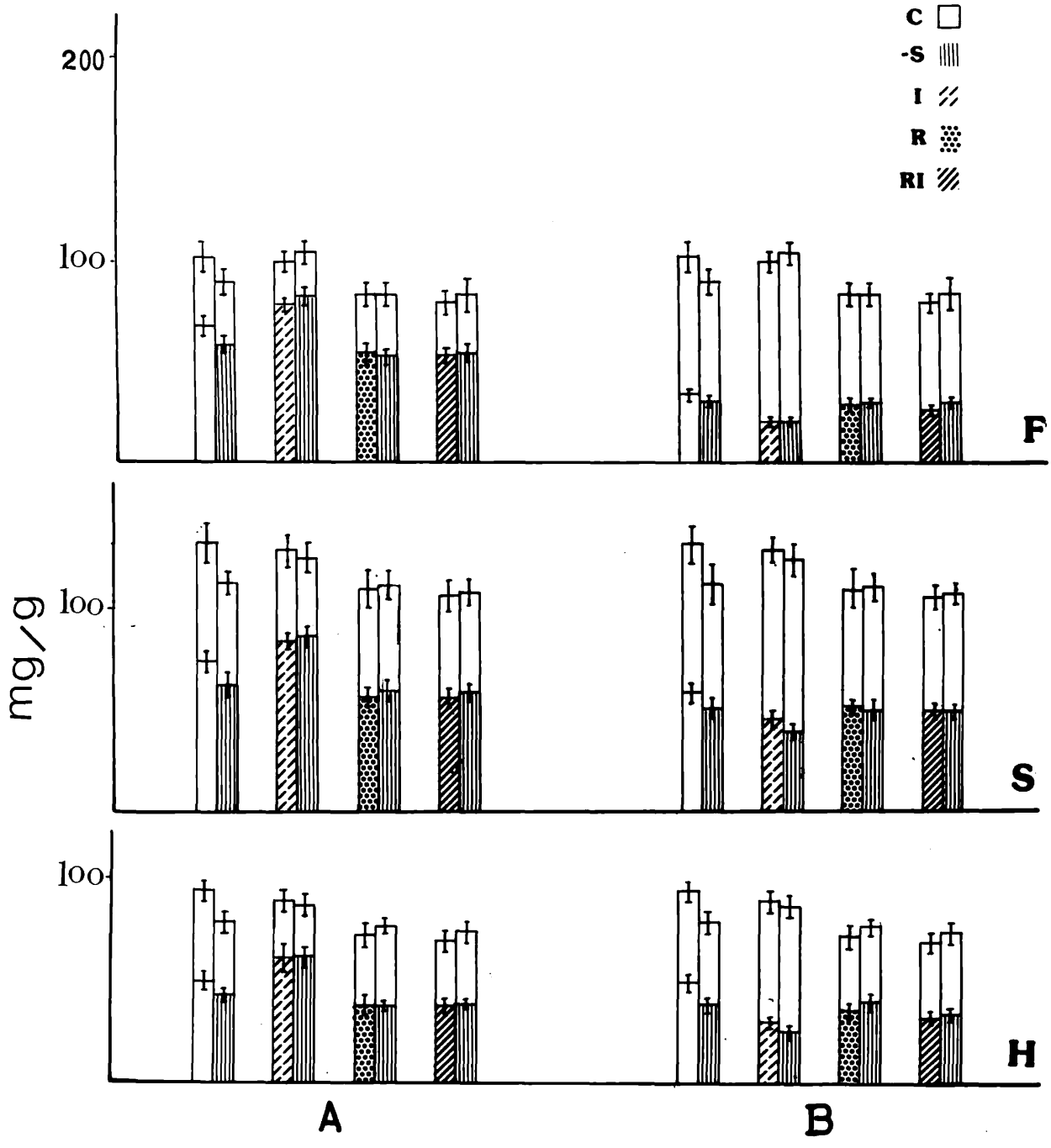
Irradiation

Although 2 Mrad irradiation did not significantly alter total available carbohydrate, starch was found to decrease (ranging from 13.4 to 14.6 mg g^{-1}), while free sugar content increased (ranging from 9.0 to 11.0 mg g^{-1}) (table 6).

It has been suggested that breakdown of starch to maltose was caused by the depolymerization action of irradiation. This was probably also the

Fig.10: Effect of different processing and storage on the free sugars, starch and total available carbohydrates of three varieties of almonds are represented graphically in this figure.

In A, the top part of each column represents starch (mg/g) and the bottom part free sugars (mg/g ± SEM), while in B the top part represents free sugars (mg/g) and the bottom part starch (mg/g ± SEM).


C
-S
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RI
200
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100
mg/g
F
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B

cause for cellulose breakdown as inferred from increases in cellobiose content.

Breakdown of sucrose, raffinose and stachyose resulted in the accumulation of the monosaccharides glucose, fructose and galactose.

A build up in sorbitol was caused by the chemical reduction of glucose or fructose and implied the presence of free hydrogen radicals in the cells (p. 89). These radicals were probably produced as a direct effect of irradiation.

One year storage did not bring about any changes in total available carbohydrates, starch and total free sugars (table 8). However, there were significant changes in the individual free sugar fractions (table 10). Increases in fructose, galactose and glucose were accompanied by decreases in sucrose, in raffinose and in stachyose. Oligosaccharides of the raffinose family cannot be digested very well by the human tract, therefore their breakdown during storage is beneficial nutritionally in terms of increasing the digestibility of carbohydrates in almonds.

Once again, there was evidence of cellulose breakdown as reflected by a further increase in cellobiose during storage. There was no direct evidence for the occurence of non-enzymic browning reactions.

After one year storage, a significant decrease in total available carbohydrate content (12.8 - 16.0 %) of untreated almond (C-S) was found (table 9). As there was no significant change in starch, the decrease in total available carbohydrate was solely due to the significant decreases observed in free sugars (13.0 - 18%).

Examination of individual free sugars (table 11) showed that there were decreases in sucrose, raffinose and stachyose which would imply, in theory, an increase in monosaccharides. This was not the case and in fact glucose, fructose and galactose decreased in amount. This would suggest that the monosaccharides, on being liberated, were immediately directed to further reaction pathways, thereby making them unavailable for analysis. These reactions may have been those of the Maillard type.

In conclusion, it can be said that one year storage of untreated almonds brings about 12.8 to 16.0% decrease in total available carbohydrate (table 9).

Roasting

Losses of between 19 and 24 mg g^{-1} in total available carbohydrate content were recorded as a result of salting and the heat treatment of almonds (table 12).

Decreases were noted in starch content, ranging from 6.3 to 9.5 mg g^{-1}, and total free sugars from 12.0 to 16.0 mg g^{-1}. Among the free sugars (table 13) only maltose was found to increase while all others decreased. This was evidence for starch breakdown, but there was no direct indication of cellulose breakdown here.

The proposed pathway for the changes occurring during heat treatment was as follows. During the soaking of the almonds in salt solution, part of the free sugar fraction may have been leached into the soak water. In addition, the influx of water into the almonds during this period provided suitable conditions for the activation of enzymes and occurrence of

chemical reactions. Subsequently heating led to the hydrolysis of starch, first, to dextrin and maltose and finally to glucose. Decrease in monosaccharides and the conditions prevailing at the time further suggest the occurrence of browning reactions.

After one year storage, there was no further starch hydrolysis, nor any changes in total free sugar content (table 14).

Among the free sugars, the only notable increases were in sorbitol, inositol and cellobiose (table 15).

The absence of an increase in cellobiose in controls during storage suggested the initial heat treatment during roasting must have been responsible for the subsequent cellulose breakdown during the storage of roasted almonds.

There was no evidence of further browning reactions. Hence it was assumed that the low moisture content of roasted almonds may have helped to prevent these reactions from taking place.

Dual Treatment: roasting-irradiation

Losses were noted in total available carbohydrate content ranging from 22.0 to 27.5 mg g^{-1} in roasted-irradiated almonds (table 16). This was due to the net result from the decrease in starch and free sugar contents ranging from 8.0 to 13.0 mg g^{-1} amd 12.0 to 17.9 mg g^{-1} respectively. These changes were very similar to those reported for roasted almonds.

Similarly, maltose was observed to increase (table 17), supporting the fact that starch breakdown had occurred. All other sugars decrease, hence

suggesting that part of the latter was lost to the soak water and part via the browning reactions.

After one year storage, total available carbohydrate content remained unchanged. Amounts ranging from 73.4 to 107.6 mg g^{-1} (table 18) were similar to those found for roasted-stored almonds (76.2 to 109.1 mg g^{-1}), but substantially lower than those found in irradiated-stored almonds (85.9 to 124.9 mg g^{-1}).

Both starch and total free sugar content were found to be unchanged.

Some degree of hydrolysis of oligosaccharides would be expected in roasted-irradiated-stored almonds, since this was observed in irradiated-stored almonds. This was, however, not the case (table 19), thus suggesting that the heat processing part of the combined treatment may have aided in stabilising oligosaccharides so that no further hydrolysis occurred during storage.

CHAPTER V

EFFECT OF IRRADIATION AND STORAGE ON PROTEINS

INTRODUCTION

Chemical changes resulting from the irradiation of protein in food have been the subject of considerable studies. In fact there are a number of comprehensive reviews on this subject by GARRISON (1972), LORENZ (1975), and URBAIN (1977). The principal reasons for this have been:

(i) the need to prevent the development of undesirable flavour and odours in products caused possibly by high dose treatment, such as radiation sterilization (Radappertization).

(ii) the need to have information on the functional properties of protein food.

Due to the complexity of proteins, the radiation chemistry of these substances is not fully understood. Nevertheless, there is value in increasing the understanding of this subject and assessing the effects on the organoleptic properties of irradiated protein foods.

Since proteins are large, asymmetric structures with folded peptide chains, held together by hydrogen bonds and containing a number of polar and non-polar side-chains, it is obvious that such labile molecules might be easily disturbed in structure or destroyed by ionizing radiation (BARRON and FINVELSTEIN, 1952). It has been shown that radiation can cause denaturation where rupture of hydrogen bonds occurs with subsequent unfolding of the molecule. However, hydrogen bonds are not only easily broken, but

also easily formed. Hence in order to detect a noticeable change in the protein structure, sufficient numbers of bonds would need to be destroyed simultaneously. Other changes in protein structure may include the modification of side chains or the formation of new functional groups. It is also possible that breakage of the polypeptide chain, due to splitting of peptide bond, can lead to fragmentation of the original molecule. The formation of inter-and intra-molecular cross-links may lead to polymerisation (CARROL et al ,1952) while degradation may bring about specific or non-specific amino acid damage (PATTEN and GORDY, 1964).

This effect on specific amino acids may be explained by the presence of many loci for interaction within the protein molecule, termed "sensitive sites." Photons of energy may be absorbed from a radiation source by large protein molecule, and this energy is transferred along to one of these 'sensitive sites' causing bond breakage accompanied by the formation of free radicals (GARRISON, 1972).

The full effect of the irradiation treatment would to a certain extent depend on the presence of water in the food and the radiation dose to which the food is subjected (URBAIN, 1977). The presence of water may affect it in two ways:

(i) ionizing radiation breaks water into a number of fragments (most of which are highly reactive chemically), and

(ii) water provides a good medium for movement of non-aqueous components and in this way permits easy interactions.

If oxygen is present in the system, free electrons ($\bar{e}$) and hydrogen ions (H^o) are both removed by fast reactions, which result in the formation of the perhydroxy radicals, $\bar{O}$ and $H\dot{O}_2$ The perhydroxy radical itself is either an oxidising or reducing agent, yielding either H_2O_2 or O_2. As a consequence of the action of O_2 to remove $\bar{e}$ and H^o from the system, this may affect the reactions of some amino acids in solution depending upon the presence or absence of O_2.

Some of the reactions of protein caused by irradiation are shown below:

1. Oxidation Deamination:

(i) $RCH(NH_2)COOH + OH^o \longrightarrow RC^o(HN_2)COOH + H_2O$

(amino acid) (amino acid free radical)

(ii) $RC^o(NH_2)COOH + O_2 + H_2O \longrightarrow NH_3 + RCOCOOH + H^oO_2$

(amino acid free radical) (keto-acid)

(iii) $H^oO_2 + H^o \longrightarrow H_2O_2$

(peroxide)

$H^oO_2 + OH \longrightarrow H_2O + O_2$

2. Oxidation and Degradation of cysteine:

The -SH group of this acid is a focal point of attack, especially in oxygenated solution. The main result is oxidation to cystine (URBAIN, 1977):

$R\text{-}SH + O^oH \longrightarrow R^oS + H_2O$

$R\text{-}SH + H \longrightarrow R^oS + H_2$

$R\text{-}SH + H_2O \longrightarrow R^oS + H_2O_2$

$$2R\text{-}SH + H_2O_2 \longrightarrow 2R^{\circ}S + 2H_2O$$

$$RS^{\circ} + R^{\circ}S \longrightarrow RSSR$$

In addition there are secondary reactions involving products of primary reactions and water radicals.

Finally, there may be reactions between radicals derived from the amino acid with water radicals. A competitive situation among the reactants may exist.

Unless one is dealing with a processed protein food, such as gelatin, a natural protein food contains non-protein components, lipids and carbohydrates. Irradiation may form radiolytic products from these non-protein components which may then react with either the protein fraction or with radiolytic products formed from it. Peroxidised lipids (p.323) may result from irradiation and have shown to attack cytochrome c (DESAI and TAPPEL,1963).

Since a primary purpose of food processing is preservation for later consumption, the effect of length and condition of storage is also an important consideration in the nutrient content of foods as they are consumed (KRAMER, 1974).

Known changes in colour, odours and flavour of food during storage suggest possible alteration in other properties which might involve protein. JONES and GERSDORFF (1938) have pointed out factors responsible for causing loss in the nutrient value of cereals and soybeans upon storage. They mentioned that the changes occurring during storage varied depending upon the amount of fat in the product, the storage temperature and the

nature of the storage container. A definite possibility of nutritive impairment was suggested under all these conditions by the abovenamed authors.

The nutritive value of any specific protein in a diet depends on its essential amino acid composition and their relative proportions. To be highly nutritious in itself, a protein must contain all the essential amino acids, and these amino acids must be present in amounts closely approximating the need for the various amino acids.

In the evaluation of the protein in a food, the chemical score for the determination of essential amino acids is often used as a criterion. It is also well known that chemical score correlates well with biological value as determined by several methods (RICE and BEUK, 1953).

In the present study chemical score was used as a guide to assess the losses in protein quality in three varieties of almond kernels after irradiation at 2 Mrad and storage at 22°C for one year.

MATERIAL AND METHODS

Estimation of Protein

Various methods for estimating protein were used, but the KJELDAHL method was chosen due to its consistency of results, despite the fact that it is more time consuming.

Dye-binding methods are based on the ability of anionic dyes to combine with protein groups of opposite ionic charge. The reduction in optical density is proportional to the protein present. The dye-binding technique actually measures the phenol containing amino acid tyrosine present in all protein. An assumption is then made for its proportion in relation to other amino acids present in the proteins.

The results were found to be unsatisfactory due to inconsistency of values obtained. Under certain conditions some amino acids may have been destroyed, hence this method would overestimate total protein. Conversely, some tyrosine may be destroyed and underestimated protein content would have been obtained.

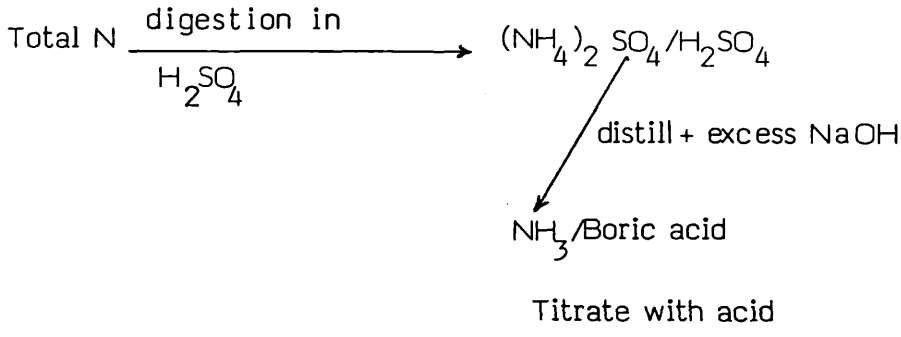
However, HART and FISHER (1971) have recommended the KJELDAHL methods for nuts and nut products.

Principle:

The product is digested with concentrated sulphuric acid using copper sulphate as a catalyst, to convert organic nitrogen to ammonium ions. Potassium sulphate is included in the digestion mixture to raise the boiling

point of the acid. Alkali is added and the liberated ammonia distilled into an excess of boric acid solution. The distillate is titrated with hydrochloric acid to determine the ammonia absorbed in the boric acid (OSBORNE and VOOGT, 1978).

The KJELDAHL method involves the following steps:



The KJELDAHL procedure does not, however, determine all forms of nitrogen e.g., nitrates and nitrites, unless they are suitably modified.

The KJELDAHL method estimates the 'crude' protein or total nitrogenous matter. This is calculated by multiplying total nitrogen (N) by an empirical factor and the result is invariably reported as 'protein.' Such factors have been calculated by considering the basic components of a large number of samples of the same food (PEARSON, 1973).

$$\text{Factor} = \frac{\text{Mean of total nitrogenous matter by difference}}{\text{Mean of total nitrogen by KJELDAHL}}$$

HART and FISHER (1971) have used a value of 5.18 for almonds.

Reagents:

1. Sulphuric acid (concentrated)
2. H_2SO_4 (0.1 N standardised).
3. Boric acid solution, 40gr boric acid (H_3BO_3) was dissolved in

distilled water and diluted to 1 litre.

4. Sodium hydroxide solution, 500g of sodium hydroxide (NaOH) was dissolved in 1 litre of distilled water.
5. Copper sulphate pentahydrate ($CuSO_4.5H_2O$)
6. Potassium sulphate (anhydrous K_2SO_4)
7. Mixed indicator solution, 2 g of methyl red and 1g of methylene blue were dissolved in 1 litre ethanol (96% V/V); colour change occuring at pH 5.4. (This was stored in a dark and cool place.)
8. Glass boiling beads.

Digestion

Most of the fat from the prepared sample was removed by extraction with petroleum ether (HART and FISHER, 1971). A suitable quantity (an amount of sample containing 0.03 - 0.04g of nitrogen) of the defatted material was weighed out on a filter paper. The filter paper and its content were rolled and dropped directly into the bottom of the flask. A similar filter paper was included in the blank. Fifteen grams of K_2SO_4 and 0.5 g of $CuSO_4$ and a few glass beads were placed in the KJELDAHL flask to which 25ml H_2SO_4 were carefully added. Gentle swirling of the liquid ensured sufficient mixing. The flask was placed on an electrically heated hot-plate at an angle of approximately 40° from the vertical. Heat was applied gently at first, but when the initial frothing had subsided, it was increased gradually until the liquid boiled at a moderate rate. The flask was shaken and swirled from time to time in order to wash down any charred material adhering to the flask, but caution was taken so as to avoid any spillage. The heat was continued for 1 hour, after the liquid became clear. The flask was cooled to 40°C and 50 ml of water was cautiously

added. After mixing, the flask was allowed to cool further.

Distillation and titration

The contentsof the KJELDAHL flask was diluted with not more than 200ml of fresh tap water and transferred to a one litre distillation flask. The mixture was washed with several small volumes of tap water until the total volume was about 400ml. To the 500ml receiving flask 50ml of boric acid solution and a few drops of the indicator solution (7) were added. One large piece of granulated zinc was added to the distillation flask and the apparatus was connected to the delivery tube dipping below the boric acid solution. After fifteen minutes, 75ml of 50% sodium hydroxide solution was added through the top funnel, so as to form two layers. Alkalinity of the liquid was shown by the liquid turning from light to dark blue owing to the effect on the copper catalyst. The alkaline liquid was boiled in the flask, and distilled over to about 300ml. The outlet was removed from the liquid just prior to terminating distillation. The outlet was rinsed with distilled water.

The cold distillate was titrated with O.I N H_2SO_4 and this volume was noted. A blank test was conducted at the same time, following this procedure exactly, except that no sample was added here.

Calculation

Weight (g) of the test protein = W

Volume (ml) of H_2SO_4 solution required for the blank test = V_1

Volume (ml) of H_2SO_4 solution required for the test protein = V_2

1 ml of O.IN H_2SO_4 = 0.0014 g (N)

Correction factor = 5.18

Non-protein nitrogen = NPN

then, the total nitrogen % = $\frac{(V_2 - V_1) \times 0.0014}{W}$

Protein nitrogen = Total nitrogen - NPN

True protein % = protein nitrogen X 5.18

Estimation of non-protein nitrogen (NPN)

The KJELDAHL method does not determine true protein in food, as the protein content is estimated from the 'N' content. However, part of this N is derived from non-protein sources, e.g. free amino acids, amides and nucleic acids. In order to calculate the true protein content in almonds, the amount of NPN needs to be determined.

Studies by BHATTY and FINLAYSON (1973) have shown that NPN extracted from soybean and oilseed meals by the use of 80% ethanol was poor. SINGH and JAMBUNATHAN (1981) working on chickpeas, tested NPN extraction using different concentrations (1-20%) of trichloro-acetic acid (TCA), and concluded that the value obtained by direct extraction using 10% TCA represented the NPN of the sample. Therefore, this method was used.

Procedures:

Five hundred mg of ground and dried (50°C overnight) almonds were dispersed in 15ml of 10% TCA and shaken in a centrifuge bottle by a mechanical shaker for 1 hour at room temperature. The insoluble material was sedimented by centrifuging at 20,000 rpm for 15 minutes. The residue was washed with another 10 ml of the solvent (30 minutes shaking) and again centrifuged to separate the insoluble material. The supernatants were

combined to give a volume of 25ml.

The nitrogen content of this extract was determined by the KJELDAHL method already described (p. 134). Instead of adding the ground kernels, the 25ml supernatant (TCA) was directly mixed with the H_2SO_4. NPN was calculated using the same formula used previously.

Identification and determination of amino acids

Free amino acids and total amino acids were extracted with slight modifications of the method of NAGUIB (1964).

Defatted almond kernels were dried in an oven at 50°C overnight, and ground to a fine powder. To 100mg of this powder, 5ml of 2% phenol and 10 ml of 30% TCA were added. The mixture was left overnight and then filtered through a Whatman No.1 filter paper. The pH of the resulting solution which contained free amino acids was adjusted to 2 ± 0.1 using N-NaOH.

Protein hydrolysis

During hydrolysis, the peptide linkages of the protein are split to give a mixture of amino acids. These mixtures of amino acids will accurately reflect the composition of the original protein. Precise amino acid analysis of the hydrolyzate will then allow the composition of the original protein to be readily obtained. A reaction which occurs during hydrolysis is the addition of water to the peptide link and the side-chain amide group; but in practice this is seldom the case, and a variety of other reactions may take place during the hydrolytic treatment. Consequently, the amino acid analysis will be influenced to a greater or lesser extent by side reactions. The hydrolytic procedure is of importance in obtaining an accurate knowledge of the protein composition. Three kinds of hydrolytic agents may be used: acids, bases and enzymes. Each has advantages in particular cases. In the present study only acid and alkaline hydrolysis were carried out.

The most usually employed acidic reagent is HCl. A number of factors

might cause the amino acid composition of the acid hydrolyzate to differ from that of the protein from which it is derived. The principal ones are artefact formation, incomplete liberation of amino acids, racemization of amino acids and destruction of the amino acids during hydrolysis.

Removal of HCl from the hydrolyzate is important. Hydrochloric acid is rapidly removed by using a rotary evaporator at a slightly elevated temperature under reduced pressure. Formation of artefacts can be minimized, but not always avoided completely by rapid evaporation on a rotary evaporator. The esters are probably formed during the final stage of drying when the amino acids are present in a highly concentrated, syrupy solution. Ester formation during sample preparation can therefore lead to unidentified peaks in such analyses. This is especially true when large amounts of carbohydrate or lipid materials are present.

Amino acids differ in the ease with which they are liberated from peptide linkage during acid hydrolysis. Different hydrolytic times are required in order to hydrolyse the protein bonds involving each amino acid (BLACKBURN, 1968). Some of the amino acids are most readily hydrolysed and it is to be expected that these amino acids will be liberated completely after hydrolysis for twenty hours.

However, amino acids are progressively destroyed under the conditions of acid hydrolysis giving rise to ammonia. There is, however, no general agreement as to the rate at which the amino acids are destroyed. Twenty-four hour hydrolysis is most commonly used.

Acid hydrolysis using HCl shows good results, but it is known that cystine, cysteine, methionine and tryptophan are labile under these condi-

-tions (OSBORNE and VOOGT, (1978). These amino acids are more stable under alkaline conditions (BLACKBURN, (1968). In order to obtain a complete picture of the total amino acids, both acid and alkaline hydrolysis of proteins must be carried out (MAURON, 1973).

Procedure

Defatted almond kernels were dried in an oven at 50°C overnight, and ground to a fine powder.

Acid Hydrolysis

To 10mg of the dried powder, 10ml of 6NHCl was added and the mixture hydrolysed in a boiling water bath for 24 hours. The hydrolyzate was filtered on Whatman No. 1 filter paper to remove humin. The solution was reduced to a small volume by rotary evaporator, and the residue was dissolved in 0.5NHCl and made up to 10ml in this acid, 1ml of the solution was taken for chromatography after bringing the pH to 2 ± 0.1 with 5N-NaOH (Fig. 12).

Alkaline Hydrolysis

Alkaline hydrolysis of protein is of limited use (BLACKBURN, 1968). It is now usually employed only in determining amino acids that are labile to acid, in particular, tryptophan. To 10mg of the dried powder, 10ml of 5N-NaOH was added. This was hydrolysed in a boiling water bath for twenty hours. After filtration through a Whatman No. 1 filter paper, the pH of the solution was adjusted to 2 ± 0.1 with 4N H_2SO_4 (Fig. 13).

Estimation of individual amino acids

The sample containing the mixture of amino acids was introduced at the top of the column of an amino acid analyser filled with ion-exchange resin [Resin (LCR-2)]

Fig.11: Column chromatographic separation of amino acids standards by automatic amino acid analyser.

1.	Tryptophan	(Try)
2.	Lysine	(Lys)
3.	Histidine	(His)
4.	Ammonia	(-)
5.	Arginine	(Arg)
6.	Buffer	(-)
7.	Aspartic acid	(Asp)
8.	Threonine	(Thr)
9.	Serine	(Ser)
10.	Glutamic acid	(Glu)
11.	Proline	(Pro)
12.	Glycine	(Gly)
13.	Alanine	(Ala)
14.	Cysteine	(CysH)
15.	Valine	(Val)
16.	Methionine	(Met)
17.	Isoleucine	(Ileu)
18.	Leucine	(Leu)
19.	Tyrosine	(Tyr)
20.	Phenylalanine	(Phe)

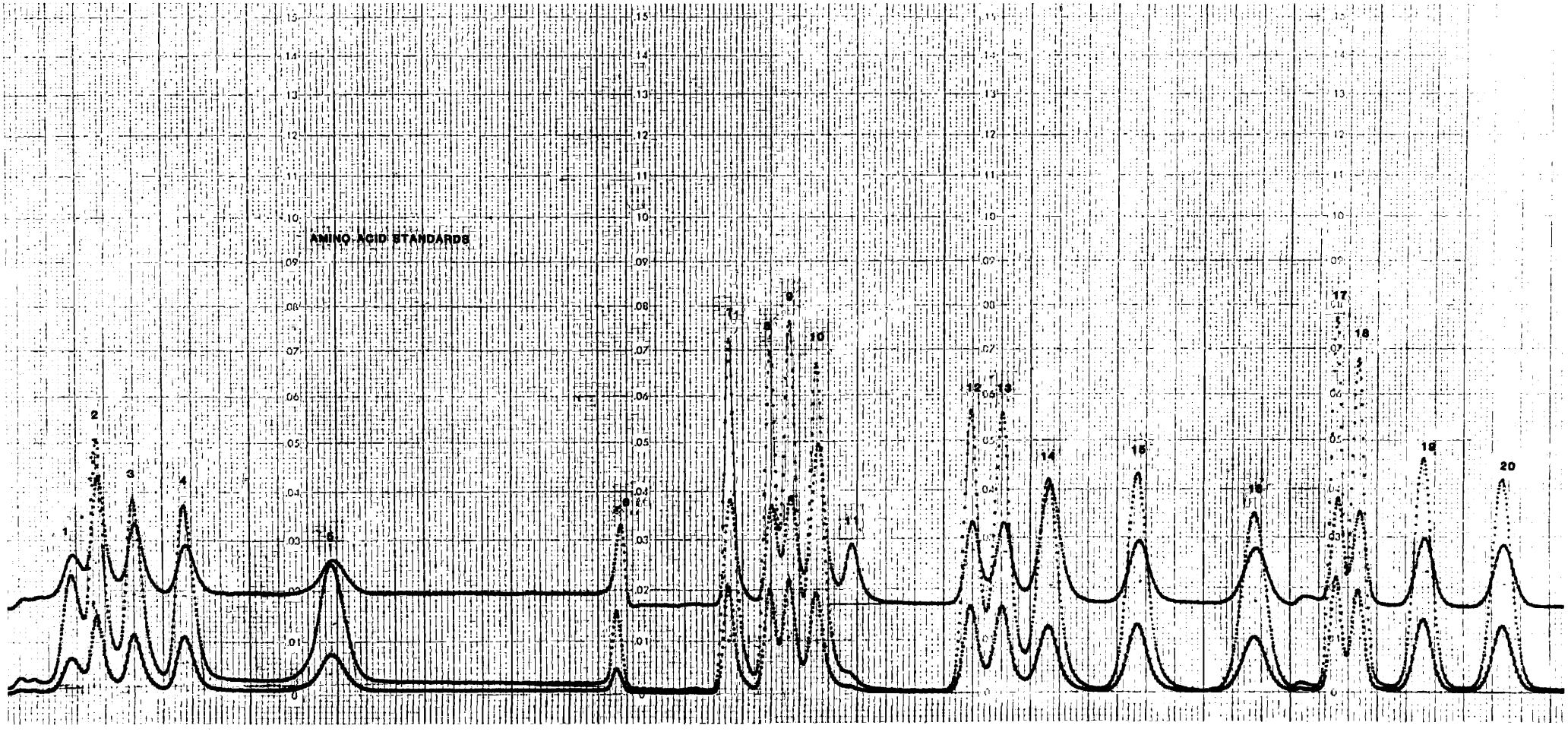

AMINO ACID STANDARDS
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Fig.12: Amino acid analysis of acid-hydrolysed.

1. Lysine
2. Histidine
3. Ammonia
4. Arginine
5. Buffer
6. Aspartic acid
7. Threonine
8. Serine
9. Glutamic acid
10. Proline
11. Glycine
12. Alanine
13. Cysteine
14. Valine
15. Methionine
16. Isoleucine
17. Leucine
18. Tyrosine
19. Phenylalanine

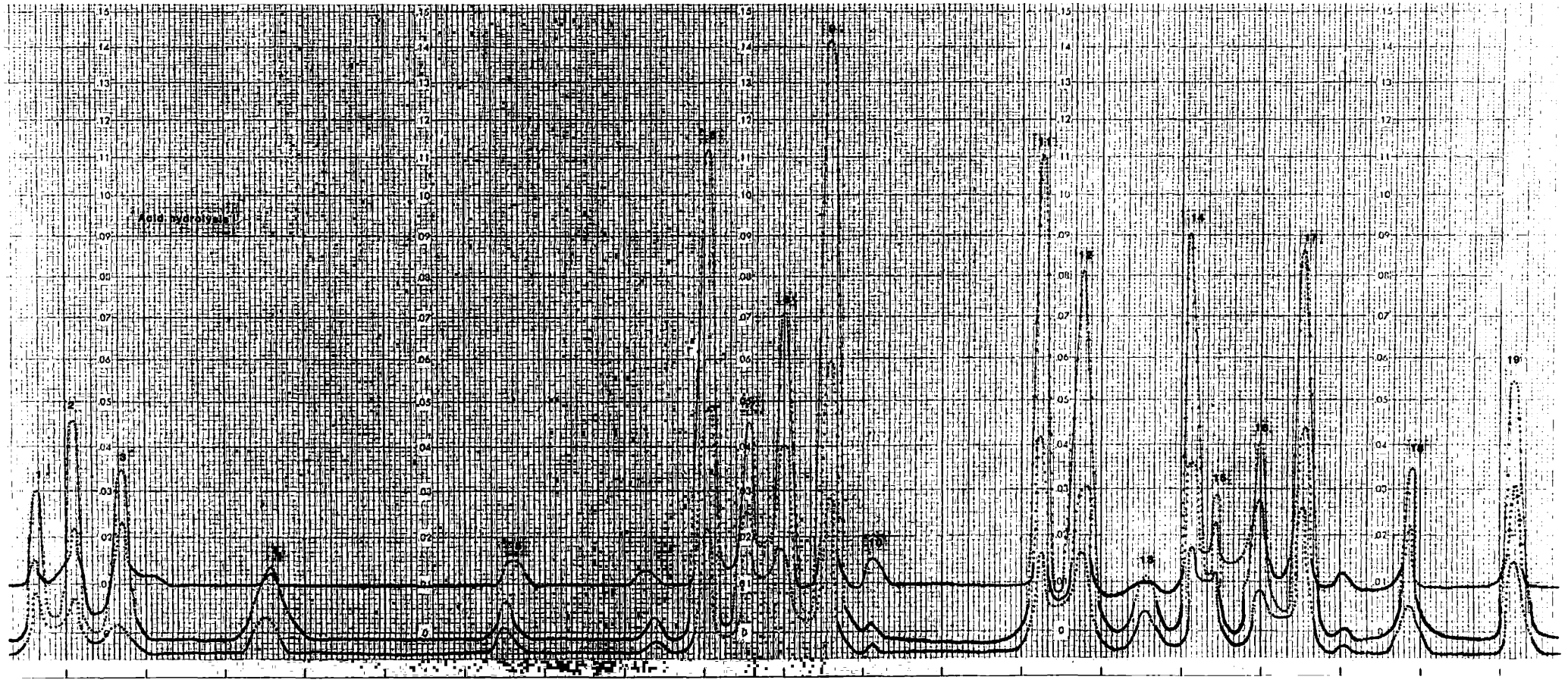
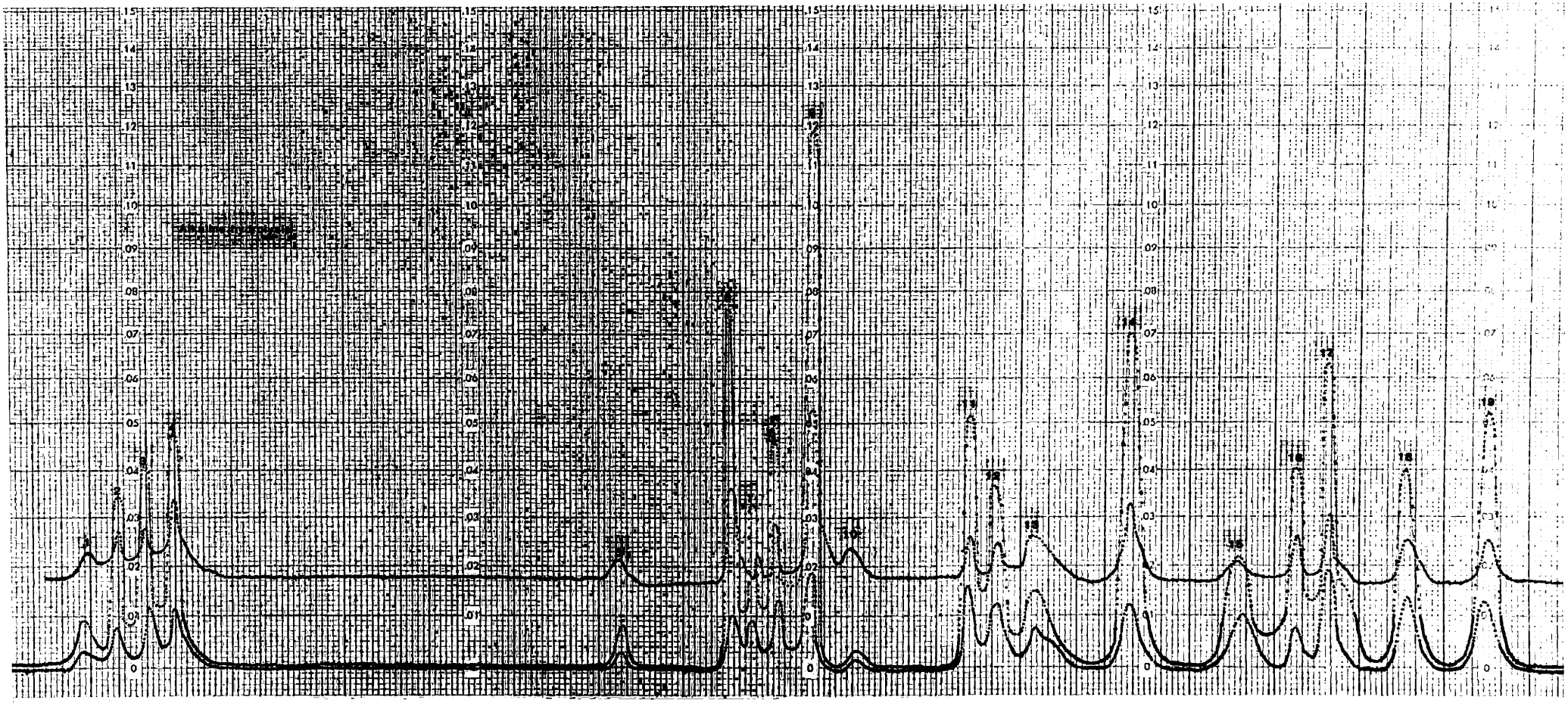

Acid hydrolysate

Fig.13: Amino acid analysis of alkaline-hydrolysed

1. Tryptophan
2. Lysine
3. Histidine
4. Ammonia
5. Buffer
6. Aspartic acid
7. Threonine
8. Serine
9. Glutamic acid
10. Proline
11. Glycine
12. Alanine
13. Cysteine
14. Valine
15. Methionine
16. Isoleucine
17. Leucine
18. Tyrosine
19. Phenylalanine



As the buffer and the sample are pumped down through the column, the resin retards the amino acids for a characteristics length of time; when the individual amino acids emerge from the bottom of the column, they are separated from each other and can be determined. As the buffer carrying the amino acids leaves the column, it is met by a stream of ninhydrin reagent from a second pump. This mixture enters a boiling water reaction bath, where the ninhydrin reacts with amino acids to form a colour. The colour density of each reaction product at 570 and 440 nm is measured as it flows through the colorimeter, and these values are plotted against time by a recorder as a curve on a moving strip chart (BLACKBURN, 1968). The peak on the recorder curves can be integrated with a precision of 100 ± 3% for loads from 0.1 to 3 μ moles for each amino acid (SPACKMAN et al, 1958). An automatic sample injector on the amino acid analyser can be loaded with four samples. A calibration standard of amino acids is always run in addition to the protein hydrolyzates. Customarily, the standard is placed in the first sample holder and the protein hydrolyzates in the other three. The standard is analysed first, when the instrument operation can be checked and malfunction can be detected and corrected before the hydrolyzates are injected. The identification and determination of the amino acids were carried out on a Jeol Model JLC 6AH fully automatic amino acid analyser. The amount of amino acid in each sample was calculated by comparison of peak areas with those obtained using a calibration mixture as described by EVELEIGH and WINTER (1970. Fig 11.

The calibration mixture of amino acids was expressed in μ moles $(g\ mixture)^{-1}$. In order to convert the values for amino acids into mg weight, a modified formula was used to include the molecular weights of

individual amino acids.

Weight of amino acid (g) in sample = μ moles amino acid x M x 10^{-6}

Where: M = Molecular weight of each individual amino acid.

Chemical score

In order to calculate the chemical score of amino acid, egg was used as the reference standard (FAO, 1970). The content of each essential amino acid in a food protein (Ax) is expressed first as a ratio of total essential amino acids (Ex) in the food $\frac{Ax}{Ex}$. These ratios are then expressed as a percentage of ratios between each amino acid in egg (Ae) and the total essential amino acids of egg (Ee) using the formula:

$$\text{Chemical score} = \frac{Ax}{Ex} \div \frac{Ae}{Ee} \times 100$$

$$= \frac{Ax}{Ex} \times \frac{Ee}{Ae} \times 100$$

(FAO, 1970)

RESULTS AND DISCUSSION

Total protein and free amino acids of almonds:

The values for total protein (N x 5.18) of almond varieties F, S and H were 174.9, 188.9 and 183.4 mg g^{-1} dry weight respectively (table 20) and they were not significantly different from each other. These results are in close agreement with those of WOODROOF (1979) who recorded a total protein value of 186 mg g^{-1} for Californian almonds, and CALIXTO et al,(1981) 205.1 mg g^{-1} for the sweet almonds of Mallorca, Spain. OSBORNE and VOOGT (1978) gave a protein value of 190 mg g^{-1} for unspecified almonds while FAO (1970) quoted a value of 168 mg g^{-1}.

The free amino acid content for F, S and H was found to be 4.6, 5.6 and 5.1 mg g^{-1} respectively. These were significantly different from each other (table 20). The total free amino acid content of the three varieties of almonds was found to be on average 2.8% of total protein.

Protein amino acids of almonds

The protein fraction of the almonds was hydrolysed and their amino acids separated, identified and estimated. The results of protein amino acids expressed as mg g^{-1} dry weight, are given in table 21 . Eighteen different amino acids were identified: tryptophan (Try), lysine (Lys), histidine (His), arginine (Arg), aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), cysteine (CysH), valine (Val), methionine (Met), isoleucine (Ileu), leucine (Leu), tyrosine (Tyr), and phenylalanine (Phe). The amino acid composition of the three varieties of almonds in the present study is similar to that reported by FAO (1970).

Table 20: The effect of 2 Mrad irradiation on the protein and free amino acids of almond nuts.

		Total protein (TP)	Free amino acids (FAA)	Ammonia	% Retention TP	% Retention FAA
		mg/g dry wt.				
F	C	174.9± 8.4	4.6±0.2	5.0±0.6	98.7	128.2
	I	172.6±9.6	5.9±0.5	12.5±1.2		
S	C	188.9±13.2	5.6±0.2	9.0±0.8	99.0	117.9
	I	187.2±12.2	6.6±0.4	11.0±1.1		
H	C	183.4±11.2	5.1±0.2	6.9±0.2	99.0	127.5
	I	181.6±11.5	6.5±0.4	10.2±1.5		

Table 21: The effect of 2 Mrad irradiation on individual protein amino acids of almond nuts.

Amino acids	F		S		H	
	C	I	C	I	C	I
	mg/g dry wt.					
Try	1.1	1.2	1.8	1.9	1.8	1.7
Lys	3.6	2.1	4.9	3.1	3.9	3.1
His	3.9	2.5	4.8	3.3	4.2	2.8
Arg	20.5	18.6	20.1	18.0	20.7	18.8
Asp	19.1	24.9	19.4	25.2	20.1	25.3
Thr	3.8	2.2	4.9	3.5	4.9	3.5
Ser	6.8	8.7	7.0	8.9	7.5	9.6
Glu	43.6	40.5	44.9	42.1	45.1	43.4
Pro	8.8	8.8	10.2	10.2	9.4	9.4
Gly	10.2	9.9	11.1	11.4	11.1	10.0
Ala	7.1	6.3	8.0	8.1	7.9	7.3
CysH	1.6	1.5	1.9	1.8	1.5	1.6
Val	10.5	10.1	10.4	11.4	10.3	10.4
Met	4.8	4.6	5.4	5.5	4.9	4.9
Ileu	7.1	6.8	8.1	7.2	6.8	6.7
Leu	12.4	12.2	13.0	13.0	12.1	12.0
Tyr	5.9	7.7	6.2	8.2	5.4	6.9
Phe	9.4	8.9	10.2	10.1	10.1	10.0

NASSAR et al, (1977) have studied eight different varieties of almonds grown in Egypt and identified eleven different amino acids in measurable amounts, with asparagine, glutamic acid and leucine being the major amounts in these cultivars while valine, arginine and methionine were found only in minor quantities. The presence of hydroxy-proline in their almond varieties was unusual for plant materials and was not found in the present study, although JOSLYN and STEPKA (1949) have confirmed the presence of hydroxyproline in plums.

In the present study, glutamic acid was found to be the most abundant protein amino acid showing on average 24% followed by arginine and aspartic acid (approximately 11% each).

Chemical score

The status of a food protein depends not only on the presence of adequate amounts of essential amino acids, but also on a specific balance between them. The latter is determined by the chemical score evaluation as assessed against the essential amino acid balance of eggs, eggs being considered as having 100% values for all essential amino acids. Table 22 shows the chemical score values for control almonds. The amino acid representing the lowest value is termed the limiting amino acid.

Varieties S and H appear to be adequate in essential amino acids apart from lysine whereas F was adequate in only valine, methionine, isoleucine, leucine and phenylalanine. Overall, it was found that lysine, with a chemical score ranging from 44-53 was the limiting amino acid for all three varieties. Moreover, tryptophan and threonine were of a lower chemical score in variety F compared to S and H. It was interesting to

Table 22: The effect of 2 Mrad irradiation on the chemical-score of essential amino acids of almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	C	I	C	I	C	I
	% compared to egg					
Tryptophan	63	76	93	103	99	98
Lysine	44	28	53	36	45	38
Threonine	63	40	72	55	78	58
Valine	130	137	115	134	123	130
Methionine	121	127	122	132	118	125
Ileucine	96	100	98	92	88	91
Leucine	119	128	113	118	112	116
Phenylalanine	139	144	135	142	143	149
Limiting amino acid (A.A.)	Lys 44	Lys 28	Lys 53	Lys 36	Lys 45	Lys 38
Total essential A.A. (T.E.A.A.)	52.7	48.1	58.7	55.7	54.8	52.3
% Retention of T.E.A.A.	91.3		94.9		95.4	

find that, although the sulphur containing amino acids were found to be low, methionine was not limiting.

EFFECT OF IRRADIATION ON PROTEIN

Total protein and free amino acids:

The protein content of F, S and H after irradiation treatment is given in table 20. The total proteins were found to be 172.6. 187.2 and 181.6 mg g^{-1} dry weight respectively for F, S and H, which were not significantly different from controls. It has thus been shown that 2 Mrad irradiation of almonds has no effect on total protein content.

Similar observations have been made on the effect of irradiation on protein in other food materials. MILNER (1961) and PAPE (1972) have both reported that irradiation of wheat with 1.5 Mrad did not affect the total protein content of wheat. Furthermore, NENE et al (1975) have found the same for red gram (Cajanus cajan) using a 3 Mrad dose of irradiation.

The free amino acid content of almonds following irradiation is shown in table 20. The values for total free amino acids increased significantly from between 4.6 to 5.6 mg g^{-1} to between 5.9 to 6.6 mg g^{-1}..Therefore it is possible that some protein hydrolysis had occurred. The increase in free amino acids was also reported by LORENZ (1975) who had irradiated cereal grains and flour with a dose of 1 Mrad and NENE et al (1975) who reported that total free amino acids of red gram was increased by about 15% with the same level of irradiation. These increases are comparable to those found in the present study, where increases in free amino acids ranged from 18% in variety S to 28% in variety F. VAKILE et al (1973) have found an increase of approximately 8% in the free amino acid content of wheat after irradiation with 1 Mrad. So, it is not surprising that the

increase is greater at double that irradiation dose.

Ammonia was found to increase substantially in all cases, suggesting that certain chemical reactions such as deamination may have been taking place.

These results reflected the quantitative value of protein as affected by irradiation, but a more detailed study of individual protein amino acid was needed in order to assess any changes in the quality of the protein.

The effect of irradiation on protein amino acids

The protein amino acid composition of irradiated almonds is given in table 21.

Irradiation treatment of F, S and H did not lead to any changes in total protein amino acid content, although minor changes were observed in certain individual amino acids. This partly confirms the finding of KAUFFMAN and HARLAN (1969), who stated that the amino acid content of beef irradiated at the 6 Mrad level resulted in no measurable changes in total amino acid content. However, PATTEN and GORDY (1964) have reported specific and non-specific amino acid damage in irradiated wheat. This was further supported by the fact that individual amino acids differed in their sensitivity to ionizing radiation, the S-containing amino acids being the most labile (JOHNSON and METTA, 1956; LEE, 1962).

It was interesting to note that in the present study cysteine and methionine were not appreciably affected, whereas losses in lysine, histidine

and threonine were clearly apparent. Furthermore, noticeable increases in serine, aspartic acid and tyrosine were recorded. The question then arose as to why were there fluctuations in individual amino acids without there being any changes in total protein amino acid content. This can be explained by the fact that often some amino acids may be converted to others through a series of intermediary compounds by the action of irradiation. Examples of these are shown below (Vide URBAIN, 1977):

Try ⟶ Asp, Gly, Ala

Lys ⟶ Asp, Glu, Gly, Ala

His ⟶ Asp, Ser, Glu, Ala

Met ⟶ Asp, Ser, Thr, Ala

Phe ⟶ Ala, Tyr

Therefore, it is possible that decrease in one amino acid may have led to an increase in another without resulting in an overall change in total amino acid content. Obviously, the above conversions may not all necessarily be possible in foods where other components such as carbohydrates and lipids would affect the end product of the reactions concerned.

In this study, the noted decreases in lysine, histidine and threonine may well have affected the increases in serine, aspartic acid and tyrosine as shown by the above conversions.

The nutritional value of the various proteins is mainly dependent on the relative amount of the essential amino acids present. Of all the amino acids supplied in the diet, eight of these are regarded as essential, therefore must be obtained directly from the food. These are tryptophan, lysine, threonine, valine, methionine, isoleucine, leucine and phenylalanine. Owing

to their sparing effect on methionine and phenylalanine respectively, cysteine and tyrosine are considered semi-essential.

Although irradiation resulted in minor changes between amino acids without any significant change in total content, the important point to be considered was whether there was any change in the quality of protein as determined by the chemical score.

Chemical score

Table 22 represents the chemical score of irradiated almonds. Losses in lysine have resulted in a lowering of its score, making lysine even more severely limiting.

In general, it may be said that an irradiation dose of 2 Mrad has no real detrimental effect on most individual protein amino acids of almonds, although there is a minor decrease in protein quality as reflected by a decrease in the chemical score for lysine.

EFFECT OF STORAGE ON THE PROTEIN OF IRRADIATED AND UNTREATED ALMONDS

Total protein and free amino acids

After irradiating the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on protein of irradiated almonds, comparisons were made between results taken immediately after irradiation and those after one year storage.

Results for total protein content of irradiated-stored almonds are shown in table 23.

Total protein content of F, S and H was found to be 173.9, 185.6 and 177.5 mg g^{-1} respectively. This indicates that no significant changes in total protein has resulted from storage of irradiated almonds.

This was supported by the fact that no significant changes in free amino acids or in ammonia (table 23) were noted in irradiated-stored almonds compared to those immediately after irradiation.

Untreated almonds (controls) were also stored at 22°C for one year (control-stored) and the results of protein content of stored almonds are given in table 24. It was found that total protein in F, S and H was 156.0, 165.5 and 160.8 mg g^{-1} dry weight respectively. Comparison of total protein of control with that of control-stored indicated that one year storage resulted in significant losses ranging from 18.9 in variety F to 23.4 mg g^{-1} in variety S. These losses on a % basis range from 10.8 to 12.4%. Further evidence for protein breakdown in control-stored samples was given from the significant increase noted both in free amino acids and

Table 23: The effect of one year storage on the protein and free amino acids of irradiated almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	Retention (%) FAA
		mg/g dry wt ± SE				
F	I	172.6 ± 9.6	5.9 ± 0.5	12.5 ± 1.2	100.7	84.7
	I-S	173.9 ± 12.2	5.0 ± 0.5	15.8 ± 2.2		
S	I	187.2 ± 12.2	6.6 ± 0.4	11.0 ± 1.1	99.0	114.0
	I-S	185.6 ± 13.9	7.5 ± 1.1	13.2 ± 1.7		
H	I	181.6 ± 11.5	6.5 ± 0.4	10.2 ± 1.5	97.7	96.9
	I-S	177.5 ± 10.3	6.3 ± 0.9	7.4 ± 1.3		

Table 24: The effect of one year storage on total protein and free amino acids of untreated almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	Retention (%) FAA
		mg/g dry wt. ± SE				
F	C	174.9 ± 8.4	4.6 ± 0.2	5.0 ± 0.6	89.2	126.0
	C-S	156.0 ± 9.8	5.8 ± 0.5	10.2 ± 0.9		
S	C	188.9 ± 13.2	5.6 ± 0.2	9.0 ± 0.8	87.6	132.1
	C-S	165.5 ± 9.4	7.4 ± 0.7	11.6 ± 1.1		
H	C	183.4 ± 11.2	5.1 ± 0.2	6.9 ± 0.2	87.7	119.6
	C-S	160.8 ± 10.6	6.1 ± 0.1	14.5 ± 0.8		

in ammonia content (table 24).

When irradiated-stored samples (table 23) are compared with control-stored, it is easy to observe that irradiated-stored samples not only prevent spoilage but also that protein content was better retained than in control-stored almonds. In fact, irradiated-stored almonds retained on average 10.2% more protein than control-stored samples.

Protein amino acids

The protein amino acids of irradiated-stored almonds are shown in table 25. Although there was no change as noted previously in the total protein content after storage, some fluctuation in the amino acid constituents were observed.

A significant increase was found in phenylalanine, while decreases occurred in arginine, serine and cysteine. The reason for these results is unknown.

In controls stored for one year, losses were observed in most individual amino acids (table 26). The amino acids most affected include lysine, histidine, threonine, arginine, serine, proline, methionine, leucine and tyrosine. Some of these losses are probably the result of the low and slow respiration of almonds due to their low moisture content, in which case some of the amino acids may have been metabolised.

Another possibility is that oxidation products of lipids (p.323) such as peroxides may have undergone reactions with protein amino acids, thereby reducing their availability.

Table 25: The effect of one year storage on individual protein amino acids of irradiated almond nuts.

Amino acids	F		S		H	
	I	I-S	I	I-S	I	I-S
	mg/g dry wt.					
Try	1.2	1.1	1.9	1.6	1.7	1.5
Lys	2.1	2.6	3.1	4.0	3.1	3.2
His	2.5	2.5	3.3	5.1	2.8	3.1
Arg	18.6	14.8	18.0	13.2	18.8	13.5
Asp	24.9	22.5	25.2	21.3	25.3	22.5
Thr	2.2	2.6	3.5	4.2	3.5	4.0
Ser	8.7	6.5	8.9	5.9	9.6	6.3
Glu	40.5	43.8	42.1	44.6	43.4	45.9
Pro	8.8	8.3	10.2	10.8	9.4	7.5
Gly	9.9	9.2	11.4	10.9	10.0	10.2
Ala	6.3	6.1	8.1	8.3	7.3	7.1
CysH	1.5	0.9	1.8	0.5	1.6	1.1
Val	10.1	10.2	11.4	11.3	10.4	10.9
Met	4.6	4.2	5.5	5.8	4.9	5.0
Ileu	6.8	7.7	7.2	6.8	6.7	6.6
Leu	12.2	11.0	13.0	12.7	12.0	11.9
Tyr	7.7	6.3	8.2	6.5	6.9	6.9
Phe	8.9	11.4	10.1	13.1	10.0	12.9

Table 26: The effect of one year storage on individual protein amino acids of untreated almond nuts.

Amino acids	F		S		H	
	C	C-S	C	C-S	C	C-S
	mg/g dry wt.					
Try	1.1	0.5	1.8	1.5	1.8	1.3
Lys	3.6	2.2	4.9	3.6	3.9	2.8
His	3.9	2.4	4.8	3.3	4.2	2.5
Arg	20.5	15.3	20.1	15.7	20.7	20.1
Asp	19.1	19.0	19.4	19.2	20.1	19.7
Thr	3.8	2.9	4.9	3.3	4.9	3.2
Ser	6.8	4.6	7.0	4.2	7.5	4.7
Glu	43.6	42.6	44.9	42.4	45.1	43.1
Pro	8.8	4.7	10.2	5.8	9.4	5.5
Gly	10.2	9.1	11.1	10.5	11.1	9.8
Ala	7.1	7.2	8.0	7.2	7.9	6.5
CysH	1.6	1.3	1.9	1.1	1.5	1.2
Val	10.5	9.3	10.4	9.3	10.3	8.9
Met	4.8	3.3	5.4	3.5	4.9	2.9
Ileu	7.1	6.2	8.1	7.6	6.8	5.9
Leu	12.4	9.6	13.0	10.2	12.1	8.8
Tyr	5.9	3.2	6.2	4.3	5.4	3.1
Phe	9.4	8.6	10.2	9.8	10.1	8.9

Indirect evidence for peroxidative damage of amino acids has been given by TAPPEL and DESAI (1963) who showed that histidine, serine, proline, arginine, methionine and cysteine were the most labile. In the present study, it was shown that of the above amino acids, control-stored samples had large losses in histidine, serine, proline and methionine. There was also a significant loss in total essential amino acid content (average of 19.4%, table 28.) It was clear that protein amino acids in general and total essential amino acid as a group were better retained in irradiated-stored samples (table 27) as compared to control-stored samples (table 28). The difference in total essential amino acids between irradiated-stored samples and control-stored samples ranged from 8.2 mg in F to 13.3 mg/ g in H (tables 27 and 28) corresponding to decreases of 16% and 24% respectively.

Chemical score

Table 27 shows the chemical scores for essential amino acids in irradiated-stored almonds. No significant changes in total essential amino acid content was noted when compared to irradiated samples before storage. In addition, it was found that although there were minor fluctuations among individual amino acids, no overall changes in chemical score resulted, hence protein quality was the same, while lysine remained the limiting amino acid yet again.

The chemical score for control-stored almonds is given in table 28. It was found that there is a decrease in the chemical score of lysine (the limiting amino acid) in varieties F and S as compared to their controls.

It was interesting to note that in variety F the score for tryptophan decreased while it was threonine which had decreased in varieties S and H.

Table 27: The effect of one year storage on the chemical score of essential amino acids of irradiated almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	I	I-S	I	I-S	I	I-S
	% compared to egg					
Tryptophan	76	66	103	81	98	81
Lysine	28	33	36	43	38	37
Threonine	40	44	55	61	58	62
Valine	137	131	134	124	130	127
Methionine	127	110	132	130	125	119
Ileucine	100	107	92	81	91	84
Leucine	128	109	118	108	116	107
Phenylalanine	144	175	142	172	149	180
Limiting amino acid (A.A.)	Lys 28	Lys 33	Lys 36	Lys 43	Lys 38	Lys 37
Total essential A.A. (T.E.A.A.)	48.1	50.8	55.7	59.5	52.3	56.0
% Retention of T.E.A.A.	106.0		107.0		107.0	

Table 28: The effect of one year storage on the chemical score of essential amino acids of control almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	C	C-S	C	C-S	C	C-S
	% compared to egg					
Tryptophan	63	36	93	93	99	92
Lysine	44	33	53	47	45	42
Threonine	63	59	72	59	78	65
Valine	130	143	115	125	123	136
Methionine	121	103	122	96	118	91
Ileucine	96	103	98	110	88	98
Leucine	119	114	113	106	112	104
Phenylalanine	139	158	135	157	143	163
Limiting amino acid (A.A.)	Lys 44	Lys 33	Lys 53	Lys 47	Lys 45	Lys 42
Total essential A.A. (T.E.A.A.)	52.7	42.6	58.7	48.8	54.8	42.7
% Retention of T.E.A.A.	80.0		83		77.9	

This suggests that there was some loss in protein quality of control-stored almonds.

When control-stored almonds were compared to irradiated-stored almonds, lysine was still shown to be limiting in all cases. Irradiated-stored almonds contained a higher content of total essential amino acids, hence were of a better quality in this respect.

The balance of these amino acids, however, was similar in both irradiated-stored and control-stored almonds.

CHAPTER VI

EFFECT OF SALTING-ROASTING, DUAL TREATMENT AND STORAGE ON PROTEIN AND AMINO ACIDS

INTRODUCTION

Considerable recent progress has been made on the effect of heating on protein and some accounts have already appeared in general reviews by RICE and BEUK (1953), HARRIS and VON LOESECKE (1960) and BENDER (1960, 1966).

It is well known that heat affects native protein in changing the spacial arrangement of the protein molecule. Heat increases the thermal molecular oscillation that disrupts the bonding forces and causes an unfolding of the molecule, which is then followed by a disruption of the disulfide bridge (TANFORD, 1968). The whole sequence of events is referred to as "heat denaturation" (Fig 14). It can be defined simply as a major change from the original native structure (quaternary, tertiary and secondary) without alteration of the amino acid sequence (primary structure). In principle, denaturation is a reversible process, but when it is brought about by heat, the irreversible stage is quickly reached and heat denaturation of protein often appears irreversible.

In food chemistry the term "denaturation" is often used to describe changes in spacial arrangement of the protein molecule (i.e. disulfide bonds). Irreversible chemical modification of the amino acid side-chains (primary-structure) is termed "denaturation" (MAURON, 1977). It is worth mentioning

that minor conformational changes may occur prior to denaturation; these are referred to as "predenaturation transition," which is known to be reversible. It is generally agreed that the disruption of the native protein structure by heat tends to increase the nutritive value of protein, because its susceptibility to enzymic attack is augmented. Actually, the first step in protein digestion is denaturation by proteolytic enzymes. Heat denaturation of protein facilitates this first step of digestion and must therefore be considered a positive factor in nutrition. However, often following denaturation the proteins may interact either with themselves or with other molecules, to form aggregates (precipitates) and these reactions are virtually irreversible. On excessive heat supplied to protein, covalent bonds may rupture, causing "thermal degradation" of the molecule (LEDWARD, 1979). Fig 14 shows a diagrammatic representation of the effect of heat on protein.

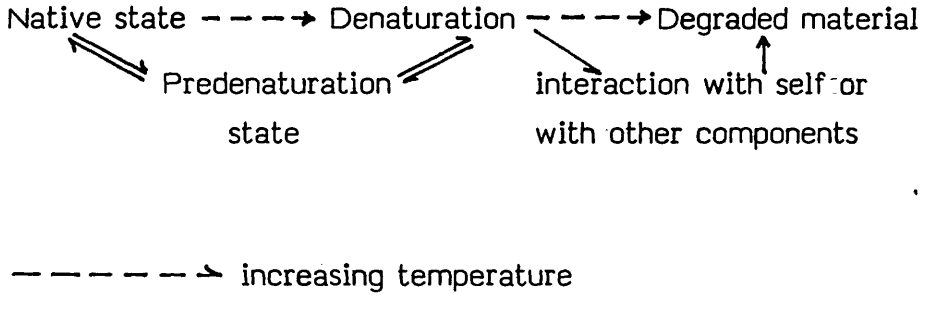


Fig. 14:
Schematic representation of stages in denaturation of proteins

In the absence of carbohydrates or fats, heat damage is largely of a protein-protein nature without other groups being involved, and the availability of all amino acids being affected (CARPENTER and BOOTH, 1973). MILLER et al (1955) have shown that in severely heated cod muscle, cysteine was heavily destroyed, while lysine barely so. These post denaturation reations are virtually irreversible. Heating such as applied to the outside

of roasted foods leads to the destruction of amino acid by complete decomposition or by racemisation and the formation of cross-linkages forming poly amino acids (HAYASE et al 1975). GREAVES et al (1938) studying the amino acids of casein, have shown that lysine is damaged by heating at 130°C for thirty minutes, while histidine required a temperature of 140°C and cysteine, tyrosine and tryptophan appeared unaffected. In general, provided the temperature does not exceed about 100°C and the period of heating is no more that one hour, little damage occurs, but as these conditions are exceeded, lysine, arginine, methionine, cysteine, leucine, tryptophan and histidine may become metabolically unavailable to various degrees (OSNER and JOHNSON, 1968).

Various proteins respond differently to processing depending on the composition and properties of the protein and the state of the food. Proteins are ordinarily mixed or combined with other compounds such as carbohydrates, lipids and water. When considering the effect upon proteins, these non-protein materials frequently are of as much importance as the severity of the processing treatment. With mild heat in the presence of reducing sugars, the Maillard reaction or non-enzymic browning reaction occurs between the reducing sugar and the free amino groups of amino acids, peptides and protein (p. 100). Other carbonyl groups such as aldehydes and ketones may also react in the same way. This reaction is encountered very frequently in food under storage. The Maillard reaction decreases the availability of certain amino acids in the food, thereby reducing the nutritive value in the case of essential amino acids.

At high temperatures, lipid peroxides (p.323)can react with the amino acid residues of proteins and decrease their availability (CUQ et al 1973 ;

VENOLIA and TAPPEL, 1958).

At a moisture level of between 5-14%, maximum amino acid binding may occur for lysine, methionine, arginine and tryptophan (CARPENTER et al, 1962). In another investigation, a greater loss of lysine occurred with 14% moisture than with 50%, although the reverse was true for methionine, tryptophan and leucine (MILLER et al, 1965).

In addition to moisture, the length of storage has some effect on the damage done to protein. Lowering of moisture has some effect on decreasing loss of protein, but no simple generalisation can be made.

In summary, the following points may be made on the effect of heat on proteins:

1. The nutritive value of the protein is often improved by moderate heating, especially in the case of vegetable protein, whereas it is impaired by intensive heat treatment.

2. Heat damage to the protein will not become apparent in a biological test, when the most damaged amino acid is not the limiting amino acid of the protein.

3. The presence of autoxidising fats also increases heat damage.

4. At a given temperature the damage is proportional to the length of heating time.

5. High water content reduces heat damage, whereas intermediate moisture content augments the latter (MAURON, 1982).

RESULTS AND DISCUSSION

Total protein and free amino acids:

After roasting, the total protein content of varieties F, S and H were 155.4, 159.1 and 155.2 mg g^{-1} dry weight respectively (table 29). Losses when compared to unroasted samples were significant ranging from 11.1 to 15.8% (19.5 to 29.8 mg g^{-1}).

A significant decrease in free amino acids ranging from 30-47% was accompanied by a significant increase in ammonia. This suggests that a large amount of protein and amino acids may have undergone deamination leading to the release of ammonia. However, it is not possible at this stage to rule out the possibility of the occurrence of non-enzymic browning and peroxidative damage of the amino acids.

NEWELL et al., (1967) studying roasted peanuts found that within replicates of the same sample the total free amino acid losses varied from 20 to 56%, which is comparable to the losses observed in almonds.

Presoaking the almonds in salt solution prior to heating would have increased the possibility of hydrolysis, thereby increasing the amount of free amino acids which would be more susceptible to further reactions. The reason for this may be explained by the relationship between the salt in the soak-media and the protein of the seed coat. Certain types of proteins such as globulins and albumins are both soluble in dilute salt solution (5-15%) but albumins are readily soluble in water. However,

Table 29: The effect of salting and roasting on the protein and free amino acids of almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	FAA
		mg/g dry wt. ± SE				
F	C	174.9 ± 8.4	4.6 ± 0.2	5.0 ± 0.6	88.8	69.6
	R	155.4 ± 10.3	3.2 ± 0.4	7.4 ± 0.3		
S	C	188.9 ± 13.2	5.6 ± 0.2	9.0 ± 0.8	84.2	62.5
	R	159.1 ± 9.4	3.5 ± 0.3	15.7 ± 1.2		
H	C	183.4 ± 11.2	5.1 ± 0.2	6.9 ± 0.2	84.6	52.9
	R	155.2 ± 11.2	2.7 ± 0.3	14.2 ± 1.0		

in concentrated salt solution, all protein becomes less soluble. The increase in solubility of globulins in dilute salt solutions known as 'salting in' may be explained in terms of the relative affinity of the protein molecules for each other and for the solvent. The ions of the neutral salt will interact with bonds in the protein, thereby decreasing protein-protein interactions and consequently increasing the solubility of globulins.

Therefore, it appears that in the case of almonds, soaking in 15% salt solution could have resulted in the weakening of bonds between protein molecoles in the seed coat, hence allowing the leaching of water-soluble constituents in the cells.

The effect of roasting on protein amino acids

The data for individual protein amino acids in almonds after roasting is given in table 30. Significant losses were observed in roasted almonds compared to non roasted ones in almost all amino acids. The amino acids appearing most affected in terms of absolute weight losses were lysine, histidine, threonine, serine, proline, cysteine, methionine, isoleucine and tyrosine. There were, however, instances where certain amino acids (aspartic acid and phenylalanine) had increased. Although increases in amino acids during heat processing are not expected normally, this phenomenon is not uncommon. PHADKE and SOHONIE (1962) have reported increases in threonine, histidine, methionine and tyrosine for double beans autoclaved for five minutes, while KAKADE and EVANS (1963) have reported increases in isoleucine, leucine, threonine and valine in kidney beans autoclaved for five minutes. These authors have not tried to explain the reasons for increases in these amino acids.

Table 30: The effect of salting and roasting on individual protein amino acids of almond nuts.

Amino acids	F		S		H	
	C	R	C	R	C	R
	mg/g dry wt.					
Try	1.1	0.9	1.8	1.8	1.8	1.0
Lys	3.6	1.5	4.9	2.7	3.9	2.2
His	3.9	1.7	4.8	2.3	4.2	1.9
Arg	20.5	14.3	20.1	13.6	20.7	15.3
Asp	19.1	24.8	19.4	24.3	20.1	25.4
Thr	3.8	2.3	4.9	2.8	4.9	2.6
Ser	6.8	3.9	7.0	3.3	7.5	3.7
Glu	43.6	39.8	44.9	42.2	45.1	42.1
Pro	8.8	4.2	10.2	4.4	9.4	3.9
Gly	10.2	11.5	11.1	11.5	11.1	10.3
Ala	7.1	6.5	8.0	7.5	7.9	6.8
CysH	1.6	0.9	1.9	1.0	1.5	0.8
Val	10.5	9.8	10.4	9.5	10.3	9.1
Met	4.8	2.2	5.4	2.6	4.9	2.4
Ileu	7.1	5.1	8.1	5.9	6.8	3.5
Leu	12.4	11.5	13.0	13.3	12.1	10.1
Tyr	5.9	2.7	6.2	3.4	5.4	2.4
Phe	9.4	12.1	10.2	12.9	10.1	13.5

The main problem encountered was in identifying the pathways by which amino acids were lost. The principle reactions most discussed, revolve around the "non-enzymic" browning and peroxidative damage by lipids. HURRELL and CARPENTER (1977) have reported that the Maillard reaction mainly affects lysine and arginine and to a lesser extent tryptophan, cysteine and histidine.

Maximum losses found in the present study for the latter were lysine (58%), arginine (32%), tryptophan (44%), cysteine (47%) and histidine (56%). This may indicate the occurrence of non-enzymic browning between free sugars and protein amino acids.

However, the brown pigments (melanoidins) produced by the Maillard reaction have been shown not to adversely affect the nutritional properties of the remaining protein. ATKINSON and CARPENTER (1970) and BOCTOR and HARPER (1968) have shown that heated cod-glucose and egg white-glucose mixture that had lost more than 80% of their nutritive value after the Maillard reaction, when supplemented to well balanced diets, did not depress the growth of rats.

Arginine, cysteine and histidine together with serine were also implicated by DESAI and TAPPEL (1963) as being most susceptible to peroxidative damage. The large losses of these amino acids observed in this study may also provide indirect evidence for the occurrence of these reactions. Lipid-protein interaction products may adversely affect the flavour of almonds.

NEWELL et al (1967) have stated that amino acids can give rise to aldehydes by Strecker degradation and can serve as the source of nitrogen

for the formation of pyrazine compounds. That these compounds were produced during the roasting of peanuts was indicated by JOHNSON (1966) and MASON et al (1967) in which pyrazine and aldehydes were shown to represent the major classes of organic compounds produced from peanuts during roasting. Although the decrease in protein and free amino acids found in the present study may be disadvantageous from a nutritional point of view, part of this protein may have undergone reactions as suggested by NEWELL et al (1967) which could have improved the flavour of the product, and therefore in the present study, the flavour of almonds.

From NEWELL et al (1967) studies, it was assumed that glutamic acid, phenylalanine and alanine were the most important amino acids in terms of flavour precursors. It would be expected that if the above amino acids were the major flavour precursors, then maximum loss of these should be observed on roasting. In this study, however, large losses were observed in a number of amino acids other than those reported by NEWELL et al (1967), suggesting that any of these may be involved in the production of flavour.

Chemical score

It may be supposed that since there is a decrease in total protein and in free amino acids as a result of roasting, protein quality may be affected to some extent.

The limiting amino acid continued to be lysine (table 31), as its chemical score dropped significantly. The other essential amino acids were also severely affected. The score for methionine decreased as much as 56 in variety F, while the score for tryptophan fell by 31 in variety H. The

Table 31: The effect of salting and roasting on the chemical score of essential amino acids of almond nuts, expressed as a % of that found in egg.

Essential amino acids	F		S		H	
	C	R	C	R	C	R
	% compared to egg					
Tryptophan	63	60	93	106	99	68
Lysine	44	21	53	34	45	32
Threonine	63	44	72	47	78	51
Valine	130	141	115	121	123	134
Methionine	121	65	122	67	118	72
Ileucine	96	80	98	81	88	56
Leucine	119	128	113	130	112	115
Phenylalanine	139	208	135	196	143	237
Limiting amino acid (A.A.)	Lys 44	Lys 21	Lys 53	Lys 34	Lys 45	Lys 32
Total essential A.A. (T.E.A.A.)	52.7	45.4	58.7	51.5	54.8	44.4
% Retention of T.E.A.A.	86.0		87.7		81.0	

chemical score for threonine also decreased significantly in all three varieties.

EFFECT OF STORAGE ON THE PROTEINS OF ROASTED ALMONDS

Total protein and free amino acids:

After salting and roasting the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on protein of salted and roasted almonds, comparisons were made between results taken immediately after processing and those after one year storage.

Table 32 represents the total protein, free amino acid and ammonia content of roasted almonds after one year storage. Total protein content of the roasted-stored almonds was 153.8, 153.5 and 153.3 mg g^{-1} for F, S and H respectively, which does not reflect any significant loss in protein. Values for free amino acid content for the above were 4.5, 4.7 and 4.1 mg g^{-1} respectively. The free amino acid represented a significant increase ranging from 34 to 52%. This indicates that although the loss in total protein was not significant, a small amount of protein hydrolysis may have led to an increase in free amino acids. However, increase in ammonia was not significant suggesting that there was little, if any, destruction of protein.

In general, it was shown that total protein content was not affected in roasted almonds after one year storage.

It was found that there was no significant difference in protein content between roasted-stored (table 32) and control-stored almonds (table 24).

A significantly greater increase in free amino acids and in ammonia was found in control-stored almonds in comparison to roasted-stored samples. Evidently, more protein breakdown occurred in control almonds during storage. One possible explanation for this difference may have been due to enzymic action

Table 32: The effect of one year storage on total protein and free amino acids of salted and roasted almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	FAA
		mg/g dry wt. ± SE				
F	R	155.4 ± 10.3	3.2 ± 0.7	7.4 ± 0.3	98.9	140.6
	R-S	153.8 ± 12.3	4.5 ± 0.3	7.8 ± 0.3		
S	R	159.1 ± 9.4	3.5 ± 0.3	15.7 ± 1.2	96.5	134.3
	R-S	153.5 ± 11.5	4.7 ± 0.3	16.2 ± 0.9		
H	R	155.2 ± 11.2	2.7 ± 0.3	14.2 ± 1.0	98.8	151.8
	R-S	153.3 ± 10.5	4.1 ± 0.4	14.6 ± 0.7		

in the latter.

Protein amino acid of roasted-stored almonds:

Table 33 gives the results for individual protein amino acids for roasted and roasted-stored almonds.

Although it was found previously that total protein content was not significantly changed, it appears that this observation was not reflected by individual amino acids. Significant decreases were noted in a number of amino acids, mainly the essential amino acids: typtophan, lysine and threonine.

The overall profile of the protein amino acids of control-stored almonds is similar to that of roasted-stored almonds apart from fluctuations in a few individual amino acids.

Chemical Score

The chemical score for the limiting amino acid lysine has decreased, on average by 9 (table 34). The distribution of chemical-score for essential amino acids was affected to a certain extent in all three varieties of roasted-stored almonds.

The balance of amino acids immediately after roasting (roasted-sample) was substantially better than in roasted-stored samples. This was especially so in the case of tryptophan, lysine and threonine, where chemical scores were lower in roasted-stored samples.

Comparison of chemical scores of control-stored and roasted-stored showed an interesting picture that although lysine was found to be the limiting amino acid in both cases, its chemical score was lower in roasted-stored almonds than in control-stored ones. Scores for tryptophan and threonine were, however, generally higher in control-stored than in roasted-stored almonds.

Table 33: The effect of one year storage on individual protein amino acids of salted and roasted almond nuts.

Amino acids	F		S		H	
	R	R-S	R	R-S	R	R-S
	mg/g dry wt.					
Try	0.9	0.3	1.8	0.9	1.0	0.4
Lys	1.5	0.9	2.7	1.6	2.2	1.3
His	1.7	2.0	2.3	2.7	1.9	2.2
Arg	14.3	16.2	13.6	16.2	15.3	16.3
Asp	24.8	20.4	24.3	20.3	25.4	21.2
Thr	2.3	1.5	2.8	1.9	2.6	1.5
Ser	3.9	4.2	3.3	3.7	3.7	4.4
Glu	39.8	40.1	42.2	40.5	42.1	41.5
Pro	4.2	3.8	4.4	4.1	3.9	4.4
Gly	11.5	10.5	11.5	11.4	10.3	9.3
Ala	6.5	6.3	7.5	6.5	6.8	6.5
CysH	0.9	1.1	1.0	0.9	0.8	1.0
Val	9.8	8.9	9.5	8.5	9.1	8.3
Met	2.2	1.9	2.6	2.1	2.4	1.5
Ileu	5.1	4.9	5.9	4.6	3.5	5.2
Leu	11.5	10.3	13.3	12.4	10.1	9.4
Tyr	2.7	2.3	3.4	4.1	2.4	2.9
Phe	12.1	11.5	12.9	10.8	13.5	11.7

Table 34: The effect of one year storage on the chemical score of essential amino acids of salted-roasted almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	R	R-S	R	R-S	R	R-S
	% compared to egg					
Tryptophan	60	23	106	64	68	31
Lysine	21	15	34	23	32	21
Threonine	44	32	47	39	51	33
Valine	141	145	121	130	134	138
Methionine	65	63	67	65	72	51
Ileucine	80	86	81	76	56	94
Leucine	128	129	130	146	115	121
Phenylalanine	208	223	196	197	237	233
Limiting amino acid (A.A.)	Lys 21	Lys 15	Lys 34	Lys 24	Lys 32	Lys 21
Total essential amino acid (T.E.A.A.)	45.4	40.2	51.5	42.8	44.4	39.3
% Retention of T.E.A.A.	88.5		83.0		88.5	

THE EFFECT OF THE COMBINED TREATMENT: SALTING-ROASTING AND IRRADIATION ON PROTEIN & AMINO ACIDS

Total proteins and free amino acids

Total proteins, free amino acids and ammonia content for roasted irradiated almonds are represented in table 35. The values recorded for F, S and H were 158.3, 162.2 and 160.2 mg g^{-1} respectively, which were significantly lower than controls. This observation was not contrary to what was expected due to the fact that a significant loss in total protein was noted in roasted almonds as shown earlier (p. 176). On a percentage basis the losses in the case of combined process were approximately 12%, which was slightly lower than that recorded for the same varieties after roasting only (14%).

Irradiation alone was shown not to have any effect on the total protein content of almonds (table 20), therefore, it would be expected that in a combined process, such as roasting followed by irradiation, the irradiation would not be the significant factor in protein breakdown for the combined process.

URBAIN (1977), on the other hand, has suggested that a heat-denatured protein, with its structure order disrupted, is less capable of recombining with itself after irradiation has induced disruption. Therefore after irradiation, the protein molecule experiences a greater amount of free-radical formation than does its native counterpart. From this argument it may

Table 35: The effect of dual treatment on protein and free amino acids of almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	FAA
		mg/g dry wt ± SE				
F	C	174.9 ± 8.4	4.6 ± 0.2	5.0 ± 0.6	90.5	76.1
	RI	158.3 ± 7.2	3.5 ± 0.2	10.3 ± 0.4		
S	C	188.9 ± 13.2	5.6 ± 0.8	9.0 ± 0.8	85.9	69.6
	RI	162.2 ± 6.9	3.9 ± 0.5	13.9 ± 0.8		
H	C	183.4 ± 11.2	5.1 ± 0.2	6.9 ± 0.2	87.4	56.9
	RI	160.2 ± 8.3	2.9 ± 0.3	11.6 ± 0.5		

be inferred that the roasting-irradiation process should lead to greater free radical production and hence augmentation of protein breakdown. However, this was not the case in the present study.

It was found previously that the roasting process brought about losses in total protein ranging from 19.5 to 29.8 mg g^{-1} in almonds, while for the roasting-irradiation process the losses ranged from 16.6 to 26.7 mg g^{-1}. So, contrary to the statement made by URBAIN (1977), it appears that the irradiation part of the combined process has helped to reduce the loss in total proteins rather than to increase it. However, the reason why irradiation has caused less loss in protein is at present unknown.

The free amino acid content of roasted-irradiated samples ranged from 2.9 to 3.9 mg g^{-1} (table 35). These values correspond to significant losses of 1.1 to 2.2 mg g^{-1} when compared to controls, suggesting that free amino acids may have entered into certain reactions, thereby, making them unavailable. Some of these reactions have already been discussed briefly in the roasting process; these may involve non-enzymic browning and the occurrence of peroxidative damage.

Observations made previously for irradiated and roasted samples showed that the former induced an increase, while the latter a decrease in free amino acids. From this it may again be confirmed that it is the roasting process of the combined treatment which seems to have the major effect in bringing about a loss in free amino acids. However, these losses due to the combined process, were less than those found for roasted samples only, indicating that the irradiation treatment may have aided in preventing loss of free amino acids, resulting in a lower net decrease.

Increase in ammonia content confirmed the fact that a significant amount of protein degradation had taken place.

Protein amino acids

The profile of protein amino acids (table 36), reveals some interesting details.

A number of similarities exist between changes in individual amino acids of roasted-irradiated samples and those reported for roasted samples (table 30). The increase in phenylalanine is almost identical to that found in roasted almonds. This supports earlier discussion that in the combined treatment, roasting has a greater effect on protein than has irradiation.

Aspartic acid is a common end-product of a number of other amino acids (e.g. proline, tryptophan, tyrosine, glycine and methionine) subjected to the irradiation process hence it was not surprising to note an increase in aspartic acid accompanying losses in the amino acids mentioned above. This has also been reported for roasted almonds. The increase in valine was unexpected, since it had not been affected by either irradiation or by roasting.

The amino acids most affected were lysine, threonine, serine, proline, methionine and tyrosine, which were all found to decrease (table 36). These amino acids may be involved in non-enzymic browning and peroxidative damage and their loss may suggest the occurrence of these reactions as the main cause for protein loss.

Total amounts of both S-containing and aromatic amino acids after the

Table 36: The effect of dual treatment on individual protein amino acids of almond nuts.

Amino acids	F		S		H	
	C	RI	C	RI	C	RI
	mg/g dry wt.					
Try	1.1	1.0	1.8	1.5	1.8	1.2
Lys	3.6	1.2	4.9	2.2	3.9	2.4
His	3.9	1.9	4.8	2.1	4.2	2.7
Arg	20.5	15.1	20.1	14.2	20.7	16.1
Asp	19.1	25.6	19.4	24.9	20.1	26.2
Thr	3.8	2.4	4.9	2.3	4.9	2.2
Ser	6.8	3.3	7.0	3.7	7.5	2.4
Glu	43.6	40.4	44.9	41.5	45.1	42.0
Pro	8.8	3.1	10.2	4.3	9.4	3.4
Gly	10.2	7.0	11.1	8.2	11.1	7.6
Ala	7.1	6.5	8.0	7.4	7.9	7.4
CysH	1.6	0.8	1.9	0.9	1.5	0.9
Val	10.5	13.7	10.4	14.1	10.3	12.9
Met	4.8	2.5	5.4	2.1	4.9	2.2
Ileu	7.1	5.2	8.1	5.3	6.8	3.7
Leu	12.4	11.3	13.0	12.3	12.1	10.1
Tyr	5.9	2.9	6.2	3.1	5.4	2.3
Phe	9.4	12.2	10.2	13.2	10.1	13.5

dual process (table 36) ranged from 3.0 to 3.3 mg g^{-1} in the former, which was similar to the total amounts of 3.1 to 3.6 mg g^{-1} found in roasted almonds (table 30) but much lower than in irradiated almonds (6.1 - 7.3 mg g^{-1}, table 21). The total amount of aromatic amino acids in the dual process ranged from 15.1 to 16.3 mg g^{-1} (table 36) which was again almost identical to that found in roasted almonds (14.8 to 16.3 mg g^{-1}, table 30) but again lower than that found in irradiated almonds (16.6 to 18.3 mg g^{-1}, table 21).

The range of total essential amino acids in three varieties after the dual process was found to be from 49.5 to 53 mg g^{-1} (table 37) as compared to the range of 44.4 to 51.5 mg g^{-1} for roasted almonds (table 31) and the range of 48.0 to 55.7 mg g^{-1} for irradiated almonds (table 22). It may be seen that irradiating the almonds after roasting may have helped to maintain protein quality as compared to solely roasting them.

Chemical score

Protein quality as judged by the balance of total essential amino acids showed that there was a substantial decrease in the chemical score for lysine, the limiting amino acid, in all three varieties; the decrease being 29, 26 and 13 points respectively (table 37).

The chemical score for threonine, methionine and isoleucine also decreased in all three varieties. These decreases ranged from 21 to 38 points in the case of threonine, from 54 to 69 points for methionine and from 21 to 34 points for isoleucine.

In general, it may be concluded that the protein quality of roasted-

Table 37: The effect of dual treatment on the chemical score of essential amino acids of almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	C	RI	C	RI	C	RI
	% compared to egg					
Tryptophan	63	61	93	86	99	75
Lysine	44	15	53	27	45	32
Threonine	63	42	72	38	78	40
Valine	130	181	115	174	123	175
Methionine	121	67	122	53	118	61
Ileucine	96	75	98	71	88	54
Leucine	119	115	113	117	112	105
Phenylalanine	139	192	135	194	143	219
Limiting amino acid (A.A.)	Lys 44	Lys 16	Lys 53	Lys 27	Lys 45	Lys 32
Total essential amino acid (T.E.A.A.)	52.7	49.5	58.7	53	54.8	48.2
% Retention of T.E.A.A.	94.0		90.3		88.0	

irradiated almonds was lower than that of untreated almonds. This observation was similar to that for roasted almonds.

EFFECT OF STORAGE ON PROTEIN OF DUAL TREATED ALMONDS

Total protein and free amino acids:

After salting-roasting and irradiating the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on total protein and free amino acids of dual treated almonds, comparisons were made between results taken immediately after processing and those after one year storage.

Total protein content for roasted-irradiated almonds stored for one year was found to be 150.7, 165.7 and 158.0 mg g^{-1} for varieties F, S and H respectively (table 38). From this it can be seen that storage for one year has no significant effect on the total protein content of roasted-irradiated almonds.

Free amino acid content was found to be 5.1, 5.6 and 4.9 mg g^{-1} for varieties F, S and H corresponding to an increase of 46%, 44% and 69% respectively. These significant increases in free amino acids suggest that some hydrolyses of protein had occurred within the almond despite the fact that there had not been any significant overall change in the content of the latter. That only a small degradation of protein had occurred may be correlated with the apparent small increase in ammonia content in all cases.

When considering total protein and free amino acid content together and comparing that of roasted-irradiated-stored (table 38) with control-stored almonds (table 24). it was found that no significant difference existed.

From this it may be concluded that by the combined treatment, it is

Table 38: The effect of one year storage on the protein and free amino acids of dual treated almond nuts.

		Total protein (TP)	Free amino acid (FAA)	Ammonia	Retention (%) TP	Retention (%) FAA
		mg/g dry wt. ± SE				
F	RI	158.3 ± 7.2	3.5 ± 0.2	10.3 ± 0.4	95.2	145.7
	RI-S	150.7 ± 8.1	5.1 ± 0.3	11.5 ± 0.9		
S	RI	162.2 ± 6.9	3.9 ± 0.5	13.9 ± 0.8	102.2	143.6
	RI-S	165.7 ± 9.2	5.6 ± 0.3	14.6 ± 1.0		
H	RI	160.2 ± 8.3	2.9 ± 0.3	11.6 ± 0.5	98.6	168.9
	RI-S	158.0 ± 7.1	4.9 ± 0.4	12.9 ± 0.9		

possible to improve flavour, prevent spoilage and also maintain equal amounts of protein after one year storage as compared to storing unprocessed almonds for the same period.

The evaluation of the combined treatment over roasting and irradiation separately depends mainly on the purpose of the use of almonds. If the almonds are to be roasted for improving flavour, and then stored for one year it is found that the combined treatment does not offer any further advantage in terms of maintaining total protein, due to the fact that retention of the total protein in both cases was similar.

In the case of the combined treated samples, significantly less total protein was retained after one year storage than in irradiated treated almonds. This implies that for the purpose of storage only (if flavour is not to be taken into account), irradiating the almonds with 2 Mrad is sufficient to maintain the total protein content for a shelf-life of one year.

Protein amino acids

On storage, the amino acid profile has not been affected appreciably, although changes in some amino acids have been observed. Some of the individual amino acids which decreased significantly were lysine, threonine, alanine and tyrosine (table 39). This supports previous results in which it was shown that an increase in free amino acids was observed, thus indicating some breakdown of protein.

When the amino acid profile for roasted-irradiated-stored samples (table 39) was compared with that of control-stored (table 26) fluctuations between individual amino acids were observed but no consistent pattern

Table 39: The effect of one year storage on individual protein amino acids of dual treated almond nuts.

Amino acids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	mg/g dry wt.					
Try	1.0	1.0	1.5	1.3	1.2	0.9
Lys	1.2	0.7	2.2	1.4	2.4	1.2
His	1.9	2.1	2.1	1.9	2.7	2.8
Arg	15.1	17.6	14.2	17.6	16.1	19.5
Asp	25.6	21.4	24.9	26.7	26.2	24.5
Thr	2.4	1.5	2.3	1.4	2.2	0.9
Ser	3.3	3.4	3.7	3.5	2.4	2.6
Glu	40.4	40.4	41.5	39.9	42.0	41.9
Pro	3.1	3.5	4.3	4.5	3.4	3.8
Gly	7.0	7.6	8.2	8.6	7.6	8.4
Ala	6.5	3.4	7.4	3.8	7.4	4.1
Cys H	0.8	0.7	0.9	1.0	0.9	1.1
Val	13.7	14.1	14.1	12.2	12.9	11.4
Met	2.5	2.4	2.1	2.3	2.2	1.8
Ileu	5.2	5.1	5.3	5.1	3.7	3.9
Leu	11.3	11.0	12.3	12.4	10.1	9.9
Tyr	2.9	1.8	3.1	2.1	2.3	2.1
Phe	12.2	11.6	13.2	10.8	13.5	11.1

emerged. It was noted, however, that tyrosine was significantly lower in roasted-irradiated-stored than in control-stored almonds. When tyrosine was considered together with phenylalanine as total aromatic amino acids, it was found that there was no significant difference between the total aromatic amino acids of these two samples. This is possibly due to the fact that tyrosine may be converted to phenylalanine during processing or during storage.

In general, then, it can be said that, discounting minor fluctuations of individual amino acids, there are no significant losses incurred by the combined treatment on almonds when stored for one year at 22°C.

Chemical score

Table 40 shows the chemical score for roasted-irradiated treated samples before and after one year storage.

It was found that both lysine and threonine of roasted-irradiated-stored almonds were severely limiting in all three varieties. The chemical score for lysine ranged from 10 to 19, while that for threonine ranged from 19 to 28.

When comparing chemical-score of roasted-irradiated-stored (table 40) with that of control stored (table 28) the first common point to emerge was that the essential amino acids most affected by processing and storage were tryptophan, lysine and threonine. The chemical score for these amino acids were generally higher in control-stored almonds. This was partially true in the case of F, where chemical score for tryptophan in roasted-irradiated-stored samples was higher than in control-stored ones.

Table 40: The effect of one year storage on the chemical score of essential amino acids of dual treated almond nuts, expressed as % of that found in egg.

Essential amino acids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	% compared to egg					
Tryptophan	61	64	86	84	75	66
Lysine	16	10	27	19	32	19
Threonine	42	28	38	26	40	19
Valine	181	194	174	170	175	181
Methionine	67	68	53	65	61	58
Ileucine	75	76	71	77	54	67
Leucine	115	117	117	133	105	121
Phenylalanine	192	191	194	180	219	211
Limiting amino acid (A.A.)	Lys 16	Lys 10	Lys 27	Lys 19	Lys 32	Lys 19
Total essential A.A. (T.E.A.A.)	49.5	47.4	53	46.9	48.2	41.1
% Retention of T.E.A.A.	95.8		88.5		85.3	

It was therefore concluded that, although the dual treatment may lengthen the shelf-life of almonds, the protein score was generally lower.

There does not appear to be much difference between the protein score of roasted-irradiated-stored almonds (table 40) and that of roasted-stored ones (table 34). While there were higher chemical scores for tryptophan and lower ones for lysine in the former, the reverse was observed in the latter.

A different situation exists when the essential amino acids of roasted-irradiated-stored almonds are compared with those of irradiated-stored ones; the latter containing higher amounts than the former. The protein score was also higher in the latter because it possessed higher scores for the limiting amino acid (lysine).

If the FAO (1970) values for chemical-score of almonds (Appendix 2) are taken to mean a well-balanced profile, then the values for irradiated-stored almonds are closer to them than are those of roasted-irradiated-stored almonds. This is especially so in the case of the main limiting amino acid (lysine) and the second most limiting one (threonine).

SUMMARY

Control:

Total protein content in the three varieties of almonds ranged from 174.9 to 188.9 mg g^{-1} dry weight (table 20). These values are not unlike those given by FAO (1970) of 168 mg g^{-1} dry weight for unspecified almonds and those of Californian almonds by WOODROOF (1979) of 186 mg g^{-1} dry weight. The content of free amino acids varied from 4.6 to 5.6 mg g^{-1} (table 20).

Eighteen amino acids were identified on protein hydrolysis: glutamic acid, arginine and aspartic acid collectively making up 46% of total protein amino acids (table 21).

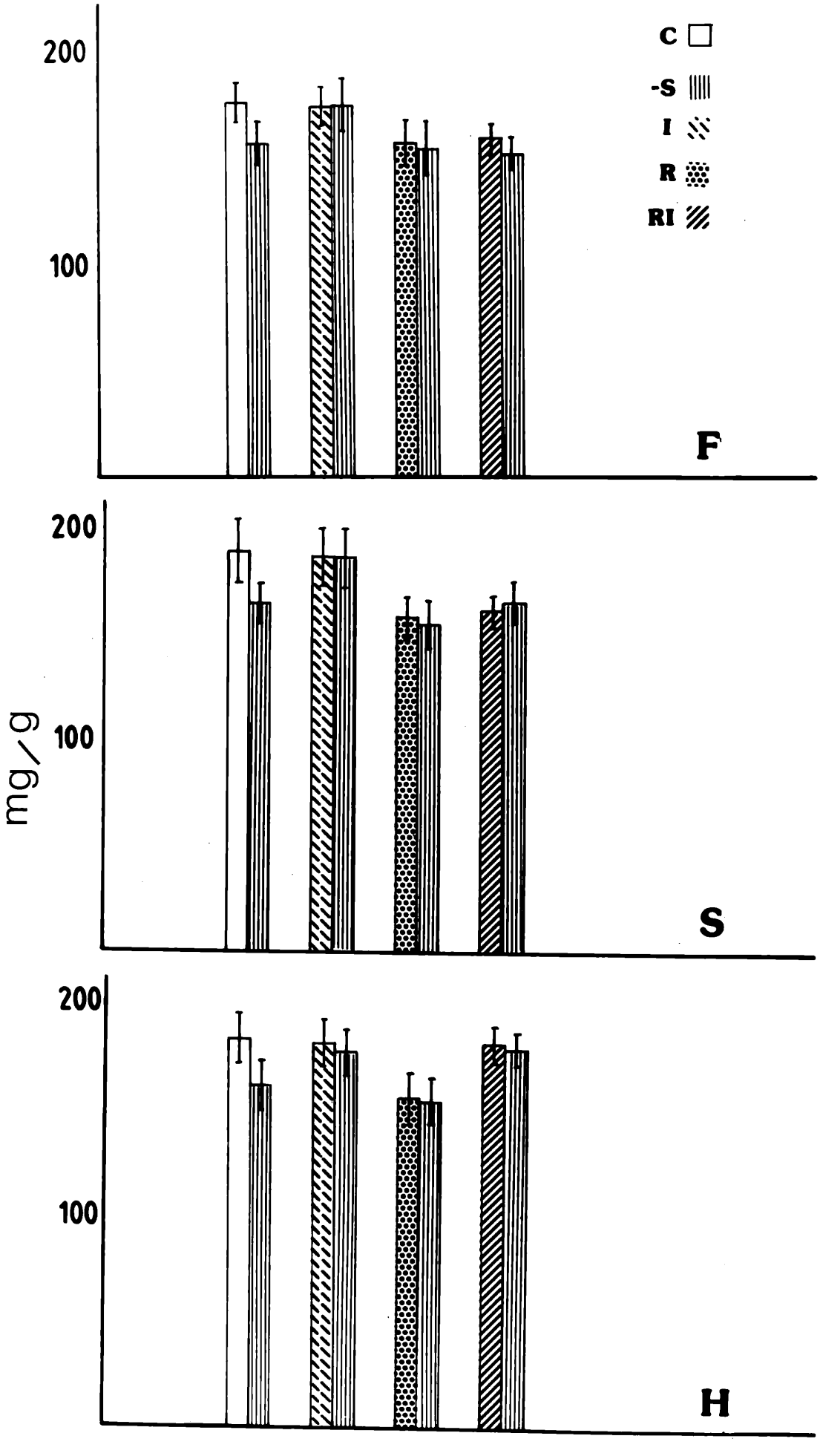
Total essential amino acids ranged from 52.7 to 58.7 mg g^{-1} dry weight. The limiting amino acid in terms of the chemical score was found to be lysine, while threonine also possessed low chemical score in variety F.

One year storage at 22°C resulted in a significant decrease in total protein content (table 24). Protein breakdown was also indicated by an increase in both free amino acids and in ammonia. The decrease in total protein was mainly in lysine, histidine, threonine, serine, proline, methionine, leucine and tyrosine and may have been due to the continuous respiration of the almonds during storage. Reactions with oxidised lipid products and sugar have also been suggested.

Total essential amino acids also decreased on storage, indicating a loss in protein quality.

Fig 15: Effect of different processing and storage on protein of three varieties of almonds are represented graphically in this figure.

The second of each pair of columns represents total proteins (mg/g ± SEM) of the stored products of the first column.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Irradiation

In the present study, irradiation treatment (2 Mrad) did not cause significant change in total protein content, while free amino acids and ammonia were found to increase (table 20). These observations were similar to those of MILNER (1961) and of PAPE (1972) who had investigated the effect of irradiation on wheat protein. Furthermore, minor changes in individual amino acids, e.g. lysine, histidine and threonine were also observed here (table 21).

The increases in serine, aspartic acid and tyrosine were probably due to their being formed from other amino acid groups as a result of irradiation (table 21). Decreases in lysine and in threonine effectively lowered the protein quality to a certain extent.

There was no significant change in total protein, in free amino acids or in ammonia content after irradiated almonds were stored for one year (table 23). Among the protein amino acids, phenylalanine was observed to increase, while arginine, serine and cysteine decreased (table 25).

Total essential amino acid content was unchanged as was the quality of the protein.

Roasting

Salting and roasting significantly decreased the total protein and free amino acid content of almonds (table 29). The increase in ammonia may be indicative of protein breakdown. Part of the protein and amino acids losses may have been due to protein-salt interactions during the pre-soaking step prior to heating. Aqueous salt solution possesses

the property of solubilising proteins such as albumin and globulin. Losses were found in a number of individual protein amino acid which included lysine, histidine, threonine, serine, proline, methionine, isoleucine, cysteine and tyrosine, while increases were observed in aspartic acid and in phenylalanine (table 30). These results are not unlike those of PHADKE and SOHONIE (1962) and VAKILE and EVANS (1963) who have shown increases in a number of protein amino acids after cooking beans.

It was also suggested that losses may have resulted from Maillard reactions and by peroxidative damages brought about by lipid-protein interactions.

No further losses in total protein content were reported after one year storage (table 32). The increases in free amino acids suggested some hydrolysis of protein.

Analysis of protein amino acids showed that on the whole, essential amino acids decreased, which was confirmed by decreases in the chemical score for lysine, tryptophan and threonine.

Dual treatment: Roasting -Irradiation

The dual processing of almonds brought about a significant loss in total protein and free amino acids and an increase in ammonia (table 35). It was obvious that protein degradation had taken place, possibly due to either non-enzymic browning (p. 100), to peroxidative damage (p.323) or to both reactions.

As in the case of roasted almonds, aspartic acid and phenylalanine again

increased (table 36). Aspartic acid may be the end-product of a number of other amino acids, proline, tryptophan, tyrosine, lysine, glycine and methionine, which may be affected by irradiation and were all observed to decrease in the present study.

Total essential amino acids decreased, which therefore affected the chemical score of lysine. Protein quality of roasted-irradiated almonds was calculated to be lower than that of controls.

In general, the dual treatment was assessed to be better than roasting in terms of maintaining essential amino acids.

There was no further losses in total protein after one year storage, but free amino acids and ammonia increased (table38).

It was concluded that with the dual treatment it was possible to improve the flavour, prevent spoilage and maintain equal amounts of protein after one year storage when compared to storing unprocessed almonds.

Of the protein amino acids, lysine, threonine, alanine and tyrosine decreased (table 39).

It was noted, however, that tyrosine in roasted-irradiated-stored almonds was significantly lower than that of control-stored almonds. When tyrosine was considered together with phenylalanine as total aromatic acids, it was found that there was no significant difference between the total aromatic amino-acids of these two samples. This is possibly due to the fact that tyrosine may be converted to phenylalanine during processing or during storage.

Both lysine and threonine were severely limiting in all three varieties. The chemical score for lysine ranged from 10 to 19 while that for threonine ranged from 19 to 28 (table 40).

CHAPTER VII

EFFECT OF IRRADIATION AND STORAGE ON LIPIDS

INTRODUCTION

The effects of irradiation on lipids have been investigated under widely varied conditions, such as the temperature of irradiation, the dose rate and the effect of post-irradiation conditions. However, the effect of ionizing radiation on lipids is to some extent not unlike changes brought about by heat and oxidation.

The chemical components which appear as a result of irradiation on lipid fractions in complex foods are qualitatively similar to those formed in model systems containing fats.

The changes which may take place in lipids upon exposure to high energy radiation are generally grouped as follows:

(1) gross changes in physical or chemical properties

(2) auto-oxidative changes

(3) non-oxidative radiolytic changes.

There is evidence, however, that under normal conditions several of these events may occur simultaneously and their effects may become superimposed (NAWAR, 1977).

(1) Gross changes:

Only very slight changes in the usual indices for fat quality have been observed when natural fats are irradiated below 5 Mrad. Definite increases in acid number, in trans fatty acid content and peroxide values have been

observed at doses between 10 and 100 Mrad (LUECK and KOHN, 1959; 1961; 1963).

(2) Autoxidative changes:

The spontaneous non-enzymic oxidation of lipids exposed to air is termed autoxidation. The autoxidation of unsaturated fatty acid occurs largely via a mechanism of free radical chains. The mechanism involves the removal of hydrogen atoms at position ' α ' to double bonds, followed by oxygen attacking at these locations, leading to the formation of peroxy radicals. These further abstract hydrogen from α — methylenic groups of other molecules to form hydroperoxides. The hydroperoxides decompose readily and give rise to a variety of breakdown products including aldehydes, aldehyde esters, oxo acids, hydrocarbons, alcohols, ketones, hydroxy-keto acids and dimeric compounds.

Irradiation accelerates this autoxidative process, by enhancing one or more of the following reactions:

(a) formation of free radicals which can combine with oxygen

(b) breakdown of hydroperoxides

(c) destruction of antioxidants.

It is not surprising therefore that oxidation products found by several investigators in irradiated model systems and in irradiated natural fats are identical to those usually present in non-irradiated but oxidised fats. The extent of irradiation induced oxidative changes depends on factors which, with the exception of dose and rate, are also typical of oxidation in the absence of irradiation, e.g. temperature, presence or absence of oxygen, composition of the fat, pro-oxidant and anti-oxidants (NAWAR, 1977).

TRUBY et al (1957) have also stated that irradiation produces free radicals in fats. The types of free radicals formed and their decay are influenced by temperature, since fats are more stable at low temperatures.

Among the methods most commonly used to determine the extent of autoxidation are:

(1) the peroxide value which is a measure of oxygen accumulation and
(2) the amount of carbonyl compounds formed, a measure of peroxide decomposition.

The carbonyl compounds produced in fats by irradiation have received considerable attention, particularly in view of their relevance in producing flavour and off-flavour. The carbonyls found in irradiated fats are thought to be largely derived from the normal decomposition of the hydroperoxides (MILCH and KLASSEN, 1965; MONTY et al, 1961). Higher quantities of carbonyls are produced with increasing doses or unsaturation. Changes in peroxide values and the formation of carbonyl compounds, esters, acids and hydrocarbons as a result of the irradiation of the various foods have been repeated by many workers. Of course, the results vary considerably, but this is not surprising in view of the complexity of food systems. The environment in which the fat fraction exists and the physical form of the lipid molecule in the various foods may influence their sensitivity to radiation. The rate of peroxide formation in dry fats is known to be different from that in dilute fat solution or in emulsion (WILLS and ROTBLAT, 1964).

In general, the factors which influence oxidative changes in irradiated

lipids include the type of material being irradiated, the total dose used, the presence or absence of oxygen, the temperature during irradiation, and the length of time under post-irradiated storage (CHIPAULT, 1962).

Certain interaction between the lipid and the other food constituents may also take place during irradiation. Protein (e.g. gelatin or serum albumin) was found to cause a marked reduction in the peroxides formed from pure fatty acids. BATZER et al (1957) have suggested that the carbonyls produced by irradiation of meat and meat fats do not contribute directly to the off-odours, but by reacting with compounds such as sulphydryl compounds and amines do impart off-flavours.

(3) Non-autoxidative radiolytic changes:

The major compounds formed when a saturated fatty acid is exposed to ionizing radiation in the absence of oxygen are hydrogen gas (H_2), carbon dioxide (CO_2), carbon monoxide (CO) and a series of hydrocarbons and aldehydes.

Among the radiolytic products of free fatty acids, CO_2 is formed in relatively large amounts. In general, the pattern of radiolytic compounds formed from unsaturated fatty acids is similar to that of saturated acids. Some hydrogenation of the unsaturated acid may also occur during irradiation, producing a small amount of the corresponding saturated fatty acid. In general, saturated fatty acids of low and high molecular weights are less affected by irradiation than are unsaturated fatty acids (NAWAR, 1977).

For the purpose of the present study, lipids were subdivided into two major groups, namely simple and complex lipids, in order to facilitate

analysis and to simplify the final discussion.

Fatty acids, one of the simple lipids, may accumulate as a result of the hydrolysis of either simple or complex lipids. The significance of these fatty acids in food is the development of a harsh acrid taste as a result of their liberation. This type of deterioration known as hydrolytic rancidity is quite common in olives, milk, cream, butter and nuts (BERK, 1976). Furthermore, the susceptibility of these fatty acids to oxidation is associated with the presence of their unsaturated bonds.

Similar to the fact that certain amino acids are considered essential, so are certain fatty acids. Acids with two or more double-bonds are known as "polyunsaturated acids" (e.g. linoleic acid). Polyunsaturated fatty acids perform certain important physiological functions, but they cannot be synthesized in the body fast enough and must be supplied in the food. They are referred to as "essential fatty acids." In the present study linoleic acid is the only essential fatty acid found in almonds.

Due to the importance and large contribution made by fatty acids in off-flavour development, this class of simple lipids is discussed in greater detail.

Detailed analyses of almond oil were carried out in the present study in order to assess the overall effect of irradiation on them. Furthermore, changes produced in these components after one year storage have also been studied here.

MATERIALS AND METHODS

The term LIPID is often used to cover a wide variety of substances which:

- are insoluble in water
- are soluble in organic solventssuch as chloroform, ether, hexane, benzene and methanol
- contain long chain hydrocarbon group in their molecules
- are present in or derived from living organisms

This definition covers a wide range of compounds including fatty acids and their derivatives, steroids, terpenes, carotenoids, wax esters, aldehydes, 'fat soluble' vitamins and bile acids, which all have in common a ready solubility in organic solvents.

CHRISTIE (1973) has used a more specific definition and the term nowadays is generally restricted to fatty acids and their derivatives or metabolites. It is in this sense that the term is used in this study.

The principal lipid classes consist of fatty acid moieties linked by an ester bond to an alcohol, principally the trihydric alcohol glycerol, or by amide bonds to long chain bases. Also, they may contain phosphoric acid, organic bases, sugars and more complex components that can be liberated by various hydrolytic procedures.

Lipids may be subdivided into two broad classes - SIMPLE, which contain one or two of these hydrolysis products per mole, and COMPLEX, which contain three or more types of hydrolysis products per mole. The terms

NEUTRAL and POLAR respectively are used more frequently to define these classes, but are less precise, and may occasionally lead to confusion, for example, unesterified fatty acids (free fatty acids) are normally classed as neutral lipid despite the presence of the carboxyl groups in their molecules.

Lipid extraction

Quantitative isolation of lipids free of non-lipid contamination must ideally be achieved before the lipid analysis itself can begin. Carelessness at this preliminary stage may result in the loss of specific components or in the production of artefacts. Lipids can be readily extracted from tissues by a number of organic solvents, but special precautions are necessary to ensure that the recovery is complete. Non-lipid contamination must then be eliminated from the extract by washing or by column chromatography procedures before the sample is ready for analysis. Precaution must be taken at each stage to minimise the risk of autoxidation of polyunsaturated fatty acids or of hydrolysis of lipids.

There are several procedures for lipid extraction in which different organic solvents are involved. All these methods have their own advantages and disadvantages. When choosing a procedure, consideration needs to be taken on the nature of the sample and the purpose of the experiment.

To extract lipids from tissues the following consideration should be taken into account:

(1) The solvent mixture should be sufficiently polar to remove all lipids from their association with cell membranes, and should not be

so polar that triglycerides and other non-polar simple lipids are not dissolved and are left adhering to the tissue. There must be complete recovery of all the lipids of the sample.

2. The non-lipid compounds must be eliminated from the extract.

3. The chemical used should not react chemically with these lipids.

4. Artefacts which may be produced under some circumstances must be prevented.

Taking these considerations into account, there are many different solvent systems suggested by different authors for extracting lipids from tissues. However, the extractibility of a tissue under investigation is variable and depends on the nature of the tissues and of the lipids.

Satisfactory methods for the preparation and purification of lipids have been described by FOLCH et al (1957) and by BLIGH and DYER (1959). These methods have been widely used and will be used in this study, except that certain necessary modifications have had to be made.

CHRISTIE (1973) has shown that a mixture of chloroform : methanol (2:1 V/V) extracted lipid more exhaustively from plant, animal or bacterial tissues than most other simple solvent systems. BLIGH and DYER (1959) have mentioned that this method recovered 95-99% of the lipid content of the sample. After comparing several methods, FISHWICK and WRIGHT (1977) found a chloroform-methanol mixture to be the most efficient.

LOUGH et al (1962) have found that if chloroform-methanol or any other alcoholic extract which contains lipids is stored for long periods in the presence of a very small amount of tissue, trans-esterification of many of

the lipids may occur and large amounts of methyl esters are found in the extracts. So, most extractions should be completed fairly quickly to avoid this trans-esterification.

It is well known that most polar organic solvents used to extract lipids from tissues also extract significant amounts of non-lipid contaminants (FOLCH et al, 1957) and a dilute salt solution (0.88% sodium chloride solution) at approximately a quarter of total solvent volume will help to remove almost all non-lipid contaminating compounds.

Procedures:

Ten grams of almond kernels were homogenised in 200 ml chloroform and 100 ml methanol in a Waring blender for 2 minutes. As almond kernels are too hard and cannot be properly crushed in the blender, they have to be ground in a mortar first (the mortar was washed thoroughly with the solvent). For filtration, Whatman No.1 filter paper and a sintered glass funnel were used. The extraction was carried out again on the residue with the same amount of solvent. Then the filtrates of both extractions were combined and 150 ml (a quarter of the total solvent volume) 0.9% of NaCl in distilled water was used for washing. After shaking, the phases were allowed to separate. This procedure has removed all extractable lipids after the second extraction, since further extraction did not yield any more lipids. The lower chloroform layer contains total lipids, while the upper aqueous methanol layer containing lipid contaminants was discarded.

Calculation:

From the total chloroform extract a certain volume (100ml) was removed and evaporated under vacuum at approximately 25°C to determine the total lipid content. This was calculated using the following formula:

$$\text{Total lipid content (\% dry weight)} = \frac{W \times V_1 \times 100}{a \times V_2}$$

in which:

W = weight of the oil after evaporation

V_1 = total volume of extract

V_2 = volume removed for evaporation

a = weight of the sample (dry weight)

The rest of the extract was evaporated to dryness and redissolved in 10 ml of benzene containing butylated hydroxy toluene (100 mg/l), which acted as an anti-oxidant. The lipid sample was stored at -18°C.

Fractionation and identification of Simple lipid classes

In general, plant lipids commonly contain triglycerides as the predominant simple lipid class together with sterol esters, sterols, partial glycerides and free fatty acids in addition to complex lipids. Apart from common simple lipids, trace amounts of hydrocarbons, methyl esters, wax esters and glyceryl esters may be found (HITCHCOCK and NICHOLS, 1971).

Two preliminary techniques used in an attempt to obtain the

different classes present in almond kernels were:

(a) solvent partition

and (b) thin layer chromatography

(a) Solvent partition (counter current distribution)

Solvent partition or counter current distribution is so often an effective preliminary step in fractionation of lipids which are particularly rich in glycerides, such as seed oil. In the counter current distribution procedure the mixture is partitioned between a suitable pair of immiscible solvents, the material in each phase being then repeatedly partitioned or distributed between the same solvent pair for as many times as is necessary to obtain a separation of the components. With simple mixtures of two or three components differing greatly in polarity, about four distributions will suffice.

Procedure: (GALANOS and KAPOULAS, 1962)

The distribution was carried out in two separatory funnels by the single withdrawal procedure (CRAIG and CRAIG, 1950) using the solvent system petroleum ether (b.p. 40-70°C) - 87% ethanol. Ten g. of total lipid extract were dissolved in 45 ml petroleum ether (upper) phase of the solvent system (pre-equilibrate) equal volume of petroleum ether and 87% ethanol); the solution was transferred to a separatory funnel and shaken with 15 ml of the lower ethanol phase. After 3 - 4 minutes, the equilibrated lower phase was transferred to a second funnel containing 45ml of upper (pre-equilibrated) phase, and 15 ml of (pre-equilibrated) lower phase was added to the first funnel. Both funnels were shaken and after 3-4 minutes the lower phase withdrawn from the second funnel to a flask, and the

lower phase of the first funnel transferred to the second one.

This partitioning procedure was repeated six times, except that no lower-phase solvent is added to the first funnel during the last partitioning step. The combined ethanol phases (8 x 15 ml) are then diluted with benzene and concentrated in vacuo on the rotary evaporator to yield >97% of the polar lipids contaminated with 0.02 - 0.03% of the neutral lipids. The bulk of the latter was recovered completely by evaporation of the combined petroleum ether phases.

Fractionation into simple and complex lipids

The above method of GALANOS and KAPOULAS (1962) was therefore used in the separation of lipids into simple and complex lipids.

(b) TLC using silica gel coated plates was found to be the most successful in terms of separating and identifying different lipid classes. For this purpose, the solvent systems of CHRISTIE (1973), hexane: diethyl ether: formic acid (80:20:2, V/V) and those of STORRY and TUCKLEY (1967), benzene: diethyl ether: ethyl-acetate: acetic acid (80:10:10:0.2, V/V/V/V) were tried. Since there was a better separation with the first solvent system (Fig. 16) this was used in the present study.

Experimental:

Thin layer plates (20 x 20 cm) plastic-backed, coated with a layer of 0.25 nm thick silica gel G, supplied by Mackery-Nagel and Co. Ltd, Germany, were used.

The samples, together with a number of different standard compounds

Fig 16: TLC analysis of simple lipids of almond oil on silica gel G layers.

F, S and H are samples
A, B and C are standards

Bands were identified as follows:

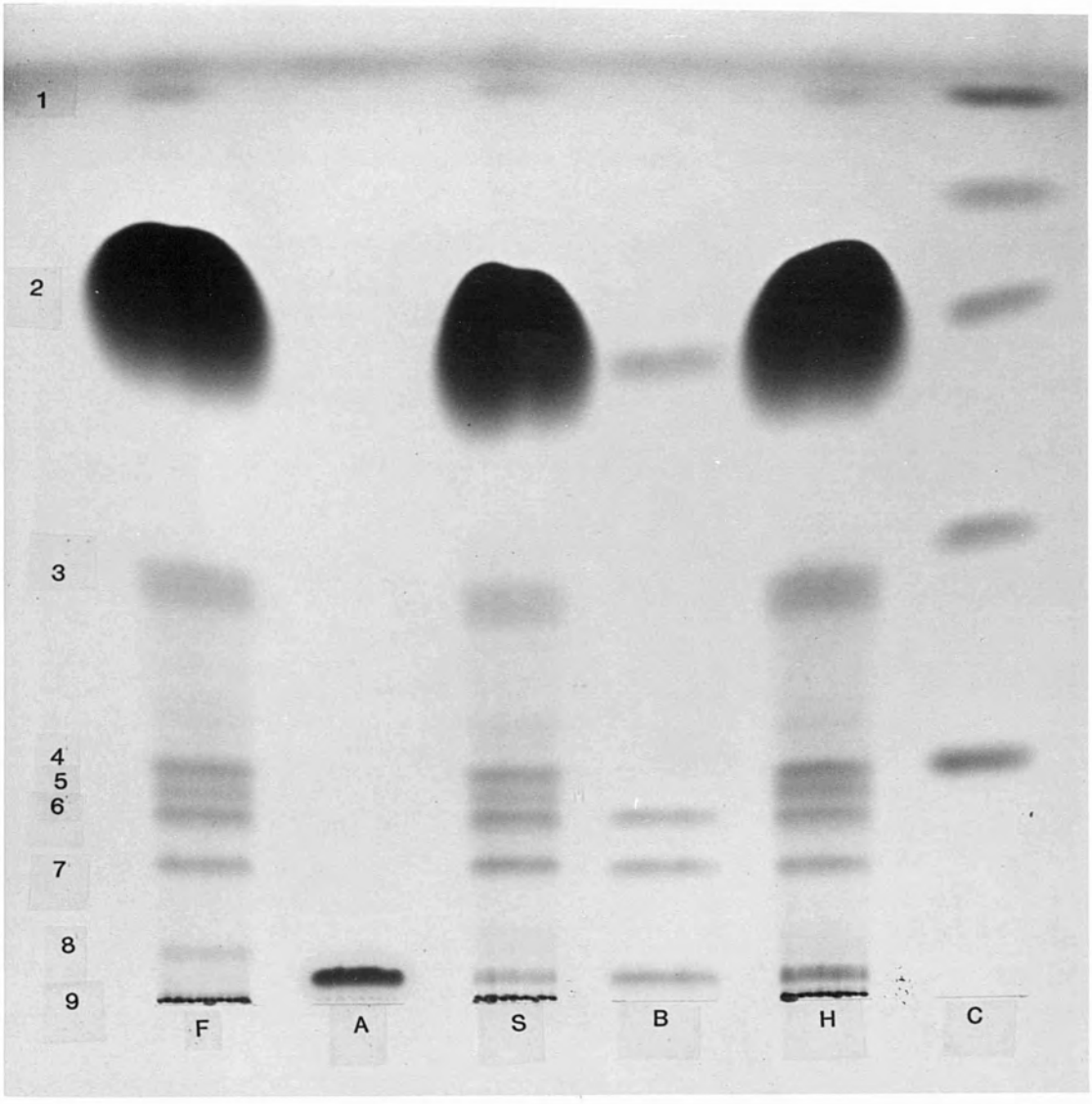
1. Sterol esters
2. Triglycerides
3. Free fatty acids
4. β -sitosterol
5. Free sterols
6, 7 Diglycerides
8. Monoglycerides
9. Origin

Developing solvent:

Hexane - diethyl ether - formic acid
(80 : 20 : 2, V/V)

Spray:

cupric acetate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were applied to the chromatoplates by graduated micro-syringes as bands 1.5 cm long with space of 0.5 cm between them and 2 cm from the edge of the plates. These were placed in an airtight chamber containing the already mentioned solvent system of CHRISTIE (1973).

The best way of obtaining a straight solvent front was by inserting plates in vertical positions with the back almost in complete contact with the chamber wall. It was found that if only one plate per chamber was used, good reproducibility could be obtained.

The solvent front was allowed to move to 4 cm from the top of the plate, which was then air-dried at room temperature for 4 hours.

The plates were ready for identification by using appropriate sprays (p.232).

FRACTIONATION AND IDENTIFICATION OF COMPLEX LIPID CLASSES

Complex lipids can be subdivided into three classes:

(i) Phospholipids which on hydrolysis yield glycerol, fatty acids, inorganic phosphate and an organic base or polyhydroxy compound.

(ii) Glycolipids which on hydrolysis yield glycerol, fatty acids and sugars.

(iii) Sphingolipids which contain a long chain base, fatty acids and inorganic phosphate, carbohydrate or other complex organic compounds.

Due to the difficulty of separating all complex lipid fractions by a single dimension TLC procedure (CHRISTIE, 1973), and because glycolipids tended to overlap phospholipids, several methods were used which will be discussed presently.

Separation of Glycolipids:

The technique used by GARDENER (1968) for isolating glycolipids gave good results, hence this was adopted. The TLC plates were developed in the following solvent:

acetone - acetic acid - water (100 : 2 : 1., V/V/V)

The simple lipids moved with the solvent front, while the phospholipids remained at or near the origin. The glycolipids were the compounds

that separated out under these conditions, and these were identified (p236).

Separation of complex lipids other than glycolipids

In order to separate these complex lipids, a two-step developing system was required. After trying various solvent systems it was found that those recommended by NICHOLS et al (1965) gave the best results.

The TLC plates were run first in the following solvent:

Petroleum ether: acetone (2 : 1, V/V) and then air-dried for 2 hours.

The plates were then run in the same direction in the second solvent of:

Chloroform - methanol - acetic acid - water (170:25:25:6, V/V) Fig. 17

The plates were air-dried overnight before spraying.

Two-dimensional TLC for complex lipids:

The problem of separating all phospholipids as well as glycolipids has previously been mentioned. In order to confirm that the separation was successful, and that there were no lipids masked by others (overlap), two-dimensional TLC was adopted. Different combinations of solvents were used but those of NICHOLS (1964) were found to give the best possible separation.

The method was as follows: ten µml of lipid sample was applied to a corner of a silica gel G plate as a single spot 2 cm from each edge. The plate was then developed in chloroform-methanol - 7

Fig 17: One-dimension two-step separation of complex lipids of almond oil on silica gel G-layers.

F, S and H are samples
A, B and C are standards

Bands were identified as follows:

1. Phosphatidic acid
2. Steryl glycoside
3. Sulpholipid
4. Phosphatidyl ethanolamine
5. Digalactosyl diglyceride
6. Phosphatidyl choline
7. Phosphatidyl inositol
8. Lysophosphatidyl choline

First developing solvent:
Petroleum ether - acetone
(2 : 1, V/V)

Second developing solvent:
Chloroform - methanol - acetic acid - water
(170 : 25 : 25 : 6, V/V)

Spray:
cupric acetate

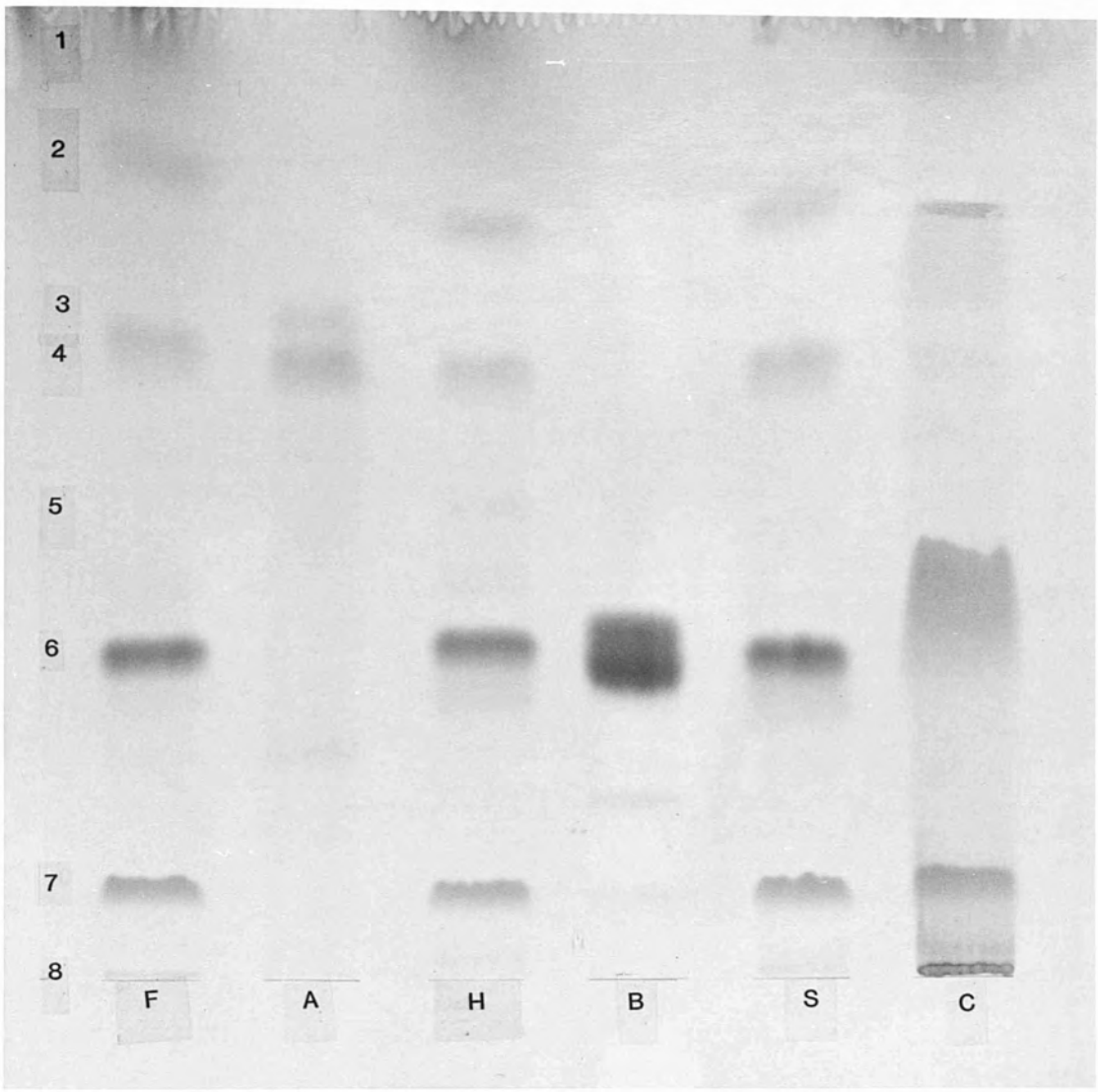
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Fig. 18: Two-dimensional TLC of complex lipids of almond oil on silica gel G layers.

1. sulpholipids
2. steryl glycoside
3. Phosphatidyl ethanolamine
4. Digalactosyl diglycerides
5. Phosphatidyl choline
6. Phosphatidyl inositol
7. Phosphatidic acid
8. Lyso phosphatidyl choline
9. Origin

First developing solvent:

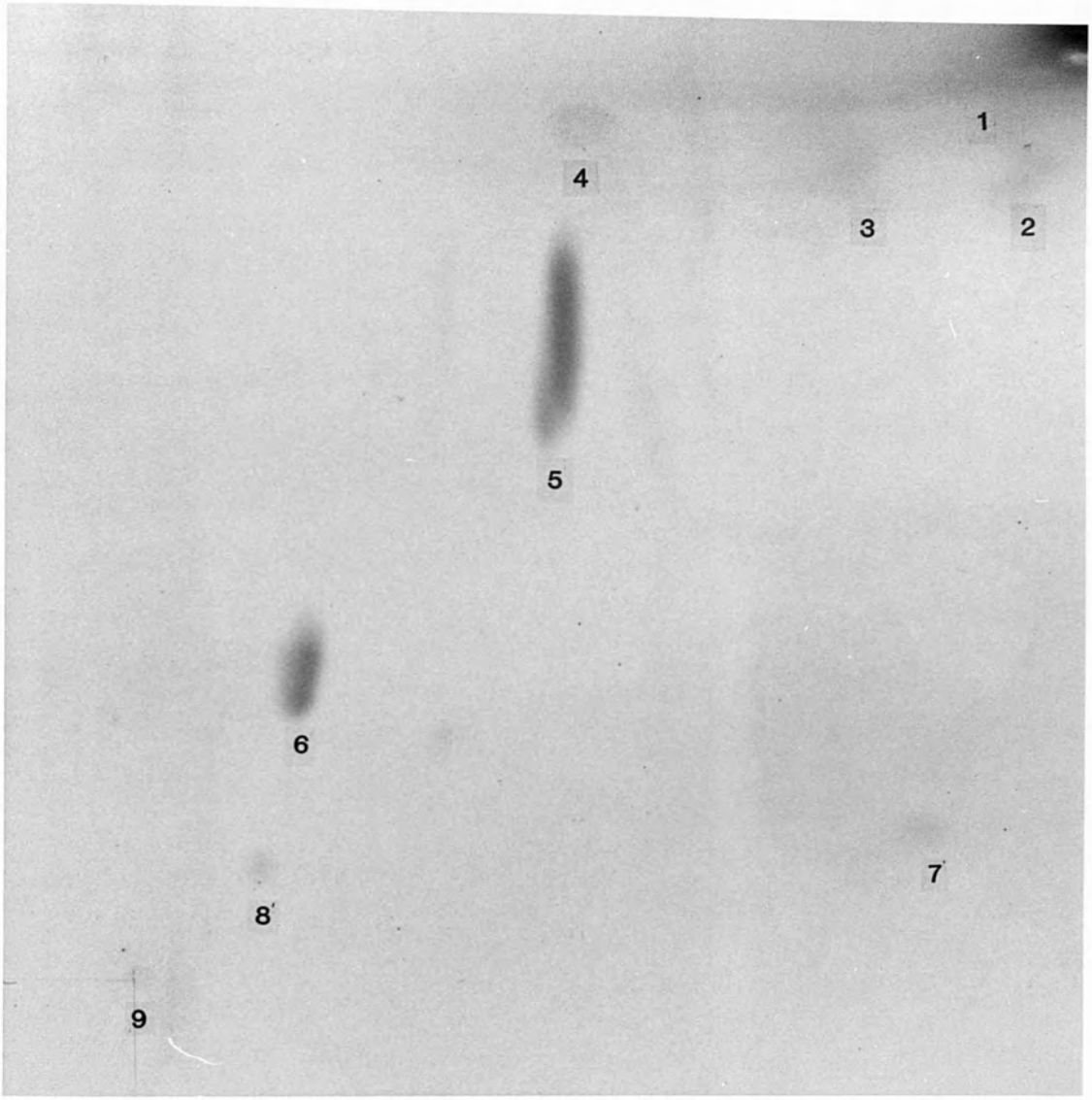
Chloroform - methanol - 7N ammonia
(65 : 30 : 4, V/V)

Second developing solvent:

Chloroform - methanol - acetic acid - water
(170: 25 : 25 : 6, V/V)

Spray:

cupric acetate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normal ammonia (65:30:4, V/V) in the first dimension. The plate was air-dried until all traces of ammonia had been removed (detected by odour). The plate was then turned 90° anticlockwise and placed in the second solvent system of chloroform-methanol-acetic acid-water (170:25:25:6, V/V/V/V) for developing in the second direction. The plate was then vacuum dried to remove traces of solvent.

Detection and Identification of lipid components

Detection of unknown lipid components on developed thin-layer plates was carried out by destructive or non-destructive, specific or non-specific reagents. They were identified by their developed colour or migration characteristics relative to authentic standards that were chromatographed alongside the unknown lipid mixtures on the same plate.

General Detection Tests

All the following are non-specific reagents, hence give positive reaction with all lipid classes:

1. Cupric acetate:

The chromatoplates were sprayed with cupric acetate solution (3g of cupric acetate in 100 ml of 8% phosphoric acid) until the surface became wet. The plates were then heated at 180°C for 25 minutes. This procedure minimises the evaporation of the volatile material and therefore there is little difference in the intensity of spots between saturated and unsaturated compounds (PRIVETT et al, 1973). This procedure was used for the quantification of lipids in the scanning densitometer.

2. Sulphuric acid

The chromatoplate was sprayed lightly with 50% sulphuric acid followed by heating at 180°C for 20 minutes. All lipids, as well as other organic non-volatile compounds formed dark brown spots on the white background (PRIVETT and BLANK, 1962).

3. 2', 7' - Dichlorofluorescein

The chromatoplates were sprayed with a 0.1% (W/V) solution of 2', 7' - dichlorofluorescein in 95% methanol. The lipids showed up immediately as yellow spots under U.V. light.

4. Iodine vapour

The chromatoplates were hung in a developing tank containing a few crystals of iodine. After a few minutes most lipids appeared as brown spots on a pale yellow background (SKIPSKI and BARCLAY, 1969).

Specific Reagents

1. Sterols and their esters:

Fifty mg ferric chloride ($FeCl_3$, $6H_2O$)were dissolved in 90 ml of distilled water with 5 ml acetic acid (glacial) and 5 ml concentrated sulphuric acid added. The developed plate was sprayed with the reagent, then heated at 100°C for 10 minutes when the presence of sterols and their esters was indicated by the appearance of a red-violet colour. The colour for sterols appeared slightly before that of their esters (CHRISTIE, 1973).

2. Free Fatty acids:

The developed plate was sprayed in turn with a 0.1% (W/V) solution

of 2', 7' - dichlorofluorescein in 95% methanol, a solution of 1% aluminium chloride in ethanol and finally with 1% aqueous ferric chloride, warming the plate briefly after each spray. Free fatty acids gave a rose-violet colouration (CHRISTIE, 1973).

3. Phospholipids

A spray reagent specific for phosphate esters on developed thin-layer plates was particularly useful in the identification of phospholipids. A modification of the molybdenum blue reagent of ZINZADZE (1935) as described by DITTMER and LESTER (1964) was used to identify phospholipids.

Solution I: To 1 litre of 25 NH_2SO_4, 40.11 g of molybdic anhydride (MoO_3) was added and the mixture was boiled gently in a fume cupboard until the MoO_3 dissolved.

Solution II: To 500 ml solution I, 1.78g of powdered molybdenum was added and the mixture was boiled gently for 15 minutes. The solution was cooled and decanted from any residue that may have been present.

Solution III: Equal volumes of solution I and II were mixed and the combined solution was mixed with two volumes of water. The final solution was greenish yellow in colour.

Procedure:

The developed plate was sprayed lightly until the absorbent was

uniformly damp. Components containing phosphate ester showed up immediately as blue spots on a white or light blue-grey background. The intensity of the colour increased on standing. After several hours the background darkened to a deep blue and the spots were obscured; therefore, plates could not be kept as a permanent record.

4. Phospholipids containing free amino groups

Phospholipids such as phosphatidyl ethanolamine, phosphatidyl serine and related lyso compounds that have free amino groups can be detected with the aid of a ninhydrin spray. The developed plate was sprayed with a solution of 0.25% ninhydrin in acetone, diluted with an equal volume of water immediately before use. Lipid fractions having free amino groups appeared red-violet when the plate was heated in an oven at 100°C for 10 minutes (DITTMER and LESTER, 1964).

5. Phospholipids containing choline

Phosphatidyl choline and lyso phosphatidyl choline gave a positive reaction with the following reagents:

Reagent I: 1.7g basic bismuth nitrate was dissolved in 100 ml of acetic acid.

Reagent II: 40g of potassium iodide was dissolved in 100 ml water.

Reagent III: 20 ml reagent I was mixed with 5 ml reagent II and 70 ml water added just before use.

Procedure:

After the plate was sprayed with reagent III the choline-containing

phospholipids appeared as orange to orange-red spots immediately or after warming at 40°C for 5-10 minutes (WAGNER et al, 1961).

6. Sphingolipids

Cerebrosides and sphingolipids give positive reaction with the clorox-benzidine spray.

Reagent I: 50 ml benzene was mixed with 5 ml clorox bleach (trade name of commercial bleach; active reagent sodium hypochlorite) and 5 ml of glacial acetic acid.

Reagent II: 200 ml of benzidine dihydrochloride and a small crystal of potassium iodide were dissolved in 50 ml of 50% ethanol and filtered.

Procedure:

The plate was sprayed immediately with freshly-prepared reagent I, left at room temperature for 30 minutes, dried under hot air (this was done in the fume cupboard) for 10 minutes and then sprayed with reagent II (this reagent was used within two hours of preparation and was protected from direct light). Sphingolipids appeared as blue spots almost immediately (SKIPSKI and BARCLAY, 1969).

7. Glycolipids

As well as the rather negative use of iodine in conjunction with non-specific reagents to detect glycolipids, a number of more positive highly-specific reagents are available for the detection of the carbohydrate moieties. The reagents most widely used are: (i) an orcinol sulphuric acid

mixture, and (ii) α naphthol.

(i) Orcinol spray

The reagent was prepared by dissolving 200g orcinol in 100 ml of 75% sulphuric acid. The whole surface of the developed plate was wetted by spraying and then heated in an oven at 100°C for 10 minutes. Glycolipids appeared as blue-violet spots on a white background. The solution is stable for about one week if refrigerated and kept in the dark (SVENNERHOLM, 1956).

(ii) α — Naphthol

Half a gram α- naphthol was dissolved in 100 ml methanol-water (1:1, V/V) and sprayed on the developed plate until the surface was wet. After air drying at room temperature, the plate was sprayed lightly with 95% sulphuric acid, then heated at 120°C in an oven when glycolipids appeared as purple-blue spots and other complex lipids as yellow spots (SIAKOTOS and ROUSER, 1965).

Quantification of lipid components

Simple lipid and complex lipid fractions were quantified by densitometry after they were separated on thin-layer plates, sprayed with cupric acetate reagent and charred in an oven at 180°C for 20 minutes. All lipid classes were charred (MARSH and WEINSTEIN, 1966). Each series of zones were cut out and scanned in a chromoscan double beam recording and integrating densitometer (JOYCE LOEBL and Co. Ltd.) with quartz iodine light source, blue filter and 20cm x 1cm light slide and sample-holder (drive in gear ratio was 1:1). The area of the peaks on the recorder trace is proportional to the amount of lipid originally present in the sample (CHRISTIE, 1973) and in authentic standards. Individual bands were estimated

using the following formula:

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

There are considerable doubts that there is a linear relationship between the density of a spot and the amount of lipid present (CHRISTIE, 1973). However, in the present study, results with the densitometer scan were generally reliable as they compared favourably with those of the weighing method.

Gas-liquid Chromatogarphy (GLC) of fatty acids:

Gas-liquid chromatography, a technique first introduced by JAMES and MARTIN (1956), is the method of choice for a rapid, quantitative analysis of volatile lipid components such as fatty acid esters, fatty alcohol, etc.

In this procedure the fatty acids are first converted to a volatile form like methyl esters. The commonest chromatographic stationary phases used for fatty acid analyses are polyester-packing materials: polyethylene - glycol - adipate (PEGA) and diethylene - glycol succinate (DEGS) (HITCHCOCK and NICHOLS, 1971).

Polyester columns resolve fatty acid esters according to both chain length and their degree of unsaturation. The increase in chain length gives increased retention times. Two components with identical chain lengths elute according to the number of double bonds they contain, increasing the number gives increasing retention times.

When components of methyl ester fraction are unknown, they can frequently be identified by a comparison of their retention time with those of reference substances analysed under the same conditions.

Procedures:

Total fatty acid saponification and esterification

Saponification of lipid classes leading to the release of fatty acid components, and their subsequent methylation was carried out using the rapid technique of METCALFE et al, (1966) as modified by VAN -

WIJNGAARDEN (1967).

Approximately 150 mg of lipid was added to a 50 ml round bottom flask equipped with a ground glass joint. Two ml of 0.5 normal methanolic sodium hydroxide was added and the flask connected to a helicoil condenser. The mixture was boiled under total reflux on an electrically heated hot-plate until the fat globules went into solution (2-5 minutes). Two ml of boron trifluoride - methanol (14% boron fluoride in methanol BF_3) was added via the condenser and the boiling continued for 2 minutes. Two ml of heptane was then added via the condenser and the mixture boiled for another two minutes before removing the flask for cooling. Enough saturated sodium chloride solution was poured in to bring the liquid level up to the neck of the round bottom flask. One ml of the upper (heptane) layer was pipetted into a glass stoppered sample tube, dried over Na_2SO_4 and ready for direct injection into the gas chromatographic column.

Free fatty acid esterification

Essentially the same method as already described for total fatty acid esterification was used, with one modification, namely that the 2-5 minutes boiling of lipid with 0.5 normal methanolic sodium hydroxide was omitted.

Gas-liquid chromatography

Fatty acid methyl esters were determined with a PYE series 104 gas chromatograph equipped with a hydrogen flame detector. Glass column (1.5m in length and 2mm in diameter) was packed either with 10% polyethylene glycol-adipate (PEG-A) on Chromosorb W. AN-DMCS, 60-80 mesh or with 15% diethylene-glycol succinate (DEGS) on Chromosorb 60-80 mesh, both

supplied by Phase-Separation Ltd., Clwyd, Great Britain. The gas chromatograph was operated with H_2 pressure of 12 p.s.i. and air pressure of 10 p.s.i., with chart speed of 76.2 cmh^{1} on a Speedomax W recorder (Leeds and Northrup Co., England).

Two temperature conditions were investigated:

(a) temperature programme from 80°C to 190°C at 8 min^{-1}

(b) isothermally at 190°C.

No difference was observed using those two temperature conditions and better resolution was obtained with PEG-A column, so all work was therefore carried out on a PEG-A column isothermally at 190°C as it was the faster of the two. Fig 19 shows the GLC of fatty acid methyl esters.

Identification and Quantification

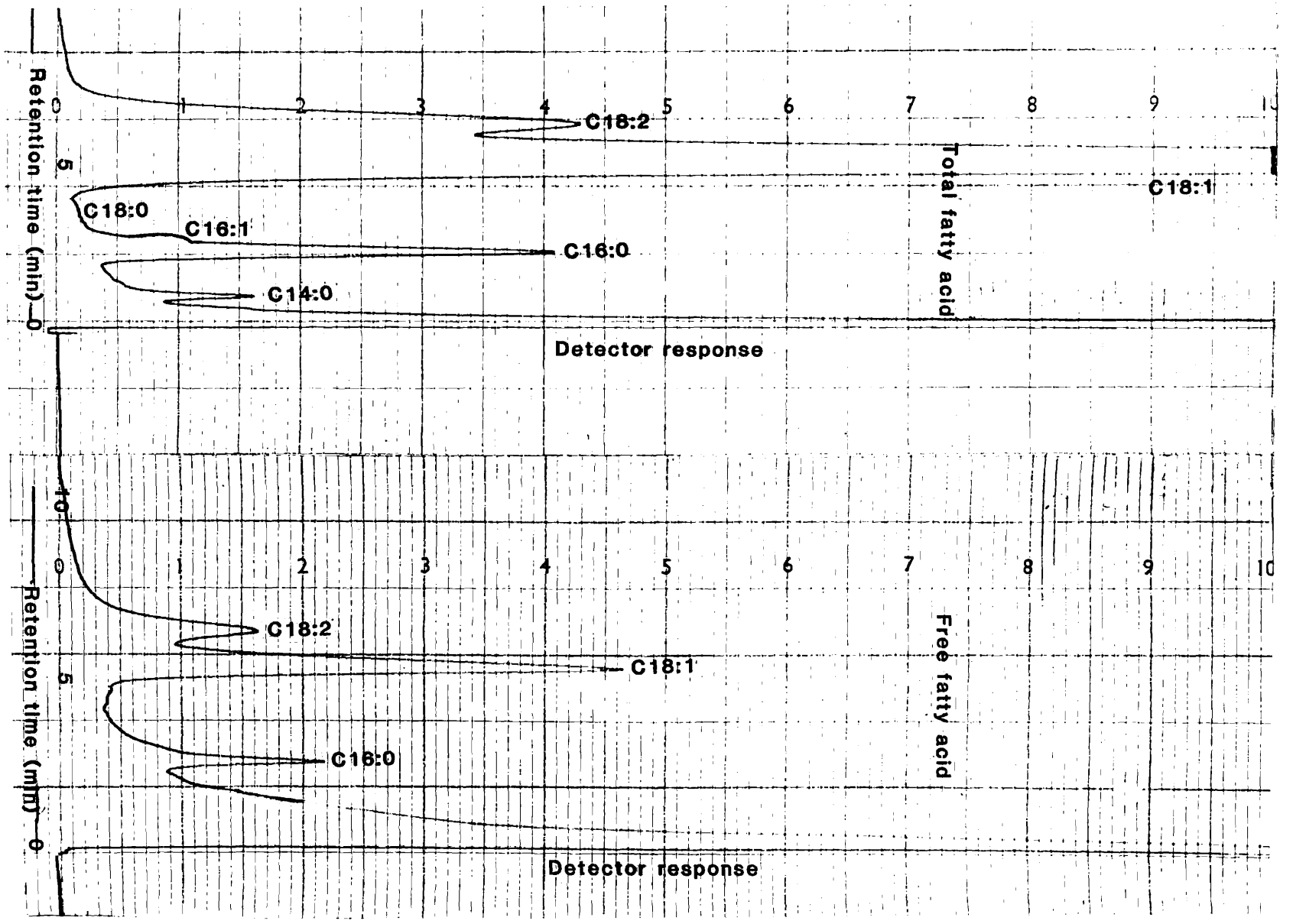
Standard mixtures of known fatty acid methyl esters obtained from Applied Science Products (Pierce and Warriner (UK); Chester, UK) were used for comparison of retention times, identification and the quantification of fatty acid methyl esters in each sample. In every case unsaturated acids emerged later than the corresponding saturated ones, and the degree of retention depended on the number of double bonds present (NICHOLS et al, 1966). The amount of each fatty acid ester was calculated as follows:

$$\frac{\text{Peak area of standard}}{\text{Peak area of sample}} \equiv \frac{\text{Amount of fatty acid in standard}}{\text{Amount of fatty acid in sample}}$$

Other calculations used were:

relative concentration of each fatty acid which was calculated by

Fig. 19: The separation of free and total fatty acids' methyl esters by Gas-Liquid Chromatography on a 150 cm PEGA column at 190°C.


Retention time (min)
0
1
2
3
4
5
6
7
8
9
10
C18:2
C18:1
C18:0
C16:1
C16:0
C14:0
Detector response
Total fatty acid
Retention time (min)
0
1
2
3
4
5
6
7
8
9
10
C18:2
C18:1
C16:0
Detector response
Free fatty acid

triangulation of the peak area on the chromatogram and was expressed as a percentage of the total peak area (DOGRASS et al, 1977):

$$\text{Percentage peak i} = \frac{\text{Peak area i}}{\text{Peak area}} \times 100$$

Iodine value :

The iodine value of an oil or fat is defined as the weight of iodine absorbed by 100 parts by weight of the sample. The glycerides of the unsaturated fatty acids present (particularly of the oleic acid series) unite with a definite amount of halogen and the iodine value is therefore a measure of the degree of unsaturation. It is constant for a particular oil or fat, but the exact figure obtained depends on the particular technique employed.

The iodine value is often the most useful figure of identifying an oil or at least placing it into a particular group. It should also be noted that the less unsaturated fats with low iodine value are solid at room temperature or conversely, oils that are more highly unsaturated are liquids (showing that there is a relationship between the melting points and the iodine values). A further point of interest is that, in general, the greater the degree of unsaturation (i.e. the higher the iodine value), the greater is the liability of the oil or fat to go rancid by oxidation.

The iodine number is determined by dissolving a weighed sample of fat or oil (0.1g to 0.5g) in chloroform or carbon tetrachloride and adding an excess of halogen. After standing in the dark for a controlled period

of time, the excess, unreacted iodine is measured by thiosulphate titration.

Two methods in general use are (1) the Hanus and (2) the Wijs methods. The AOAC gives both methods, while the AOCS has endorsed the WIJS method. However, the Hanus method is stated as an alternative procedure in BS 684 (PEARSON, 1976).

In the Hanus method the standard iodine solution is made up in glacial acetic acid and contains not only iodine but iodine bromide which accelerates the reaction. The Wijs method uses an iodine solution made up in glacial acetic acid, but contains iodine chloride as the accelerator. The excess iodine reacts with sodium thiosulphate according to the following equation:

$$2\,Na_2SO_3 + I_2 \longrightarrow 2\,NaI + Na_2S_4O_6$$

The end point is determined by disappearance of blue-starch-iodine colour.

Both methods were used in preliminary studies, and since negligible numerical differences were found between the two methods, throughout this study, the Hanus method was used, as it was the more rapid of the two.

Hanus Method

1. Hanus iodine solution: 13.2g pure iodine was dissolved in one litre glacial acetic acid with slight heating. After cooling 3 ml Br_2 was added to produce the double halogen.

2. Potassium iodide solution: 15g KI was dissolved in 100 ml distilled water.

3. Sodium thiosulphate solution; 0.1N and 0.01N accurately prepared.

4. Starch indicator solution; 1% starch in distilled water.

Procedure:

In a 500ml glass stoppered flask, approximately 0.2g of almond oil was weighed accurately and dissolved in 10 ml chloroform. Then 25 ml of Hanus iodine solution was added by pipette(carefully)and allowed to stand for exactly 30 minutes in the dark with occasional shaking. Ten ml of 15% potassium iodide and 100 ml of freshly boiled and cooled distilled water was then added, washing down any free iodine on the stopper. The iodine in the solution was titrated with standard 0.1N and 0.01N thio-sulphate, which was added gradually with constant shaking until the yellow solution turned almost colourless. Starch indicator (0.5 ml) was added and the titration continued until the blue colour disappeared. Towards the end of the titration the flask was shaken vigorously, so that any iodine remaining in the chloroform layer might be taken up by the potassium iodide solution.

Calculation

Two blank determinations were carried out along with determination on the sample. The number of ml 0.1N $Na_2 S_2O_3$ required by blank minus ml used in the determination on the sample, gives $Na_2S_2O_3$ equivalent of iodine absorbed by the oil. Then:

$$\text{Iodine number} = \frac{(B - S) \times N \times 12.69}{W}$$ where

B = blank titration

S = sample titration

N = normality of $Na_2S_2O_3$

W = weight of sample

Peroxide Value

The peroxide value is a measure of the peroxides contained in the oil. During storage, peroxide formation is slow at first (during an induction period), which may vary from a few weeks to several months according to the particular oil or fat. The temperature and other variables must be borne in mind when interpreting quantitative results.

The peroxide value in usually determined volumetrically (PEARSON, 1976). These depend on the reaction of potassium iodide in acid solution with the bound oxygen followed by titration of the liberated iodine with sodium thiosulphate (MEHLENBACHER, 1960). Chloroform is normally used as solvent. The following method gives rapid results.

Reagents

1. Acetic acid - chloroform(3:2 V/V) solution
2. Saturated potassium iodide solution in recently boiled distilled water. The critical point is that the solution must remain saturated as indicated by the presence of undissolved crystals.
3. Sodium thiosulphate solution O.1N and 0.01N accurately standardised.
4. Starch indicator solution, 1% of starch in distilled water.

Procedure

In a 250 ml glass stopper flask, approximately 5.0 g of almond oil

was weighed accurately and then 30 ml of the acetic acid - chloroform (3:2, V/V) solution was added. The flask was shaken until the sample was dissolved in the solution. Then 0.5 ml of saturated potassium iodide was added and the solution was allowed to stand with occasional shaking for exactly 1 minute, at which point 30 ml of distilled water was added. Titration was carried out on the solution using 0.1N and 0.01N sodium thiosulphate with constant and vigorous shaking until the yellow colour has almost disappeared. Then 0.5 ml of starch indicator solution was added and the flask was shaken vigorously to liberate all the iodine from the chloroform layer. Titration was continued until the blue colour just disappeared.

Calculation

The peroxide values of samples were calculated using the following formula:

Peroxide value as milli-equivalent of peroxide per 100 g of sample

$$= \frac{S \times N \times 1000}{W}$$ where:

S = titration of sample

N = normality of sodium thiosulphate solution

W = weight of sample

Malonaldehyde

Malonaldehyde (MA) has generally been associated with oxidative rancidity in food (SINNHUBER and YU, 1958; KONING and SILK, 1963). However, it has also been found in small amounts in a number of fresh supermarket products including meats (BIDLACK et al, 1972), fish (KUUSI et al, 1975), vegetable oil (ARYA and NIRMALA, 1971) and orange juice essence (BRADDOCK and PETRUS, 1971). So an increase in MA is an index of rancidity.

Recently, interest in the possible significance of MA in human health has been stimulated by reports that it is mutagenic (MUKAI and GOLDSTEIN, 1976) and carcinogenic (SHAMBERGER et al, 1974). BROOKS and KLAMERTH (1968) found evidence that MA reacts with deoxyribonucleic acid (DNA) suggesting a possible rationale for its mutagenic effects.

While it is still too early to definitively assess the oncogenic significance of MA in humans, its chemical and toxicological properties are impressive enough to warrant serious consideration regarding its effects on health.

The reaction between thiobarbituric acid (TBA) and lipid oxidation products yield a red colour which has been used as a measure of oxidative deterioration in food.

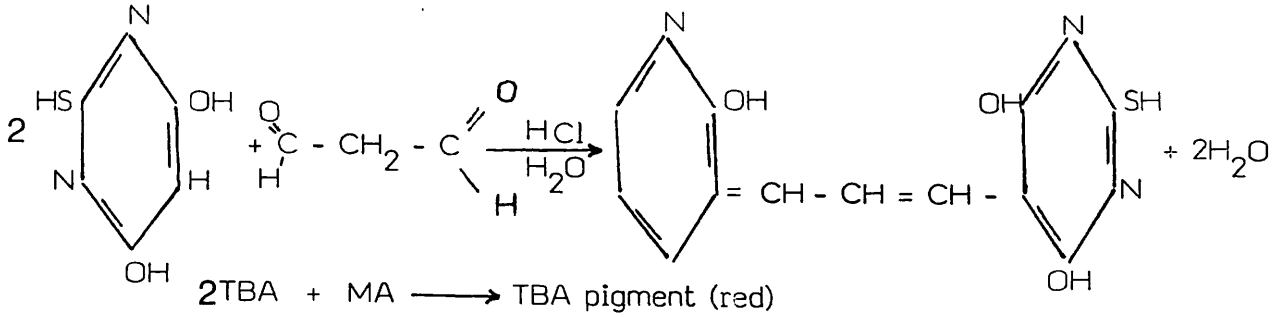


FIG 20: PROPOSED FORMATION OF TBA PIGMENT FROM MA

(SINNHUBER et al., 1958)

The chemistry of the reaction is largely unknown, but several workers have noted the correlation between the intensity of the colour and the degree of fat oxidation.

MA can be determined by the distillation method and by a modification of the filtration methods of TARLADGIS et al, (1964). The distillation method gives higher values than the filtration method. This finding suggests several possibilities:

(a) the filtration method gives incomplete extraction of endogenous MA

(b) the hot condition associated with the distillation method promotes the release of MA.

Distillation of ground almond nuts from a slightly acidified concentrated NaCl solution gives a high percentage recovery of MA which is associated with rancidity in nut meat products. Heating an aliquot of the distillate in an acetic solution of 2 thiobarbituric acid yields an intense red colour with a maximum absorbance at about 532 nm. As little as 0.1 µg MA/ml can be measured. Recoveries of MA added to samples ranged from

92 to 97%. No MA was detected in fresh nut meats.

In this study, MA was determined as an index of oxidative rancidity in nuts. The tests most commonly used for determining rancidity in food are the peroxide value test, the carbonyl determination test, the Kreis test, TBA test and organoleptic test (THIEBOLD and AURAND, 1963; MEYER, 1978).

The 2-thiobarbituric acid (TBA) test has been the subject of a number of papers during the last few years (SINNHUBER and YU, 1958 and SIDWELL et al.,1955).

The reactant substance present in the rancid nut meat has been identified as the dicarbonyl compound, malonaldehyde (YU and SINNHUBER, 1964).

A literature review of the numerous procedures employing the TBA test was conducted. It was found that the basic steps consist of:

(a) heating the food product in the presence of a strong acid, and

(b) condensing the liberated MA with TBA (YU and SINNHUBER, 1962 and TARLADGIS et al, 1960)

The procedure used in the present study had

(a) an additional step in the TBA procedure of TARLADGIS et al. (1960).

(b) the boiling point of the solution was elevated by the addition of sodium chloride in order to obtain better than 90% recoveries of MA.

Reagents:

1. TBA reagent: 0.3% 2-thiobarbituric acid (BDH Chemicals Ltd.) in 90% acetic acid; dissolved by warming in a hot water bath.

2. Malonaldehyde tetraethyl acetal standard (1, 1, 3, 3 tetra-ethoxy propane, TEP)(BDH Chemicals Ltd).

3. Stock solution A: 0.31g TEP was dissolved in 10 ml 95% alcohol. This solution contains 1 mg/ml as MA and can be kept several days if stored in a refrigerator.

4. Stock solution B: 10 ml stock solution A was transferred to 250 ml volumetric flask and diluted to volume with distilled water and mixed. This solution contains ca.40 µg/ml as MA.

5. Acetic acid,90%.

6. Hydrochloric acid, 1.5N.

7. Antifoam, DOW Corning

Standard Curve

1.0, 2.0, 3.0, 4.0 and 5.0 ml stock solution B were pipetted into each of five 200 ml volumetric flask and diluted to volume with distilled water (working standards range from ca. 0.2 to 1.0 µg/ml. Five ml from each of the five working standard solutions were transferred to 50 ml glass-stopper tubes, mixed thoroughly with 5 ml of TBA reagent and immersed in a boiling water bath until maximum colour was developed (ca.35 min at 95°C). The tubes were cooled to room temperature and

absorbance of each standard solution was determined in 1 cm cell against a reagent blank by scanning from 400 to 600 nm (maximum occurring at ca·532 nm). Absorbance at 532 nm was plotted against concentration of final solution (final solution range from 0.1 to 0.5 µg ml).

Distillation

Depending upon the degree of apparent rancidity, 1.5 g ground almond meats were weighed on ashless filter papers [Whatman 42]. The paper containing the sample was transferred to a 500ml Kjeldahl flask containing 25g NaCl. Two hundred and fifty-five ml distilled water containing 5 ml 1.5N HCl was added. The reagent blank was treated similarly and to both a few drops of antifoam added. One hundred and ninety five ml distillate was collected in ca. 25 min. using a 200 ml volumetric flask, ensuring that the tip of the delivery tube was almost touching the bottom of the flask.

Determination

Five ml distillate was pipetted into 50 ml glass-stoppered tube to which 5 ml TBA reagent was added. Tubes were then stoppered and after mixing thoroughly were immersed in a boiling water bath until maximum colour was developed (ca. 35 min, at 95°C). They were then cooled to room temperature, and a portion of the solution transferred to a 1 cm spectrophotometric cell and the absorbance determined against a reagent blank. The concentration of MA was calculated from a prepared standard curve:

$$\text{mg MA / 1000 g sample} = \frac{\text{µg MA/ml final solution} \times 400}{\text{(g) sample}}$$

A standard curve prepared from 1, 2, 3, 4 and 5 ml of aliquot of stock solution B through the distillation procedure indicated that losses during distillation were small.

The standard curve also shows that the red TBA-MA complex follows Beer's law over the concentration and range stated.

RESULTS AND DISCUSSION

Total lipids of almonds

The term 'total lipids' refers to the cumulative value of all lipid classes extracted by the procedures mentioned in the section 'materials and methods.' Although the procedures used are reported to be exhaustive, small amounts of lipids may be tightly bound to other molecules (e.g. protein) which makes their complete extraction difficult.

Results of table 41 show that the lipid content of the three varieties of almonds range from 460.0 to 560.0 mg g^{-1} dry weight. This implies that by far the most important constituent of almonds is the oil which makes up approximately half of the kernels by weight (46 to 56%).

MEHRAN and FILSOOF (1975) have shown that the lipid content of nine varieties of Iranian almonds ranged from 55.4 to 61.7%. These results do not differ greatly from those reported in the present study. Similarly, NASSAR et al, (1977) have found that total lipids range from 53.7 to 59.3% in eight different varieties of almonds grown in Egypt. OSBORNE and VOOGT (1978) have stated the lipid value to be 55% without specifying the variety of almonds studied. However, very low lipid content of 35% was reported by GUTFINGER et al, (1972) for the Ne-Plus Ultra variety of almonds.

In the present study, although the range of total lipid content lies within that reported in the literature, there is a significant difference between the values of the three varieties. Whereas S contains the lowest

Table 41: The effect of 2 Mrad irradiation on total simple, total complex and total lipids of almond nuts, with % retention compared to controls.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt ± SE					
F	C	506.8 ± 28.7	18.4 ± 1.2	525.2 ± 32.5	95.6	88.0	95.4
	I	484.7 ± 29.5	16.2 ± 1.1	500.9 ± 33.7			
S	C	443.3 ± 27.2	17.3 ± 0.9	460.6 ± 26.0	95.1	96.5	95.2
	I	421.7 ± 23.6	16.7 ± 1.2	438.4 ± 27.3			
H	C	542.8 ± 30.8	17.7 ± 1.1	560.5 ± 32.8	96.2	95.5	96.2
	I	522.3 ± 31.5	16.9 ± 1.1	539.2 ± 34.9			

mean lipid value (460 mg g^{-1}), H contains the highest (560 mg g^{-1}) and F lies in between (525 mg g^{-1}). However, there is no significant difference between the latter two values (table 41).

Effect of irradiation on total lipids

The lipid content of the three varieties of irradiated almonds are represented in table 41. There was no significant change in the total lipid content in any of the varieties used after being irradiated with a dose of 2 Mrad. However, it is not possible to say that lipids are not affected by 2 Mrad dose without considering individual lipid constituents.

It is to be noted that all the degradative products after lipid irradiation such as carbonyl compounds and peroxides are soluble in these solvents which are normally used in lipid extraction, therefore one would not expect to observe a significant difference in total lipids after irradiation.

Simple lipids of almonds

Simple lipids made up most of the total lipids, as F, S and H contained 506.8, 443.3 and 542.8 mg g^{-1} respectively (table 41), corresponding to approximately 96% total lipids. Further it is to be noted that the ratio of simple lipids to total lipids remained fairly constant irrespective of almond variety.

Seven individual simple lipid classes were identified (table 42). These were monoglycerides, diglycerides, sterols, free fatty acids, triglycerides, sterol esters and the hydrocarbons. Diglycerides were further divided into 1, 2 and 1,3- diglycerides by comparison with standards, while sterols were regarded as either

Table 42: The effect of 2 Mrad irradiation on individual simple lipids of almond oil.

Simple lipids	F		S		H	
	C	I	C	I	C	I
	mg g^{-1} dry wt.					
MG	15.4	11.2	10.3	7.1	12.3	9.2
1,2 DG	28.3	30.1	28.1	31.2	30.1	32.9
1,3 DG	38.7	39.9	39.3	41.7	40.2	43.7
FS	3.4	3.3	2.6	2.7	2.2	1.8
β -SI	19.1	18.0	17.1	16.2	18.2	16.7
FFA	3.1	5.3	3.5	5.1	2.8	4.9
TG	391.7	371.5	333.6	317.9	432.5	413.7
SE	1.6	1.7	1.2	1.5	1.3	1.3
HC	T	T	T	T	T	T

β- sitosterol, and other sterols which were not identified further.

Triglycerides were by far the major class comprising on average 78.3% simple lipids. These results are not unlike those of NASSAR et al, (1977). Diglycerides and sterols form an average of 13.8% and 4.2% simple lipids. All other simple lipids were found in smaller quantities. β -sitosterol, the major sterol, formed approximately 86.7% total sterols. GUTFINGER et al (1972) have also found that β-sitosterol was the major sterol (97%) of almond oil.

Effect of irradiation on simple lipids

The results in table42show that total simple lipids are not significantly affected after 2 Mrad irradiation treatment in any of the almond varieties studied.

Due to the similar trend followed by the simple lipids in all three varieties of almonds these are discussed on a general basis.

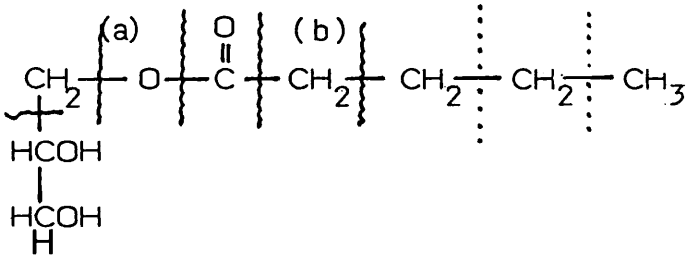
Although the free fatty acid content increased and monoglycerides tended to decrease, other components were not significantly different. These results are not unlike those of RAO and NOVAK (1973) who found that a chicken-based wet pet food subjected to 4.5 Mrad did not change the relative composition of the total lipid extracts or of the triglyceride fraction compared to non-irradiated controls.

Also, RAO et al, (1978) have reported that no gross change in chemical composition of wheat lipids occurred when wheat kernels were irradiated up to 1 Mrad. This was further supported by TIPPLES

and NORRIS (1965) who stated that lipids in cereal are degraded generally at very high dose level.

The significant increase in free fatty acids may be indicative of some changes due to irradiation. In the present study as there were no changes in triglycerides and in diglycerides, but a significant decrease in monoglycerides, then there is a possibility that cleavage of ester linkage occurred in the monoglyceride molecule. This in effect may have caused the release of the component free fatty acid molecules.

Based on the results available to date, the mechanisms of radiolysis in monoglycerides appear to proceed largely via a specific route in agreement with the concept of WILLIAMS (1962) concerning the location of the primary ionization events in oxygen-containing compounds. In a monoglyceride molecule,



radiolytic cleavage occurs preferentially at five locations in the vicinity of the carbonyl group (solid lines), and randomly at all the remaining C-C bonds (dotted lines) in the fatty acid moiety. The resulting free radicals are terminated by hydrogen abstraction, and to a lesser degree by loss of hydrogen with the formation of an unsaturated linkage. Cleavage at site 'a' and 'b' would result in the release of fatty acids and hydrocarbons respectively.

It would appear probable that a build up ot hydrocarbons would also

be indicative of damage to lipid caused by irradiation. Studies by SHEPPARD and BURTON (1946) have indicated that irradiating free fatty acids resulted in the formation of CO_2, H_2, fatty acids and hydrocarbons among the products. KAVALAM and NAWAR (1969) found that the major radiolytic products (essentially hydrocarbons) were few in number but were dependent largely on the fatty acid composition of the lipids. These observations were confirmed by BEKE et al, (1974 and 1975).

Results of the present study show trace amounts of hydrocarbons in the raw and irradiated almonds, suggesting that the effect of irradiation on the simple lipid fraction was very small. The stability of these lipids to irradiation may be attributed to the presence of natural anti-oxidants in almond oil, possibly in the form of α—tocopherol. Moreover, GREEN and WATTS (1966) have suggested that irradiation in certain cases may result in the formation of a new antioxidant factor which can improve product stability. It is thought that this was due to the possible induced formation of an aldehyde-amine complex. Other workers, on the other hand, have concluded that irradiation reduces the stability of lipids in food by destroying antioxygenic factors. TIPPLES and NORRIS (1965) have found that 52% of the tocopherol content was retained in Manitoba wheat and 88% in English wheat after irradiation with 1 Mrad. However, it may be possible that the tocopherol content of almond oil is above the optimum concentration for antioxidant effect and that even though irradiation destroyed a large proportion of tocopherol, a sufficient amount was left to give the oil a near maximum stability.

Fatty acids of almonds

Free fatty acids of almonds

Table 43 shows the individual free fatty acids of almond oil. Three fatty acids were identified: C16:0 (palmitic), C18:1 (oleic) and C18:2 (linoleic) acids. C18:1 was the most abundant, averaging 46.5% total free fatty acids followed by C18:2 with 34.9% and finally C16:0 making up the remaining 18.5%.

The ratio of unsaturated to saturated fatty acids is defined as the desaturation or unsaturation ratio (HITCHCOCK and NICHOLS, 1971). Any changes in this value after treatment may indicate either that hydrogenation of unsaturated fatty acids was occurring or that saturated fatty acids were being degraded.

The unsaturation ratio for free fatty acids in varieties F, S and H was found to be 5.2, 6.0 and 3.0 respectively.

Total fatty acids of almonds

Table 44 gives the total fatty acids found in almond oil on a percentage basis. Five fatty acids were identified: myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2). In general, taking all three varieties of almonds into account, the average value for myristic, palmitic, stearic, oleic and linoleic were 2.6%, 14.4%, a trace, 49.8% and 33.1% respectively.

In contrast to the results obtained with free fatty acids, the unsaturation ratio for total fatty acids in all three varieties of almonds had a narrower range of between 4.7 and 5.0. The iodine value ranged from

Table 43: The effect of 2 Mrad irradiation on free fatty acids and unsaturation ratio of almond oil.

Fatty acids	F		S		H	
	C	I	C	I	C	I
	mg/g dry wt.					
C16:0	0.5	0.7	0.5	0.7	0.7	1.0
C18:1	1.5	2.7	1.7	2.3	1.2	2.3
C18:2	1.1	1.9	1.3	2.1	0.9	1.6
Total	3.1	5.3	3.5	5.1	2.8	4.9
Unsaturation ratio	5.2	6.6	6.0	6.3	3.0	3.9

Table 44: The effect of 2 Mrad irradiation on (a) fatty acids, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of almond oil

Fatty acids	F		S		H	
	C	I	C	I	C	I
	% total fatty acids					
C14:0	2.8	5.6	2.5	4.3	2.6	4.8
C16:0	14.7	19.4	14.2	20.2	14.3	21.5
C16:1	T	T	T	T	T	T
C18:0	T	T	T	T	T	T
C18:1	49.3	43.9	50.2	45.7	49.8	41.6
C18:2	33.2	30.7	32.9	29.5	33.2	31.5
U.R.	4.7	3.0	5.0	3.1	4.9	2.8
I.V. **	98.2±6.1	93.6±6.3	99.3±6.5	94.5±5.1	98.5±3.1	92.5±4.6

(** $cgI_2\ g^{-1}$ oil)

98.2 to 99.3 (table 44).

NASSAR et al (1977) studying eight different varieties of almonds grown in Egypt, found that the unsaturation ratio for total fatty acids ranged from 3.1 to 12.6 with a corresponding iodine value of between 94.5 and 100.7. The values in the present study are well within this range. These are similar to the values cited for almond oil by WOODROOF (1979), namely 5.1 (unsaturation ratio) and 99.4 (iodine value). In contrast, MEHRAN and FILSOOF (1975) have found that the unsaturation ratio for total fatty acids of nine different varieties of Iranian almonds were at the top end of the range observed by NASSAR et al (1977) (i.e. from 10.7 to 12.4) and therefore much higher than the values obtained in the present study, while the iodine value was very similar. The highest values for the unsaturation ratio of almond was that of GUTFINGER et al., (1972), who recorded a value of 14.6, while no iodine value was reported. On the other hand, MAYER (1978) has reported an iodine value between 93 and 103.4, with the unsaturation ratio was not given.

It is apparent from all these results that on the whole, the iodine value is not related to the unsaturated ratio of total fatty acids: the iodine value is fairly constant, while the unsaturation ratio varies from 3.1 to 14.6.

Effect of irradiation on fatty acids:

Free fatty acids

The results of the effect of 2 Mrad irradiation on the free fatty acids are given in table 43. Increases in all three of the individual fatty acids (C16:0, C18:1 and C18:2) were noted in all cases. The increases in individual fatty acids is not accompanied by any change in the unsaturation ratio of all three varieties. Since the total increase in C18:1 and C18:2 were greater than the increase in C16:0, it was expected to observe increases in unsaturation ratio, which, in fact, was not the case in the present study.

Although an increase in free fatty acid content generally reflects the onset of deterioration in lipid, other tests namely iodine value, peroxide and malonaldehyde determinations are needed to be carried out in order to determine the extent of deterioration in the lipids or irradiated almonds.

Total fatty acids

The effectsof 2 Mrad irradiation on fatty acids as a percentage of total fatty acids, unsaturation ratio and iodine value for all three varieties of almonds are given in table 44.

A significant increase in C14:0 and C16:0 with a decrease in C18:1 and C18:2 are noted. Although the decreases in C18:1 and C18:2 were not significant at the 5% level, it had been proposed that the breakdown of C18:1 and C18:2 may have been as in Fig 21 , either through:

(1) hydrogenation:

$$C18:2 \xrightarrow{H_2} C18:1 \xrightarrow{H_2} C18:0$$

and/or through

(2) degradation:

$$C18:0 \longrightarrow C16:0 \longrightarrow C14:0 \longrightarrow \text{smaller units}$$

FIG 21: HYDROGENATION AND SUBSEQUENT DEGRADATION OF LINOLEIC ACID (BERK, 1976)

It also follows from the sequence in Fig·21 that hydrogenation reactions could have occurred prior to breakdown. It is also possible that hydrogenation of the unsaturated acid may occur at the same time as breakdown. This latter explanation is consistent with the absence of a C18:0 build up.

NAWAR (1977) has found that irradiating herring oil at 1 Mrad caused the complete disappearance of C20:2, C20:3, C20:4 and C20:5 fatty acids. No change in the qualities of the short-chain saturated fatty acids C12:0, C14:0 or C16:0 were found. It seems therefore that oil of lower M.W. fatty acids (in this case almond oil) shows better stability to irradiation than oils containing higher M.W. fatty acids (e.g. herring oil) since even at 2 Mrad irradiation almond oil is less affected than herring oil at 1 Mrad irradiation. The results of the present study show without doubt that 2 Mrad irradiation has but very little effect on the nutritional value of almond oil.

Complex lipids of almonds:

Total complex lipids (table 41) amounted to 18.4, 17.3 and 17.7 mg g^{-1}

in varieties F, S and H respectively, which were not significantly different from one another, and corresponded to an average value of 3.5% total lipids. The low contribution of complex lipids to total lipids may explain the absence in the literature of data concerning complex lipids. To the author's knowledge the complex lipids of almond oil have not been studied to date.

Eight separate classes of complex lipids were identified by the use of one and two dimensional TLC (table 45). These consisted of five phospholipids namely lyso-phosphatidyl choline (LPC), phosphatidyl inositol (PI), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidic acid (PA), and three glycolipids namely digalactosyl diglyceride (DGDG), sulpholipid (SL) and sterol glycosides (SG).

Effect of irradiation on complex lipids

The total complex lipids of the three varieties of almonds were not significantly affected by 2 Mrad irradiation (table 41). Among the individual complex lipids only phosphatidyl inositol decreased significantly (table 45). This may explain the observed increase in free inositol within the sugar fraction reported previously in chapter 3.

MIRONOVA et al (1970) have observed that when sunflower oil phospholipids were treated with 58 Krad, the inositol fraction was the least resistant to breakdown. The latter would, in theory, lead to a build up of phosphatidic acid. This however was not the case in the present study, as there was no increase in phosphatidic acid after 2 Mrad irradiation. The reason for this is unknown, although, of course, some of the phosphatidic acid could have been degraded further. So, there may have been a breakdown

Table 45: The effect of 2 Mrad irradiation on individual complex lipids of almond oil.

Complex lipids	F		S		H	
	C	I	C	I	C	I
	mg/g dry wt.					
LPC	2.2	2.1	2.1	2.2	1.9	1.9
PI	3.2	1.2	2.3	1.6	2.5	1.7
PC	6.3	6.4	5.9	5.5	6.6	6.4
DGDG	0.3	0.2	0.3	0.4	0.4	0.4
SL	1.2	1.1	1.1	1.1	0.9	1.1
PE	2.3	2.4	2.4	2.5	2.2	2.1
SG	2.1	1.9	2.2	2.2	2.3	2.2
PA	0.8	0.9	1.0	1.2	0.9	1.1

of phosphatidic inositol which may have resulted in the release of fatty acids, alcohol and free phosphate residues.

Quality indices

The immediately recognisable effect of lipid oxidation in food is the development of undesirable odours and off flavours. The chemical identity of a large number of 'rancid' products of lipid oxidation has been determined. These are largely short chain carbonyl compounds formed as a result of peroxide decomposition. The overall organoleptic nature of rancidity depends somewhat on this system.

Rancidity tests have been developed over the years in order to establish sensitive control of the stability of fats or the foods in which they are used (DAUBERT and O'CONNELL, 1953). The common tests are peroxide value and determination of carbonyl compounds (e.g. malonaldehyde).

Peroxide value of almonds:

This index indicates the amount of all substances which oxidise potassium iodide in terms of milli-equivalent of peroxide per 1000 g of sample. These substances are generally assumed to be peroxides or other similar products of lipid oxidation.

Table 46 shows the peroxide values for the different almond samples. Those reported for varieties F, S and H were 2.9, 4.6 and 4.2 milli-equivalents per 1000 g of almond oils respectively. Peroxide values for F and H were significantly different from each other. It is possible that this may be due to the different handling procedures before the samples arrived in the laboratory.

Table 46: The effect of 2 Mrad irradiation on (a) peroxide (PV) and (b) the malonaldehyde (MA) value of almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1} of oil and (b) mg MA 1000 g^{-1} of nuts.

		Peroxide	MA
		meq. $1000g^{-1}$	mg $1000g^{-1}$
F	C	2.9 ± 0.2	5.6 ± 0.4
	I	3.5 ± 0.2	7.4 ± 0.5
S	C	4.6 ± 0.3	4.4 ± 0.3
	I	5.3 ± 0.3	6.3 ± 0.4
H	C	4.2 ± 0.3	4.6 ± 0.3
	I	5.1 ± 0.4	6.4 ± 0.6

It was interesting to find that for the almond samples studied by MEHRAN and FILSOOF (1975) the peroxide values were found to range from 0 to 0.4. These values were substantially lower than those found in the present study. It may be that low peroxide values are directly related to the presence of low quantity of free fatty acids. In the sample of MEHRAN and FILSOOF (1975) free fatty acid amounts ranged from 0.02 to 0.06% of the oil. This was much lower than the 0.5 to 0.7% free fatty acid content found in the varieties F, S and H.

Malonaldehyde value of almonds:

In recent years the malonaldehyde colour reaction with 2-thiobarbituric acid (TBA) has been widely used to estimate oxidative rancidity in fatty materials (SIU and DRAPER, 1978; NEWBURG and CONCON, 1980).

The formation of malonaldehyde in food during processing seems to be dependent on many factors, including the degree of unsaturation of fatty acids and the time that the lipids are in contact with oxygen. However, the amounts found in samples of processed food also depend very much on the loss of malonaldehyde due to its volatility or to its reactivity.

In this study malonaldehyde content of F, S and H were found to be 5.6, 4.4 and 4.6 mg/1000 g almonds. The amount of malonaldehyde mg / 1000 g in two different dried raisins were 1.4 to 2.1, tomato ketchup contained 1.0 mg while French dressing 1.0 to 1.4 mg. Chopped walnuts were found to contain 2.0 to 6.4 mg / 1000 g and a freshly opened jar of peanut butter had no malonaldehyde, but after the jar had

been opened and in use for an unspecified amount of time, 1.2 mg $1000g^{-1}$ malonaldehyde was found. Fresh fruit generally contained little or no malonaldehyde (SHAMBERGER et al, 1977).

The studies of ARYA and NIRMALA (1971) have shown that malonaldehyde values may differ considerably even within samples of the same food products. They found that the malonaldehyde content of 2 samples of ground nut oil were 4.4 and 0.5 mg respectively. A similar case was observed here since the means of the malonaldehyde values of variety F were significantly different from those of variety S and H which were very similar (table 46).

Effect of irradiation on peroxide and malonaldehyde content

Table46also shows the peroxide and malonaldehyde values for the three varieties of almonds after 2 Mrad irradiation.

It was found that the average increase in peroxide after treatment was 0.7 milli equivalent / 1000g oil. This was a significant increase and suggested that irradiation treatment had induced some lipid deterioration in almonds.

Malonaldehyde content also showed a significant increase of between 1.8 to 1.9 mg / 1000g nut and added further support to the suggestion that some lipid deterioration had actually taken place.

KONING and SILK (1963) have stated that the peroxide and malonaldehyde values were useful criteria of lipid decomposition during the early stages of oxidation. However, as both these products are labile, they may be decomposed during storage hence may be of less importance subsequently.

The carbonyl compounds produced in lipids by irradiation have received considerable attention, particularly in view of their recognised flavour and importance in causing off-flavours. The carbonyls found in irradiated fats are thought to be largely derived from the normal decomposition of hydroperoxides which themselves originate from peroxides. MONTY et al., (1961) have shown that a low dose irradiation (0.5 Krad) in a model system induced autoxidation of linoleic acid and the formation of peroxide. It is worth mentioning that the chemicals which arise as a result of irradiation on lipids in food are qualitatively similar to those formed by irradiating lipid in model systems. However, they may be quantitatively different due to the other food components present in food but absent in model systems. Also similarities exist between the autoxidation products formed by irradiated fats in model system and in natural fats, and by non-irradiated but oxidised fats (NAWAR, 1977). The difference lies in the fact that irradiation accelerates the autoxidative process, particularly if oxygen is present during or subsequent to the irradiation treatment. This may be caused by the enhancing effect of irradiation on one or more of the following reactions:

formation of free radicals which can combine with oxygen, and destruction of antioxidants.

It is therefore not surprising that in the present study the increase in peroxide and malonaldehyde was significant, thus suggesting that some lipid deterioration had taken place after 2 Mrad irradiation.

EFFECT OF STORAGE ON LIPIDS OF IRRADIATED ALMONDS

Total Lipids

Immediately after irradiating the almond samples, they were stored in plastic bags at a temperature of 22°C. In order to determine the effect of storage on lipids of irradiated almonds, comparisons were made between results taken immediately after irradiation and those after one year storage.

No changes in total lipid content of irradiated-stored almonds were observed after one year storage (table47).

The total lipid content of control-stored almonds was found not to be significantly different from that of controls (table 48). It may therefore be said that storage of irradiated almonds has no detrimental effect in terms of maintaining total lipid content.

Simple lipids

Total simple lipid content of irradiated-stored almonds was not significantly changed after one year storage (table 47). Individual simple lipids are given in table 49. From this table it may be observed that there were no significant changes in any of the individual simple lipids in irradiated-stored almonds. This was unexpected, as from the report of BERK (1976) subsequent storage of irradiated food had resulted in magnifying small differences observed after irradiation. He has also stated that certain oils, which normally exhibit high stability are strongly affected by irradiation. Thus, rancidity can be a problem in irradiation preserved lard and bacon. The effect of irradiation is mainly connected with increased free radical

Table 47: The effect of one year storage on the total simple, total complex and total lipids of irradiated almond nuts.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt. ± SE					
F	I	484.7 ± 29.5	16.2 ± 1.1	500.9 ± 33.7	97.0	107.0	97.0
	I-S	470.2 ± 28.9	17.4 ± 1.3	487.6 ± 25.3			
S	I	421.7 ± 23.6	16.7 ± 1.2	438.4 ± 27.3	96.0	101.0	96.5
	I-S	406.3 ± 27.5	16.9 ± 0.9	423.2 ± 26.5			
H	I	522.3 ± 31.5	16.9 ± 1.1	539.2 ± 34.9	96.0	102.9	96.4
	I-S	502.5 ± 29.6	17.4 ± 1.0	519.9 ± 30.0			

Table 48:The effect of one year storage on the total simple, total complex and total lipids of control almond nuts.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt., ± SE					
F	C	506.8 ± 28.7	18.4 ± 1.2	525.2 ± 32.5	97.3	95.0	97.2
	C-S	493.1 ± 34.4	17.5 ± 1.1	510.6 ± 34.6			
S	C	443.3 ± 27.2	17.3 ± 0.9	460.6 ± 26.0	97.8	94.2	97.6
	C-S	433.5 ± 35.1	16.3 ± 0.8	449.8 ± 30.8			
H	C	542.8 ± 30.8	17.7 ± 1.1	560.5 ± 32.8	96.9	95.5	96.8
	C-S	525.9 ± 30.6	16.9 ± 0.9	542.8 ± 31.4			

Table 49: The effect of one year storage on individual simple lipids of irradiated almond oil.

Simple Lipids	F		S		H	
	I	I-S	I	I-S	I	I-S
	mg g^{-1} dry wt.					
MG	11.2	10.3	7.1	8.3	9.2	9.9
1,2 DG	30.1	31.7	31.2	31.9	32.9	34.9
1,3 DG	39.9	38.5	41.7	39.8	43.7	40.6
FS	3.3	2.9	2.7	2.8	1.8	2.1
β -SI	18.0	17.4	16.2	16.9	16.7	17.2
FFA	5.3	5.7	5.1	5.9	4.9	5.3
TG	371.5	353.2	317.9	279.8	413.7	372.5
SE	1.7	1.5	1.5	1.6	1.3	1.3
HC	T	T	T	T	T	T

Table 50: The effect of one year storage on individual simple lipids of almond oil.

Simple lipids	F		S		H	
	C	C-S	C	C-S	C	C-S
	mg g^{-1} dry wt.					
MG	15.4	16.7	10.3	11.9	12.3	12.9
1,2 DG	28.3	30.4	28.1	29.2	30.1	32.5
1,3 DG	38.7	39.9	39.3	41.5	40.2	43.6
FS	3.4	3.2	2.6	2.4	2.2	2.0
β-SI	19.1	19.7	17.1	16.9	18.2	18.7
FFA	3.1	5.2	3.5	6.1	2.8	4.1
TG	391.7	343.7	333.6	291.5	432.5	384.2
SE	1.6	1.4	1.2	0.9	1.3	1.1
HC	T	T	T	T	T	T

formation which is evident from the observation that rancidity develops not only during irradiation, but upon storage of irradiated food.

Total simple lipid content of control-stored almonds was not significantly changed after one year storage (table 48). Individual simple lipids are given in table 50. After one year storage the amount of triglycerides in control-stored almonds appeared lower but showed no significant difference when compared to controls. Among the other simple lipid fraction only free fatty acids increased significantly. From this, the assumption made (p261) that the presence of natural anti-oxidants acted as protective agents by preventing the deterioration of lipid is not likely to be true. Compounds such as tocopherol would also be present in untreated almonds. So, the lipids of irradiated almonds seem to be better preserved than those of control-stored almonds. Similar results have been obtained by GREEN and WATT (1966) who have shown that irradiated beef, pork and poultry meat stored in air tight conditions for several months showed less tendency to deterioration than non-irradiated meat. Also the lipid component of irradiated wheat flour was affected less during six months' storage than that of non-irradiated flour (TIPPLES and NORRIS, 1965). As stated earlier (p.261)they suggested the possibility that irradiation induced formation of other antioxidant factors, the latter acting in lipid preservation. However, different food may be affected differently by similar treatments.

One of the few studies of the nutritional consequences of eating irradiated oil has been reported by PLOUGH et al (1957). After feeding pork (irradiated at 2.79 Mrad and stored for one year at room temperature) to human subjects, identical apparent digestibility values for treated and untreated fats were obtained. Similarly, MOORE (1961) has reported that

after feeding corn oil irradiated at doses of 2.79 and 5.58 Mrad to rats the digestibility of lipids was not adversely altered.

Fatty acids:

Free Fatty acids

Table 51 represents the data for free fatty acids of irradiated-stored almonds. No significant changes were observed as a result of storing irradiated almonds for one year compared to irradiated samples before storage. This supports the previous observation that no changes appeared in simple lipid components due to the storage of irradiated almonds.

It was found that there was a significant increase in the free fatty acids of control-stored almonds when compared to controls, (table 52). The unsaturated fatty acids were more affected than were saturated ones. The source of these fatty acids may tentatively be said to come from triglycerides (table 50).

Total fatty acids

Individual fatty acids of irradiated-stored almonds were expressed as a percentage of total fatty acids and are shown in table 53.

One of the interesting features which emerged after storage of irradiated almonds was that both C16:1 and C18:0 increased significantly from trace amounts. The build up of C18:0 would suggest that hydrogenation of C18:1 and C18:2 may have occurred during storage. However, the unsaturation ratio was not significantly altered, nor was iodine value.

NAWAR (1977) has stated that the introduction of one double bond

Table 51: The effect of one year storage on free fatty acids and unsaturation ratio of irradiated almond oil.

Fatty acids	F		S		H	
	I	I-S	I	I-S	I	I-S
	mg/g dry wt.					
C16:0	0.7	0.7	0.7	0.8	1.0	0.9
C18:1	2.7	2.9	2.3	2.7	2.3	2.4
C18:2	1.9	2.1	2.1	2.4	1.6	2.0
Total	5.3	5.7	5.1	5.9	4.9	5.3
Unsaturation ratio	6.6	7.1	6.3	6.4	3.9	4.9

Table 52: The effect of one year storage on free fatty acids and unsaturation ratio of almond oil.

Fatty acids	F		S		H	
	C	C-S	C	C-S	C	C-S
	mg/g dry wt.					
C16:0	0.5	0.6	0.5	0.6	0.7	0.6
C18:1	1.5	2.7	1.7	3.0	1.2	1.9
C18:2	1.1	1.9	1.3	2.5	0.9	1.6
Total	3.1	5.2	3.5	6.1	2.8	4.1
Unsaturation ratio	5.2	7.7	6.0	9.2	3.0	5.8

Table 53: The effect of one year storage on (a) fatty acids, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of irradiated almond oil.

Fatty acids	F		S		H	
	I	I-S	I	I-S	I	I-S
	% total fatty acids					
C14:0	5.6	6.7	4.3	5.7	4.8	6.8
C16:0	19.4	21.3	20.2	22.4	21.9	20.7
C16:1	T	1.2	T	2.2	T	1.2
C18:0	T	3.5	T	3.2	T	2.9
C18:1	43.9	38.9	45.7	39.9	41.6	38.6
C18:2	30.7	28.2	29.5	26.2	31.5	29.3
U.R.	3.0	2.2	3.1	2.2	2.8	2.3
I.V.**	93.6±6.3	84.6±5.2	94.5±5.1	89.9±5.6	92.5±4.6	86.5±3.3

(** $cgI_2\ g^{-1}$ oil)

Table 54: The effect of one year storage on (a) fatty acid, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of control almond oil.

Fatty acids	F		S		H	
	C	C-S	C	C-S	C	C-S
	% total fatty acids					
C14:0	2.8	4.1	2.5	3.8	2.6	3.4
C16:0	14.7	16.3	14.2	16.9	14.3	15.7
C16:1	T	1.3	T	0.9	T	1.0
C18:0	T	2.5	T	1.2	T	1.5
C18:1	49.3	45.6	50.2	46.7	49.8	47.2
C18:2	33.2	29.8	32.9	30.2	33.2	31.1
U.R.	4.7	3.3	5.0	3.6	4.9	3.9
I.V.**	98.2±6.1	93.7±7.1	99.3±6.5	91.5±5.7	98.5±3.1	89.7±6.5

(** $cgI_2\ g^{-1}$ oil)

in fatty acids is not unexpected and is often reflected in radiolytic compounds produced after irradiation treatment. Hence there are two possible sources of C16:1 formation: either from C16:0 or from C18:1. However, if the iodine value is maintained as is the case in the present study, then it would be logical to assume that C16:1 was produced from C16:0 while C18:0 resulted from the hydrogenation of C18:1.

Table 54 shows individual fatty acids as a percentage of total fatty acids in control-stored almonds. Increases in C16:1 and C18:0 are noted once more. This suggests that the above observation regarding changes produced after storage of irradiated almonds was not necessarily due to irradiation. These changes were probably induced by enzymes acting on fatty acids.

Complex lipids

Total complex lipids (table 47) were not significantly changed in one year stored irradiated almonds compared to those of irradiated almonds before storage. This correlates directly with the absence of any noticeable changes in total and simple lipids in the corresponding samples. The fact that one of the free sugars, inositol, did not accumulate further after storage of irradiated almonds reported earlier (p.95) is added evidence that lipids remain stable during storage, especially since it is known that phosphatidyl inositol is one of the most sensitive of phospholipid compounds to irradiation.

Table 56 gives the content of individual complex lipids in control-stored almonds. Although total complex lipid content was not significantly changed (table 48), phosphatidyl inositol was the only lipid which was significantly

Table 55: The effect of storage on individual complex lipids of irradiated almond oil.

Complex lipids	F		S		H	
	I	I-S	I	I-S	I	I-S
	mg/g dry wt.					
LPC	2.1	2.0	2.2	2.2	1.9	2.1
PI	1.2	1.3	1.6	1.7	1.7	1.7
PC	6.4	6.6	5.5	5.3	6.4	6.6
DGDG	0.2	0.4	0.4	0.3	0.4	0.3
SL	1.1	1.3	1.1	1.1	1.1	0.9
PE	2.4	2.6	2.5	2.7	2.1	2.4
SG	1.9	2.0	2.2	2.3	2.2	2.1
PA	0.9	1.2	1.2	1.3	1.1	1.3

Table 56: The effect of one year storage on individual complex lipids of almond oil.

Complex lipids	F		S		H	
	C	C-S	C	C-S	C	C-S
	mg/g dry wt.					
LPC	2.2	2.1	2.1	2.2	1.9	2.0
PI	3.2	2.4	2.3	1.3	2.5	1.5
PC	6.3	6.6	5.9	6.0	6.6	6.7
DGDG	0.3	0.2	0.3	0.2	0.4	0.3
SL	1.2	0.9	1.1	0.9	0.9	0.7
PE	2.3	2.4	2.4	2.7	2.2	2.1
SG	2.1	1.9	2.2	2.1	2.3	2.1
PA	0.8	0.8	1.0	0.9	0.9	1.1

reduced in amount during storage. This was also reflected by the accumulation of free inositol in the free sugar fraction (p.97).

Peroxide and malonaldehyde

Peroxide and malonaldehyde values for freshly irradiated almonds and for those stored for one year are given in table 57.

The peroxide values of the three varieties of almonds did not differ significantly from those of freshly irradiated almonds, nor was the malonaldehyde content significantly affected. This suggests that one year storage of almonds irradiated by 2 Mrad did not have any further effect on the breakdown of lipids of freshly irradiated almonds.

A similar situation exists with untreated almonds (control) after one year storage (table58), where neither the peroxide nor the malonaldehyde values were affected. These observations however are different from those of COLEBY et al (1962) who found that the peroxide values in sausages irradiated with 1 Mrad rose linearly to 17 mg / 1000 g after 30 days storage at 0°C, while that of untreated samples hardly changed. Apart from the fact that the two tissues used are very different indeed, the result of COLEBY et al (1962) and those of the present study may possibly be explained in terms of the temperature of storage. HANNAN and SHEPHERD (1954) have observed that the formation of peroxide in fats was markedly dependent on post-irradiated storage. Irradiated butter fat stored at 0°C increased in peroxide content slightly, while that stored at 20°C showed no significant increase.

One possibility could be that storage at low temperature immediately

Table 57: The effect of one year storage on peroxide (PV) and the malonaldehyde (MA) value of irradiated almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1}oil, and (b) MA 1000 g^{-1} nuts.

		Peroxide	MA
		meq 1000g^{-1}	mg 1000g^{-1}
F	I	3.5 ± 0.2	7.4 ± 0.5
	I-S	3.7 ± 0.2	6.6 ± 0.4
S	I	5.3 ± 0.3	6.3 ± 0.4
	I-S	4.9 ± 0.3	6.4 ± 0.4
H	I	5.1 ± 0.4	6.4 ± 0.6
	I-S	4.5 ± 0.4	6.2 ± 0.5

Table 58: The effect of one year storage on (a) peroxide (PV) and (b) the malonaldehyde (MA) of almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1} of oil, and (b) mg MA 1000 g^{-1} of nuts.

		Peroxide	MA
		meq 1000 g^{-1}	mg 1000 g^{-1}
F	C	2.9 ± 0.2	5.6 ± 0.4
	C-S	3.1 ± 0.2	4.8 ± 0.4
S	C	4.6 ± 0.3	4.4 ± 0.3
	C-S	4.2 ± 0.3	3.9 ± 0.3
H	C	4.2 ± 0.3	4.6 ± 0.3
	C-S	4.5 ± 0.4	4.5 ± 0.3

after irradiation may lower the activity of molecules within the cell, thereby slowing down the termination reactions of free radicals. This, in turn would maintain high levels of free radicals which could promote lipid deterioration with time.

CHAPTER VIII

EFFECT OF SALTING-ROASTING, DUAL TREATMENT AND STORAGE ON LIPIDS

INTRODUCTION

The physical and chemical make up of lipids in foods, whether natural or added, are altered by heat processing procedures that may be beneficial or detrimental to the product.

Lipids undergo a number of degradative changes during heat processing which have an important effect on palatability and wholesomeness of foods containing them.

The greatest changes in structure take place in the most unsaturated lipids. The degradative changes in lipids produced by heat processing such as roasting is related to the degree of unsaturation.

In heat processing of lipids, three main types of reactions can occur:

(a) Hydrolysis results in the formation of free fatty acids which at high concentration may promote rancidity of the foods, hence giving a sour taste to food.

(b) Reaction with oxygen (autoxidation) leads to the formation of hydroperoxides, epoxides and peroxides. All of these may subsequently undergo further degradation into smaller molecules.

Lipid autoxidation is very important and of much interest, because it results in the formation of off-odours and off-flavours, in the reduction or destruction of essential fatty acids.

The occurrence of off-flavours, generally described as 'rancidity' in fat containing foods is a common observation. Autoxidation is defined as the spontaneous oxidation of a substance in contact with molecular oxygen. The lipid components most susceptible to autoxidation are the unsaturated fatty acids, especially those with more than one double bond. In general, the greater the degree of unsaturation (the higher the iodine value) the greater is the fat liable to become oxidatively rancid. Although formally the process consists of a reaction between the molecular species (the lipid and oxygen) the number of possible pathways increase enormously in the course of the reaction (Fig. 22). Even in the simplest model system consisting of one fatty acid and oxygen a very large number of intermediates and final products are found and the system soon becomes very complex.

The most common type of lipid autoxidation is autocatalytic autoxidation. FARMER and SUTTON (1943) have shown that hydroperoxides are formed during the usual autoxidation of lipids. The rate of autoxidation reaction increases with time because products which are formed during the reaction tend to catalyze the rest of the reaction. Hence, as the reaction proceeds, the rate of hydroperoxide accumulation increases. The rate of oxidation can be generally accelerated by a number of factors, e.g. trace metals and biological catalysts including oxidative enzymes as well as light and temperature (LUNDBERG, 1962). Moisture is also an important factor in that rancidity develops more rapidly both at very high and at very low moisture levels. Maximum stability is observed at intermediate moisture level.

According to FARMER and SUTTON's theory (1943) the reaction proceeds through a free radical mechanism and consists of the following

steps (Fig. 22) : (RH represents a molecule of lipid)

Step 1: Initiation

$$RH \xrightarrow{\text{Activation}} R + H^{o} \qquad (1)$$

Step 2: Propagation

$$R + O2 \longrightarrow ROO \qquad (2)$$

$$ROO + RH \longrightarrow R^{o} + ROOH \qquad (3)$$

Step 3: Decomposition

$$ROOH \longrightarrow RO^{o} + OH^{o} \text{ (also } R^{o}, ROO^{o} \text{etc)} \qquad (4)$$

Step 4: Termination

$$ROO^{o} + X \longrightarrow \text{stable compounds} \qquad (5)$$

FIG. 22 : HYDROPEROXIDE FORMATION DURING LIPID AUTOXIDATION (FARMER AND SUTTON, 1943)

The hydroperoxides are important as the primary products of lipid autoxidation. They are in themselves non-volatile, odourless and tasteless. The unwanted odours and flavours of autoxidised materials (off flavour) are caused by secondary substances formed during the various reactions and possibly through further oxidations of the peroxide and their degradative products (PATTON et al , 1959). The number and variety of hydroperoxide degradative compounds are indeed quite large. Further, oxygen can act on ethylenic bonds to yield other degradative products. In addition to these, alcohols, aldehydes and other compounds formed during the initial degradation are susceptible to further oxidation. Hydroperoxide formation and accumulation measured as the increase in the "peroxide value" indicates the progress of autoxidation, but not necessarily the appearance of rancidity.

Hydroperoxides are relatively unstable. As their concentration in the system increases, they begin to decompose.

Although the onset of "rancidity" is a significant consequence of lipid oxidation, flavour deterioration is not the only damage suffered by food in this process. Colour is also affected through accelerated browning reactions. Further the nutritional value is impaired and in some cases, toxicity may be induced (Fig. 23). There is also some evidence that undesirable changes which occur in heated lipids may have a deleterious effect on human health (CRAMPTON et al, 1956; KAUNITZ et al., 1956).

The interaction between protein and the products of lipid oxidation may result in changes of texture. The mechanism of interaction involves propagation of the free radical chain to the protein system. Various groups in the protein molecule are capable of converting to free radicals by losing one hydrogen atom to a free radical of lipid origin. The protein "free radical" thus formed tends to combine by cross linkage. In short, oxidation deterioration of lipids may be considered as a spoilage factor affecting food acceptability.

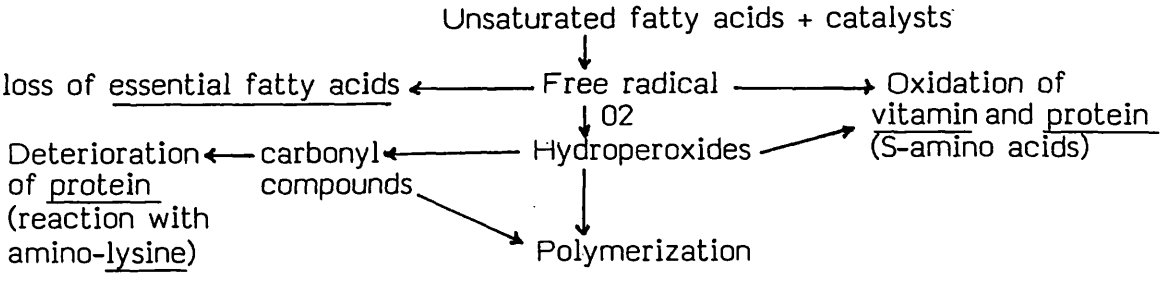


FIG. 23: LOSS OF NUTRIENTS BY LIPID OXIDATION

(MAURON, 1977)

Heating may cause cross-linking to form new carbon-carbon bonds between two triglyceride molecules, particularly in the absence of oxygen. If these bonds are formed within one fatty acid, cyclic fatty acids are produced. New bonds between two different fatty acids lead to the formation of dimeric acids either within one triglyceride molecule or between two molecules. This latter reaction is the first step in the polymerization of lipids (MORTON, 1977). This could also result in decreased digestibility of lipids in the diet.

In the processing of almonds, the operations involved may bring about changes in the total lipid content. Other changes may also take place which have a direct bearing on the storage life of the product in terms of increases in fatty acids and oxidative degradation of unsaturated lipids. In the present study these possible effects in terms of lipid degradation have been studied.

RESULTS AND DISCUSSION

Total lipids:

The total lipid content was not significantly affected in salted and roasted almonds (table 59). It must be mentioned that the mixture of chloroform-methanol solvent which has been used in this study to extract total lipids, also extracts all lipid degradation compounds produced as a result of roasting. This means that although roasting may accelerate degradation of lipids, this would not necessarily be observed in total lipid content.

Simple lipids

To determine the possible effects of roasting on almond oil, further investigations were carried out on simple and complex lipids separately. Roasting has no significant effect on total simple lipid content (table 59). It is possible, however, that individual lipids may have been affected.

Individual lipid constituents are shown in table 60. There is a significant decrease in triglycerides with a corresponding increase in diglycerides and free fatty acids. The increase in free fatty acids ranged from 117.1% in variety S to 160.7% in variety H, which may be due to hydrolysis of other lipid fractions.

As mentioned previously, during the roasting process the samples were soaked in salt solution which increased the moisture content of samples and when they were subsequently heated, hydrolysis of lipids was very likely.

These results were not unexpected since similar results have been obtained previously, for example when various oils are heated at 160°C or at

Table 59: The effect of salting and roasting on total simple, total complex and total lipids of almond nuts, with % retention compared to controls.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt., ± SE					
F	C	506.8 ± 28.7	18.4 ± 1.2	525.2 ± 32.5	93.4	95.0	93.5
	R	473.4 ± 29.9	17.5 ± 1.1	490.9 ± 31.2			
S	C	443.3 ± 27.2	17.3 ± 0.9	460.6 ± 26.0	92.6	102.0	93.0
	R	410.5 ± 28.5	17.7 ± 1.5	428.2 ± 29.8			
H	C	542.8 ± 30.8	17.7 ± 1.1	560.5 ± 32.8	93.5	101.1	93.8
	R	507.6 ± 31.7	17.9 ± 1.2	525.5 ± 34.3			

Table 60: The effect of salting and roasting on individual simple lipids of almond oil.

Simple lipids	F		S		H	
	C	R	C	R	C	R
	mg g^{-1} dry wt.					
MG	15.4	14.2	10.3	7.5	12.3	10.8
1,2 DG	28.3	36.8	28.1	41.4	30.1	44.9
1,3 DG	38.7	54.2	39.3	58.9	40.2	62.3
FS	3.4	3.3	2.6	2.7	2.2	2.0
β -SI	19.1	18.2	17.1	15.9	18.2	16.3
FFA	3.1	6.9	3.5	7.6	2.8	7.3
TG	391.7	309.5	333.6	262.6	432.5	334.4
SE	1.6	1.1	1.2	0.9	1.3	1.2
HC	T	T	T	T	T	T

180°C FLEISCHMAN et al (1963) and YUKI (1967) observed huge increases in free fatty acids. It seems that hydrolysis and therefore, the increased production of free fatty acids is an early reaction in the heat processing of lipid containing foods. KRISHNAMURTHY et al (1965) concluded that production of free fatty acids as a result of the hydrolysis of glycerides is obviously due to the presence of water in the system. YUKI (1967) has also stressed the point that presence or absence of water is a very significant factor in the production of free fatty acids.

Fatty acids

Free fatty acids

From the results of table 61 it may be observed that among the free fatty acids, C16:0 was not affected, while C18:1 and C18:2 increased significantly. This, in effect, also led to an increase in the unsaturation ratio of the free fatty acids. Roasting of almonds thereby resulted in an increase in both free fatty acid content and in the degree of unsaturation of these compounds.

Total fatty acids

Individual fatty acids expressed as a percentage of total fatty acids of almond oil are shown in table 62.

There were significant increases in both C14:0 and C16:0 with significant decreases in C18:1 and C18:2 of roasted almonds when compared to controls. There are many reports indicating a decrease in polyunsaturated fatty acids due to heat processing (KILGORE, 1969; KILGORE and BAILEY, 1970).

Table 61: The effect of salting and roasting on free fatty acids and unsaturation ratio of almond oil.

Fatty acids	F		S		H	
	C	R	C	R	C	R
	mg/g dry wt.					
C16:0	0.5	0.7	0.5	0.6	0.7	0.6
C18:1	1.5	3.6	1.7	4.3	1.2	4.1
C18:2	1.1	2.6	1.3	2.7	0.9	2.6
Total	3.1	6.9	3.5	7.6	2.8	7.3
Unsaturation ratio	5.2	8.9	6.0	11.7	3.0	11.2

Table 62: The effect of salting and roasting on (a) total fatty acids, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of almond oil.

Fatty acids	F		S		H	
	C	R	C	R	C	R
	% total fatty acids					
C14:0	2.8	10.5	2.5	7.9	2.6	12.4
C16:0	14.7	25.7	14.2	24.9	14.3	23.7
C16:1	T	T	T	T	T	T
C18:0	T	T	T	T	T	T
C18:1	49.3	36.9	50.2	40.7	49.8	38.5
C18:2	33.2	25.9	32.9	26.1	33.2	24.9
U.R.	4.7	1.7	5.0	2.0	4.9	1.8
I.V. **	98.2±6.1	86.2±4.3	99.3±6.5	83.7±3.7	98.5±3.1	89.2±5.2

(** $cgI_2\ g^{-1}$ oil)

The increase in C14:0 and C16:0 may have been due directly to the combined effects of hydrogenation and degradation reactions. If there were hydrogenation and breakdown reactions from the C18:2 to form C18:1 and C18:0, then one would have expected an eventual build-up in C18:0. As this was not so, then it may be assumed that C18:2 and C18:1 were diverted through a separate pathway, one possibility being through the oxidative reactions of Figs 22 (p. 295).

MAURON (1982) has stated that reducing the moisture content in foodstuff increases oxidation changes within the lipid fraction of foods. These oxidative changes may have been responsible for the losses observed in both C18:1 and C18:2. This may be confirmed by the observed changes in peroxide and malonaldehyde values which will be discussed on p 306

Losses in double bonds of the fatty acids were also reflected by a significant decrease in iodine value in all three varieties (table62).

Complex lipids

The total complex lipid content in roasted almonds remained unchanged (table 59). Individual complex lipids are shown in table 63.

Among the complex lipids, phospholipids appear to be the most affected by heat. Both lysophosphatidyl choline and phosphatidyl choline decreased, while the phosphatidic acid content increased sharply. This indicated that hydrolysis of some phospholipids had taken place during salting and roasting.

A similar observation has been made by KASHANI and VALADON (1983) in that on salting and roasting pistachio kernels they reported losses in phosphatidyl choline and increases in phosphatidic acid and in free fatty

Table 63: The effect of salting and roasting on individual complex lipids of almond oil.

Complex lipids	F		S		H	
	C	R	C	R	C	R
	mg/g dry wt.					
LPC	2.2	1.2	2.1	1.1	1.9	0.8
PI	3.2	3.5	2.3	2.5	2.5	2.8
PC	6.3	4.2	5.9	3.7	6.6	4.2
DGDG	0.3	0.5	0.3	0.4	0.4	0.5
SL	1.2	1.1	1.1	0.9	0.9	0.9
PE	2.3	2.2	2.4	2.6	2.2	2.4
SG	2.1	1.9	2.2	2.2	2.3	2.1
PA	0.8	1.8	1.0	3.2	0.9	2.7

acids. The increase in free fatty acids may have been derived both from complex and other simple lipids. However, the reson why phosphatidyl choline is more susceptible to heat treatment than are other phospholipids is at present unknown.

Peroxide and Malonaldehyde values:

Peroxide and malonaldehyde values are shown in table 64. The results for the three varieties of almonds followed similar trends, hence it is simpler to discuss them on a general basis. Peroxide values were found to increase sharply, while malonaldehyde values were not significantly affected. The increase in peroxide value suggests that some oxidation of the oil had taken place. Although peroxide formation of salted and roasted almonds was significantly higher than controls, yet it is not very high, an average of 2.4 meq / 1000g. This is, no doubt, due to the mild heating and the presence of natural antioxidants in the almonds.

As mentioned previously, there was no significant change in the malonaldehyde value. HOLLAND (1971) using malonaldehyde value as an index of rancidity in walnut meats stated that nuts judged to be slightly rancid by a taste panel contained approximately 40 mg malonaldehyde /1000g nut meat. In the present study the average malonaldehyde content was only 5.4 mg / 1000g almonds. Therefore, when compared to HOLLAND's (1971) results, it was assumed that the almonds were still acceptable in terms of palatability.

A certain increase in malonaldehyde is however expected, as has been shown by the following authors: SIU and DRAPER (1978) have shown that there was an increase of 3.25 mg malonaldehyde/1000 g after cooking

Table 64: The effect of salting and roasting on peroxide (PV) and the malonaldehyde (MA) value of almond nuts. The results are expressed as (a) meq of peroxide 1000 g^{-1} oil, and (b) mg of MA 1000 g^{-1} nuts.

		Peroxide	MA
		meq 1000 g^{-1}	mg 1000 g^{-1}
F	C	2.9 ± 0.2	5.6 ± 0.4
	R	5.2 ± 0.4	6.4 ± 0.4
S	C	4.6 ± 0.5	4.4 ± 0.3
	R	7.3 ± 0.5	4.6 ± 0.3
H	C	4.2 ± 0.3	4.6 ± 0.3
	R	6.5 ± 0.4	5.3 ± 0.5

beef, while NEWBURY and CONCON (1980) noted an increase of approximately 4.8mg / 1000g in skinned chicken.

In the present study, although salting and roasting caused a decrease in the unsaturatedness of almond fatty acids, yet the effect is not all that great as although there was an increase in peroxide (on average 64%), there was no significant increase in the malonaldehyde content.

It has been shown previously that there was a significant decrease in iodine value, so the loss in the fatty acids C18:1 and C18:2 was possibly due to the oxidative pathway (as described in Fig 22). It appears now that this suggestion is quite likely due to the observed increase in peroxide value.

In general, salting and roasting causes an increase in free fatty acids and phosphatidic acid, and also an increase in the unsaturatedness of the free fatty acids. Furthermore, the peroxide and iodine values are significantly changed after salting and roasting. However, salting and roasting does not have any effect on total lipids or on total simple or total complex lipids of the three varieties of almonds under study. Also, salting and roasting does not affect the malonaldehyde content. So, though it can be taken that salting and roasting do cause some degradation of the oil, this is not very high.

EFFECT OF STORAGE ON LIPIDS OF SALTED-ROASTED ALMONDS

Total lipids

After salting and roasting the almond samples, they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on lipids of roasted almonds, comparisons were made between results taken immediately after processing and those after one year storage.

The total lipid content of any of the almond varieties was not significantly changed after one year storage of the roasted almonds (table 65). This was not different to that found in controls after storage. Therefore roasting almonds prior to storage does not promote any obvious lipid deterioration.

Simple lipids

Total simple lipid content was not significantly changed after storage (table 65).

Individual simple lipids are shown in table 66. It may be observed that a further significant increase in free fatty acids occurred, while no significant changes were observed in the other simple lipid fractions. This is in agreement with the earlier suggestion that hydrolysis of certain lipid components had occurred, but the effect was not significant. It will be assumed at this stage that part of these fatty acids may have been due to breakdown of some complex lipid component.

In untreated almonds, one year storage brought about a significant

Table 65: The effect of one year storage on total simple, total complex and total lipids of salted-roasted almond nuts.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt., ± SE					
F	R	473.4 ± 30.9	17.5 ± 1.1	490.9 ± 31.2	95.1	95.4	95.1
	R-S	450.2 ± 31.8	16.7 ± 1.3	466.9 ± 28.8			
S	R	410.5 ± 28.5	17.7 ± 1.5	428.2 ± 29.8	93.6	99.4	93.8
	R-S	384.2 ± 27.9	17.6 ± 1.5	401.8 ± 19.3			
H	R	507.6 ± 31.7	17.9 ± 1.2	525.5 ± 34.3	95.9	96.0	95.9
	R-S	486.6 ± 28.6	17.2 ± 1.5	503.8 ± 30.1			

Table 66: The effect of one year storage on individual simple lipids of salted and roasted almond oil.

Simple lipids	F		S		H	
	R	R-S	R	R-S	R	R-S
	mg g^{-1} dry wt.					
MG	14.2	13.1	7.5	6.8	10.8	11.5
1,2 DG	36.8	39.3	41.4	44.2	44.9	47.9
1,3 DG	54.2	57.5	58.9	61.3	62.3	66.4
FS	3.3	2.9	2.7	2.7	2.0	2.2
β -SI	18.2	15.4	15.9	14.7	16.3	18.2
FFA	6.9	9.3	7.6	10.2	7.3	11.1
TG	309.5	287.1	262.6	237.6	334.4	301.4
SE	1.1	0.9	0.9	0.7	1.2	1.2
HC	T	T	T	T	T	T

increase in free fatty acids but no significant changes were observed in any individual simple lipids. So, the suggestion made earlier (p.281)may also be applicable here, indicating that the increase in fatty acids in control-stored samples may have come from hydrolysis of triglycerides. It is to be noted however, that although there is no significant difference in triglycerides of roasted-stored almonds compared to roasted ones, it is possible that the small increase in fatty acids could be accounted for by hydrolysis of triglycerides which are present in such large amounts compared to the other lipid components.

It is surprising to note that, on the whole, since lipids are relatively more labile to chemical changes than other major food components, they were affected less than expected after salting and roasting and also during storage,in the course of this study.

Fatty acids:

Free fatty acids

The free fatty acid content of R-S almonds is shown in table 67; C16:0 remained fairly constant, while C18:1 and C18:2 increased. This resulted in an increase in the unsaturation ratio (table 67). This observation was similar to that detected in untreated almonds after one year storage, where again the unsaturation ratio increased. The source of these unsaturated fatty acids which increased, thereby increasing the unsaturation ratio, may have been triglycerides which appeared to decrease (although not significantly). It must be pointed out that there was a very small increase in fatty acids, compared to the total amount of triglycerides. From the results of table 67 it is also apparent that the unsaturated and not the saturated fatty acids are selectively released during storage of roasted almonds.

Table 67: The effect of one year storage on free fatty acids and unsaturation ratio of salted and roasted almond oil.

Fatty acids	F		S		H	
	R	R-S	R	R-S	R	R-S
	mg/g dry wt.					
C16:0	0.7	0.7	0.6	0.7	0.6	0.7
C18:1	3.6	5.0	4.3	5.6	4.1	6.2
C18:2	2.6	3.6	2.7	3.9	2.6	4.2
Total	6.9	9.3	7.6	10.2	7.3	11.1
Unsaturation ratio	8.9	12.3	11.7	13.6	11.2	14.8

Total fatty acids

Individual fatty acids of salted-roasted-stored almonds were expressed as a percentage of total fatty acids and the results are shown in table 68.

The changes observed in the fatty acid composition of salted-roasted almonds after one year storage resemble very much those reported earlier for irradiated-stored almonds. There was a noticeable increase in both C16:1 and C18:0 fatty acids. This could be caused by rearrangement and the possible degradation of higher fatty acids (C18:1 and C18:2) which decreased. In fact, these decreases in both fatty acids support the above assumption. In view of this, it was surprising to note that the iodine value was not affected significantly under the same conditions.

It appears then, that fatty acid degradation was low otherwise the iodine value would have been severely affected. MEYER (1978) has stated that vegetable oil, particularly that from seeds, shows a marked resistance to the onset of rancidity. Some seeds, if they are not bruised or crushed, can be stored for years without any changes in their oils. In the present study, seeds which were visibly damaged were removed prior to the experiments. This may be one of the reasons why the lipids were noticed to be relatively well preserved even after one year storage.

Complex lipids:

There were no significant changes in total complex lipids after one year storage (table 65). The individual complex lipids are shown in table 69. It is observed that the phospholipids are clearly affected, while glycolipid

Table 68: The effect of one year storage on (a) fatty acids, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of salted and roasted almond oil.

Fatty acids	F		S		H	
	R	R-S	R	R-S	R	R-S
	% total fatty acids					
C14:0	10.5	13.5	7.9	7.2	12.4	15.1
C16:0	25.7	27.3	24.9	28.3	23.7	25.9
C16:1	T	3.1	T	4.4	T	4.9
C18:0	T	7.4	T	8.3	T	8.9
C18:1	36.9	28.2	40.7	31.6	38.5	25.8
C18:2	25.9	20.2	26.1	19.8	24.9	18.9
U.R.	1.7	1.1	2.0	1.3	1.8	1.0
I.V.**	86.2±4.3	83.1±5.2	83.7±3.7	82.2±3.9	89.2±5.2	94.4±5.3

(** $cgI_2\ g^{-1}$ oil)

Table 69: The effect of one year storage on individual complex lipids of salted and roasted almond oil.

Complex lipids	F		S		H	
	R	R-S	R	R-S	R	R-S
	mg/g dry wt.					
LPC	1.2	1.5	1.1	0.9	0.8	0.9
PI	3.5	2.3	2.5	1.5	2.8	1.6
PC	4.2	3.1	3.7	2.4	4.2	2.9
DGDG	0.5	0.4	0.4	0.5	0.5	0.4
SL	1.1	0.9	0.9	1.0	0.9	0.8
PE	2.2	2.2	2.6	2.3	2.4	2.6
SG	1.9	1.6	2.2	1.9	2.1	1.9
PA	1.8	3.4	3.2	5.6	2.7	4.7

remained fairly stable during storage. Among the phospholipids, phosphatidyl choline and phosphatidyl inositol decreased significantly. This has led to a sharp increase in phosphatidic acid. This greater susceptibility of phospholipids in salted-roasted-stored samples was also observed in irradiated samples. There is a possibility that, general processing treatments such as irradiation and roasting may be deactivating a number of lipid enzymes, thereby preventing further breakdown of some simple lipid fraction and glycolipids. At the same time, certain phospholipids (phosphatidyl ethanolamine) may be more resistant to breakdown, hence left unaltered. So, during storage, enzymic action on some phospholipids (i.e. phosphatidyl inositol) may be found to be more pronounced than on others.

Peroxide and malonaldehyde values:

Peroxide values of salted-roasted-stored almonds were not significantly changed, while malonaldehyde values decreased significantly (table 70). This almost contradicts the suggestion made earlier regarding fatty acid oxidation (p.314). One possible explanation for the absence of any changes in peroxide value is that offered by BERK (1976) who stated that despite accelerated rates of oxidation being sometimes observed, the fact that peroxide values are usually low is due to the rapid decomposition of the peroxides formed. Also, malonaldehyde being relatively unstable could have decomposed fairly rapidly, thereby showing a net decrease after one year storage.

In conclusion then, very little fatty acid deterioration had occurred in salted-roasted-stored almonds as the iodine value and peroxide values were not appreciably affected. On the other hand, a slight decrease in

Table 70: The effect of one year storage on peroxide (PV) and the malonaldehyde (MA) value of salted-roasted almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1} oil and (b) mg MA 1000 g^{-1} nuts.

		Peroxide	MA
		meq 1000g^{-1}	mg 1000g^{-1}
F	R	5.2 ± 0.4	6.4 ± 0.4
	R-S	5.5 ± 0.4	5.2 ± 0.3
S	R	7.3 ± 0.5	4.6 ± 0.3
	R-S	7.7 ± 0.6	3.9 ± 0.2
H	R	6.5 ± 0.4	5.3 ± 0.5
	R-S	6.6 ± 0.5	3.9 ± 0.4

malonaldehyde value was observed and since malonaldehyde is volatile, this decrease may have been due to its volatility, and therefore this value (MA) may not have been appreciably affected as well.

THE EFFECT OF THE COMBINED TREATMENT: SALTING-ROASTING AND IRRADIATION ON LIPIDS

Total Lipids

Total lipids of dual treated almonds (table 71) was not significantly changed as compared to untreated controls. This was not altogether unexpected as all individual treatments of roasting and irradiation separately (reported earlier in this study) did not significantly change total lipids in any of the varieties of almonds studied. Therefore, it appears that the dual treatment has not brought about any additional adverse effect in terms of maintaining total lipids.

Simple Lipids

Total simple lipid content was not significantly affected by the dual treatment of almonds (table 71). This was also observed in irradiated and in salted-roasted almonds separately.

Individual simple lipid content of dual treated almonds are given in table 72. A significant decrease in triglycerides was observed, while the two species of diglycerides and free fatty acids increased significantly. No other simple lipids were altered. The increase in free fatty acids in all three varieties of almonds ranged from 129.0% in F to 189.3% in H, with an average of 152.8%.

Previously, it had been noticed that irradiation treatment resulted in the hydrolysis of monoglycerides. This was not observed in the case of

Table 71: The effect of dual treatment on total simple, total complex and total lipids of almond nuts, with % retention compared to controls.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt., ± SE					
F	C	506.8 ± 28.7	18.4 ± 1.2	525.2 ± 32.5	92.2	90.0	92.1
	RI	467.3 ± 38.1	16.6 ± 1.5	483.9 ± 39.4			
S	C	443.3 ± 27.2	17.3 ± 1.3	460.6 ± 26.0	89.9	95.4	90.2
	RI	398.8 ± 29.5	16.5 ± 1.6	415.3 ± 31.0			
H	C	542.8 ± 30.8	17.7 ± 1.4	560.5 ± 32.8	89.1	96.6	89.4
	RI	483.8 ± 30.6	17.1 ± 1.1	500.9 ± 27.6			

Table 72: The effect of dual treatment on individual simple lipids of almond oil.

Simple lipids	F		S		H	
	C	RI	C	RI	C	RI
	mg g^{-1} dry wt.					
MG	15.4	15.1	10.3	8.4	12.3	10.4
1,2 DG	28.3	38.2	28.1	39.4	30.1	43.6
1,3 DG	38.7	56.2	39.3	56.9	40.2	60.3
FS	3.4	3.2	2.6	2.8	2.2	2.3
β -SI	19.1	18.9	17.1	16.5	18.2	17.2
FFA	3.1	7.1	3.5	8.4	2.8	8.1
TG	391.7	292.1	336.6	245.6	432.5	324.4
SE	1.6	1.2	1.2	0.9	1.3	1.1
HC	T	T	T	T	T	T

dual treated samples, therefore these lipid components may have been stabilised in some way against breakdown. This stabilising effect could have been brought about during the roasting treatment as observed previously (p.298). when it was noted that salting and roasting did not affect monoglycerides. Alternatively, breakdown of triglycerides may have produced some monoglycerides thereby compensating for any loss in the latter, hence no apparent net change would have been noted.

An interesting point of speculation is inferred from the report of LABUZA (1971) who found that the rate of lipid oxidation was reduced to one tenth in the presence of protein. Since oxidising fats can react with protein (Fig 24) (DESAI and TAPPEL, 1963), the complexes formed may then function as antioxidants against further reactions. It is possible therefore that roasting may have resulted in the formation of antioxidants (Fig. 24), thereby reducing the subsequent effect of irradiation in dual treated almonds.

(1) $LOO^{o} + P \longrightarrow L^{o}OOP$

Lipid peroxide + Protein $\longrightarrow$ Lipid-protein complex may undergo further cross-linkage

$LO^{o} + P \longrightarrow L^{o}OP$

Lipid alkoxide + Protein $\longrightarrow$ Lipid-protein complex

Further complex formations

(2) $LOOP + O_2 \longrightarrow OOLOOP \xrightarrow{P} POOLOOP$

FIG 24: SCHEME OF REACTION BETWEEN PEROXIDISED LIPID FREE RADICALS WITH PROTEIN

(DESAI and TAPPEL, 1963)

As mentioned previously (p.180) results of DESAI and TAPPEL (1963) suggest that the amino acids histidine, serine, proline and arginine were most labile to peroxidative damage, while the sulphur amino acids, methionine and cysteine were next in order. If peroxides or other degraded products of lipid damage are involved in reactions with protein, decreases in the above six amino acids would be expected to ensue. Results in chapter 6 for effects of dual treatment on individual amino acids indicated that the amino acids most affected were histidine, serine, proline, methionine, cysteine and to a lesser extent, arginine. This would imply that complexing between lipids and proteins might have in fact taken place. However, it is worth mentioning that DESAI and TAPPEL (1963) studied model reaction systems containing linoleic acid and cytochrome 'c'; so these results might not necessarily be applicable to all food products.

Fatty acids:

Free fatty acids

The free fatty acids of dual treated almonds are shown in table 73. It was observed that the quantity of C16:0 remained constant, while C18:1 and C18:2 increased compared to controls. This obviously also resulted in an increase in the unsaturation ratio. With such an increase in unsaturated fatty acids, especially in the free form, it is likely that there would be increased lipid deterioration during storage. This will be discussed further (p 340).

Total fatty acids

Total fatty acids expressed as a percentage of total fatty acids are shown in table 74. The changes which occurred in the three varieties

Table 73: The effect of dual treatment on free fatty acids and unsaturation ratio of almond oil.

Fatty acids	F		S		H	
	C	RI	C	RI	C	RI
	mg/g dry wt.					
C16:0	0.5	0.5	0.5	0.6	0.7	0.7
C18:1	1.5	4.2	1.7	5.1	1.2	4.9
C18:2	1.1	2.4	1.3	2.7	0.9	2.5
Total	3.1	7.1	3.5	8.4	2.8	8.1
Unsaturation ratio	5.2	13.2	6.0	13.0	3.0	10.6

Table 74: The effect of dual treatment on (a) fatty acids, (b) unsaturation ratio (U.R.) and (c) the iodine value (I.V.) of almond oil

Fatty acids	F		S		H	
	C	RI	C	RI	C	RI
	% total fatty acids					
C14:0	2.8	11.8	2.5	9.9	2.6	14.3
C16:0	14.7	28.1	14.2	25.3	14.3	25.1
C16:1	T	T	T	T	T	T
C18:0	T	T	T	T	T	T
C18:1	49.3	35.1	50.2	40.2	49.8	34.7
C18:2	33.2	24.7	32.9	24.1	33.2	25.5
U.R.	4.7	1.5	5.0	1.8	4.9	1.5
I.V.**	98.2±6.1	85.2±5.2	99.3±6.5	86.3±4.7	98.5±3.1	87.2±5.7

(** $cgI_2\ g^{-1}$ oil)

of almonds were very similar, in that there was a decrease in the unsaturated fatty acids with a corresponding increase in the saturated ones. A decrease in unsaturation ratio has resulted and evidence for the loss of unsaturatedness in almond oil is provided by the observed decrease in iodine value. These changes very much resemble those found in salted and roasted samples, indicating that the loss in unsaturated fatty acids probably occurred in dual treated almonds by a similar mechanism to that already described for salted and roasted samples

While it might be expected that the highly unsaturated almond oil would show an even greater instability than actually observed, the fact that this was not so may be due to the natural or formed antioxidants present in processed nuts.

Complex lipids

Total complex lipid content was not affected by the dual treatment in any of the varieties of almonds used in this study (table 71). Individual complex lipid contents are shown in table 75. The first point to note was that phosphatidic acid content increased sharply. This increase ranged from 187.5% for variety F to 255.5% in variety H. This certainly may indicate phospholipid breakdown. This was confirmed by the significant decrease noted in lyso phosphatidyl choline, phosphatidyl choline and phosphatidyl inositol (table 75). Again, these changes were similar to those noted for salted and roasted almonds (p.304), where decreases in lysophosphatidyl choline and phosphatidyl choline were observed. Therefore part of the increase in fatty acids in the simple lipid fraction could be due to phospholipid breakdown.

Table 75: The effect of dual treatment on individual complex lipids of almond oil.

Complex lipids	F		S		H	
	C	RI	C	RI	C	RI
	mg/g dry wt.					
LPC	2.2	1.1	2.1	0.9	1.9	0.6
PI	3.2	2.3	2.3	1.5	2.5	1.4
PC	6.3	3.9	5.9	3.4	6.6	4.2
DGDG	0.3	0.2	0.3	0.3	0.4	0.5
SL	1.2	1.3	1.1	1.2	0.9	1.1
PE	2.3	2.4	2.4	2.5	2.2	2.7
SG	2.1	1.8	2.2	2.0	2.3	2.4
PA	0.8	2.3	1.0	3.3	0.9	3.2

In general, it may be said that of the dual treatment, it is the salting and roasting process which has the most pronounced effect on the lipid composition of almonds.

Peroxide and malonaldehyde values:

Peroxide and malonaldehyde values of dual treated almonds are given in table 76. Both peroxide and malonaldehyde contents have increased significantly. These large increases are most probably due to the release of unsaturated fatty acids during lipid hydrolytic reactions, and these fatty acids were then subjected to oxidative degradation. The coupled effect of roasting and irradiation seemed to have increased the occurrence of oxidative reactions. Also the increase in peroxides would allow their reactions with protein (Fig 24).

Further intensive organoleptic tests will have to be carried out to determine to what extent all the above changes due to roasting-irradiating almonds have affected the flavour of the almonds under study.

Table 76: The effect of dual treatment on peroxide (PV) and malonaldehyde (MA) value of almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1} oil, and (b) mg MA 1000 g^{-1} nuts.

		Peroxide	MA
		meq 1000 g^{-1}	mg 1000 g^{-1}
F	C	2.9 ± 0.2	5.6 ± 0.4
	RI	6.2 ± 0.4	8.8 ± 0.6
S	C	4.6 ± 0.3	4.4 ± 0.3
	RI	6.8 ± 0.4	7.1 ± 0.5
H	C	4.2 ± 0.3	4.6 ± 0.3
	RI	7.1 ± 0.5	6.9 ± 0.4

EFFECT OF STORAGE ON LIPIDS OF DUAL TREATED ALMONDS

Total lipids

After salting and roasting, and irradiating the almond samples they were stored in plastic bags at a temperature of 22°C. To determine the effect of storage on lipids of dual treated almonds, comparisons were made between results taken immediately after processing and those taken after one year of storage.

The total lipid content of varieties F, S and H are shown in table 77. It may be seen that total lipids were not significantly affected by storing salted-roasted-irradiated almonds for one year. The same results have already been observed after one year storage of controls, of irradiated, and of salted-roasted almonds, in that in each case total lipid content remained unaltered.

Simple lipids

That total simple lipid content was unaffected after one year storage (table 77). This, again does not necessarily mean that there was no change in individual simple lipid components, which have been investigated and which are given in table 78. It may be seen that the triglyceride content decreased significantly. Significant increases in free fatty acids, in monoglycerides, and in diglycerides were also noted, while other simple lipids generally remained constant. Monoglycerides, diglycerides and free fatty acids are possible breakdown products of triglycerides. Therefore there is no doubt that the observed increased in the former three components were due to triglyceride breakdown (Fig 25).

Table 77: The effect of one year storage on total simple, total complex and total lipids of dual treated almond nuts.

		Total simple lipids (TSL)	Total complex lipids (TCL)	Total lipids (TL)	% retention		
					TSL	TCL	TL
		mg/g dry wt., ± SE					
F	RI	467.3 ± 38.1	16.6 ± 1.5	483.9 ± 39.4	95.0	100	95.2
	RI-S	444.2 ± 34.8	16.6 ± 0.9	460.8 ± 29.8			
S	RI	398.8 ± 29.5	16.5 ± 1.6	415.3 ± 31.0	92.5	99.4	92.7
	RI-S	368.8 ± 27.8	16.4 ± 1.2	385.2 ± 27.6			
H	RI	483.8 ± 30.6	17.1 ± 1.1	500.9 ± 27.6	88.6	96.5	88.9
	RI-S	428.8 ± 26.6	16.5 ± 1.1	445.3 ± 31.7			

Table 78: The effect of one year storage on individual simple lipids of dual treated almond oil.

Simple lipids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	mg g^{-1} dry wt.					
MG	15.1	22.6	8.4	11.9	10.4	13.3
1,2 DG	38.2	48.2	39.4	50.5	43.6	52.3
1,3 DG	56.2	71.1	56.9	71.1	60.3	75.6
FS	3.2	3.2	2.8	2.6	2.3	1.9
β -SI	18.9	17.7	16.5	16.3	17.2	16.9
FFA	7.1	9.3	8.4	10.5	8.1	11.3
TG	292.1	236.7	245.6	183.8	324.4	229.9
SE	1.2	0.9	0.9	0.7	1.1	1.2
HC	T	T	T	T	T	T

TG ⟶ DG + FA ⟶ MG + 2 FA ⟶ Glycerol + 3 FA

FIG. 25: POSSIBLE HYDROLYSIS OF TRIGLYCERIDES

It may be said that hydrolytic reactions may have been responsible for this breakdown, which may very well be true in the early stages of storage. Alternatively, due to the fact that loss in triglycerides is greater than the cumulative increase in their breakdown products, it is equally possible that there is another pathway by which breakdown of triglycerides may have occurred. NAWAR (1969) has proposed a reaction pathway, Fig 26, for the breakdown of triglycerides under high temperature in the absence of moisture. This pathway by which triglycerides may be degraded without there being a build up of either di- or mono-glycerides may have been followed in the present case since stored almonds had a low moisture content.

Taking all the above evidence into account, it appears that triglyceride decomposition may have occurred via two different routes:

(1) there may be hydrolysis in the presence of moisture, Fig 25 and

(2) breakdown in the absence of moisture, Fig 26

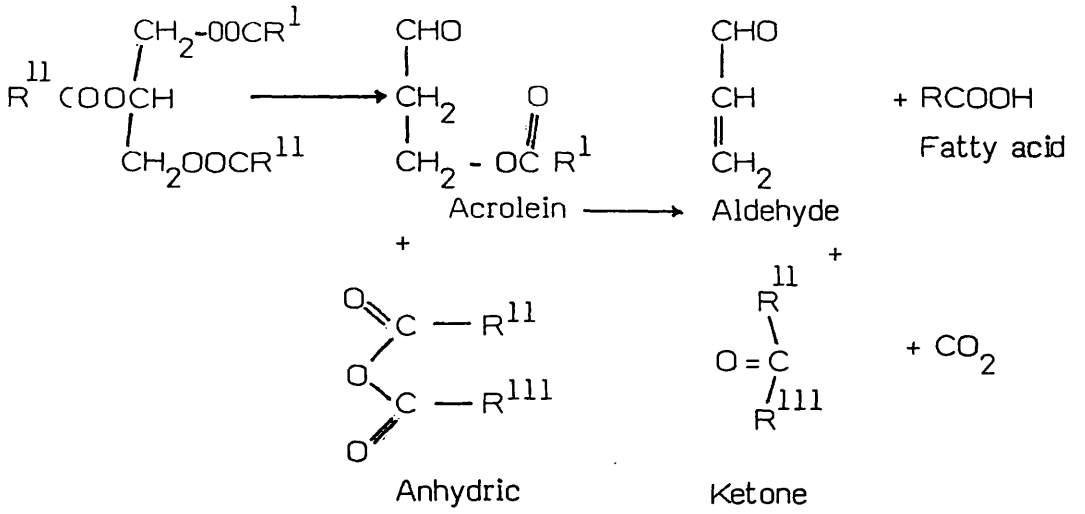


FIG 26: DECOMPOSITION OF TRIGLYCERIDES BY HEAT IN THE ABSENCE OF MOISTURE

(Modification of the scheme of NAWAR, 1969)

A proportion of the free fatty acids may have entered into further reactions resulting in their oxidations.

Fatty acids:

Free fatty acids

Individual free fatty acids of roasted-irradiated-stored almonds are shown in table 79. C16:0 remained absolutely constant. There was a significant increase in C18:1 in all three varieties. In total, there was a significant increase in unsaturation ratio for all three varieties. It seems highly likely that a proportion of esterified fatty acids may have been released in the free state. For salted-roasted and for irradiated almonds

Table 79: The effect of one year storage on free fatty acids and unsaturation ratio of dual treated almond oil.

Fatty acids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	mg/g dry wt.					
C16:0	0.5	0.5	0.6	0.6	0.7	0.7
C18:1	4.2	6.2	5.1	6.9	4.9	6.8
C18:2	2.4	2.6	2.7	3.0	2.5	3.8
Total	7.1	9.3	8.4	10.5	8.1	11.3
Unsaturation ratio	13.2	17.6	13.0	16.5	10.6	15.1

separately there was a general tendency for both C18:1 and C18:2 to increase during storage. In the present instance with the high decrease in triglycerides, it was expected that large amounts of free fatty acids would be liberated. This was not the case and suggests that free fatty acids, especially unsaturated ones may have been converted to lipid derivatives or oxidative products.

Total fatty acids:

Table 80 gives the fatty acid contents expressed as a percentage of total fatty acids in the three varieties of almonds. The trend was similar in all three varieties and, therefore, the results will be discussed on a general basis.

The C16:1 and C18:0 fatty acids which were not observed in measurable amounts prior to storage were now in larger amounts. The small changes in the other fatty acids were not high enough to affect either the unsaturation ratio or the iodine value (table 80).

The appearance of C16:1 and C18:0 implied the occurrence of hydrogenation and breakdown of fatty acids during storage. This supports the suggestion made earlier for free fatty acids, namely that these components were taking part in further reactions.

Complex lipids:

Total complex lipid content was not significantly affected after one year storage (table 77) . From table 81 it may be observed that individual complex lipids were relatively unchanged as well. It was expected that during storage the salted-roasted-irradiated samples would have followed

Table 80: The effect of one year storage on (a) fatty acids, (b) unsaturation ratio (U.R.) and (c) iodine value (I.V.) of dual treated almond oil.

Fatty acids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	% total fatty acids					
C14:0	11.8	10.3	9.9	10.5	14.3	15.5
C16:0	28.1	27.5	25.3	26.2	25.1	25.9
C16:1	T	2.9	T	2.6	T	2.1
C18:0	T	5.3	T	5.9	T	6.2
C18:1	35.1	31.4	40.2	34.1	34.7	28.3
C18:2	24.7	22.3	24.1	20.5	25.5	21.9
U.R.	1.5	1.3	1.8	1.3	1.5	1.1
I.V. **	85.2±5.2	81.4±4.7	87.3±4.7	84.7±5.8	89.2±5.7	85.6±4.6

(** $cgI_2\ g^{-1}$ oil)

Table 81: The effect of one year storage on individual complex lipids of dual treated almond oil.

Complex lipids	F		S		H	
	RI	RI-S	RI	RI-S	RI	RI-S
	mg/g dry wt.					
LPC	1.1	0.9	0.9	0.8	0.6	0.5
PI	2.3	2.4	1.5	1.6	1.4	1.5
PC	3.9	4.1	3.4	3.3	4.2	4.2
DGDG	0.2	0.3	0.3	0.2	0.5	0.7
SL	1.3	1.1	1.2	1.1	1.1	0.9
PE	2.4	2.6	2.5	2.7	2.7	2.9
SG	1.8	1.6	2.0	2.1	2.4	2.2
PA	2.3	2.5	3.3	3.4	3.2	3.0

the trend observed for roasted-stored almonds, where generally phospholipids decreased. As opposed to this, the lack of changes in phospholipids resembled those found in irradiated-stored almonds. It may be inferred from this that in some way irradiation treatment has a stabilising effect on phospholipids. Alternatively, irradiation may have deactivated certain enzymes responsible for phospholipid breakdown.

It may be seen that, while the combined salted-roasted-irradiated treatment had a destabilising effect on triglycerides, causing their further breakdown during storage, there was a stabilising effect in terms of preventing phospholipid breakdown.

Peroxide and malonaldehyde values:

Table 82 shows the peroxide and malonaldehyde values for the three varieties of salted-roasted-irradiated treated almonds after storage.

Peroxide values were not significantly altered after storage which would at first sight imply that oxidation of lipids had not taken place. However, the rate of peroxide formation may be equal to that of its breakdown, so that no net change was observed. Evidence for this comes from the fact that (i) peroxides are relatively unstable and hence their rapid breakdown to form malonaldehydes and (ii) that malonaldehyde content tended to increase significantly, thus possibly reflecting some peroxide breakdown.

It appears then, that there may be some peroxide formation. Simultaneously, peroxide breakdown leads to some increases in malonaldehyde content. Adverse flavour deterioration would not, in theory be observed in

Table 82: The effect of one year storage on peroxide (PV) and the malonaldehyde (MA) value of almond nuts. The results are expressed as (a) meq peroxide 1000 g^{-1} oil, and (b) mg MA 1000 g^{-1} of nuts.

		Peroxide	MA
		meq $1000g^{-1}$	mg $1000g^{-1}$
F	RI	6.2 ± 0.4	8.8 ± 0.6
	RI-S	5.9 ± 0.4	9.8 ± 0.3
S	RI	6.8 ± 0.4	7.1 ± 0.5
	RI-S	6.6 ± 0.4	8.2 ± 0.4
H	RI	7.1 ± 0.5	6.9 ± 0.4
	RI-S	6.7 ± 0.4	8.1 ± 0.5

almonds as a result of roasting-irradiation treatment followed by one year storage. This is due to the fact that the malonaldehyde value was much less than that recorded for rancid nuts as detected by a taste panel (HOLLAND, 1971).

SUMMARY

Controls:

The total lipid content of three varieties of almonds ranged from 460.0 to 560.5 mg g^{-1} dry weight (table 41). This shows that lipids represent the major nutrient fraction in almonds (ca. 50% of nut).

Simple lipids (app. 95%) constituted most of total lipids. The seven simple lipid classes identified were monoglycerides, diglycerides, triglycerides, free fatty acids, sterols and hydrocarbons (table 42). Of these, triglycerides made up by far the largest proportion, about 78% of simple lipids.

The free fatty acids included C16:0, C18:1 and C18:2. Total fatty acids possessed not only those found as free fatty acids but also included small quantities of C14:0 and C18:0 (table 44).

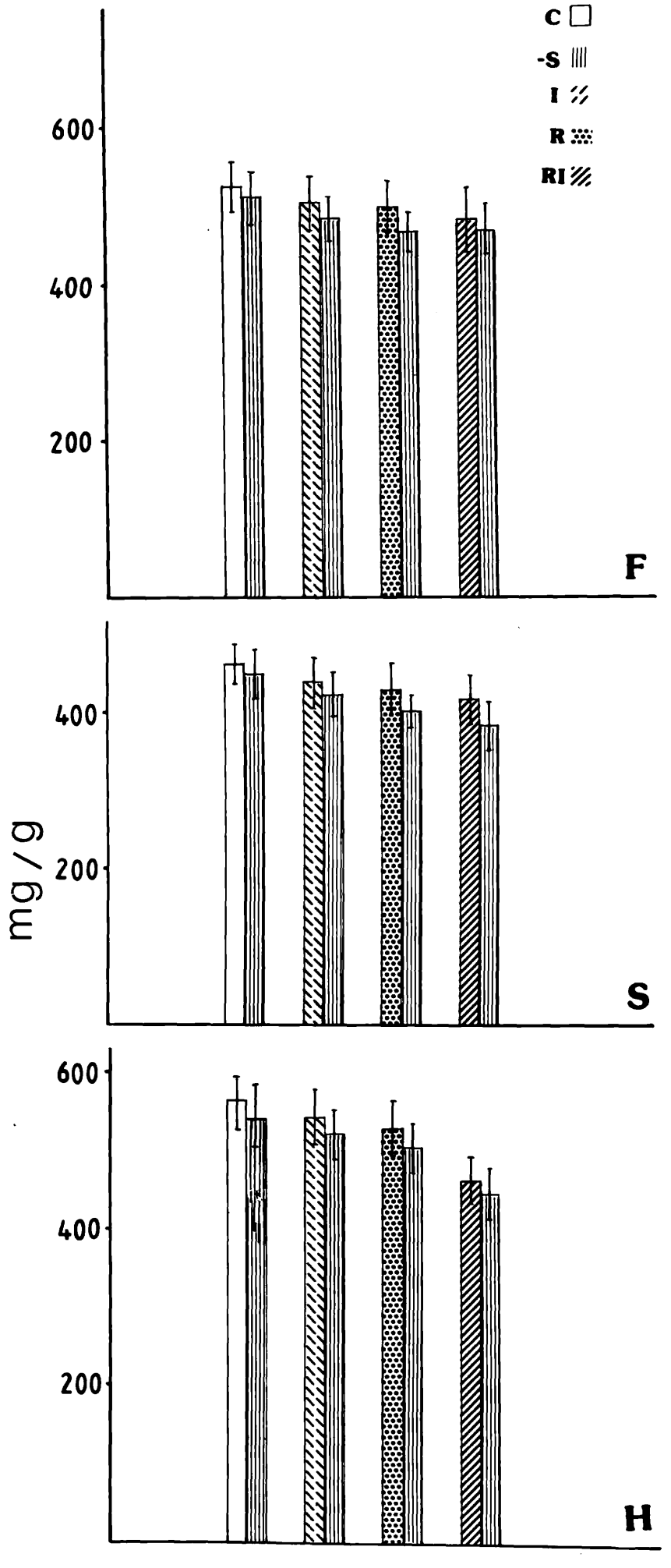
Complex lipid constituents corresponding to about 3.5% of total lipids were lyso-phosphatidyl choline, phosphatidyl inositol, phosphotidyl choline, digalactosyl diglycerides, sulpholipid, phosphatidyl ethanolamine, sterol glycosides and phosphatidic acid (table 45).

Iodine, peroxide and malonaldehyde values were found to range from (98 - 99 cg I_2 g^{-1} oil), (2.9 - 4.6 meq/1000g^{-1} oil) and (4.4 - 5.6 mg 1000 g^{-1} of almond) respectively.

One year storage did not bring about any significant changes in total lipids or in total simple lipids (table 48). However, among the simple lipids, triglycerides appeared lower but were not significantly different when

Fig. 27: Effect of processing and storage on lipids of three varieties of almonds are represented graphically in this figure.

The second of each pair of columns represents total lipids (mg/g ± SEM) of the stored products of the first column.


C
-S
I
R
RI
600
400
200
F
400
200
mg/g
S
600
400
200
H

compared to controls, while free fatty acids increased significantly.

Increases in C16:1 and C18:0 were noted (table54)suggesting that after storage the changes in C16:1 and C18:0 are brought about by enzymic action.

In general, it was found that one year storage of almonds (control) did not bring about any significant deterioration of lipids. This was inferred by the absence of changes in peroxide and in malonaldehyde values (table 58).

Irradiation

Irradiation did not bring about significant changes in the total lipid or in total simple lipid content of almonds.

Monoglyceride content decreased, while fatty acids increased, by 46 to 75% (table 42). Other simple lipid classes were not affected. It is suggested that irradiation may have brought about hydrolysis of monoglycerides thereby releasing free fatty acids. The overall stability of lipids to irradiation may be attributed to the presence of natural anti-oxidants or to the formation of new anti-oxygenic factors such as aldehyde-amine complexes.

The increasesin C16:0, C18:1 and C18:2 were observed without any significant changes in the unsaturation ratio of fatty acids (table 43).

Total complex lipid content remained stable, although phosphatidyl inositol was found to decrease significantly (table 45).

Although there was no change in iodine values, increases in both peroxide and in malonaldehyde content indicated the onset of lipid deterioration (table 46).

One year storage did not bring about any significant changes in total lipids, total simple lipids or total complex lipids. Individual simple lipids were not affected significantly.

Among the total fatty acids C16:1 and C18:0 increased significantly from trace amounts (table 53). The build up of C18:0 would suggest that hydrogenation of C18:1 and C18:2 may have occurred. However, this was not reflected by any changes in iodine value and in the unsaturation ratio.

The fact that storage did not result in any deterioration of the lipids was also supported by the fact that both peroxide and malonaldehyde values remained unchanged.

Roasting

As in previous cases, total lipid and total simple lipid contents were not affected by heat treatment. There was a significant decrease in triglycerides with a corresponding increase in diglycerides and free fatty acids (table 60). The increase in free fatty acids ranged from 117.0 to 160.7%. These increases were mainly in C18:1 and C18:2 with C16:0 remaining unchanged. It was interesting to note, however, that among the total fatty acids, increases were found in C14:0 and C16:0, while decreases occurred in C18:1 and C18:2 (table 62). Iodine value decreased hence some rearrangement of fatty acids may have occurred.

Total complex lipid content remained unchanged, but certain individual complex lipids were changed: namely phospholipids, including lysophosphatidyl choline, phosphatidyl choline and phosphatidic acid (table 63). These observed breakdowns may also explain the increase in free fatty acids

noted previously.

Oxidation of unsaturated lipids was indicated by an increase in peroxide content; malonaldehyde content remaining unchanged. (table 64).

One year storage did not bring about any significant effect on total lipids, total simple lipid or total complex lipid content. There was an increase in free fatty acids (table 66) but this was not accompanied by any significant changes in other simple lipids. It is to be noted however that although there is no significant change in the triglycerides of roasted-stored almonds compared to roasted ones, the small increase in fatty acids could be accounted for by hydrolysis of triglycerides, which are present in such large amounts.

It appears that hydrogenation and possible degradation of the higher fatty acids continued during storage.

Peroxide value was not significantly affected but malonaldehyde content decreased (table 70). This was not altogether surprising as malonaldehyde is unstable and is decomposed in time.

The stability of lipids in roasted almonds during storage may have been increased indirectly by the inactivation of degradative enzymes by heat treatment.

Dual Treatment

The dual treatment of heat and irradiation did not cause any significant changes in total lipids, total simple lipids and total complex lipids (table 71). A significant decrease in triglycerides was observed, while the two species of diglycerides and free fatty acids increased significantly

(table 72). Increases in free fatty acids ranged from 129.0% to 189.0%. Irradiation alone resulted in the degradation of monoglycerides (table 42). This was not observed in the case of dual treated samples, therefore these lipid components may have been stabilised in some way against breakdown. LABUZA (1971) has found that the rate of lipid oxidation was reduced to one tenth in the presence of protein. Since oxidising fats can react with protein, the complex formed may then function as antioxidants against further reactions: roasting may have resulted in the formation of antioxidants, thereby reducing the subsequent effect of irradiation on the dual treated almonds.

Among the free fatty acids, C16:0 remained unchanged, while C18:1 and C18:2 increased (table 73) similar results having been found for almonds subjected to roasting only. This resulted in an increase in the unsaturation ratio of the free fatty acids.

In contrast, the unsaturation ratio for total fatty acids decreased due to the percentage increases in saturated fatty acids and decreases in unsaturated ones (table 74). This was also accompanied by a decrease in iodine values from which it was assumed that some fatty acid hydrogenation or oxidation had occurred.

Due to changes observed in roasted-irradiated almonds being similar to those subjected to roasting alone, it was suggested that similar mechanisms of reactions may have occurred in both cases.

Among complex lipids, lysophosphatidyl choline, phosphatidyl choline and phosphatidyl inositol were observed to decrease while phosphatidic acid content increased (table 75). This would also have resulted in the

release of fatty acids.

Increases in both peroxide and malonaldehyde valies implied the onset of lipid oxidation and possibly rancidity (table 76).

One year storage did not result in any significant changes in total lipids, total simple lipids or total complex lipid content (table 77).

It was interesting to note that triglyceride content decreased further during storage of the dual-treated almonds (table 78). There was no change in the triglyceride content of roasted-stored or irradiated-stored samples as shown previously. This led to the opinion that the dual treatment: roasting followed by irradiation may have had a more severe effect on lipids than either roasting or irradiation alone. Breakdown of triglycerides resulted in the accumulation of diglycerides, monoglycerides and free fatty acids.

Among the free fatty acids, C16:0 and C18:2 remained unchanged while C18:1 increased. However, there was no change in the iodine value and in the unsaturation ratio of total fatty acids (table 80).

There was no change in phospholipids on storage (table 81), indicating similarities to results obtained previously for irradiated almonds, but not to those for roasted almonds. This implied that irradiation may have aided in stabilising phospholipids. There is a possibility that irradiation may have a severe effect on phospholipases, which is the enzyme responsible for phospholipid breakdown.

Peroxide value was unchanged, but malonaldehyde content increased (table 82). It appears that malonaldehyde was being formed from peroxides

hence the former increased. The fact that peroxide value still remained unchanged suggested that peroxide formation from the oxidation of unsaturated fatty acids was equal to peroxide breakdown to form malonaldehydes.

It would seem, therefore, that some lipid deterioration had occurred during the storage of roasted-irradiated almonds.

CHAPTER IX

GENERAL CONCLUSIONS

Untreated almonds (controls)

Of the three varieties of almonds used in this study, two varieties Monagha (F)and Sangi (H) were of Iranian origin, while the third, Peerless (S) was from California.

Monagha; Peerless and Sangi almonds were fragile (F), semi-hard (S) and hard-shell (H) respectively. F was the lightest, while S was the heaviest in terms of weight per 100 nuts.

The moisture content was similar in all cases ranging from 5.9 to 6.8%. All these samples were regarded as sweet almonds, since on being tested, the presence of bitter almonds was not detected.

Total available carbohydrate content of almonds ranged from 93.6 to 132.4 mg g^{-1} dry weight. Of this, starch was in the range 34.4 to 58.9 mg g^{-1}, and free sugars in the range 49.5 to 73.5 mg g^{-1}. The latter was made up of eleven components, namely glucose, fructose, galactose, sorbitol, inositol, sucrose, maltose, cellobiose, mellibiose, raffinose and stachyose.

The total nitrogen containing fraction in almonds was calculated to range from 179.5 to 194.5 mg g^{-1} dry weight. The protein part was 174.9 to 188.9 mg g^{-1}, while free amino acids were found to be 4.6 to 5.6 mg g^{-1}. On hydrolysis, the protein was observed to contain eighteen amino acids: tryptophan, lysine, histidine, arginine, aspartic acid, threonine,

serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine.

Essential amino acids amounted to 52.7 to 58.7 mg g^{-1} of which lysine was regarded as the limiting amino acid, although tryptophan and threonine were also found in low amounts.

Total lipids, making up about 50% of the almond kernel, were made up of 96% simple lipids and 3.5% complex lipids. In the simple lipid fraction, monoglycerides, diglycerides, sterols, free fatty acids, triglycerides, sterol esters and hydrocarbons were identified, of which triglycerides were the major component (78%). Constituents of the complex lipids included lysophosphatidyl choline, phosphatidyl inositol, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidic acid, digalactosyl diglycerides, sulpholipid and sterol glycosides.

The quality indices namely iodine, peroxide and malonaldehyde values ranged from 98.2 to 99.3 cg I_2 g^{-1} oil, 2.9 to 4.6 meq 1000 g^{-1}oil and 4.4 to 5.6 mg 1000 g^{-1}nuts respectively.

After one year storage there was a small but significant increase in moisture content in the almonds. This may have some bearing upon the other changes observed in some of the nutrients on storage.

Total available carbohydrate content decreased significantly but this was mainly due to a loss in free sugars of between 13 and 18%. However, due to there being a significant loss in total protein content accompanied by an increase in free amino acids and NH_3 on storage, condensation reaction between sugars and amino acids may have also occurred. This has

resulted in a drop in protein quality due to a decrease in the chemical score.

One year storage did not bring about any significant changes in total lipids or in total simple lipids. However, among the simple lipids, triglycerides appeared lower, but were not significantly different when compared to controls, while free fatty acids increased significantly.

Although phosphatidyl inositol was shown to decrease, there was no apparent change in phosphatidic acid.

In general, with the absence of any changes in peroxide and in malonaldehyde values, it is suggested that no severe deterioration of lipids had taken place during storage. There is the possibility that some reactions may have taken place between amino acids and oxidised lipid products.

Irradiated almonds

Although 2 Mrad irradiation did not alter total available carbohydrate content, it did not seem either to have a degradation effect on some of the macromolecules and free sugars. Starch was found to decrease, while free sugars increased. It was highly likely that irradiation may have had a depolymerisation action on starch and cellulose, thereby bringing about the release of maltose and cellobiose respectively.

Hydrolysis of sucrose, raffinose and stachyose was also brought about which led to the accumulation of monosaccharides. The increase in sorbitol may have been due to its formation from the chemical reduction of either glucose and/or fructose. The latter would suggest that irradiation

had resulted in the formation of free 'H' radicals.

Two Mrad irradiation also brought about minor changes in protein constituents. Ammonia, free amino acids and certain protein amino acids (serine, aspartic acid and tyrosine) increased, while others (lysine, histidine and threonine) decreased. It is possible that the irradiation treatment caused the interconversion of certain amino acids from one form to another. Further it appears that 2 Mrad irradiation reduced protein score by lowering the chemical score for both lysine and threonine.

Total lipid content was not changed by a 2 Mrad dose of irradiation. However, monoglycerides decreased to release free fatty acids (an increase from 46.0 to 75.0%). The total complex lipids of irradiated almonds were not significantly affected by 2 Mrad. Among the individual complex lipids only phosphatidyl inositol decreased significantly. This may explain the observed increase in free inositol within the sugar fraction. Other lipid groups were not severely affected therefore stability of these lipids may be attributed to the presence of natural anti-oxidants or to the formation of new antioxidant factors such as the aldehyde-amine complex. The onset of fatty acid deterioration was detected by an increase in both peroxide and in malonaldehyde contents.

During the storage of irradiated almonds, only the free sugars among the total available carbohydrates were affected. Increases in fructose, glucose and galactose were accompanied by decreases in sucrose, in raffinose and in stachyose. This would suggest that storage of irradiated almonds may have a beneficial effect nutritionally, as the oligosaccharides of the raffinose family cannot be easily digested by the human tract,

therefore, their breakdown during storage increases the digestibility of carbohydrates in almonds.

One year storage of irradiated almonds did not further change total protein, free amino acids or ammonia content. Total essential amino acids remained constant as did the protein score.

Total lipids, total simple lipids and total complex lipids were not affected significantly.

Free fatty acids were not significantly changed after one year storage.

The build up of C18:0 would suggest that hydrogenation of C18:1 and C18:2 fatty acids may have occurred during storage.

That lipids remained stable during storage was supported by the fact that there were no changes in both peroxide and in malonaldehyde values.

Salted and Roasted almonds

Roasting of almonds resulted in decreases in total available carbohydrate content from 19.0 to 24.0 mg g^{-1}. This was due to a 6.3 to 9.5 mg g^{-1} decrease in starch and to a 12.0 to 16.4 mg g^{-1} decrease in free sugars. An increase was only noticed in maltose which resulted from starch degradation.

The sequence of events taking place during the above has been proposed. The first place where sugar loss would have occurred would have been in the salt soaked solution, into which leaching would occur

directly. Moreover, the influx of water into the almonds during this period provided suitable conditions for the activation of enzymes and chemical reactions. Subsequent heating led to hydrolysis. The decrease in monosaccharides and the condition prevailing at the time further suggested the occurrence of browning reactions.

Protein and free amino acid content decreased after roasting. Breakdown was indicated by an increase in ammonia. No doubt, part of the loss was due to protein-salt interactions during the pre-soaking step, whereby protein dissolved somewhat in salt solutions. Losses mainly occurred in essential amino acids, resulting in decreased protein scores.

The above changes go together with losses in free sugars, implying that sugar-protein interactions similar to those in browning reactions may have taken place during roasting. Roasting did not affect total lipid content. Among the individual simple lipid fraction, a significant decrease in triglycerides with a corresponding increase in diglycerides and free fatty acids was observed. The increase in free fatty acids ranged from 117% to 161%.

As mentioned previously, during the roasting process the samples were soaked in salt-solution which increased the moisture content of samples and when they were subsequently heated, hydrolysis of lipids was most likely.

A decrease in iodine value and an increase in peroxide content indicated hydrogenation and oxidation of fatty acids but these were low as the malonaldehyde content remained constant. This low effect may again be attributed to the presence of natural anti-oxidants.

After one year storage of roasted almonds, there was no apparent decrease in starch or increase in total free sugar content, although certain individual sugars, e.g. sorbitol, inositol and cellobiose did increase. There is no doubt that the initial heat treatment during roasting was responsible for the subsequent breakdown of cellulose leading to the accumulation of cellobiose.

There was no evidence supporting the occurrence of browning reactions during storage.

Total protein content remained stable after one year storage but the increase in free amino acids and ammonia suggests some hydrolysis and degradation.

Essential amino acids among protein amino acids were the most affected by storage. Chemical-score of lysine, tryptophan and threonine all decreased, thereby reducing protein quality. These amino acids may have undergone protein-lipid interactions. There was no change in total lipid content after storage, nor in total simple and total complex lipid fractions. However, there was a further increase in free fatty acid content compared to that of roasted almonds before storage, without any significant changes in the other simple lipid fractions. It is suggested that hydrolysis of certain lipid components had occurred, but the effect was not significant.

Among the individual complex lipids, phosphatidyl choline and phosphatidyl inositol decreased significantly. This has led to a sharp increase in phosphatidic acid.

There was again a noticeable increase in both C16:1 and C18:0 among the total fatty acids. This reflected the occurrence of hydrogenation and the possible degradation of C18:1 and C18:2 of total fatty acids.

The overall stability of lipids during storage may have been due to the inactivation of degradative enzymes by the heat treatment.

Dual treated: roasted and irradiated almonds

The dual treatment of almonds resulted in a significant decrease in total available carbohydrates of between 22.0 and 27.5 mg g^{-1} dry weight. This was due to both (i) starch hydrolysis (decrease of 8.0 to 13.0 mg g^{-1}) with a concomitant increase in maltose, and (ii) to a loss in free sugars. Some of the free sugars were lost directly to the salt water during presoaking while the rest may have entered into reactions with proteins.

The fact that total proteins and free amino acids decreased would tend to support the above statement of protein-sugar interactions. Also, the increase in ammonia would suggest some protein degradation.

Aspartic acid increased significantly and it is suggested that the formation of this amino acid may have resulted from the action of irradiation on proline, tryptophan, lysine, glycine and methionine which were all observed to decrease in amounts.

The retention in total essential amino acids was found to range from 88.0 to 94.0%. Losses in total essential amino acids were less than those for almonds subjected to roasting alone. This implies that the dual treatment was better than roasting alone in terms of protein quality.

The total lipid content was not changed significantly. However, a significant decrease in triglycerides was observed, while the two species of diglycerides and free fatty acids increased significantly. The increase in free fatty acids ranged from 129.0% to 189.3%. Decreases in both the unsaturation ratio and iodine value for total fatty acids suggested that the hydrogenation and oxidation of bound fatty acids was taking place. Obviously, these products may have reacted with proteins which themselves were observed to decrease after the combined treatment of roasting and irradiation.

Common changes occurring in lipids after the dual treatment of almonds were as found in roasted ones alone, suggesting that reactions may have followed a similar pathway in both cases.

Among the complex lipids: lysophosphatidyl choline, phosphatidyl choline and phosphatidyl inositol decreased while phosphatidic acid increased. Therefore, part of the increase in free fatty acids may have been due to phospholipid breakdown.

Increase in peroxide and malonaldehyde content implied the onset of oxidation and possibly of rancidity.

After one year storage, total available carbohydrates remained unchanged, there being no decreases in either starch or in free sugars. In fact, the amounts left in roasted-irradiated almonds were similar to those found in roasted-stored, but less than those obtained in irradiated-stored samples. It is suggested that the heat processing part of the roasting-irradiation treatment may have aided in preventing the hydrolysis

of oligosaccharides.

Total protein content did not decrease on storage although free amino acids and ammonia increased. It is concluded that using the dual treatment it is possible to improve the flavour, prevent spoilage and yet maintain equal amounts of protein after storage when compared to stored unprocessed almonds. However, due to decreases in mainly lysine and in threonine, the protein score was affected.

Storage did not result in any significant changes in total lipids, in total simple lipids and in total complex lipids.

It was interesting to note that triglycerides decreased further during storage. No changes were observed in the triglycerides of either roasted-stored or of irradiated-stored almonds. This leads to the suggestion that the dual treatment of roasting followed by irradiation may have had a more severe effect on lipids than either roasting or irradiation alone. Breakdown of triglycerides resulted in the accumulation of diglycerides, monoglycerides and free fatty acids.

No changes in phospholipids on storage were observed, thus indicating similarities to results obtained for irradiated almonds, but not to those for roasted almonds. This implies that irradiation may have aided in stabilising phospholipids. The possibility exists that irradiation may have a severe effect on phospholipase, which is the enzyme responsible for phospholipid breakdown, and hence the stabilising influence of the dual treatment on phospholipids.

Final conclusion

When food is preserved by ionizing radiation as was done in the present study and under processing conditions proposed for commercial production, nutrient destruction is no greater than that which occurs when food is preserved by more conventional means. Because of the protective qualities inherent in food, more specifically with almonds (as has just been shown), the sensitivity to radiation of nutritional components in food is less than that of the same constituents irradiated in pure form or in artificial solutions and mixtures (BREGYADZE and BOKERIYA, 1971; METLITSKII et al., 1968). Therefore the wholesomeness question has largely been resolved and legislative aspect are now well on the road to final international acceptance within the framework of the Coden Alimentarius (POTTER et al., 1982).

It is to be noted that certain countries (e.g. Japan and Germany) have already accepted ionizing radiation as a food preservation process. The U.S.A. has started to canvass opinion for proposed regulations "for using ionizing radiation for treating food, to inhibit the growth and maturation of fresh fruits and fresh vegetables, to disinfest food of insects at doses not to exceed 100 Kilorads (Krad), and to disinfect spices of microbes at doses not to exceed 3 megarads (Mrad)"(LEVEILLE, 1984).

In this case, the dose of 2 Mrad used in the present study is well within the proposed acceptable level of 3 Mrad.

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APPENDIX 1 :

FOR CONVERTING THE DIFFERENCES IN MILLILITRES OF O.IN THIOSULPHATE NEEDED FOR THE FILTRATE AND THE BLANK DETERMINATION (Vo -V) X 10 X T TO GLUCOSE

ml of O.IN thio (Vo -V) x 10 x T	Glucose (mg)	Δ
1	2.4	
2	4.8	2.4
3	7.2	2.4
4	9.7	2.5
5	12.2	2.5
6	14.7	2.5
7	17.2	2.5
8	19.8	2.6
9	22.4	2.6
10	25.0	2.6
11	27.6	2.6
12	30.3	2.7
13	33.0	2.7
14	35.7	2.7
15	38.5	2.8
16	41.3	2.8
17	44.2	2.9
18	47.1	2.9
19	50.0	2.9
20	53.0	3.0
21	56.0	3.0
22	59.1	3.1
23	62.2	3.1

APPENDIX 2:

The chemical score of essential amino acids of unspecified almonds, calculated from values given by FAO (1970).

Try	Lys	Thr	Val	Met	Ileu	Leu	Phe
92	52	76	122	123	88	114	135

Essential amino acids (EAA) of whole egg from hens (FAO, 1970).

	Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	TEAA
weight mg $100g^{-1}$ egg	184	863	634	847	416	778	1091	709	5522
Fraction of Total E.A.A.	0.033	0.156	0.115	0.153	0.075	0.141	0.198	0.128	