CHAPTER - I

I N T R O D U C T I O N

INTRODUCTION

From the time immemorial, selection of safe and nutritious foods was made on a trial and error basis. The outcome of this selection was that, from the neolithic era to the present, the leguminous grains together with some cereal grains, were chosen as food that nourished civilization after civilization in many if not in most regions of the world. In some areas legume foods were partially or totally replaced by animal products while in others they have maintained their position as important sources of supplementary protein (Bressani and Elias, 1974).

It is now widely recognized that agro-economic considerations necessitate the use of plant foods for meeting human nutrition requirements. The major plant foods available to the poor in developing countries are cereals, millets and legumes (Hulse, 1980).

Legumes constitute one of the richest and cheapest sources of dietary proteins for large segments of the world's population, particularly in those countries in which the consumption of animal protein is limited by nonavailability or is self-imposed because of cultural or religious habits (Liener, 1962). Legumes are a major source of food in the world with an annual production in excess of 100 million metric tonnes (Olson et al, 1982). The family 'Leguminosae' comprise approximately 600 genera with around 13,000 species, out of

which only a few, about 20, are of economic importance as legume foods, which are consumed by humans either immature or dried.

Many of the cereals and millets are poor sources of protein because of their deficiency in certain essential amino acids like lysine (e.g. wheat, maize) and tryptophan (e.g. jowar, kodri, maize) (Ramachandran and Phansalkar, 1956; Kuppuswamy et al, 1958) or due to an imbalance of amino acids like leucine and isoleucine (Table-1). On the other hand, legume grains contain twice as much protein as cereal grains and are a rich source of lysine, although relatively low in total sulphur containing amino acids (Elias et al, 1964; Elias and Bressani, 1974; Bressani, 1973). Legumes can be considered as a relatively concentrated source of protein, although compared with the FAO (1973) provisional amino acid scoring pattern, they are not ideal being low in sulphur containing amino acids. However, the high lysine content of most of the legumes, except for groundnut, means that they are complementary to cereals (Aykroyd et al, 1982).

The principal species of legumes belong to the genera Phaseolus, Pisum, Cicer and Vicia, and they have relatively high protein content (20-30%) in comparison with cereals, a low fat (2-5%) and high carbohydrate (55-60%) content. The oil-seed pulses, such as soybean (Glycine max) pea nut (Arachis hypogea), winged bean (Tetragonolobus

ent of various cereals and legumes.

TABLE-1

	Essential amino acid composition (g/g N)							
	Try.	Phe.	Tyr.	Met.	Cys.	Thr.	Leu.	Ileu.	Val
		0.29	0.20	0.15	0.11	0.24	0.75	0.26	0.3
		0.30	0.18	0.10	0.09	0.21	0.88	0.27	0.3
		.43	0.-	0.18	0.11	0.20	0.65	0.36	0.4
		.29	0.24	0.12	0.10	0.28	0.72	0.24	0.3
		.28	0.29	0.15	0.09	0.23	0.50	0.30	0.3
		.28	0.18	0.09	0.14	0.18	0.41	0.22	0.2
		.36	0.18	0.08	0.08	0.22	0.58	0.32	0.3
		.31	0.14	0.09	0.08	0.22	0.50	0.34	0.3
		.35	0.10	0.08	0.06	0.20	0.51	0.35	0.3
		.28	0.17	0.05	0.07	0.24	0.43	0.28	0.3
		.46	0.13	0.16	0.06	0.20	0.45	0.25	0.2
		.30	0.21	0.08	0.10	0.24	0.48	0.32	0.3
		.18	0.18	0.14	0.13	0.18	0.31	0.27	0.2

1. (1978).

purpureus) and a part of lupine (lupinus spp.) are distinct in their group, because of their higher oil and/or protein content (Saio, 1980). Data on nutritional quality of various legumes are given in Table-2, which suggest that the fortification with limiting amino acids improves their protein quality and a considerable portion of pulse protein is not hydrolyzed into amino acids in the digestion (Saio, 1980).

Legumes are not only a good supplement to cereals because of their favourable amino acid composition (Table-3) (Phansalkar et al, 1957; [illegible], 1957; Daniel et al, 1965; 1970; Bressani et al, 1974; Hallab et al, 1974) but also form a valuable source of nutrients like thiamin, niacin and iron (Table-4). Thus in poorer parts of the world, legumes act as a valuable supply of protein, calcium, iron, thiamin and riboflavin. Added to western diets, the contributions of legumes as supplements for animal products in terms of dietary fibre and complex carbohydrates would bring these diets closer to recommended dietary goals (Walker, 1982).

Given the economic compulsions of the situation prevailing in India, the average per capita income is Rs.2340 per annum with a substantial proportion getting less than Rs.1000-1200 per annum and land available being very less (Cf. R.R.D., 1987), a solution in terms of increased supply of milk, eggs, meat and industrially processed foods, is unthinkable and one has to rely mainly on foods of plant

TABLE-2 : Protein qu

	Isoleucine
Common Bean	105
Broad Bean	100
Chick pea	111
Cow pea	96
Lentil	108
Lima bean	124
Lupine	110
Pea	107
Pigen pea	78
Vetch	91
Peanut	84
Soybean	114

(a) - Percentage

(b) - Methionine

(c) - Phenylalani

(d) - Grams of to
The E/T r
oats, 2.3

(e) - Biological
in the body

(f) - Digestibil

(g) - 'NPU' is t
'o' - Firs
'oo' - Seco

TABLE-3 : Essential amino acid composition of cereal-legume combinations.

	Essential amino acid composition g/g nitrogen								
	Lys.	Try.	Phe.	Met.	Cys.	Thr.	Leu.	Ileu.	Val.
Jowar + Bengal gram	0.23	0.06	0.32	0.10	0.09	0.21	0.79	0.29	0.33
Wheal + Bengal gram	0.23	0.07	0.29	0.09	0.12	0.18	0.43	0.24	0.27
Bajra + Bengal gram	0.26	0.09	0.31	0.13	0.10	0.24	0.71	0.28	0.32
Rice + Bengal gram	0.31	0.08	0.29	0.12	0.09	0.23	0.51	0.32	0.35
FAO reference protein	0.27	0.09	0.18	0.14	0.13	0.18	0.31	0.27	0.27

- The ratio of cereal to legume was 4:1.
- Values for amino acids calculated from data given by Gopalan et al. (1978).

TABLE-4 : Nutrient content of cereal-legume combination.

	Protein (g)	Calcium (mg)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Rice (400 g)	27.2	40.0	12.4	0.24	0.24	7.6
Red gram (100g)	22.3	73.0	5.8	0.45	0.19	2.9
Rice (400g) + red gram (100g)	49.5 (95)*	113.0 (28)	18.2 (90)	0.69 (95)	0.43 (45)	10.5 (91)
RDA**	45-60	400-500	20-25	0.72-0.90	0.91-1.20	9.6-12.

- Values derived from Gopalan et al. (1978).

* Values in parentheses are percent of RDA.

** RDA = Recommended Daily Allowances.

origin, while making recommendations for improving the nutrition of the poor (Table-5). India has the largest area in the world under pulse crops. In this country, bengal gram, green gram, red gram and black gram are the chief legumes cultivated and consumed, all of which are easily dehusked and decorticated. The whole legumes are referred to as 'pulses' and the split ones as 'dhals'. Lathyrus sativus or 'kesari dhal' also forms a staple item in the diet of a major part of rural population in parts of Madhya Pradesh, Uttar Pradesh and Bihar, in India.

For reasons, which scientists have yet to fathom, nature has seen fit to endow many plants with the capacity to synthesize a wide variety of chemical substances which are known to exert a deleterious effect when ingested by man and animals. Included among such plants are the many varieties of legumes (Liener, 1975).

Raw pulses contain a wide range of antinutritional (antiphysiological) factors and toxic substances such as hemagglutinins, protease inhibitors, amylase inhibitors, allergens, cyanogens, lathyrus factors, goitrogenic factors, oestrogens, saponins, antivitamin factors, metal binding constituents, flatus factors etc. (Liener, 1962; [illegible] 1969; 1976a; Jaffe, 1969; Pusztai, 1967; Liener, 1980; Walker, 1982; Elkowicz and Sosulski, 1982) (Table-6). According to Liener (1976a) presence of antinutritional factors is one of the main drawbacks limiting the nutritional and food qualities of the legume.

TABLE-5 : Relative efficiency of vegetable foods and animal foods as suppliers of nutrients[a].

Foodstuff	Gross yield per acre (Kg)	Approximate amount producible per acre (b)					
		Calories x 10^5	Protein (kg)	Calcium (kg)	Iron (g)	Vitamins	
						-carotene (ug x 10^5)	Riboflavi (g)
Cereals	350	12.0	35	0.10	21.0	2.1	0.35
Pulses	250	8.6	60	0.20	20.0	3.3	0.75
Oilseeds	300	16.5	78	0.15	4.8	1.1	0.90
Milk[c]	360	2.4	12	0.63	0.6	0.9	0.30
Animal foods[c]	20	0.4-0.8	4-8	0.05-0.10	0.4-0.8	0.3-0.6	0.06
Leafy vegetables	5000-10,000	24-48	200-400	125-250	750-1500	2100-4200	5-10
Root vegetables	5000-10,000	50-100	100-200	10-20	30-60	30-60	5-10
Other vegetables	2500-5000	10-20	50-100	1-2	50-100	45-90	1.5-3.0
Fruits	10,000-20000	50-100	80-160	3-6	120-140	30-60	2-4
Sugar	2000	80	-	-	-	-	-

(a) Rajalakshmi and Ramakrishnan (1969)

(b) Calculated from figures for yeild and average nutrient composition of different food groups derived from values given in food tables for commonly consumed foods. (Aykroyd, 1966).

(c) Assuming that efficiency in the conversion of plant proteins is 3:1 in the case of milk and 10:1 in the case of other animal foods.

TABLE-6 : Antinutritional factors and toxins in cereals and legumes.

Antinutritional factor	Source	Content	Reference
Flatulence producing oligosaccharides	All legumes	2-10%	Cristofaro et al.(19
Phytohemagglutinin	Soybean, kidney beans, field beans, moth beans, lima beans, peas, wheat	2-10% of total protein	Jaffe (1969); Liener (1974).
Saponins	Soybean, groundnut	0.5-1.0%	Birk (1969).
Phytate-phosphorus	All cereals and legumes	50% of all total phosphorus	Gopalan et al.(1971)
Cyanogen glycosides	Limabeans, sorghum, cassava.	Equivalent to 10-250 mg HCN/100 g.	Montgomery (1969)
Protease inhibitors	All legumes	9-26 TIU per 100 gms.	Sohonie and Bhandarkar (1955); Liener and Kakade (19
Osteolathyrogen	Sweet pea (Lathyrus odoratus)	0.1 = 2.5%	Rao et al. (1964)
Neurolathyrogen	Kesari-dhal (Lathyrus sativus)		

It is perhaps ironic to note that although legumes have assumed such an important role in human diet, they have at the same time wide variety of substances which may be considered toxic to the human body (Liener, 1962). Through trial and error man has not only learned to avoid those foods which produce immediate ill effects, but he has also devised ways and means of eliminating them from others.

Osborne and Mendel (1917) observed that young rats given a diet containing heat treated soybean flour grow better than rats given raw soyflour. Subsequently, this was confirmed by a number of investigators using a variety of experimental animals such as mice, pigs, chicks and ducklings (Liener et al, 1949; Kayambashi and Lyman, 1966; Gertler et al, 1967; Liener and Kakade, 1969; 1980; Hewitt et al, 1973; Kakade et al, 1973; Madar and Klein, 1979). The factors related to the improvement of the nutritional value by heat treatment were : 1) trypsin inhibitors and 2) hemagglutinins, primarily. Feeding soybeans and other legumes containing active trypsin inhibitors to the animals causes deleterious effects like growth inhibition, reduction in digestibility, hyperplasia and hypertrophy of pancreas (due to hypersecretion of pancreatic enzymes) and interference with the utilization of sulphur amino acids, partly or fully due to antitryptic activity of inhibitor (Liener, 1962; 1976a; Rackis, 1965; Anderson et al, 1979; Rackis and Gumbmann, 1981; Gates and Morgan, 1982; Gallaher and Scheenman, 1984; Gumbmann et al, 1986). Edible grade soyproducts containing

residual heat resistant trypsin inhibitor activity have been shown to increase the risk of pancreatic neoplasia in rats (Gumbmann et al, 1985; Morgan et al, 1986). Recently it has been suggested that foods with trypsin inhibitors do not initiate pancreatic cancer, but rather they promote the development of pancreatic cancer initiated by chemical other than trypsin inhibitors (Roebuck, 1987). On the other hand, there are other reports that various protease inhibitors including trypsin inhibitors may actually prevent some forms of cancer (Troll et al, 1984; Weed et al, 1985). The correlation between the nutritive value of edible seeds and the content of protease inhibitors present is the subject of much controversy.

Lectins (hemagglutinins) constitute a class of proteins characterized by their property to bind to carbohydrates and glycoproteins, and have been identified as the principal component responsible for the poor nutritive value of many legumes when consumed in raw state (Liener, 1986).

The presence of these phytohemagglutinins in legumes which have the ability to agglutinate the red blood cells from various species of animals has long been recognised (Goodard and Mendel, 1929; Russel et al, 1946; Liener and Pallansch, 1952; Jaffe, 1969). These hemagglutinins are toxic when administered orally or intravenously to mammals and birds (Jaffe, 1973; Liener, 1974). Regarding the effect of hemagglutinin on animals, many investigators have reported

growth retardation and an increased mortality rate in rats fed hemagglutinin containing legumes such as soybeans (Osborne and Mendel, 1917; Liener and Kakade, 1969; Nistan and Liener, 1976a,b), kidney beans (Jaffe and Brucher, 1972; Coffey et al, 1985) and navy beans (Kakade and Evans, 1965a,b). Pancreatic hypertrophy, increase in weight of the kidney and heart, and fatty metamorphosis of the liver are the major symptoms in animals fed raw beans containing hemagglutinins (de Muelenaera, 1964; Kakade et al, 1965; Liener, 1976b). Hemagglutinins are found to get bound to many of the structural and functional glycoproteins of various tissues including specific receptor sites on the surface of the intestine, a phenomenon that would account for the decreased absorption of nutrients like glucose in the intestine due to change in membrane permeability (Jaffe, 1960; Jaffe and Camejo, 1961; Clarke and Denbough, 1971; Steinman and Stryer, 1973; Etzler, 1974; Culvenor and Weidmann, 1976; Jaffe, 1980). Pusztai et al. (1981) observed that in rats fed kidney bean, the poorly digestible lectins react with the intestinal cells in vivo causing destruction of many of the brush borders of the deodenal and jejunal enterocytes which leads to the abnormal absorption of potentially harmful substances. Recently it has been established that the toxicity of lectins has been attributed to the fact that they bind to glycoconjugates located on the luminal surface of the gut, causing morphological changes, leading to an impairment in the normal physiological functions of the intestine (King et al, 1980; 1982; Rossi et al, 1984; Donatucci et al, 1987).

Phytic acid, commonly called myo-inositol hexaphosphate or 1,2,3,4,5,6-hexakis (dihydrogen phosphate) myo-inositol (IUPAC-IUB, 1968) is present as phytin (a mixture of calcium, magnesium and potassium salt of phytic acid) in almost all the legumes and some cereals upto a level of approximately 5% by weight (de Boland et al, 1975). Phytic acid chealtes divalent cations like calcium, magnesium, iron and zinc to form insoluble complexes which are not readily absorbed in the intestine (Davies and Nightingale, 1975; Davies, 1979; Davies and Olphin, 1979; Taylor and Coleman, 1979; Reinhold et al, 1980). The prolonged ingestion of a diet containing high content of phytate has been implicated in theaetiology of certain skeletal disorders (Ford et al, 1972a,b; Reinhold et al, 1973). Rickets and Osteomalacia are reported to be common among populations in Northern India and Pakistan where wheat bread is consumed in excess, suggesting that high content of phytate in wheat may interfere with calcium absorption (Vaishnava and Rizvi, 1971). The interaction of phytate with zinc (Zn^{2+}) has been reported in the rats, chicks and man (Davies and Nightingale, 1975; Ranhotra et al, 1978; Solomans et al, 1979). Phytate has also been shown to have an inhibitory action against enzymes such as pepsin, α-amylase and trypsin (Kanaye et al, 1976; Madhav Singh and Krikorian, 1982; Desphpande and Cheryan, 1984; Knuckles et al, 1985). Phytate affects the body by forming protein-phytic acid, phytic acid-mineral-protein and other related complexes (Cheryan, 1980; Wise, 1983; Knuckles et al, 1985). Zinc deficiency produced by dietary phytate is implicated in the loss of immune functions in mice (Leucke, 1978; de Pasquale et al, 1979). However, it has been reported

that over a period of time adaptation to high phytate diet may occur in people taking high phytate diet (Bhaskaran and Reddy, 1979). Studies on germ free and conventional rats by Wise and Gilbert (1982) indicate that only bacterial phytase is involved in the digestion of phytate in gastrointestinal tract. Tannins (which are present in Sorghum and several legumes)are known to impair utilization of proteins in human and animal diets by binding with and coagulating protein (Butler et al, 1984; Reddy et al, 1985). A number of anti-nutrients may reduce the bioavailability of plant seed proteins through their inhibitory activities towards the digestion or absorption (Ikeda et al, 1986).

Among the legumes, soybeans and peanuts have been reported to produce goitrogenic effects in animals(Van Etten, 1969). Several workers (Van Wyk et al, 1959; Hydowitz,1960) have reported a number of cases of goiter in human infants fed soybean milk. The goitrogenic principle from soybeans has been partially purified and characterized as a low molecular weight oligopeptide composed of two or three amino acids or a glycopeptide consisting of one or two amino acids and a sugar (Konijn et al, 1972; 1973). Raw kidney beans are believed to contain an antagonist of vitamin E as evidenced by liver necrosis in rats and muscular dystrophy and low levels of plasma tocopherol in chicks (Hintz and Hogue, 1964; Desai, 1966). Edelstein and Guggenheim (1970a,b) demonstrated that unheated soyflour is deficient in vitamin B_{12} and contains a heat labile substance that

increases the requirement for vitamin B_{12}. Broad beans contain substances viz. divicine and isouramil which catalyze the oxidation of glutathione and cause a disease known as 'favism' (Donoso et al, 1969; Mager et al, 1969). Saponins present as triterpend glycosides in significant amounts in legume grains (Oakenfull, 1984) impart bitter taste to plant foods and cause physiological disturbances and toxicity to human system (George, 1965). Saponins, also lowers levels of plasma cholesterol (Oakenfull et al, 1979; Cheeke, 1976).

The indigestible substances present in cereals and legumes are cellulose, hemicellulose, mucillages, pectins, saponins, glycosides and alkaloids (McCance and Widdowson, 1946; Aykroyd and Doughty, 1964; Swaminathan, 1967; Liener, 1969). They are not acted upon by the digestive enzymes of the gastrointestinal tract and are mainly excreted in the feces.

Among all the toxins and antinutritional factors which are present in legumes, flatulence producing ~~ef~~ oligosaccharides (which are present in almost all the legumes) and neurotoxin (which is present in L. sativus) are described further in detail.

Flatulence producing oligosaccharides :

Carbohydrate serves as the major source of fuel or energy in the average human diet (approximately 46%) of this, much is in the form of polysaccharides, 20% as starches and dextrins, largely derived from plant foods.

The utilization of carbohydrate part of the diet is governed by three major factors viz. its breakdown by digestive enzymes in intestinal tract, transport of monosaccharides across the mucosal membrane of small intestine and finally intracellular phosphorylation allowing entry into the metabolic processes. Hence only carbohydrates which have structure compatible with structural requirement of each of the above systems will be of nutritional value. Thus the carbohydrates which we consume can be divided into dietary and non-dietary. Major dietary carbohydrates of man consists of starch, sucrose, maltose, lactose, glucose and fructose. Free sugars are not attacked by digestive enzymes, but are absorbed intact through small intestine. Cellulose hemicellulose, pentosans and raffinose family of oligosaccharides are nondigestible carbohydrates of humans. However, due to absence of necessary tissue enzymes the galactosaccharides of the raffinose family (flatulence producing oligosaccharides) are microbially metabolized in the distal part of the intestine.

Oligosaccharides are numerous in plants and can be classified into two classes: the primary and secondary oligosaccharides (Kandler and Hopf, 1980). Primary oligosaccharides comprise those oligosaccharides synthesized *in vivo* from a mono- or oligosaccharide and a glycosyl donor by the action of a glycosyltransferase. They occur free in significant amounts in plants and are of metabolic relevance, e.g. primer function, energy storage, translocation, frost resistance etc. Secondary oligosaccharides comprise all

oligosaccharides arising by hydrolysis of higher oligosaccharides, polysaccharides, glycoproteins and glycolipids in vivo or in vitro which exert their function as structural components. Oligosaccharides based on sucrose are primary oligosaccharides which are formed by the transfer of galactopyranosyl, glucopyranosyl or fructofuranosyl residues to sucrose. Thus raffinose family of oligosaccharides which are formed by the transfer of galactopyranosyl residues to sucrose are parimary oligosaccharides. Sucrose is also an important precursor for the synthesis of D-glucosyl esters of nucleoside diphosphates and thus takes part in the biosynthesis of complex oligo and polysaccharides (Dey, 1980).

The raffinose family of oligosaccharides is constituted of sugars related to raffinose by the fact of having one or more ∝-D-galactopyranosyl groups in their structure. Fig.1 shows the structural interrelationship of three important members of this family viz. verbascose, raffinose and stachyose, where the basic unit is sucrose and D-galactose units are bound to glucose moiety. These D-galactosyl groups are found in nature joined to sugars such as D-glucose, to sucrose, to certain polysaccharides, and to a few non-sugars such as glycerol and inositol (French, 1954).

The oligosaccharides of raffinose family are widely distributed in higher plants (French, 1954; Kandler and Hopf, 1982; Dey, 1985). These galactosyl derivatives of sucrose are accumulated in leaves during photosynthesis (Senser and Kandler, 1967) and in seeds during maturation (Amuti and Pollard, 1977). When raffinose is accumulated in

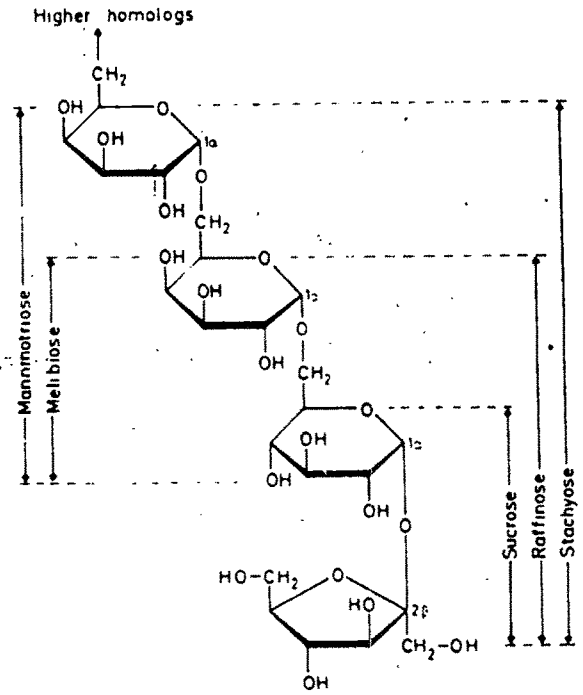


Fig. 1 The raffinose series of oligosaccharides. Raffinose: *O*-α-D-galactopyranosyl-(1-6)-*O*-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside; melibiose: *O*-α-D-galactopyranosyl-(1-6)-D-glucopyranose; stachyose: *O*-α-D-galactopyranosyl-(1-6)-*O*-α-D-galactopyranosyl-(1-6)-*O*-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside; manninotriose: *O*-α-D-galactopyranosyl-(1-6)-*O*-α-D-galactopyranosyl-(1-6)-D-glucopyranose.

higher concentrations, it is virtually always accompanied by stachyose and at least traces of verbascose (Kandler and Hopf, 1980). Among the oligosaccharides, raffinose and stachyose are wide spread in plants, mainly accumulated in storage organs, whereas verbascose and ajugose are less abundant in plants (Nigam and Giri, 1961; Jeremias, 1962; lehman and Mc Ilory, 1967). These members of raffinose family of oligosaccharides are present in appreciable concentrations in the nutritionally important food legumes (Table-7). Soybeans have the highest levels of raffinose (1.9%) and stachyose (5.2%) while chickpeas and pigeon peas have the high levels of verbascose (4.2%) (Hardinge et al, 1965; Cristofaro et al, 1974; Goel and Verma, 1981).

The kinetics of the synthesis of these oligosaccharides are typical of reserve substances. The formation of oligosaccharides during photosynthesis has been demonstrated by several workers (Trip et al, 1963; Senser and Kandler, 1967; Tanner, 1969; King and Zeevart, 1974). It was found that in the leaves of several plants subjected to photosynthesis in presence of $^{14}CO_2$, radioactive incorporation takes place in raffinose, stachyose and an unidentified oligosaccharide that has four D-galactosyl residues (Nikaido and Hassid, 1971). Sufficient in vivo and in vitro evidences have accumulated to describe the biosynthesis of the raffinose series of oligosaccharides as a sequential action of series of α-D-galactosyl transferases (GT) catalysing the reactions as shown in Fig.2. According to Dey (1981), galactinol is known to serve as a D-galactosyl donor in D-galactosylation reactions catalyzed

TABLE-7 : Distribution of flatulence producing oligosaccharides in seeds of various legumes and cereals.

Legume/Cereal	Total carbo-hydrate (%)	Oligosaccharide content (gm/100g of total dry wt.		
		Raffinose	Stachyose	Verbascose
Chick pea	60.9	1.0	2.5	4.2
Cow pea	54.5	0.4	2.0	3.1
Field beans	60.6	0.5	2.1	3.6
Horse gram	57.2	0.7	2.0	3.1
Lentils	59.0	0.6	2.2	3.0
Lima beans	57.0	-	0.2	-
Green gram	56.7	0.8	2.5	3.8
Black gram	59.6	0.5	1.8	3.7
Peas (yellow)	56.5	1.1	2.7	4.1
Soybeans	20.9	1.9	5.2	-
Groundnuts	38.0	0.89	1.5	-
Kidney beans	60.6	0.39	5.6	-
Red gram	57.6	1.10	2.70	4.10
Horse beans	-	0.023	1.07	1.14
Barley	69.6	0.79	-	-
Wheat	71.2	0.71	-	-
Corn	66.2	0.31	-	-
Rice	78.2	-	-	-
Rye	-	0.71	-	-

Values derived from Hardinge et al.(1965); Cristofaro et al.(1974); Sathe et al.(1983) and Kuo et al.(1988)

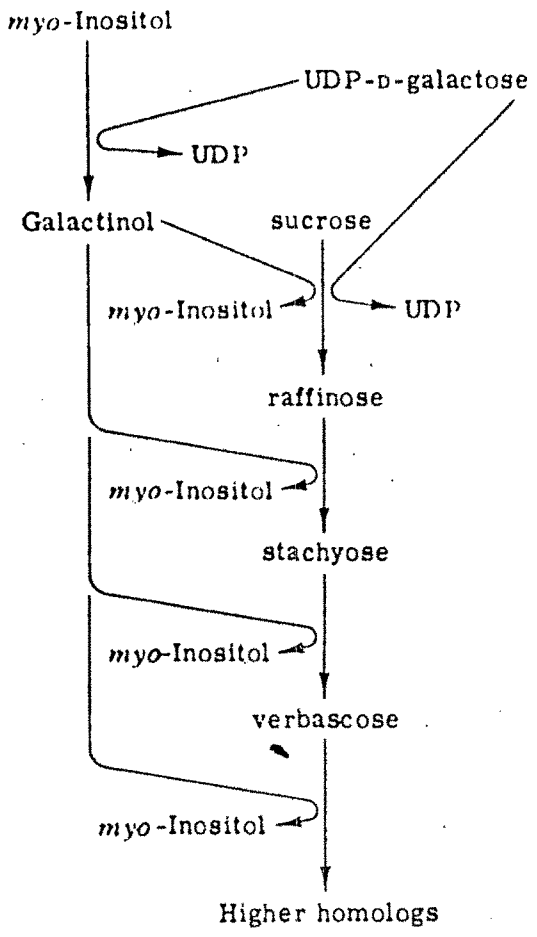


Fig. 2.—Postulated Pathway for the Biosynthesis of the Raffinose Family of Oligosaccharides.

by specific enzymes and a pathway involving galactinol as the D-galactosyl donor is the most important for the biosynthesis of raffinose family of oligosaccharides in plants.

In 1962, Korytnyk and Metzler showed the formation of raffinose and its higher homologs in the maturing beans of Phoseolus lanatus, and suggested the participation of UDP-D-galactose as the D-galactosyl donor in the biosynthesis. Bourne and coworkers (1962) demonstrated that an enzyme preparation from the mature seeds of Vicia faba is able to synthesize raffinose when incubated with a mixture of sucrose, ∝-D-galactosyl phosphate and UTP. It was suggested that the later two compounds react enzymatically and produce UDP-D-galactose, D-galactosyl group of which is then transferred to sucrose to form raffinose. Although the two enzymes galactinol sucrose--6-galactosyl transferase (GST) and galactinol : raffinose-6-galactosyl transferase (GRT) exhibit some hydrolytic activity, they differ clearly from ∝-galactosidases according to some group of workers (Kandler and Herbert-Hopf, 1980). However, earlier studies carried out by Dey and Pridham (1972) suggested that ∝-galactosidases from seeds catalysed transfer of the galactosyl group of ∝-D-galactosides to sucrose, raffinose and other higher oligosaccharides.

The important functions of oligosaccharides in plants are : good source of carbohydrate reserve in vegetative storage organs (Wamer, 1958; Jeremias, 1969; Dey and Pridham, 1977), role in cold-acclimation (Levitt, 1962; 1972;

Alden and Herman, 1971; Larcher et al, 1973; Dey, 1985), photoperiodism (Kandler et al, 1979), primer for the synthesis of higher oligosaccharides (Dey, [illegible], 1980) and importance in sugar translocation (Ziegler, 1975). Kandler et al. (1979) showed that raffinose synthesis and cold acclimation are strictly coupled even under the influence of unnatural combinations of different photoperiods. By in vitro experiments, it has been shown that protoplasmic membranes of plant cells are damaged in frost and drought and this damage can be protected by some mono and oligosaccharides (Heber and Santarius, 1964; Santarius and Heber, 1967). It has been suggested that affinity of the hydroxyl groups for water may influence the microenvironment of the labile membrane bound proteins, thus stabilizing them against different stress conditions by binding directly to the protein molecules preventing their denaturation (Levitt, 1972; Santarius and Milde, 1977).

In several plant ps species, the levels of raffinose saccharides in seeds have been shown to decrease during germination (Teufel et al, 1960; Pazur et al, 1962; Aman, 1979; Reddy and Salunkhe, 1980b; Doman et al, 1982; Abdullah et al, 1984; Ologhobo and Fetuga, 1986). Detailed information regarding the mobilization of these oligosaccharides in seed components during germination is limited. However, recent studies done by Kuo et al. (1988) suggest that raffinose saccharides are hydrolyzed directly in cotyledons during germination rather than being transported to embryonic axes for metabolism.

The enzymes involved in the degradation of the raffinose series of oligosaccharides are α-galactosidase and β-D-fructofuranosidase, the levels of which increase during germination of seeds (Dey, 1978; Pridham and Dey, 1974). Melibiose, the splitting product of raffinose after the action of β-D-fructofuranosidase has been found in storage organs and even in areas which contain large amount of raffinose.

The metabolism of the members of the raffinose family of oligosaccharides in humans would require the presence of α-galactosidase in the intestine. However, such an enzyme does not seem to be present in man and other animals (Gitzelmann and Auricchio, 1965; Rutloff et al, 1967; Cristofaro et al, 1974). A significant problem associated with the use of legumes in developed countries is poor consumer acceptability due to gastrointestinal discomfort and flatus production (Rackis et al, 1970; Calloway et al, 1971; Cristofaro, et al, 1974; Olson et al, 1981). The α-galactosides principally raffinose, stachyose, and verbascose have been identified as important contributors to these adverse physiological effects.

Blair et al. (1947) were among the first investigators to quantitate the production of flatus in man and to show that soybean increased gas production. Steggerda (1962, 1965, 1968) working with human subjects indicated that when beans

are consumed, the normal physiology of the gastrointestinal tract is altered so that flatulence production is markedly stimulated. He observed that with a pork-bean diet, fed to human subjects, flatus production increased from 16 cm^3/hr on a normal diet to an average volume of 190 cm^3/hr. Further experiments suggested that α-galactosides (raffinose and stachyose) were the principle sources of gastrointestinal gases from soybean ingested by either man or dog (Steggerda et al, 1966; Steggerda, 1968). Members of raffinose family of oligosaccharides are not digested by man and other animals mainly because the intestinal mucosa lack the hydrolytic enzyme α-galactosidase and the oligosaccharides themselves are unable to pass through the intestinal wall due to their high molecular weights.(Taufel et al, 1965; 1967; Krause et al, 1968). The microflora in the lower intestinal tract (colon) then metabolize the oligosaccharides and produce nitrogen, oxygen, methane, hydrogen and carbon dioxide and the pH is also lowered (Hardinge et al, 1965; Calloway et al, 1966; 1971; Hellendoorn et al, 1969; Cristofaro et al, 1974; Olson et al, 1975). Evidently, the oligosaccharides are first broken down to their constituent monosaccharides before the gas producing mechanism can operate. In 1967-68 Steggerda, performing experiments both on man and animals, investigated that clostridia group of organisms usually inhdbit the gastro-intestinal tract of man and animals and this flora is responsible for flatus with high concentration of carbon dioxide following ingestion of bean products. These results were confirmed by Richards et al. (1968) who proved that bacteria must be Clostridium perfringens. Later on Sacks and Olson

(1979) have shown that different strains of C. perfringens vary greatly in their ability to utilize and grow on raffinose and stachyose when these sugars were added to a casein digest medium in vitro.

In vitro experiments of Rockland et al. (1969) and Rackis et al. (1970) found that the gas producing factor is closely associated with the oligosaccharides of the leguminous seeds. Steggerda et al. (1966) and Rackis et al. (1970) studied the effect of crude 70% ethanolic extract of soybean on flatulence production in man and showed that it is largely due to the oligosaccharides present in this fraction, while soybean protein fraction was found to be non-flatulent. Thus the presence of raffinose family of oligosaccharides in the diet contribute to gastrointestinal problems which can cause a person to have flatulence and the problems associated with flatus, viz, rectal gas expulsion, [illegible], headache, dizziness, abdominal rumblings, pain, cramps, nausea and diarrhoea (Cristofaro et al, 1974; Laseer et al, 1975; Tobin and Carpenter, 1978; Olson et al, 1981). The gas that develops from the microbial activity in the colon is more likely to be trapped in the pockets where it is formed producing pain during intestinal contractions. According to Olson et al. (1981) belching, bloating, constipation, diarrhea, overeating, anxiety and specific food responses have all been associated with symptoms known as flatulence. Fleming (1981) in a study of seven different types of legume seeds showed significant

positive correlation between hydrogen production and the following chemical components : stachyose, raffinose plus stachyose, glucans and pentosans hydrolysable in diluted acids. Significant, negative correlations were determined between hydrogen production and starch or lignin contents.

Although raffinose family of sugars are held responsible for flatulence, there are reports on flatus production by starch and other unavailable carbohydrates as well (Hellendoorn, 1978; Jaya, 1978). Results of *in vitro* and *in vivo* studies by Faki *et al* (1983) indicated that apart from oligosaccharides, both the starch and hemicelluloses contributed substantially to the total flatulence effect of chick pea, cow pea and horse gram. According to Olson *et al*.(1981) in California small white beans, the removal of the α-galactosides from beans does not remove all of the flatus producing capability of the beans and component of the extracted beans that could be involved in flatus formation is the food fiber. Hickey *et al*. (1972) and Meyer and Calloway (1977) reported flatus production in young men and elderly women, when they were fed bran and bran products, which contain large amounts of food fiber, specifically hemicelluloses. Salyers *et al*. (1978) demonstrated the *in vitro* degradation of hemicellulose by intestinal bacteria. The food fiber content of dry beans is considered to be mainly a hemicellulose which is presumed to be fermentable in the colon (Hellendoorn, 1976; 1978). Cellulose and lignin are either not present in very large amounts in the plant part ingested or they are removed before

the food is eaten (Hellendoorn, 1978). Additional work is needed to verify the indigestibility of the fiber fraction, its ability to be fermented in the gut to form flatus and its possible synergism with α-galactosides. Still the composition and behaviour of intestinal microflora and their important role in intestinal fermentation requires further research.

Since raffinose family of sugars are present in food raw materials such as legumes, known as staple foods in many countries, various approaches have been suggested in order to lessen the flatulence factors from food legumes. The important ones are (a) development of special varieties of legumes by genetic manipulations that may lower the level of oligosaccharides (Dey, 1980), (b) removal of oligosaccharides from the legume products using solvents, (c) use of several food processing methods viz. soaking, boiling, parching and germination (Calloway et al, 1971; Kim et al, 1973; Ku et al, 1976; Cristofaro et al, 1974; Olson et al, 1981; Sathe et al, 1983) and (d) use of enzymes that hydrolyze the oligosaccharides (Shallenberger et al, 1967; Suzuki et al, 1969; Sugimoto and Buren, 1970; Reynold, 1974; Cruz et al, 1981). Among these methods, enzymatic treatments could be applied either with a preparation rich in α-galactosidase or with micro-organisms capable of utilizing the oligosaccharides of the raffinose family.

Neurotoxin present in Lathyrus sativus :

Although proteins in the seeds have various biological functions such as enzymic, hormonal, structural, reserve etc, the quality of proteins particularly storage protein depends on their amino acid content (Roy, 1981). It may also be possible that quality of protein may be good, but because of the presence of toxins, such as hemagglutinins, protease inhibitors, favism factors, alkaloids and free toxic amino acids, they are not acceptable. Besides the 20 amino acids that are necessary for the formation of proteins in plants, there are many unusual amino acids found in free state, viz. γ-aminobutyric acid (GABA), homoserine, citrulline, ornithine etc. Some of the free amino acids have deleterious effects on animals by feeding or on administration by different routes. A number of free but unusual amino acids having structural similarities to regular protein amino acids have been recognized (Fowden, 1962). They act in a competitive way in the biological system as antimetabolites, showing sometimes growth retardation and other abnormalities.

Table-8 summarizes some of the free toxic amino acids found in legumes. Legumes belonging to Lathyrus and Vicia species are known to be toxic to man and animals due to the presence of free unusual ninhydrin reacting toxic amino acids (Roy, 1981). Lathyrus sativus, Lathyrus cicera and Lathyrus clymenum seeds, consumption of which is implicated in

TABLE-8 : Naturally occurring toxic amino acids and derivatives in Leguminosae.

Toxic amino acid and derivative	Source	Toxicity	Reference
1) L- ∝,γ -diaminobutyric acid or L-2,4-diaminobutyric acid and γ -N-oxalylderivative.	L. latifolius (perennial pea) L. sylvestris (Flat pea) and some Lathyrus species.	Neurotoxic	Ressler et al.(1961); Bell (1973.
2) β -cyanolalanine and γ -glutamyl- β-cyanoalanine	V. sativa (common vetch) V. augustifolia and 15 other species of Vicia	Neurotoxic	Ressler (1962), Bell & Tirimanna (1965) Ressler et al.(1969).
3) β -(N-γ-Glutamyl)-amino-propionitrile.	L. pusillus (Singletary pea) L. odoratus (sweet pea)	Ostectoxic	Dupuy and lee (1954); Schilling and Strong (1954)
4) β -(N)-oxalyl-L-∝,β diamino-propionic acid (ODAP) or β (N)-oxalylamino L-alanine (BOAA)	L. sativus (Chickling vetch) L. cicera (Flat podded vetch) L. latifolius.	Neurotoxic	Murti et al. (1964); Rao et al. (1964); Bell (1973).
5) Canavanine	Canavalia ensiformis (Jack bean) and Vicia sp.	Inhibits growth of Neurospora and other microorganisms.	Bell and Tirimanna (1965)
6) Djenkolic acid	Pithecolobium lobatum (Djenkol bean)	Renal toxic	Veen and Hynan (1933); Gmelin (1959).
7) β -Nitropropionic acid	Indigofera endecaphylla (creeping or trailing indigo)	Hepatotoxic and Neurotoxic.	Britten et al.(1959)

classical lathyrism, a neurological disease (Ganapathy and Dwivedi, 1961) contain a high concentration of β -N-oxalyl-L- ∝,β-diaminopropionic acid (ODAP). Excessive oral intake of lathyrus sativus (khesari dhal or chickling pea) legume (which is known by several vernacular names such as 'teora', 'lakh' or 'lang' dhal) in remote areas of the world causes humans and animals to develop a type of spastic paraparesis or a crippling disease known as "neurolathyrism" (Seyle, 1957; Dwivedi and Prasad, 1964; Rao et al, 1969). Disease is characterized by slow or more rapid onset of spastic paraparesis associated with degeneration of corticospinal pathways which results into muscular rigidity, paralysis of leg muscles and in extreme cases death (Buzzard and Greenfield, 1921; Filiminoff, 1926; Puig and Devinals, 1943; Ganapathy and Dwivedi, 1961; Sachdev et al, 1969; Streifler et al, 1977). The major symptoms of the neurotoxicity in humans after excessive ingestion of kesari dhal are weakness, spasticity of leg muscles, convulsions and death in extreme cases, as described in Table-9. Epidemics of lathyrism, chickling-pea poisoning, frequent in the past, still occur in endemic form in some Asian and African countries (Streifler and Cohn, 1981). 'Neurolathyrism' outbreaks have been reported in Ethiopia, Bangladesh, Pakistan, Germany, Greece, Italy, Algeria, Iran and India (ICMR Report, 1964; Rutter and Percy, 1984). 'Neurolathyrism' referred to in the ancient Hindu treatise 'Bhavaprakasha' and by Hippocrates (Cf. Padmanabhan, 1980), 'Pliny' and 'Galen' continues to be a

TABLE-9 : The four stages of neurolathyrism.

(Patwardhan, 1961)

Stage	Symptoms
I	Weakness of lower limbs with spasticity of several muscles. Pain in ankles, knees. Swing round of the foot while walking.
II	Flexion of knee. Inversion of foot with tendency to walk with a stick.
III	Two stick stage prevails. Other symptoms become chronic.
IV	Knees completely flexed. Atropy of thigh and leg muscles. No motor disturbance in upper extrimeties. No sensory disturbance in affected limbs.

public health problem in the central part of India, especially during famine, when L. sativus seeds form the main part of the diet. The main reasons for continuing the cultivation of this species are that, unlike other grain legumes, (a) it can be grown with remarkable ease and does not involve complicated managerial services, (b) it has a high degree of adaptability under extreme conditions,and (c) it is a traditionally favoured item of food and feed among economically handicapped segments of the society. Moreover, under extreme conditions, such as drought and water logging, this is the only crop that remains available to the poor because of its hardy character (Misra et al, 1981)

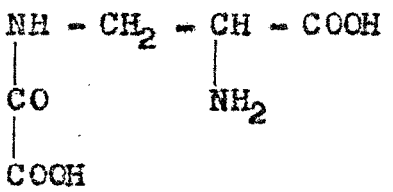
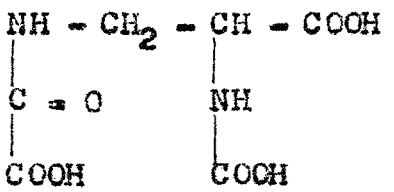
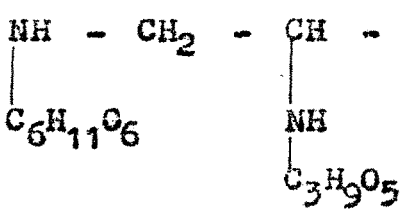
Extensive studies have led to the recognition that lathyrism in human beings, which is characterized by spastic paralysis of the legs as a result of neurological lesions of the spinal cord degeneration, is quite different from the skeletal abnormalities induced in experimental animals by feeding L. odoratus or β -aminopropionitrile (BAPN). Leyle (1957) clearly distinguished between the two forms of lathyrism, he referred to the latter manifestation, as "osteolathyrism" and to the human disease as "neurolathyrism".

Neurolathyrism generally appears when a diet consisting of one-third to one-half of L. sativus seeds is consumed for 3-6 months (Dwivedi and Prasad, 1961). The disease afflicts adult humans who have consumed a nutritionally inadequate diet

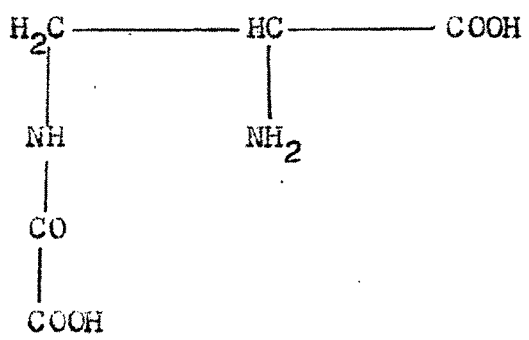
containing 200g or more per day of L. sativus seeds for several months (Dwivedi and Prasad, 1964; Sarma and Padmanaban, 1969). Passios and Demopoulos (1962) reported that the consumption of L. cicera or L. sativus or both by semistarved people for a period of 10 years resulted in neurological symptoms. Streifler et al.(1977) reported that the symptoms of neurolathyrism were apparent after an imprisoned person was kept on a diet of L. sativus for 30 years. But a recent outbreak of the disease in Madhya Pradesh, India showed that the onset can occur much earlier, around 20 days (cf. Rutter and Percy, 1984).

Various claims have been made regarding the factors responsible for the disease neurolathyrism. The various factors reported to cause the disease include (i) an acidic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (Rao et al, 1964; Murti et al, 1964), (ii) a phenol compound of unknown structure whose neurotoxic effects are potentiated by certain amino acids (Nagarajan et al, 1966), (iii) a glycoside which may be N-β-D-glucopyranosyl-N-α-D-arabinosyl α,β-diaminopropionitrile (Rukmini, 1968), (iv) deficiency of tryptophan (Basu et al, 1936) and high selenium content (Rudra et al, 1952) in L. sativus seeds. Finally studies carried out by Rao et al. (1964) and Bell and Tirimanna (1965) led to the identification of unusual amino acids in L. sativus seeds out of which β-N-oxalyl-L-α,β-diaminopropionic acid which is present along with the other toxic components (Table-10).

TABLE-10 : Different toxic factors present in Lathyrus sativus.

Name of Compound	Structure	Concentration (gm %)	Reference
ODAP (β-N-Oxalyl-L-α,β-Diaminopropionic acid)		0.1 - 2.5	Rao et al. (1964)
DODAPA (Dioxalyl diamino-propionic acid)		0.25	Rajamohan and Ramachandran (1972)
N-β-D Glucopyranosyl N-α-L-arabinosyl α- β-diaminopropionitrile.		0.1	Rukmini (1968)
Phenolic compound		0.05	Nagarajan et al. (1966)

According to Spencer and Schaumburg (1983) the chemical entity responsible for lathyrism is still unknown, but for studies on neurolathyrism, focus is at present placed on this dicarboxylic diamino acid, which is also known as β -N-oxalyl-amino-L-alanine (BOAA) (Roy, 1981) with the following structure:



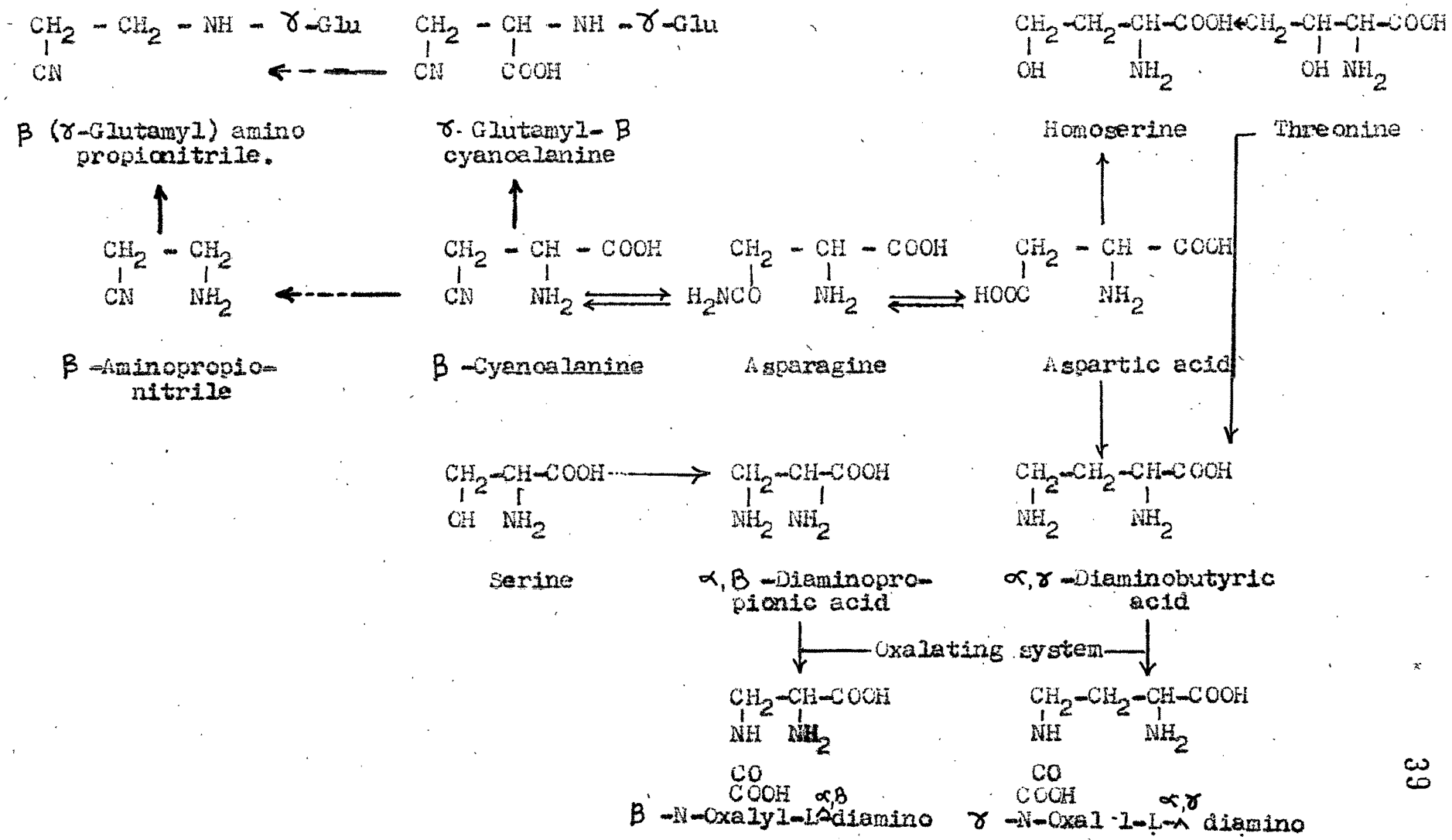
Three research groups in India (Murti et al,1964; Roy et al, 1963; Rao et al, 1964) have reported the isolation of a neuro-toxin (ODAP) from L. sativus seeds. Bell and O'Donovan (1966) observed that the naturally occuring neurotoxin (ODAP) exists in two isomeric forms alpha and beta, which are separable by high voltage electrophoresis. Roy and Rao (1968) showed that irrespective of the concentration of ODAP in different varieties of L. sativus, the proportion of alpha and beta isomers of the amino acid remains unchanged, the beta isomer in L. sativus ranges from 92 to 96% of the total ODAP content.

There have been several attempts to establish the bio-synthetic pathway of ODAP in plants. Based on the structural relationship of ODAP and its occurrence in high concentrations in L. sativus, serine had been suggested to be the precursor

of the diaminopropionic acid moiety in the biosynthesis of ODAP (Murti and Seshadri, 1964). On the contrary, Roy (1969) observed that when L. sativus seeds were germinated in the presence of DL-serine-3-^{14}C and ODAP was isolated and purified, no radioactivity was found to be associated with the ODAP fraction, suggesting that serine was not a precursor for ODAP synthesis.

It is interesting to note that the naturally occuring osteolathyrogen β-(γ-glutamyl) aminopropionitrile, and the neurotoxic amino acids including ODAP are biosynthetically inter-related as shown in Fig.3 (Nigam and Ressler[5], 1964; 1966; Ressler et al, 1961). With regard to the biosynthesis of ODAP, evidence has been obtained to indicate that (U-^{14}C) oxalic acid is incorporated as an unit into ODAP in the germinating seedling of L. sativus (Malathi et al, 1968). It has been demonstrated that ^{14}C-labelled ODAP is produced when the dibasic acid α,β-diaminopropionic acid is incubated with ^{14}C-labelled oxalic acid in presence of oxalyl activating enzyme system requiring CoA, ATP and Mg^{+2} (Johnston and Lloyd, 1967). Malathi et al. (1967) have confirmed that the transfer enzyme in L. sativus seeds reacts specially with the beta amino group of α,β-diaminopropionic acid. However, other investigators (Bell and O'Donovan, 1966; Roy and Narasinga Rao, 1968; Wu et al, 1976) suggested that isomeric rearrangement may be non-enzymic.

FIG.3 : A biosynthetic scheme for lathyrogens.
Dashed arrow, experimental proof not available,
Solid arrows, experimentally confirmed.



Ganapathi and Dwivedi (1961) on the basis of their epidemiological survey, concluded that symptoms of the adverse effects of neurolathyrism do not manifest themselves in the same manner in both males and females suggesting that the hormones might play an important role in the females resistance to the disease. These conclusions were supported by Das et al. (1974) who found the ratio of patients to be 16.9 males to 3.3 females.

To find out the actual toxic reactions involved during neurolathyrism, different animal models were tried out to create the exact neurotoxic symptoms as summarised in Table-11. Experiments by Roy et al.(1963) and Nagarajan et al. (1965) established that the injection of crude 30% ethanolic extract of L. sativus seed meal caused neurological symptoms in one day old chick. This toxin was shown to be nontoxic to adult animals (Adiga et al, 1963; Rao et al, 1965; Nagarajan et al, 1965). This led to the hypothesis that blood brain barrier (BBB) in the CNS may not be allowing entry of toxin (Curtis and Watkins, 1965). In support of their claim, these researchers (Rao et al, artificially created an acidotic condition in the adult animals through oral administration of acid forming salts such as calcium chloride and ammonium chloride, and of drugs such as dimox, sulfanilamide or salicylic acid. Under such conditions the "BBB" system was broken, thereby enabling ODAP to penetrate into the brain. During their experiments when ODAP at the level of 0.5 mg/g body weight was administered intraperitoneally in acidotic adult rats and mice, the typical neurotoxic

TABLE-11 : Effect of ODAP administration into different animals.

Reference	Type of injection and dose	Results obtained
1) Roy et al. (1963)	Injection of alcoholic extract of L.sativus seeds to one day old chick.	Production of neurotoxic symptoms.
2) Nagarajan et al. (1965)	Injection of alcoholic extract of L.sativus seeds to baby pigeons and ducklings at the dose of 1.4 mg/gm.	Retraction of head, twisting of neck.
	Intraperitoneal injection to adult birds.	No neurological symptoms.
3) Adiga et al.(1963)	Injection of alcoholic extract of L.sativus seeds to one day old chick at the dose of 10-20 mg.	Acute neurological symptoms for 8-12 h.
4) Rao and Sarma (1967)	Parenteral administration of ODAP to young rats, mice, guinea pigs and monkeys at the dose of 20-100 mg/kg body weight.	Typical neurological symptoms.
5) Rao et al. (1967)	Injection of ODAP through lumber route at interval of 2-3 days to adult monkeys.	All animals showed weaknes and sporadic jerks in lumb region and lower limbs, destruction of nerve cells and death.
	Intracranial and intracerebral injections to adult rats and mice.	Typical paralytic symptoms
	Intraperitoneal injections to adult rats, mice and monkeys when previously fed with $CaCl_2$ ammonium chloride and Dimox.	Showed neurological sympto

TABLE-11 (Contd.....)

Reference	Type of injection and dose	Results obtained
6) Rao (1978a)	Injected both acidotic and normal adult monkey with (H^3) labelled ODAP, intraperitoneally.	Detection of (H^3) labelled ODAP in CNS of both the types of animals.
7) Mehta et al.(1976)	Injected radioactive ODAP intraperitoneally to young squirrel monkey with mature blood brain barrier.	A tracer dose of ODAP enters the CNS of subhuman primate.
8) Parker et al.(1979)	Intraperitoneal injection of ODAP to adult squirrel monkeys at the dose of 0.75-2.0 mg/g body weight.	Production of acute neurological symptoms viz. drowsiness, vomiting, muscle tremors, convulsion and death.
9) Mehta et al.(1979)	Intraperitoneal injection of (^{14}C) ODAP to adult mouse at the dose of 7.67 µmol/g body weight.	Severe neurologic signs, ODAP enters CNS.

symptoms were observed (Rao et al, 1967). However, Mehta et al. (1976) questioned the existence of a blood brain barrier for neurotoxin in the young squirrel monkey with a mature blood brain barrier. These workers showed that a tracer dose (about 250-500 µg/kg body weight) of the ^{14}C-ODAP can enter the CNS of the subhuman primate. It was further confirmed by Rao, et al. (1978a) by injecting H^3-labelled ODAP that the toxin enters the CNS irrespective of age, species and conditions like acidosis. However, the uptake of ODAP is more in immature brain as compared to the adult mouse brain (Mehta et al, 1979). Earlier studies carried out in this laboratory also showed that ODAP is toxic to adult rats at a dose of 60 mg/100 g body weight when injected intraperitoneally (Ramchand, 1984). Thus the concept of a blood brain barrier to the neurotoxin (ODAP) needs further investigations.

ODAP was found to affect the adult rats and mice administered intravenously, intracisternally, intrathecally, intracerebrally or lumbar route (Rao et al, 1967). Curtis and Watkins (1963) have found that ODAP is a potent excitant of spinal interneurons and betz cells. Watkins et al. (1966) reported that the neurotoxin (ODAP) is the most potent amino acid excitant of the spinal interneurons and Betz cells in the cat spinal cord when administered micro-electrophoretically into the immediate extracellular environment of single neurons. Olney et al. (1976) found that ODAP injected intraperitoneally to immature mice

induces lesions in the retina, hypothalamus, and lower medulla and that this pattern of damage is similar to that demonstrated in animals after oral or subcutaneous administration of glutamate.

Table-12 summarize the various experiments done to understand the mode of action of ODAP, when injected into different animals, which did not give any conclusive mechanism for neurolathyrism but the other possible mechanism of ODAP action has emerged as a result of some recent investigations. Mehta et al. (1972) found that ODAP is a potent antagonist of L-glutamic acid transport in resting yeast cells and these workers also suggested possibility of such an antagonism at the synaptic level in the animal brain. Duque Magalhaes and Parker (1972) found ODAP to inhibit glutamate transport in isolated mitochondria. BOAA is chemically and neuropharmacologically related to glutamic acid and shares the property of glutamate analogues in inducing convulsant activity and circumventricular neurological damage when administered in large single doses (Rao et al, 1969; Roy, 1973; Olney et al, 1971, 1974; Olney, 1980). Ikeda and Haskell (1972) found that ODAP blocks the transmission of nerve impulse from nerve to muscle in the fleshfly, a synapse where L-glutamic acid is believed to be the neurotransmitter. When applied electrophoretically, ODAP is more potent than glutamate as an excitant of cat spinal motor neurons and nearly twice as active as kainic acid in producing depolarization of ventral

TABLE-12 : Mechanisms of ODAP toxicity.

Experimental animal	Results obtained	Conclusion	Reference
1) Cat	Effect on Betz cells and spinal cord.	ODAP is a potent excitant.	Watkins et al, (1966)
2) One day old chick	With onset of symptoms, a decrease in activity of L-glutamate NAD oxidoreductase in the brain.	Toxicity due to an increase in ammonia concentration.	Jacob et al. (1967)
3) Adult rats	Increased levels of glutamine and ammonia in brain. - No change in urea, aspartic acid and glutamic acid.	Toxicity due to effect on ammonia fixing and ammonia generating system of the brain.	Cheema et al. (1968, 1969)
4) Intraperitoneal injection into variety of animals.	- Head retractions, tremars and convulsions. - Increased rate of respiration.	ODAP is a typical convulsant.	Cheema et al.(1970)
5) Mouse	Damage to the tissues of central nervous system.	Effect similar to toxic effect of glutamate.	Olney et al. (1976)
6) Ingestion of *L. sativus* for 30 years by humans.	Loss of axon and myelin, degeneration of horn cells.	Total site of action of ODAP is the spinal cord.	Striefler (1977) et al,
7) Rat	- Changes in lysosomal enzymes, - Increased levels of degradative enzymes.	Toxicity due to lysosomal damage.	Quereshi et al.(197
8) Cat	When applied electrophoretically, BOAA acted as an excitant of cat spinal motor neurons.	ODAP acts by stimulating postsynaptic glutamate receptors.	Pearson and Nunn (1

TABLE-12 (Contd...)

Experimental animal	Results obtained	Conclusion	Reference
9) Rat	ODAP inhibited a high affinity transport of glutamate and aspartate in rat brain and spinal cord synaptosomes.	—	Ross et al.(1985).

root neurons in hemisected frog spinal cord (Pearson and Nunn, 1981). These properties of ODAP suggest that the compound acts by stimulating postsynaptic glutamate receptors (Olney et al, 1976; Pearson and Nunn, 1981).

Ross et al. (1985) suggested that ODAP might produce convulsant activity and neuronal damage by inhibiting synaptic reuptake of excitatory neurotransmitters. It is also reported (Lakshmanan and Padmanabhan, 1974a,b; Ross et al, 1985) that ODAP (1 mM) competitively inhibits high affinity uptake of glutamate and aspartate in rat brain and spinal cord synaptosomes. Thus from all these experiments, it can be concluded that the net result of ODAP action could be an accumulation of glutamate/aspartate in the synaptic environment of brain since it is well established that glutamate is both neuroexcitatory and neurotoxic (Johnson, 1972; Padmanabhan, 1980). The other possibility is that ODAP behaves like glutamate and mimics the effects (Padmanabhan, 1980).

Although considerable effort has been expanded to understand the mechanism of ODAP action, the actual mechanism is still quite unclear or none of these studies are able to pinpoint the exact biochemical abberation responsible for paralysis and death at the administration of neurotoxin to rats. When L. sativus diets was fed to different animals, viz. guinea pigs, dogs, ducks, monkeys and birds (Bhagwat, 1946;Anderson et al, 1924; Stockman, 1929; Patwardhan, 1952; Panda et al, 1972) symptoms resembling neurolathyrism were

not produced. Oral administration of synthetic ODAP at higher dose of 300 mg/100g body weight to monkeys did not result into neurological manifestation (Rao, 1975a). ODAP is not found to be attacked by the common digestive enzymes (Mehta, 1972). In vitro experiments carried out using everted intestinal sac showed that ODAP can pass from mucosal to serosal fluid without any hydrolysis (Mehta et al, 1976). Studies carried out by injecting radiolabelled ODAP into adult rats and monkeys showed that the toxin could be detected in CNS tissue as well as other tissues (Kidney having the highest concentration) but the metabolites of ODAP could not be detected (Mehta et al, 1976; Rao, 1978a) suggesting that these tissues do not posses the enzymes for the hydrolysis of neurotoxin. Mehta et al. (1976) reported the presence of an unidentified radioactive 2,4-dinitrophenylhydrazone derivative of ODAP in the stomach of squirrel monkey. These workers also reported the detection of the α-isomer of ODAP, which is nontoxic, that could arise as a result of nonenzymatic rearrangement during extraction and isolation of ODAP (Bell and O'Donovan, 1966; Wu et al, 1976). Neither ODAP nor oxalyl-CoA synthetase activity has been detected in extracts of cat and rat brain (Johnston and Lloyd, 1967). ODAP appears to be a substrate for rat kidney L-aspartate-2-oxoglutarate aminotransferase and some 5-10% of ODAP administered to adult rats undergo transamination to yield N-oxalyl-β-aminopyruvic acid, while 50-70% is extracted unchanged in the urine within 24 hr (Cheema et al, 1971). Rao (1978a) also found that neurotoxin (ODAP) remains metabolically inert in the rhesus monkey.

Earlier studies carried out in this laboratory by Ramchand (1984) also showed that even a very high dose of synthetic neurotoxin fed orally to rats did not show the symptoms of lathyrism, whereas when injected intraperitoneally at a concentration of 60 mg/100g body weight the animal showed typical symptoms of lathyrism and died within 10 minutes. All these studies on oral feeding raises the question whether neurotoxin is metabolized in the intestine and detoxified. Although the acute signs of ODAP intoxication have been produced in several species including the chick (Rao et al, 1964), mouse (Mehta et al, 1979) and monkey (Parker et al,1979), no successful attempt to produce chronic ODAP toxicity in an experimental animal has been reported. (Nor is there any reliable evidence that experimental neurolathyrism can be produced by feeding 'lathyrus' peas (Misra et al, 1981)). However, recent studies carried out by Mehta et al. (1983) suggest that the squirrel monkey is highly resistent to oral chronic poisoning with a neurotoxin dose of 0.6 to 6.0 mg/gm body weight/day. According to these group of workers the major factors in resistance of squirrel monkeys to ODAP intoxication may have been the availability of nutritionally complete stock diet which was given to them along with synthetic ODAP. Human lathyrism typically occurs in the semistarved individuals whose principal source of calories is the lathyrus pea. Epidemiological studies implicate nutritional deprivation, physical exhaustion and pre-existing disease in susceptibility to the toxic effects of lathyrus peas (Kessler, 1953; Meineertz, 1953; Dwivedi and Prasad,1964).

Ramchand (1984) incubated rat intestinal contents with radiolabelled ODAP for 24 hr and detected radioactive metabolites of ODAP degradation suggesting that intestinal microflora may metabolise the compound. ODAP hydrolysing bacteria were also detected in other species including man and this may be the reason why rats are able to tolerate ODAP when fed orally. Studies carried out by this investigator proposed that multiplication of ODAP hydrolysing bacteria in the elaboration of the enzymes involved in breakdown of ODAP *in vivo* will depend upon the environmental factors in the intestine in different species such as the composition and content of microflora, products of digestion of dietary constituents etc.

Detoxification of neurotoxin :

As mentioned earlier the *Lathyrus* species are generally cultivated for preparation of food, fodder, green manure and ornament. In India, *L. sativus* occupies 4% of the total area under pulse crops and constitutes 3% of the total pulse production. Recent studies carried out by Gopalan and Dwivedi (1983) showed that in Madhya Pradesh (India) still 10-20% of the total grain production is occupied by *L. sativus*. The pulse is used principally to prepare unleavened bread and is sometimes eaten as paste balls or as a cooked preparation. Because of its cheapness and ease of cultivation, it is used as an adultrant of other pulses (Sastri, 1962). Because of the intermittant outbreak of neurolathyrism, Government of India imposed a ban on its cultivation and consumption

(Ganapathy and Dwivedi, 1961). However, an effective ban on the cultivation of L. sativus is not possible in view of the lack of suitable alternative crop acceptable to the poor farmers (Padmanabhan, 1980). The cultivation and total production of Lathyrus sativus in India is not still declined (Gopalan, 1982).

Thus it is of interest whether L. sativus itself can be processed suitably so as to free it from toxin and render it fit for human consumption because of its other nutrients. Data given in Table-13 show that the proximate composition of L. sativus dhal compares well with the other legumes regarding protein, minerals and vitamins. On germination, the available minerals and vitamins also increases (Śastri, 1962). Amino acid analysis of L. sativus indicated that its protein was much higher than any other traditional pulse protein (Sarma and Padmanabhan, 1969), viz. 21 to 33% or next to soybean. It can be seen from the Table-14 that the pulse is rich in lysine and contains fairly good concentrations of other essential amino acids except methionine and tryptophan. A protein concentrate was prepared by Padmanabhan (1980) containing 70% protein (free of neurotoxin) which can be used as a lysine supplement. It has been reported (Shastri, 1962) that at a 10% level of protein intake, L. sativus protein has high digestibility (90%) but a low biological value. Autoclaving of the seed and supplementation with methionine are reported to greatly enhance the nutritive

TABLE-14 : Amino acid composition of L. sativus seed meal and protein concentrate.

Amino acid	Content (% of sample)	
	Seed meal(a)	Protein concentrate (b)
Lysine	1.85	5.99
Histidine	1.10	2.18
Arginine	1.41	5.69
Aspartic acid	1.80	7.41
Threonine	0.85	2.78
Serine	1.20	4.02
Glutamic acid	2.25	8.79
Proline	1.46	4.22
Glycine	0.72	2.30
Alanine	0.80	2.92
Half-Cystine	Trace	Trace
Valine	0.81	2.76
Methionine	0.35	0.99
Isoleucine	1.01	3.32
Leucine	1.45	5.30
Tyrosine	0.62	2.54
Phenylalanine	1.03	3.29

(a) - The protein content of the seed meal based on nitrogen estimation was 24.5%.

(b) - The protein content of the protein concentrate based on nitrogen estimation was 70%.

Ref.: Spackman et al.(1958); Padmanaban (1980).

value of the protein. The crippling condition of 'neurolathyrism' was believed to be totally irreversible until Ahmad and Jahan (1980) reported that in certain instances the damage could be repaired by the daily administration of 500 to 1000 mg of ascorbic acid for a week or so. It is recognized, however, to be unlikely that the population that eats L. sativus as a staple would eat enough foods rich in ascorbic acid to prevent the condition.

Along with neurotoxins, L. sativus also contains other antinutritional factors as mentioned in Table-15. Roy (1972a,b) purified the trypsin inhibitor and its biological effects such as hypertrophy of pancreas and growth retardation were demonstrated in animals. Roy and Rao (1971) have shown that heat treatment of L. sativus while making 'Chapatis' destroys only 48% of the trypsin inhibitor.

Attempts have been made to process the L. sativus seeds so as to free them of the toxin by various workers as shown in Table-16. Swaminathan (1969) inferred that mutations might be carried out in the gene that regulates the synthesis of the toxin in seeds. Considerable variation in ODAP content was observed in the M_2 generation in five varieties of L. sativus treated with mutagens. A different approach was followed by Somayajulu et al. (1975) for the removal of ODAP from seeds viz. development of pure lines from low ODAP containing plants. They were able to identify only four lines of L. sativus containing a low ODAP content

TABLE-15 : Antinutritional factors and toxins present in L. sativus seeds.

	Antinutritional factor/toxin	Content	Reference
1)	Phytate	108 mg/100 g	Gopalan et al. (1971)
2)	Trypsin inhibitor	22.0 TIU/100 g	Roy (1972a,b); Roy and Rao (1971)
3)	Flatulence producing raffinose family of oligosaccharides	4.0 g/100 g	Kalindi et al. (unpublished).
4)	Lathyrogens	0.1 to 2.5 g/100 g	Rao et al. (1964)

TABLE-16 : Effect of different food processing on neurotoxin (ODAP) content of L. sativus.

Reference	Method of processing	Degradation of ODAP	Remarks
Anonymous (1968)	Soaking the seeds overnight followed by steaming for 30 min or sun-drying the grains.	80%	No loss of thiamin, bu substantial loss of riboflavin and nicotir acid.
Mohan et al.(1966)	- Cooking the pulse in excess of water and then draining off the excess water.	70%	Loss of water soluble 'B' vitamins.
	- Steeping the dehusked seeds in hot water.	100%	Removal of essential amino acids.
Rao et al. (1969)	Roasting the seeds at 150° for 20 min.	85%	- Not economical for the poor.
			- Binding capacity of th flour is lost.
Shiv Prakash et al. (1977)	Germination.	Significant reduction	--
(Cf. Rutter and Perry, 1984).	Parboiling the dhal	80-90%	--
Jahan and Ahmad (1984)	Soaking in lime water overnight followed by boiling.	100%	Removal of trypsin inhibitor.

of which P-24 was recommended as being relatively safe for human consumption. Dahiya (1976) suggested certain morphological characteristics as criteria for the selection of varieties of L. sativus low in ODAP contents. However, no correlation was found to exist between morphology and ODAP content by Nagarajan and Gopalan (1968). Misra and Barat (1981) have used micronutrient salts of cobalt and molybdenum in very low concentrations as foliar spray at the maximum flowering stage or at the emergence of 1-2 pods per plant and observed reduction in the toxin level in those plants.

From the foregoing discussion it is evident that the reduction of ODAP content in L. sativus seeds by various techniques, such as steeping, parboiling, roasting, breeding and use of mutagens either causes loss of soluble proteins and vitamins or involves a cumbersome technology.

Thus from the earlier discussion it can be concluded that the diet consumed by the people of low socio-economic group is of poor nutritive value and there is always a risk of provision of antinutritional factors and toxins in substantial amount. In spite of all these, the clinical and biochemical status of the poor is not as poor as one would expect from the nutrients available to them (Oomen, 1961; Bailey, 1963; Adams and Shreta, 1974; Gupta et al, 1977; Jacob et al, 1976; Nutr. Rev., 1980. Energy requirement : how much is enough)[9]. There are conflicting reports also regarding the adverse

effects of antinutritional factors and toxins present in legumes. Though phytate present in plant food is supposed to interfere with intestinal absorption of Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}, either rat experiment carried out in this laboratory or human studies carried out by others (Walker et al, 1948; Leitch, 1964; Bhaskaran and Reddy, 1979) did not confirm this. Eventhough raffinose family of oligosaccharides present in the diet causes flatulence and leads to abdominal discomfort, work on Asian and Middle Eastern people (who consume good amount of chickpeas and soybeans) does not show analogous effects of these oligosaccharides (Cristofaro et al, 1974). Geervani and Theophilus (1979) have shown that preschool children from low income population of Madras city did not experience any discomfort when fed different legume diets. Feeding experiments on preschool children showed no difficulty in the digestion of legume preparations (Ramakrishnan, 1979).

These adaptation to diets containing food toxins can be explained in two ways : one is based on different food processing methods and second is based on the knowledge of degradation or modification of these toxins undergoing in gastrointestinal tract. Gastrointestinal microflora play an important role in the host nutrition (Coman, 1970, 1972; Combe et al, 1976; Deguchi et al, 1978). The microflora present in the gut can degrade endogenous proteins of the intestinal enzymes, shed cells, saliva, mucosa and complex

plant polysaccharides like cellulose and pectin (Salyers et al, 1977; Cummings et al, 1979). Many studies have reported the ability of intestinal microflora to synthesize vitamins such as thiamin, riboflavin, niacin, folic acid, biotin, B_6, K and B_{12} (Broberg et al, 1953; Daft et al, 1963; Klipstonn and Lipton, 1970; [illegible]; Dr^sar and Hill, 1974; Ramakrishnan et al, 1983). Earlier studies carried out in this laboratory also have shown the role of microflora from rat small intestine in the degradation of phytate, trypsin inhibitor, hemagglutinin and flatulence producing oligosaccharides, but it is not known whether these bacteria can grow in presence of other normal flora in the small intestine. Thus from all these studies it can be suggested that intestinal microflora also might help in improving the utilization of foods by effecting the breakdown of deleterious factors and augmenting the supply of vitamins. Neverthless, diet has been considered to play a major role in determining the nature of gastrointestinal flora (Nath et al, 1948; Aries et al, 1969; Hill et al, 1971; Dr^sor and Hill, 1972).

Although there are numerous examples of so-called toxic constituents in legumes, they have nevertheless provided man over the centuries with a valuable source of protein. This can be attributed in part to the fact that man has learned how to detoxify them by suitable preparative measures. Nevertheless, there is the ever present possibility that the prolonged consumption of a particular legume which may be improperly processed could bring to the surface toxic effects which would otherwise not be apparent (Liener, 1975).

In many developing countries protein rich plant foods should be produced locally and they should be acceptable, because protein calorie malnutrition is a more serious health problem than that of contageous disease (Van Veen et al, 1967; Streinkraus, 1985). Protein-calorie malnutrition is common in the developing world, and it leads not only to stunting of physical growth but to retardation of brain growth and mental development (Hegsted, 1978). The major deficiency in the diet are food energy and protein with associated deficiency of calcium, vitamin A, riboflavin and other nutrients (Rajalakshmi and Ramakrishnan, 1977). Such deficits can be met by increasing the proportion of protein rich foods and the total amount of food consumed. To improve the nutritive value of protein rich legumes, the different methods of food processing are employed before they are consumed. The different processing methods used for cereals and legumes in India are roasting, parching, boiling, germination and fermentation. The effect of different processing methods on biological value and chemical composition of foods are summarized in Table-17. These processing methods increases the nutritive value of foods as well as food intake due to increase in palatibility (Rajalakshmi and Ramakrishnan, 1977).

Among all the methods of food processing, fermentation is one of the most important method used from ancient times in different parts of the world, specially in India. Fermentation and germination are found to bring about almost identical changes in the chemical composition of the food but the texture

TABLE-17 : Effect of processing on the nutritive values of cereals and legumes.

Parameters used	Roasting	Parching	Soaking	Boiling/cooking	Germination
1. PER	Increased (Badenhop and Hackler, 1971)	-	-	No effect (Olson et al, 1982)	Increased (Jaya et al, 1975)
2. Biological value	-	Increased (Acharya et al, 1942)	-	-	Increased (Chattopadhyay and Banerjee 1953).
3. Digestibility	Increased (Bendor, 1970)	Increased (Kuppuswami et al, 1958; Walker et al, 1970).	-	-	Increased (Palmer et al, 1973; Subbalakshmi et al, 1976).
4. Phytate	-	-	Decreased (Rajalakshmi, 1970; Kokhar & Chandran, 1986).	-	Decreased (Peers, 1953; Mandal and Biswas, 1970; Mandal et al, 1972; Lilian et al, 1985; Lu et al Sathe et al, 1983).
5. Trypsin inhibitor	Decreased (Liener and Kakade, 1969).	Decreased (Kuppuswami et al, 1958).	Decreased (Kakade and Evans, 1966; Ologhobo and Fetuga, 1983; Bakir et al, 1982)	Decreased (Bakir et al, 1982; Ologhobo and Fetuga, 1983; Marikar and Pattabiraman, 1988)	Decreased (Kakade & Evans, 1966; Wang et al, 1972; Ologhobo & Fetuga, 1983; Anna Tanwilson et al, 1982 Sathe et al, 1982; Sathe et al, 1983).
6. Hemagglutinin	-	-	Decreased (Kakade and Evans, 1966)	Decreased (Liener, 1962)	Decreased (Subbalakshmi et al, 1976). No change (Kakade and Evans, 1966)

TABLE-17 (Contd...)

Parameters used	Roasting	Parching	Soaking	Boiling/cooking	Germination
7. Flatulence producing oligosaccharides.	Not affected (Faki et al, 1983).	--	Decreased (Silva and Braga, 1982; Jood et al, 1985; Olson et al, 1981).	Decreased (Ku et al,1976; Bianchi et al, 1983; Silva & Braga, 1982; Olson et al, 1982; Jood et al, 1985). Increased (Rao & Belavady, 1978).	Decreased (Reddy & Salunkhe, 1980b; Jaya & Venkataraman, 1981; Gupta & Wagle, 1980; Rao & Belavady, 1978; Sathe et al, 1983; Jood et al,1985)
8. Thiamin	Decreased (Altschul, 1958)	No effect (Rajalakshmi, 1970)	Increased (Dhand,1964; Rajalakshmi, 1970).	--	Increased (Burkholder, 1943; Dhand, 1964; Rajalakshmi, 1970).
9. Riboflavin	Decreased (Dun and Goodard,1948)	Not affected significantly (Rajalakshmi, 1970)	Increased (Dhand,1964; Rajalakshmi, 1970).	Decreased (Raghunath & Belavady,1979)	Increased (Burkholder et al, 1943; Shastri et al, 1975; Lay and Fields, 1981).
10. Niacin	Not affected significantly (Rajalakshmi, 1970).	Availability increased (Rajalakshmi, et al, 1964).	Increased (Rajalakshmi et al,1964; Rajalakshmi, 1970).	--	Increased (Burkholder, 1943; Goldberg & Thorp, 1946; Lay and Fields, 1981)

as well as flavour are found to be improved to great extent during fermentation and food intake almost doubled when fed to preschool children (Rajalakshmi, 1976).

Fermented foods derived from cereals and legumes are essential components of diets in all parts of the world especially South East Asia, the Near East and parts of Africa (Table-18). They are also important in the diets of the western world, because of their high nutritive value and their interesting organoleptic characteristics which add variety to the diets (Steinkraus and Van Veen, 1971). The indigenous fermented foods constitute a group of foods that are produced in homes, villages and small cottage industries at prices within the means of a majority of the consumers in the developing world (Steinkraus, 1985). Some indigenous food fermentations such as "Soy Sauce" (Shoyu"), Japanese "miso", Indonesian "tempeh", Indonesian "tape ketan", Japanese "sake", Indian "idli", and fish and shrimp sauces and pastes have been extensively studied to determine optimum conditions for fermentation, the essential micro-organisms, the biochemical, nutritive, and flavour/texture changes that occur during the fermentation, and possible toxicological problems that can arise (Steinkraus, 1983a).

Indigenous food fermentations have been classified as follows according to Steinkraus (1983b) :-

TABLE-18 : Ingredients used and micro-organisms involved in legume-based fermented foods.

Name	Substrates	Microorganisms involved	Use of food	Areas
Dawadawa	Locust bean	Unknown	Supplement to soups and stews	West Africa
Dhokla	Bengal gram and wheat	Unknown	Breakfast food	India
Dosai	Black gram and rice	Yeast and bacteria	Breakfast food	India
Idli	Black gram and rice	Yeast, *Leuconostoc mesenteroides*	Breakfast food	India (south Indi
Kecap	Soybeans and wheat	*Aspergillus oryzae*, *Lactobacillus* sp., *Hansenula* sp., *Saccharomyces* sp.	Drink	Indonesia and vicinity
Kenima	Soybeans	Unknown	Snack	Nepal, Sikkim, an Darjeeling distri of India.
Ketjap	Black soybeans	*A. oryzae*	Seasoning agent	Indonesia
Khaman	Bengal gram	Unknown	Pancake (breakfast food)	India
Meitauza	Pressed soyben cake	*Actinomucor elegans* or *Mucor meitauza*	Cake used as a a snack	China, Taiwan

TABLE-18 (Contd.....)

Name	Substrates	Microorganisms involved	Use of food	Areas
Meju	Soybeans	A. oryzae, Rhizopus sp.	Seasoning agent	Korea
Miso	Soybeans and wheat	A. oryzae, S. rouxii, lactic acid bacteria	Flavouring agent, soup base.	East Asia, Japan, China
Natto	Soybean	Bacillus natto	Cake, as a meat substitute.	Northern Japan
Oncom	Peanut press cake	R. oligosporus, Neurospora sitophila.	Snack	West Java
Papadam	Black gram	Saccharomyces sp.	Condiment	India
Soy milk	Soybeans	Lactic acid bacteria	Drink	China and Japan
Soy sauce	Soybean and wheat	A. oryzae, S. rouxii, Pediococcus halophilus, L. delbrueckii.	Seasoning agent	Japan, China and Taiwan
Sufu (Chinese soybean cheese)	Soybeans	Mucor sp. or Actinomucor elegans	Cheese	Far East

TABLE-18 (Contd...)

Name	Substrates	Microorganisms involved	Use of food	Areas
Tao-si	Soybeans and	Aspergillus oryzae	Seasoning agent	Phillipine
Taotjo	Soybeans and roasted wheat meal or glutinous rice.	A. oryzae	Condiment	East Indie
Tauco	Soybeans and cereals	R. oligosporus	Drink	West Java
Tempeh	Soybeans	R. oligosporus or R. oryzae	Cake used as snack	Indonesia, New Guinea Surnam and Vicinity.
Waries	Black gram	Candida sp., Saccharomyces sp.	Condiment	India

(Ref. Reddy et al, 1984).

- fermentations involving proteolysis of vegetable proteins by microbial enzymes in the presence of salt and/or acid with production of amino acid and peptide mixtures with a meat like flavour (e.g. 'Soy sauce', Indonesian 'kecap', 'Miso' and "tauco");

- fermentations involving enzymic hydrolysis of fish and shrimp or other marine animals in the presence of relatively high salt concentrations to produce meat-flavoured sauces and pastes;

- fermentations producing, a meat-like texture in a cereal-grain legume substrate by means of fungal mycelium that knits the particles together (e.g. Indonesian and Malaysian "tempeh kedela", Indonesian "oncom kacang", "oncom tahoo" and "tempeh bongkrek");

- fermentations involving 'koji' principle, in which micro-organisms with desired enzymes are grown on a cereal-grain or legume substrate to product "koji - a crude enzyme concentrate" that can be used to hydrolyse particular components in the desired fermentation (e.g. 'soy-sauce', 'miso', 'kecap', 'tempeh, 'oncom', 'tape' etc.);

- fermentation in which organic acids are major products (e.g. Korean "kimchi", "Sauerkreut", fermented milk and 'cheese', African 'ogi' and Indian 'idli';

- fermentations in which ethanol is a major product (e.g. "rice wines", "palm toddies", "sugar cane wines", "beers" and "tape ketan".

Fermentation is found to improve texture and flavour for better acceptability of foods (Hesseltine, 1965; Hesseltine and Wang, 1967; Van Veen and Steinkraus, 1970; Rajalakshmi, 1970; Rajalakshmi and Ramakrishnan, 1977). Fermented foods like Indonesian 'tempeh', 'oncom' and 'tempeh bongkrek' which have meat like textures are good substitute for meat. Indonesian 'tempeh' is found to be a good substitute for meat in the diets of American vegetarians (Steinkraus et al, 1960; Hesseltine, 1961; Saono et al, 1977; Wang and Hesseltine, 1979; Shurtleff and Aoyagi, 1980).

Biochemical changes during fermentation :

During fermentation, complex compounds are converted into their simpler digestible forms. Free sugar increases during fermentation, indicating a partial breakdown of carbohydrates. Similarly amino nitrogen increases indicating a similar breakdown of proteins. It is likely that during fermentation, intermediates of the breakdown of complex macromolecules such as simpler starches, dextrins, maltose and peptides also increase. These changes serve to make the food more palatable and digestible (Kunitaro, 1961; Christian, 1966; Van Veen et al, 1968a; Ramakrishnan et al, 1976).

One of the major biochemical changes involved in Indian fermented foods is the conversion of some of the carbohydrates to lactic acid. Thus many of the Indian fermented foods have low pH and sour taste which is acceptable to even poor pregnant women who suffer from loss of appetite, nausea and hyperemesis (Ramakrishnan, 1979). Fermented foods with acidic pH also lend themselves to the incorporation of lime (a mixture of $CaCO_3$, CaO and $Ca(OH)_2$ and leafy vegetables, which help to increase the nutritive value of food with regard to calcium, iron, β-carotene, vitamin-C and riboflavin and the losses which result in the cooking of vegetables by conventional procedures are minimized by such incorporation (Ramchandran, 1968; Aykroyd and Doughty, 1964; Rajalakshmi et al, 1973; Rajalakshmi and Ramakrishnan, 1977; Rajalakshmi, 1976).

The other chemical changes reported during fermentation include an increase in vitamins and some essential amino acids. Significant increase in thiamin, niacin, riboflavin and vitamin-C are reported during fermentation of 'tempeh' (Steinkraus et al, 1961; Murata et al, 1967; Robinson and Kao, 1977; Roelofsen and Thalens, 1964), chick pea 'miso' (Kao and Robinson, 1978), 'oncom' (Van Veen et al, 1968b, Beuchat, 1976; Quinn et al, 1978), 'khaman' (Rajalakshmi and Vanaja, 1967), 'dawadawa' (Platt, 1964), 'idli' (Rajalakshmi and Vanaja, 1967), 'natto' (Sano, 1967) and 'soyidli' (Ramakrishnan, 1977). In contrast to this decrease in the content of thiamin and riboflavin were reported during 'idli' (Steinkraus et al, 1967) and "tempeh kecipir" (Cadjar, 1978). Increase in folic acid, choline and methionine have

been reported during fermentation of certain foods (Radhakrishna Rao, 1961; [illegible] Steinkraus et al, 1967; Murata et al, 1970). Even an increase in the vitamin B_{12} (usually lacking in vegetarian foods) has been reported in several fermented foods viz. 'tempeh' (Roelofsen and Thalen, 1964; Steinkraus, 1976; Curtis et al, 1977; Liem et al, 1977), 'miso' (Robinson and Kao, 1977), 'natto' (Sano, 1961), peanut press cake (Beuchat, 1976; Liem et al, 1977), 'idli' (Ramakrishnan et al, 1983) and fermented 'corn meal' (Chung and Fields, 1986). During the fermentation of cassava and rice (which are the substrates rich in starch but too low in protein), the nutritive value of the fermented product is increased due to increase in lysine and thiamine content (Kao, 1972; Cronk et al, 1977). Chemical changes reported in Indian fermented foods are summarized in Table-19.

Detoxification of toxins during fermentation of foods :

As an added advantage, fermentation of foods seems to breakdown toxins and antinutritional factors present in raw materials used for preparation of fermented foods (Collard and Levi, 1959; Liener, 1962; Smith et al, 1964; Van Veen et al, 1968b). Consumption of soybeans which contain trypsin inhibitors lead to the enlargement of pancreas in the rats(Konjin and Guggenheim, 1967), whereas 'tempeh' which is a fermented product prepared from soybeans does not produce pancreatic hypertrophy (Smith et al, 1964). This suggests that certain toxic constituents present in soybean are broken down during fermentation.

However, Wang et al. (1972) have reported an increased trypsin inhibitor activity in the initial stages of fermentation, due to the release of bound trypsin inhibitor. Kamalakanan and Motlag (1982) have detected the presence of a factor in the culture filtrate of Rhizopus oligosporus (used for fermentation) which inactivates the trypsin inhibitor. During the fermentation of cassava in the preparation of Nigerian "gari", cyanogenic glycosides are broken down (Collard and Levi, 1959; Pratap, 1973). Van Veen et al.(1968b) have reported 50-70% reduction in aflatoxin-B_1 content during fermentation of 'ontjem' (fermented pea-nut press cake). When cowpeas and chick peas were allowed to undergo natural fermentation, no toxic substance was detected as determined by the in vivo tests in chicken embryo (Zamora and Fields, 1979b).

The most abundant antinutrients viz. phytate and flatulence producing are also broken down during fermentation as described in Table-20. Thus fermentation augments the nutritive value of legumes and cereals because of the favourable chemical changes.

Rat experiments carried out by Van Veen et al (1967) have shown an increased food intake and body weight gain in rats fed fermented foods. Akolkar (1977) showed that rats, pair fed fermented 'soyidli' showed higher PER, better growth and increase in liver and bone status compared to rats fed unfermented products. Rat studies carried out with Indian fermented foods like 'khaman' and 'idli' also suggested their greater digestibility with increased liver concentrations of protein, thiamin and

TABLE-20 : Effect of fermentation on some antinutritional compounds and toxins of cereals and legumes.

Type of fermented food	Trypsin inhibitor	Hemagglutinin	Flatulence producing oligo-saccharides.	Phytate	Aflatoxin
1	2	3	4	5	6
Tempeh (Soybean)	Decreased (Wang et al, 1972).	-	Decreased (Shallenberger et al, 1967, [illegible])	Decreased (Sudaramji and Markakis, 1977)	-
Tempeh (Chick pea and horse beans)	Decreased (Robinson and Kao, 1977).	-	Decreased (Robinson and Kao, 1977)	-	Decreased (Kao and Robinson, 1978)
Miso	Decreased (Kao and Robinson, 1978)	-	-	-	Decreased (Kao and Robinson, 1978)
Soymilk	-	-	Decreased (Mital and Steinkraus, 1975; Mital et al, 1974; Sugimoto and Van Buren, 1970).	-	-
Oncom (Pea nut cake)	-	-	Decreased (Fardiaz and Markakis, 1981b; Worthington and Beuchat, 1974).	Decreased (Fardiaz and Markakis, 1981a)	Decreased (Steinkraus an Veen, 1971).

TABLE-20 (Contd....)

1	2	3	4	5	6
Idli	No change (Padhye and Salunkhe, 1978)	Decreased (Reddy, 1981)	Decreased (Reddy and Salunkhe, 1980b)	Decreased Reddy and Salunkhe, 1980a; Rajalakshmi and Vanaja, 1967)	-
Soyidli	Reduced by steaming but increased during fermentation	Decreased (Ramakrishnan, 1977)	-	Decreased (Ramakrishnan, 1977).	-
Dhokla	No change (Ramakrishnan, 1977)	-	-	Decreased (Ramakrishnan, 1977; Rajalakshmi and Vanaja, 1967).	-
Iru	-	-	Decreased (Odunfa, 1985b)	-	-

riboflavin as compared to unfermented foods (Rajalakshmi and Vanaja, 1967). Zamora and Veum (1999) reported that fermentation of cooked soybean with Rhizopus oligosporus greatly improved the apparent digestibility and net proteins utilization in rats when fed diets containing fermented soy products. True digestibility, protein efficiency ratio, net protein utilization and biological value of fermented locust beans were also found to be higher than that of raw seeds when rats were used as test animals (Fetuga et al, 1973a, 1973b).
It was concluded by Ramakrishnan (1979) that fermented foods prepared from legumes can play a valuable role in the alleviation of malnutrition and undernutrition in groups who are most vulnerable to it.

Apart from changes in flavour, texture and nutritive value, fermentation also brings about changes in the keeping and cooking quality of foods. "Kishak", a fermented food from the near East can be preserved without spoilage for about an year (Van Veen et al, 1969). Acid production during fermentation prevents the growth of pathogenic organisms and prevents food spoilage (Van Veen and Steinkraus, 1970).

Thus in summary, fermentation of legumes improves their digestibility for humans, enhances keeping quality of the product, improves flavour and appearance of the final fermented product and reduces cooking time (Reddy et al, 1984).

Though fermented foods were used from time immemorial the agent of fermentation was not known till the discovery of microscope by Leuwenhoek in 1680. In 1837, Cagniard-Latour, Schwann and Kuttzing, independently proposed that yeast appears during alcoholic fermentation and the conversion of sugar to alcohol is a physiological function of yeast cells. Later, Louis Pasteur was able to show that each particular type of fermentation process is accompanied by the development of a specific type of micro-organisms (Cf. Stanier et al,1986).

Van Veen (1957) defined fermented foods as "they are fermented in so far that they (or at least one of their constituents) have been subjected to the action of micro-organisms (bacteria, yeast or fungi) for a period, so that the final products have often undergone very considerable changes in chemical composition and other respects. Sometimes these changes are due not only to microbial action but also to autolytic processes brought about by the enzymes of the product itself".

Microbial communities with their combined physiology and interactions and their enzymatic activities are responsible for the major biochemical and nutritional changes that occur in the substrates of most fermented foods and beverages (Steinkraus, 1982). The major micro-organisms which are present during fermentation of various foods are listed in Table-18, from which it can be seen that the fermentation of foods used in India is affected mainly by bacteria or yeast or both but not by fungi.

Different micro-organisms grow on different foodstuffs depending upon their ability to breakdown different compounds such as carbohydrates, proteins and lipids. Different products are formed depending upon the substances broken down and some of these products give flavour and texture to the fermented foods (Hesseltine, 1965; Hesseltine and Wang, 1967). From Table-21, it can be seen that the chemical composition of raw material used and the types of the microorganisms used will decide the type of fermented food formed, because of the enzymes elaborated by the micro-organisms. For instance, R. oryzae and R. oligosporus ferment soybean to produce 'tempeh'-a fermented food common in Indonesia. R. oligosporus elaborates a great amount of lipase and protease which will breakdown fat and protein in soybean and some cereals and impart a particular texture and flavour. On the other hand, R. oryzae can produce 'tempeh' from soybean but not from wheat and other cereals, because cereals contain a higher percentage of starch and R. oryzae produces amylase which converts starch to sugars which in turn are converted to acid, which gives an undesirable fermented product (Hesseltine, 1965). Increase in amylolytic, lipolytic and proteolytic activity of micro-organisms are reported during fermentation of 'hama natto' (Beuchat,1978), 'sufu' - a fermented product in China (Fukushima, 1979), 'soy-sauce' (Yong and Wood, 1977; Yamamoto et al, 1972; Yokutsuka, 1977; Fukushima, 1981), 'oncom' (Beuchat et al,1975; Beuchat, 1975), Japanese 'miso' (Hesseltine and Shibasaki,1961) etc. Hemicellulose type of material is partially solubilized by mold enzymes resulting in a fine textured product which

TABLE-21 : Ability of various microorganisms to bring about changes observed during fermentation.

1	Substrate/ Raw Material	Hydrolysis of				Synthesis of				Reference
		Starch	Protein	Fat	Oligo-saccharides	Thiamin	Riboflavin.	Niacin	Vitamin B_{12}	
	2	3	4	5	6	7	8	9	10	11
Bacillus natto	Soybean	-	+	+	-	-	+	-	-	Kunitaro (1961); Kiuchi et al. (1976).
Bacillus subtilis	Rice	+	+	-	-	-	+	-	-	Van Veen et al. (1968)
	African oil bean	-	+	-	+	-	-	-	-	Odunfa and Oyeyiola (1985)
lactobacillus bulgaricus	Wheat	+	+	-	-	-	+	+	-	Van Veen et al. (1969)
	Soybean milk	-	-	-	+	-	-	-	-	Mital et al. (1973)
lactobacillus fermenti	Glucose + peptone + yeast extract	-	-	-	-	-	+	+	-	Peterson and Peterson (1945)
	Soybean milk	-	-	-	+	-	-	-	-	Mital and Steinkraus (1975
Neurospora sitophila	Peanut press cake	-	+	-	+	+	+	+	-	Worthington and Beuchart (1974); Quinn et al. (1975).
Rhizopus oligosporus	Soybean	-	+	+	-	-	-	-	-	Heseltine (1965); Wang and Hesltine (1970).
	Peanut	-	-	-	-	+	+	-	-	Quinn et al. (1975).
Aspergillus oryzae	Rice (Japanese sake)	+	+	-	-	-	-	-	-	Kodama and Yoshizawa

TABLE-21 (Contd...)

1	2	3	4	5	6	7	8	9	10	11
Aspergillus oryzae	Koji preparation in soy sauce fermentation	+	+	-	-	-	-	-	-	Yamamoto *et al.*(197 Yong and Wood (1977
Corynebaecterium sp.	Nigerian 'ogi'	+	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	Tempeh fermentation	-	-	-	-	-	-	-	+	Liem *et al.* (1977)
Amylomyces rouxii	Cassva tubers	+	-	-	-	-	-	-	-	Ko (1972); Cronk *et al.* (1977)
Rhizopus oryzae	Soybean ('tempeh' fermentation)	+	+	+	-	-	+	+	+	Van Veen and Steinkraus (1970); Quinn *et al.* (1975)
	Peanut press cake	-	-	-	+	+	+	-	-	Wagenknecht *et al.* (1961).

increase digestibility during the fermentation of pea nut press cake (Beuchat et al, 1975).

A significant increase in phytase activity is reported during fermentation of various foods for the breakdown of phytate into inorganic phosphorous (Reinhold, 1975; Akolkar, 1977; Harland and Harland, 1980; Lopez et al, 1983). Phytase has widespread distribution in plants and animal tissues (Peers, 1953; Courtois, 1945; Meyer, 1958; Kindl, 1969; Mandal and Biswas, 1970; Bitar and Reinhold, 1972; Mandal et al, 1972) and numerous types of micro-organisms (Nelson et al, 1968; Tanner, 1969; Wang et al, 1980). Major micro-organisms which produce phytase are Aspergillus ficcum (Han et al, 1987; Han and Wilfred, 1988), Aspergillus oryzae (Wang et al, 1980), Neurospora sp. (Shieh and Ware, 1968), Aspergillus niger (Tadeusz, 1978), Aerobacter aerogenes (Greaves et al, 1967), Bacillus subtilis (Powar and Jagannathan, 1967) etc. Microbial degradation of phytate has been studied in detail by various workers (Greaves et al, 1967; Powar and Jagannathan, 1967; Coggrove, 1970; Irwing and Cosgrove, 1972; 1974) which showed that phytase catalyses the successive dephosphorylation of phytate to finally yield myo-inositol and inorganic phosphorous.

Earlier studies carried out in this laboratory showed that hemagglutinin is broken down by L. mesenteroides (HA) (which is present during 'soyidli' fermentation) due to production of enzymes viz. protease, β-N-acetyl-glucosaminidase and mannosidase to liberate final products of hemagglutinin hydrolysis (Rao, 1978).

Raffinose family of oligosaccharides, which causes flatulence have been reported to decrease during fermentation of "peanut press-cake" (Worthington and Beuchat, 1974), 'soymilk' (Mital et al, 1974; Mital and Steinkraus, 1975), 'tempeh' (Calloway et al, 1971; Shallenberger et al, 1967), 'iru' (Odunfa, 1982b) etc. due to the elaboration of microbial enzymes viz. α-galactosidase and invertase.

Enzymes for the breakdown of raffinose family of oligosaccharides :

During germination and fermentation of legumes, raffinose family of oligosaccharides are degraded into monosaccharides by α-galactosidase and β-D-fructofuranosidase as shown in Fig.4. α-Galactosidase (α-D-galactoside galactohydrolase E.C. 3.2.1.22) is widely distributed in nature and it catalyzes the disruption of the α-D-galactosidic bond of both simple and complex oligo- and polysaccharides (Dey and Pridham, 1977). Micro-organisms isolated from different sources are known to produce α-galactosidase (Table-22). This enzyme has been reported to occur in various plants (Dey and Pridham, 1969b; Barham et al, 1971; Dey and Dixon, 1974; Mc Clery and Matheson, 1974; Malhotra and Singh, 1976; Samuel and Joseph, 1977; Williams et al, 1977) and animal tissues like liver, placenta, brain, adrenal gland, spleen, stomach, heart, uterus, lung, ovary, testis, pancreas, small and large intestine of various mammals (Rao and Pieringer, 1970; Ho, 1973; Mayer and Beautler, 1977; Bishop and Sweeley, 1978; Bishop et al, 1978).

-Galactosidase is a useful tool not only in the determination of raffinose and the structural studies of carbohydrates, but also in the sugar-beet industry to reduce the raffinose content (Suzuki et al, 1969; Reynolds, 1974; Kobayashi and Suzuki, 1975; Linden, 1982). This enzyme has a wide application in food processing for removal

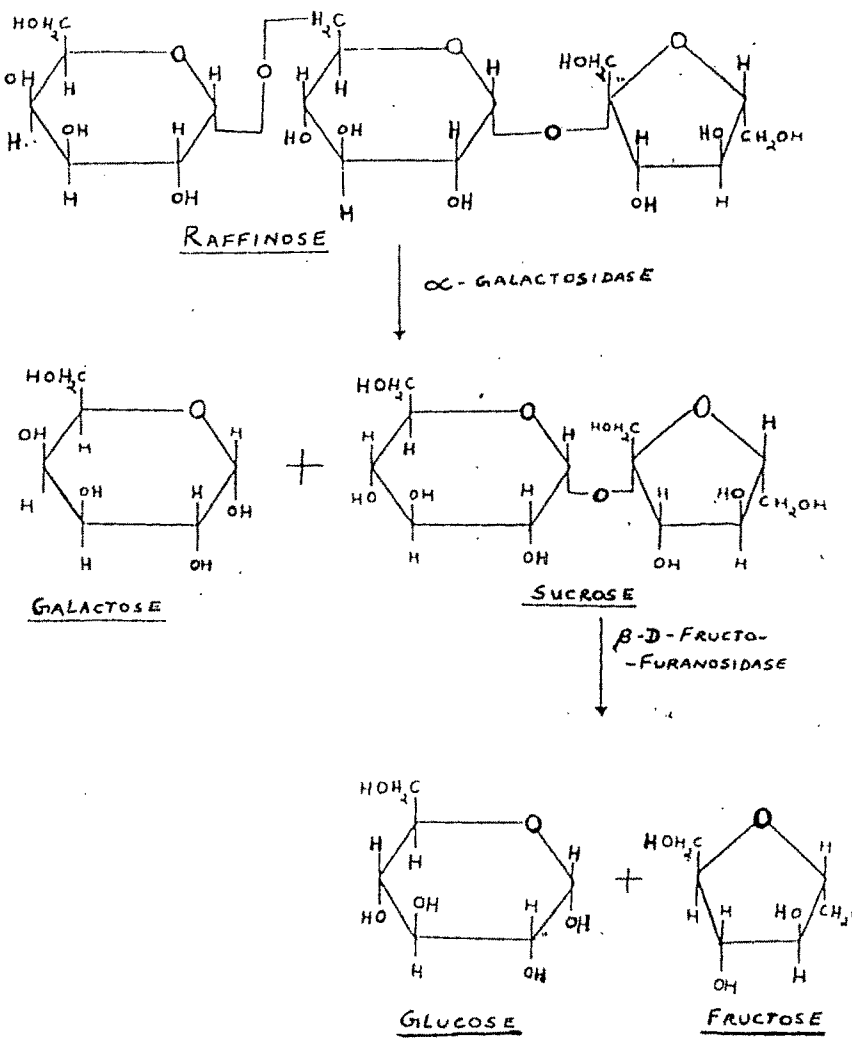


FIG.4 : Pathway for the degradation of raffinose.

TABLE-22 : Micro-organisms producing α-galactosidase.

Micro-organism	Source	Reference
Aspergillus niger	-	Bahl and Agarwal (1969); Lee and Wacek, 1970).
Aspergillus awamori NRRL 4859	-	Smiley et al. (1976).
Mortierella vinacea	Soil	Kobayashi and Suzuki (1975); Suzuki et al. (1970).
Saccharomyces sp.	-	Clancy and Whelan (1967); Maria (196:
Saccharomyces carlsbergensis	-	Lazo et al. (1978).
Cladosporium cladosporioides	Soymilk (fermented)	Cruz et al. (1981).
Bacillus sp. No. 7-5	Soil	Akiba and Horikoshi (1976)
Bacteroides fragilis	-	Berg et al. (1980)
Bacillus stearothermophilus	Hotspring	Pederson and Goodman (1980)
Bacillus stearothermophilus	Soil	Delente et al. (1974).
Corticum rolfsii	-	Kaji and Yoshihara (1972)
Diplococcus pheumoniae	-	Li, and Shetlar (1963)
Escherichia coli	-	Lester and Bonner (1957); Schmitt and Rottman (1966)
Escherichia coli subsp. Cummunior IAM-1272.	Gastrointestinal tracts of humans	Kawamura et al. (1976)

TABLE-22 (Contd...)

Micro-organisms	Source	Reference
Micrococcus sp. No. 31-2	Soil	Akiba and Horikoshi (1976)
Streptococcus bovis	Rumen	Bailey (1963)
Streptomyces 9917 S_2	Soil	Oishi and Aida (1972)
Lactobacilli	Fermented soysauce	Mital et al. (1973); Rutloff et al. (1967).
Clostridium spp.	-	Watkins and Morgan (1955)
Neurospora sitophila	Oncom (fermented peanut press cake)	Worthington and Beuchat (1974)
Monascus pilosus	Fermented food	Wong et al. (1986)

of flatulence producing oligosaccharides e.g. soymilk processing (Sugimoto and Van Buren, 1970; Smiley et al, 1976; Thananunkul et al, 1976; Cruz et al, 1981)

α-Galactosidase from Bacillus stearothermophilus (Reynolds, 1974) and Mortirella vinacea (Linden, 1982) have been immobilized on nylon and within mycelial pellets, respectively, and the immobilized enzymes have been used for the hydrolysis of raffinose in beet sugar molasses. Immobilization of this enzyme isolated from figs (Schram et al, 1978), Turbo cornulus (Shepard et al, 1983) and Pycnoporus cinnabarius (Mitsutomi et al, 1985) using Sepharose-4B, BSA and colloidal chitin with glutaraldehyde respectively have been reported for industrial applications.

The primary role of α-galactosidase in the storage organs of plants is to mobilize the reserve D-galactosyl containing oligo and polysaccharides (Dey and Pridham, 1972; Dey, 1978; Dey, 1980), which is a compartmentalized enzyme. On germination, the enzyme is undoubtedly involved in the hydrolysis of these oligosaccharides, which serve as a soluble and readily metabolizable energy reserve. Alpha-galactosidases are also involved in the metabolism of plant galactolipids in spinach (Gatt and Baker, 1970; Mc Clery and Matheson, 1974; Williams et al, 1977) and sugarcane chloroplasts (Dey and Pridham, 1972). Another possible role for the enzyme is protecting plants from α-D-galactosidic phytotoxic

substances produced by invading micro-organisms (Strobel, 1974). The ∝-galactosidase in brain tissues is involved in the hydrolysis of digalactosyl diglycerides (Subba Rao and Pieringer, 1970). Metabolism of glycosphingolipid is disturbed in patients with Fabry's disease which is characterized by deficiency of ∝-galactosidase and it leads to accumulation of ceramide trihexoside (Meyer and Beautler, 1977). ∝-Galactosidase may be used in the future for medical purpose as enzymotherapy (Ulezlo and Zaprometova, 1982).

Production of ∝-galactosidase can be induced during growth of microorganisms using raffinose, melibiose, thiogalactoside, galactose, phenyl ∝-D-galactoside, O-nitrophenyl-∝-D-galactosides and some β-D-galactosides (Koppel et al, 1953; Hogness and Battley, 1957; Lestler and Bonner, 1957; Sheimin and Crocker, 1961; Schmid and Schmitt, 1976; Lazo et al, 1981).

Synthetic ∝-D-galactosides like methyl, ethyl (Hogness and Battley, 1957), n-propyl, o-cresyl (Malhotra and Dey, 1967), m.p.o-nitrophenyl (Malhotra and Dey, 1964), 1-naphthyl, 2-naphthyl- and 6-bromo-2-naphthyl-∝-D-galactosides (Tsou and Su, 1964), galactinol (Suzuki et al, 1970) and digalactosyl glycerol (Subba Rao and Pieringer, 1970) are known to be hydrolyzed by the hydrolytic action of ∝-galactosidases. Naturally occuring oligosaccharides viz. melibiose, melibitol, melibionic acid, raffinose, umbeliferose, plantose, manninotriose, stachyose, verbascose, lychnose and polysaccharides

like galactomannans are also attacked by ∝-galactosidases from various sources (Cf. Dey and Pridham, 1972). Alpha-galactosidases from coffee beans and soybeans (Zarnit and Kabat, 1960; Harpaz et al, 1977), B. cereus (Iseki and Ikeda, 1956) and Streptomyces sp. (Oishi and Aida, 1976) can also remove nonreducing terminal ∝-D-galactosyl residues from blood group substances. Thus type 'B' erythrocytes, which contain 3-O-∝-D-galactopyranoside, can be transformed into type 'O' erythrocytes by exposure to ∝-galactosidase (Ulezlo and Zaprometova, 1982).

Alpha-galactosidases also show trans-∝-galactosylation activity in addition to hydrolytic activity and the products are complex carbohydrates in plants (Malhotra and Dey, 1967; Dey, 1969; 1979; Dey and Pridham, 1972), fungi (Itanu et al, 1982; Ohtakara et al, 1984) and bacteria (Kawamura et al, 1976;).

An active site directed irreversible inhibitor of ∝-galactosidase, conduritol-C-trans-epoxide (1,2-anhydroneo-inositol) was synthesized (Legler and Herrchen , 1981) to understand the mechanism of action of ∝-galactosidase. This compound binds initially to the enzyme active site because of its structural similarity to the substrate; the epoxide function is then activated by an acidic functional group of the site, forming a covalent bond. The mechanism of enzyme action involves two steps : the glycosyl-enzyme intermediate formation and its subsequent breakdown (Dey and Pridham, 1972; Dey, 1969; Carchon and De Bruyne, 1975).

A complete amino acid sequence analysis of only a few ∝-galactosidases viz. V. sativa, G. max (Pridham and Dey, 1974; Harpaz et al, 1977; Dey et al, 1983), A. niger (Adya and Elbein, 1977) and Saccharomyces carlsbergensis (Lazo et al, 1978) and E. Coli (Liljestrom and Liljestrom, 1987) are available. A comparison of amino acid sequence of ∝-galactosidase proteins from E. Coli with ∝-galactosidase from yeast and human origin showed that these proteins have limited homology, the yeast and human proteins being more related. However, regions common to all three proteins (E. Coli, yeast and human) were found indicating sequences that might comprise the active site of ∝-galactosidase (Liljestrom and Liljestrom, 1987). The enzyme from Vicia faba (Petek et al, 1969) and G. max (Harpaz et al, 1977) were reported to have L-alanine at their N-terminal end. Several legume ∝-galactosidases and yeast ∝-galactosidases are glycoproteins with affinity for Con-A (Hankins and Shannon, 1978; Lazo et al, 1977; 1978; Dey et al, 1982; Dey et al, 1983) unlike bacterial ∝-galactosidases, which are not glycoproteins. Aspartic acid, threonine and serine are the amino acids which act as bridges between the protein and carbohydrate were maximum (35%) in the purified yeast ∝-galactosidase (Lazo et al, 1978).

Alpha-galactosidases from various sources viz. mung bean (Hankins and Shannon, 1978), soybean (del Campillo and Shannon, 1982) and Vicia faba (Dey et al, 1982; Dey et al, 1983) display a unique activity namely agglutination of red blood cells. Vicia faba ∝-galactosidases are unique enzymes

(form I, II[1], II[2]) possessing independent lectin (glucose/ mannose specific) and catalytic sites in the same protein molecule (Dey et al, 1982) but at different sites. The physiological significance of lectin nature of ∝-galactosidase is that the enzymes bind via their lectin sites to a variety of cell components of carbohydrate and glycoprotein nature of cell membrane and cell wall, for compartmentation or regulation by stearic interaction (Basha and Roberts, 1981; Lorence-Kubis et al, 1981).

The comparison of amino acid sequences of the classical lectins with the enzymic lectins of ∝-galactosidase showed good correlations and homologies (Bauman et al, 1981; 1982; Shannon et al, 1981) suggesting that classical legume lectins are precursors of these enzymes and they are genetically related.

Multiple forms of ∝-galactosidase from a number of plant sources are known, these have been separated on the basis of well defined protein characteristics as shown in Table-23. Among the molecular forms differentiated by their molecular weights, a monomeric/tetrameric relationship generally exists, whose association and dissociation is generally pH dependent (Harpaz et al, 1977; del Campillo and Shannon, 1982). The molecular forms of Vicia faba are very well studied, which has 3 forms, I (209,000), II[1] (43,000) and II[2] (41,000) separated by gel filtration and CM-cellulose chromatography (Dey and Pridham, 1968; Dey et al, 1982). Immunological evidences suggested that all the three enzyme forms are closely related because they all cross

TABLE-23 : Molecular forms and properties of some plant α-galactosidases.

	Source	Form	Method of preparation	Mol. wt.	pH optimum	Km (mM)	Reference
1.	Cajanus indicus (Seed)	I II	Gel filtration	High Low	5.0 4.5	6.3 1.2	Dey and Dixon (1974)
2.	Caragana arborescence (seed).	I II	Gel filtration	1,35,000 34,000	- -	0.80	Hankins et al. (1980a)
3.	Cocas nucifera (Kernal)	I	Gel filtration	1,23,000	3.4 & 5.7	0.43	Balasubramaniam et al. (1976).
		II		21,000	5.7	0.43	
4.	Cyamopsis tetragnolobus (seed)	I	DEAE-cellulose	34,000	5.0	0.42	McCleary and Matheson (1974)
5.	Glycine max (seed)	I	Gel filtration	1,60,000	6.8	0.15	del Campillo and Shannon (1982).
		II		40,000	5.6	0.39	
6.	Phaseolus limensis (seed)	I	Gel filtration	1,80,000	-	0.38	Hankins et al. (1980b
		I		39,000	-	-	
7.	Vicia faba (seed)	I	Gel filtration	1,60,000	2.0 & 5.5	0.44	Dey et al. (1982)
		II_1	and SDS electrophoresis	43,000	3.0 & 5.2	0.97	Pridham and Dey (1974)
		II_2		41,000	2.0 & 5.5	0.33	

TABLE-23 (Contd...)

Source	Form	Method of preparation	Mol. wt.	pH optimum	Km (mM)	Reference
9. *Vigna radiata* (seed)	I	Gel filtration	1,60,000	5.6 & 7.2	0.2	del Campillo *et al.* (1981)
	II		40,000	5.6		
10. *Ulex europaeus*	I	Gel filtration	1,60,000	-	0.67	Hankins *et al.*(1980a)
	II		42,000	-		
11. *Trifolium repens* (seed)	I	Hydroxylapatite	41,000	3.8-4.2	6.6	Williams et al.(1977, 1978
	II		41,000	3.6-4.4	7.0	
	III		41,000	4.2-4.8	2.7	
	IV		41,000	4.2-4.6	11.1	

reacted with each other and form I is a tetramer of units of enzyme II[2]. The major possible role of different forms of ∝-galactosidase is in the regulation and utilization of storage carbohydrates (Dey and del Campillo, 1984) e.g. extracts of green and immature Vicia faba seeds showed a low level of the monomeric form of ∝-galactosidase, but during maturation, tetrameric form a was observed (Pridham and Dey, 1974).

Invertase/D-fructofuranoside fructohydrolase (β-D-fructofuranosidase) (E.C. 3.2.1.26) has been shown to occur in microbial, plant and animal sources which acts typically on sucrose and related glycosides producing hydrolysis or, in varying degree, fructosyl transfer. It is one of the oldest known enzymes, first isolated from yeast by Berthelot in 1860. Amongst micro-organisms, β-D-fructofuranosidase activity of yeasts, particularly of Saccharnomyces sp. has been extensively studied by various workers (Berthelot, 1860; Neuberg and Mandel, 1950; Lampen, 1971).

Multiple forms of invertase have been found in plants as shown in Table-24. The existence of soluble and insoluble forms of this enzyme has been reported in raddish in which the transformation of the cytosolic soluble form into the bound form was phytochrome mediated (Zouaghi et al, 1979; Largitte et al, 1981). Presence of acid invertase and alkaline invertase is a very common feature of this enzyme in plants

(Dey and Campillo, 1984), which has a role in the regulation of sucrose metabolism of plants. It was suggested that acid invertase directs sucrose to hexoses in tissues where demand of the monosaccharides is high, whereas alkaline invertase hydrolyzes sucrose in tissues that lack acid invertase e.g. in cells that store sucrose (Lyne and Rees, 1971).

The multiple forms of yeast invertase differ in their molecular weight and cellular location (Iglesias et al, 1980). Yeast invertase is an attractive system to study glycoprotein synthesis and secretion (Gascon and Ottolenghi, 1967). The subunit structure and active site of this enzyme has been investigated (Neuman and Lampen, 1969; Braun, 1977; Trimble and Maley, 1977). The heavy enzyme of yeast is a glycoprotein with 50% mannose and 3% glucosamine (Iglesias et al, 1980) whereas the light enzyme has no carbohydrate. Intermediate forms, representing varying stages of enzyme-protein glycosylation were also present, thus the light enzyme is possibly the precursor of the other forms of invertases in yeast. Many plant invertases are also glycoproteins; for example, those from grape (Arnold, 1966), barley (Prentice and Robbins, 1976). Convolvulus (Klis and Akster, 1974) and radish (Faye and Berjonneau, 1979). In plants, invertase actively participates in the breakdown of sucrose, especially in those parts of plant, where the conversion of sucrose to starch is not an important feature e.g. storage organs (Dey and Campillo, 1984).

Among micro-organisms, the regulation of invertase synthesis in yeast and neurospora is well documented (Lampen, 1968; Iglesias et al, 1980) which is under catabolite repression. Derepression by exhaustion of glucose from the medium, resulted in 90% increase in the total activity of heavier enzyme, the level of the lighter enzyme remained unaltered irrespective of the presence of glucose.

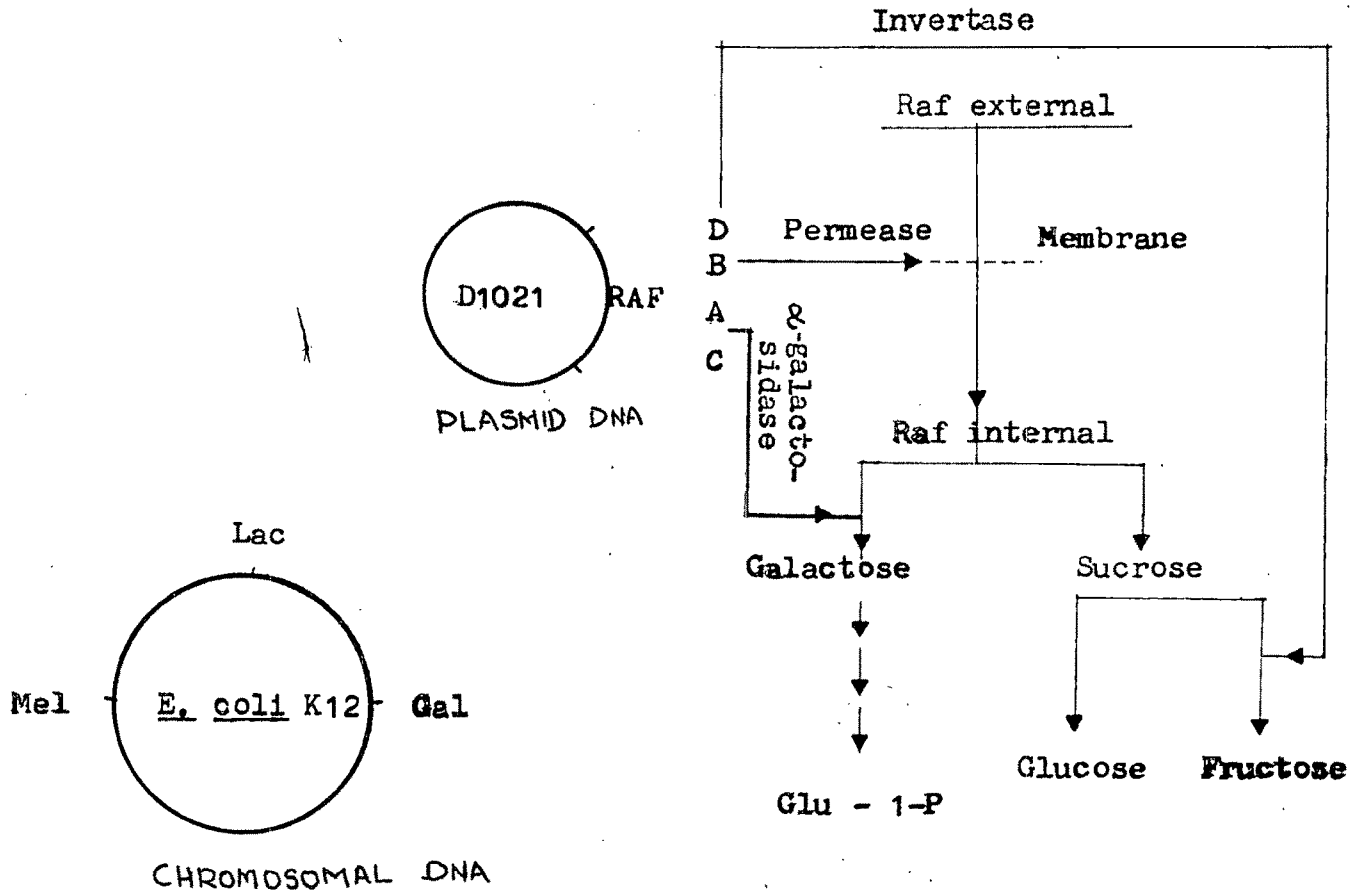
Invertase activity and the levels of the multiple forms are regulated in plants by various factors, such as stage of tissue growth (Jones and Kaufman, 1975), plant hormones, sucrose, glucose, fructose (Kaufman et al, 1973; Weston and Chin, 1975), nitrogen source (Matsumoto et al, 1976) and NaCl concentration (Hawker, 1980). Invertase inhibitors also have been implicated in the regulation of this enzyme in several plant tissues (Jaynes and Nelson, 1971; Malik and Sood, 1976). Multiple forms of invertase, I and II, from Fusarium oxysporum are distinguishable in immunological relationship, amino acid composition, molecular weight, isoelectric point, pH optima and heat stability (Nishizawa and Maruyama, 1979) and the synthesis of the two forms is regulated via gene expression during cellular differentiation.

Micro-organisms which produce α-galactosidase are isolated from various sources as described in Table-22. Among bacteria, genetics of raffinose and melibiose utilization is studied in detail in E. Coli. The transmissible nature of extrachromosomal genetic element which mediates hydrogen sulphide

production (Hys) in E. Coli was recognized by Lautrop et al. (1971). Since then Orskov and Orskov (1973) have reported that some H_2S-positive strains are also able to transfer the determinant responsible for raffinose-fermenting ability (Raf) and have shown that both markers are transmitted as a single linkage group. Thus, raffinose utilization has been added to the list of those bacterial degradative characters that are governed by plasmids (Magalhaes and Veras, 1977).

It was found that raffinose plasmids enable strains of E. Coli to use the trisaccharide raffinose as sole 'C' source by the action of three inducible plasmid coded functions namely (a) transport system, raffinose permease, (b) α-galactosidase and (c) an invertase. Their structural gene form an operon negatively controlled by a repressor gene (Schmid and Schmitt, 1976; Schmitt et al, 1979). The system consisting of a regulatory gene and the structural genes for the enzymes raffinose permease, α-galactosidase and invertase has been designated as the "raf" system (Fig.5). In the cells of E. Coli K12, the inducible functions of "mel" operon namely melibiose permease and α-galactosidase determine the utilization of α-D-galactosides such as melibiose, melibiitol and galactinol (Schmitt, 1968). The trisaccharide raffinose cannot be catabolized in this strain, since the permease is not induced by raffinose (Schmid and Schmitt, 1976). After the characterization of the proteins coded by "mel" operon and "raf" operon in E. Coli Schmid and Schmitt (1976) made a very interesting observation in E. Coli. Studies carried out by

FIG. 5 : Gene Enzyme relationship in Raffinose metabolism by *E. coli* K12 harbouring the Raf plasmid D1021.
[Ref: Schmid and Schmitt, 1976]



them proved that the plasmid coded ∝-galactosidase differs from the homologous E. Coli enzyme in substrate affinities, cofactor requirements, stability and toluene resistance. It can, therefore, be used as a marker enzyme suitable for the detection in vivo of "Raf"-plasmids.

Plasmids coding for utilization of raffinose have been found in enterobacteria isolated from man (Buissierf et al, 1977; Magalhaes and Veras, 1977) and domestic animals (Smith and Parsell, 1975) under enteropathogenic conditions. Genetic determinants of the K-88 antigen (which facilitates colonization in the anterior small intestine) have been found on plasmids together with 'raf' genes (Smith and Parsell, 1975; Shipley et al, 1978; Mooi et al, 1979). Immunochemical relationships were established among 'raf' plasmids of 39 independent isolates of man and domestic animals by using antiserum against ∝-galactosidase by Schmid et al. (1979). Immunodiffusion studies revealed three serological subclasses of ∝-galactosidase, which are correlated with the biological and geographical origin of the host strains. It was concluded from this experiment that 'raf' determinants of all 'raf' plasmids tested have evolved from a common ancestor.

In E. Coli cells, melibiose is transported by active transport system. Because of the similarity of substrate specificity to the lactose transport system (TMG permease-I), the melibiose transport system has been termed TMG permease-II

(Prestidge and Pardee, 1965). Melibiose, utilization in E. Coli is dependent on the melibiose locus 'mel' which is located at 93 min on the genetic map (Bachmann, 1983). The 'mel' locus induced by several ∝-galactosides (Prestidge and Pardee 1965; Schmitt, 1968) and its expression is controlled by the cAMP-CRP regulatory circuit (Okada et al, 1981). Melibiose is also an inducer of the 'lac' operon and can be transported into the cell by lactose permease protein 'Lac-Y'. In fact, as expression of 'mel B' is temperature sensitive, influx of melibiose at 38-40°C is dependent on Lac-Y (Beckwith, 1963; Prestidge and Pardee 1965). The 'mel' operon in E. Coli consists of atleast two structural genes, 'mel-A' and 'mel-B', coding for ∝-galactosidase and melibiose carrier respectively (Hanatani et al, 1984). The 'mel-A' and 'mel-B' gene products were identified using maxicells harbouring plasmids carrying the melibiose operon by Hanatani et al. (1984). The apparent molecular weight of 'mel-A' coded ∝-galactosidase was about 50,000 and that of the melibiose carrier coded by 'mel-B' was about 30,000 as estimated by SDS-gel electrophoresis. The structure and regulatory mechanism of melibiose operon seem to be similar to that of the lactose operon which contains structural genes 'lac-Z' and 'lac-Y' (Beckwith, 1970). Moreover, the inducibility of the operon indicates that there might exist a repressor gene similar to 'lac-I' (Hanatani et al, 1984).

Thus as described earlier there are reports of variety of micro-organisms which elaborate enzymes for the breakdown of certain toxic compounds of plants. Even though there is a diverse number of micro-organisms that can be used to rapidly degrade a variety of antinutritional factors and toxins, inherent problems exist when they are used simultaneously to ferment legume based foods. This is because of the problems of interaction among different micro-organisms leading to the survival of only a few dominant strains. An ideal solution for the rapid microbial degradation of antinutritional compounds and toxins would be to construct novel strains, which would have the genetic potentialities to degrade a wide variety of toxins simultaneously. The use of one such strain would eliminate the interaction among different micro-organisms and yet would lead to an efficient degradation of various antinutritional factors and toxins. This can be achieved by transferring various degradative genes, whichever is responsible for the degradation into one strain. This will involve DNA cloning strategies which essentially consists of the following four parts : (i) a method for generating DNA fragments; (ii) a method for joining the foreign DNA fragments to vector DNA; (iii) a means of introducing a composite DNA (artificial recombinant DNA) molecule into a host cell in which it can replicate; and (iv) a method of selecting or screening for a clone of receipient cells that has acquired the recombinant. (Cohen, 1975; Primrose, 1977; Sinsheimer, 1977).

Thus there is a potential to prepare a novel strain, which is capable of growing during fermentation and breakdown toxins. The genes coding for the enzymes responsible for the breakdown of antinutritional factors and toxins could be present either on the main bacterial chromosomes or on plasmids. Therefore, it would be interesting to study the genetic basis of degradation of food toxins in bacteria.

The genome of any prokaryotic species consists essentially of a single major linkage group, the chromosome, containing all or nearly all the cell's genes. Many bacteria, are known to have additional facultative linkage groups, separate from their chromosome, of a much smaller size, which are collectively referred to as extrachromosomal elements or plasmids (Novick, 1969).

Plasmids are DNA molecules which are :

a) <u>replicons</u> : DNA molecules which contain an origin of replication and only such DNA is capable of transforming other cells;
b) stably inherited in an extrachromosomal state;
c) <u>genetically homogenous</u> : Thus the heterogeneous circular DNA molecules which are found in <u>B. megaterium</u> (Carlton and Helinski, 1969) are not necessarily plasmids;
d) of a constant monomeric size;
e) capable of replicating independently of the chromosome
f) dispensable to the host under certain conditions.

according to Old and Primrose (1986).

Plasmids are widely distributed throughout the prokaryotes and vary in size from less than 1 x 10^6 daltons to greater than 200 x 10^6 daltons. Most of the plasmids found in bacteria are circular double stranded molecules. However, there are reports of existence of linear plasmid DNA in yeast (Gunge, 1982) and sprirochetes (Barbor and Garon, 1987). Plasmids can be classified as follows according to Novick et al. (1976) :

1) Plasmids can be categorized into one of the major types :

   (a) Conjugative or (b) non-conjugative - depending on whether or not they carry a set of genes called 'tra' genes that promote bacterial conjugation. Generally conjugative plasmids are of relatively high molecular weight and are present as 1-3 copies per chromosome whereas non-conjugative plasmids are of low molecular weight and present as multiple copies per cell (Table-25).

2) Plasmids can also be classified on the basis of their being maintained as multiple copies per cell (relaxed plasmids) or as a limited number of copies per cell (stringent plasmids).

3) Plasmid incompatibility is the inability of two different plasmids to coexist in the same host cell, in the absence of a selection pressure. Various incompatibility classes of plasmids have been defined in which groups of plasmids which are mutually incompatible are considered to belong to the same incompatibility class.

TABLE-25 : Properties of some conjugative and nonconjugative plasmids.

Plasmid	Size (Mdal)	Conju-gative	No. of copies/cell	Phenotype
Col E1	4.2	No	10 - 15	Colicin E1 production
RSF 1030	5.6	No	20 - 40	Ampicillin resistance
C|o DF 13	6	No	10	Cloacin production
R6K	25	Yes	13 - 38	Ampicillin and Streptomycin resistance
F	62	Yes	1 - 2	--
R - I	62.5	Yes	3 - 6	Multiple drug resistance
Ent P 307	65	Yes	1 - 3	Enterotoxin production

Cf. Old and Primrose (1986)

4) Plasmids also can be classified on the basis of the phenotypic traits which they confer onto the host cell. Some of the phenotypes are listed in Table-26. Most of the plasmids have been classified on the basis of their phenotypic properties which initially led to their detection (Brodą, 1979). Thus, there are the R-factors (for drug resistance), col-factors (for colicinogeny) of the gram negative bacteria, penicillinase plasmids of S. aureus and degradative plasmids of Pseudomonas sp. as described in Table-27. Plasmids coding for resistance to antibiotics, degrade or modify the active structures by forming less active or inactive derivatives. Many plasmids of both gram positive and gram negative bacteria have genes determining resistance to a wide variety of toxic inorganic cations and anions, including ions of mercury, cadmium, arsenic, chromium, silver and tellurium (Summers and Silver, 1976). Evidence that plasmid DNA is involved in carbohydrate fermentation (Efstuthiou and McKay, 1976; Le Blanc et al, 1980) and other phenotypic characters (Kempler and McKay, 1979; Neve et al, 1984) expressed by various lactic streptococci is well documented.

The physicochemical properties of chromosomal and plasmid DNA differ widely, a fact which facilitates the detection and isolation of the latter. The resistance to denaturation, sedimentation velocity in neutral and alkaline solutions and the buoyant density in alkaline condition are

TABLE-26 : Phenotype traits exhibited by plasmid-carried genes.

Antibiotic resistance,	Heavy metal resistance,
Antibiotic production,	Bacteriocin production,
Degradation of aromatic compounds,	Induction of plant tumors,
Haemolysin production,	Hydrogen sulphide production,
Sugar fermentation,	Host controlled restriction and modification.
Enterotoxin production.	

cf. Old and Primrose (1986)

TABLE-27 : Degradative plasmids in bacteria.

Bacterial species	Plasmid	Apparent primary substrate	Size (Milli-daltons)	Reference
Pseudomonas putida	CAM	Camphor	300	Chakrabarty (1980)
P. putida	NAH	Naphthalene	46	Dunn and Gunsalus (1973
P. putida	OCT	n-octane	250	Chakrabarty (1980)
P. putida	SAL	Salicylate	55,48,42	Chakrabarty (1972)
Pseudomonas sp.	TOL	Xylene/toluene	76	Chakrabarty (1980)
Pseudomonas sp.	NIC	Nicotine/nicotinate	-	Chakrabarty (1980)
Pseudomonas sp.	$pOAD_2$	6-Aminohexanoic acid	29	Negoro et al. (1980)
Pseudomonas sp.	pAC 25	3-Chlorobenzoate	68	Chatterjee et al. (1981
Pseudomonas sp.	pAC 27	3- and 4- chlorobenzoate	59	Chatterjee and Chakrabarty (198
Alcaligenes sp.	p JP4	Pesticides (2-4 MCPA and 3-CB)	-	Fisher et al. (1978) Dom and Pemberton (1981
S. lactis	PDR-1	Lactose	32	Clewell et al. (1981) Steeley & Mc Kay (1986)
S. lactis	PDR-2	Sucrose	29	Clewell et al. (1981)
L. casei	pLY101	Lactose	-	Kadota (1987)

TABLE-27 (Contd....)

Bacterial species	Plasmid	Apparent primary substrate	Size (Mega-daltons)	Reference
E. Coli	p RSD-1 or p RSD-2	Raffinose	30 83	Burkardt et al. (1978)

all enhanced in covalently closed circular plasmid DNA (Old and Primrose,1986). Being a super coiled DNA, it also adopts a more compact configuration than its released equivalent and therefore moves faster when sedimented in an ultracentrifuge or subjected to electrophoresis (Radloff et al, 1967).

Importance of plasmids :

In molecular biology, plasmids serve as excellent model system for the study of structure and function of genetic material at molecular level . Two great virtues for this purpose are:

1) their relatively small size, generally in the range between 1.5 to 100 megadaltons (which is sufficient to code for 1-2 average sized proteins) which means that they are much easier to manipulate in vitro than are intact bacterial chromosomes.

2) secondly, their host cells can usually survive without them so that the properties conferred by them (e.g. drug resistance) can be easily determined (Broda, 1979).

Thus plasmids are tools for molecular biology, especially for recombinant DNA technology. Genetic analysis of bacteria involves exchange of genes between strains, and plasmids, in particular 'F', are important agents of transfer. Certain plasmids that can transfer between different genera are being used extensively to introduce DNA into novel hosts, an example

is the introduction of nitrogen fixation genes from Klebsiella to E. Coli (Cannon et al, 1976) and to Azotobacter (Cannon and Postgate, 1976) using hybrid plasmids based on 'F' and 'RP4' respectively.

Plasmid DNA is extensively used recently in recombinant DNA technology, because it can be joined with fragments of alien DNA in vitro. Genetic recombination essentially consists of the breakage and joining of DNA molecules. The development of gene cloning procedures now permits DNA obtained from a wide variety of prokaryotic and eukaryotic sources to be cut in vitro at precise locations and the DNA fragments thereby generated to be coupled enzymatically to a self replicating genetic element, known as a cloning vehicle (either a plasmid or a virus genome). Hybrid molecules generated in this fashion are introduced into a suitable host cell in which they are propagated (Cohen, 1975) as described in Fig.6. Thus the host cells containing a hybrid molecule can serve as cellular factories for selective amplification of the cloned DNA segment and in some instances, the gene product specified by the cloned DNA.

Plasmids can be used as cloning vehicles in recombinant DNA technology, provided they have following properties (Old and Primose, 1981); (1) low molecular weight, (2) ability to confer readily selectable phenotypic trait on host cells, and (3) single sites for a large number of restriction endonucleases, preferentially in genes with a readily scorable phenotype.

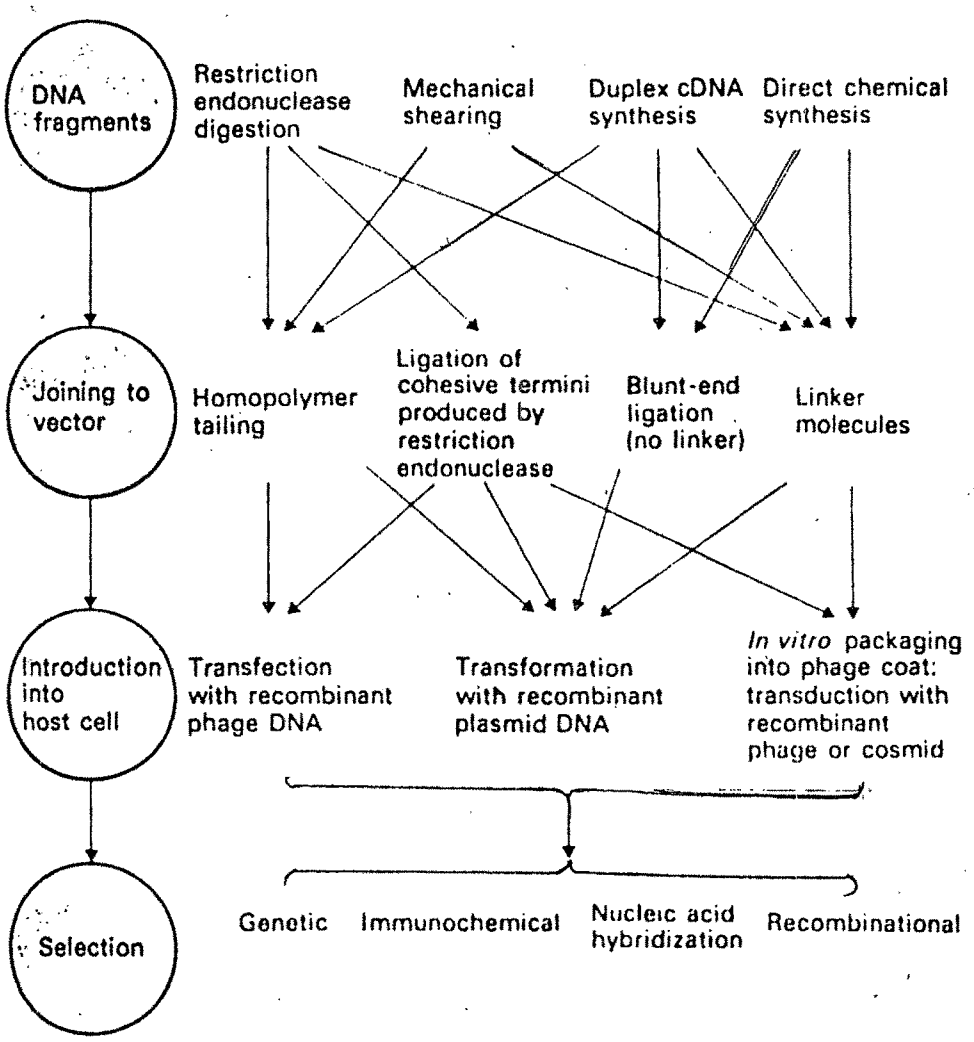


Fig. 6: Generalized scheme for DNA cloning in *E. coli*. Favoured routes are shown by arrows. (cf: Old and Primrose, 1986)

The most widely used plasmid vector is pBR-322, a plasmid under relaxed control of replication that contains both ampicillin and tetracycline resistance genes and a number of convenient restriction sites (Bolivar et al, 1977) as shown in Fig.7. The complete nucleotide sequence of pBR-322 is known (Sutcliffe, 1979). Naturally occuring plasmids which are used as cloning vehicles are listed in Table-28, alongwith their properties, Plasmid pBR-322 is also used as a vector to clone eukaryotic genes e.g. somatostatin, insulin. To date the greatest contributions of recombinant DNA research have undoubtedly been within molecular biology itself, because this technology has made possible the detailed structural and function analysis of potentially any gene (Old and Primose, 1986). Recombinant DNA probes are useful for diagnosis of genetic diseases like hemoglobin disorder (Alter, 1981), thalassaemia (Weatherall and Clegg, 1982) and for the foetal DNA analysis (Williamson et al, 1981; Old et al, 1982) in medicine. Using recombinant DNA technology, micro-organisms have been engineered to synthesize proteins normally formed only in higher animals, thus making many of these proteins commercially available as shown in Table-29.

The location of desired genes in bacteria can be established by different means, viz. by curing the strains of plasmids, transformation or cloning studies. However, most of the evidence for plasmid involvement in genetic characteristics has been based on curing experiments; demonstration of increased proportion of nonproducing strains (at frequency higher

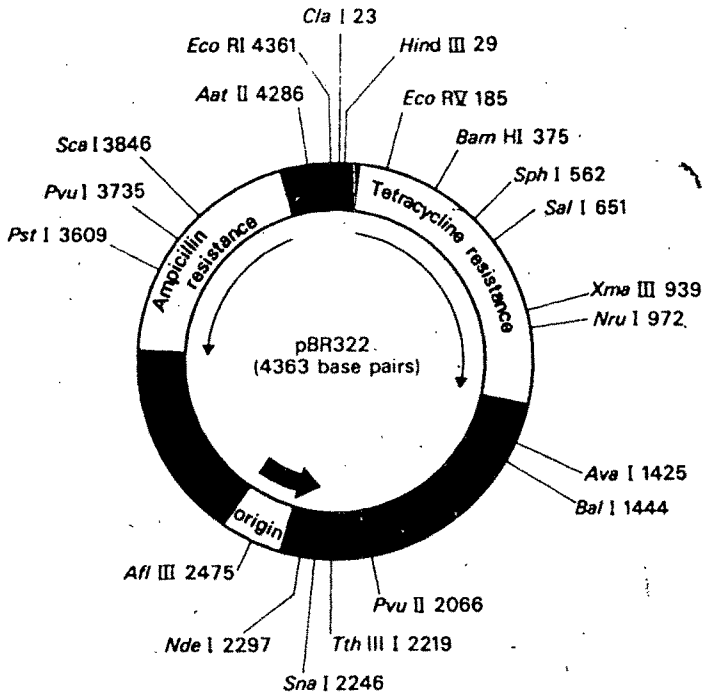


Fig. 7: The structure of pBR322 showing the unique cleavage sites. The numbers relate to the coordinate of the base which corresponds to the 5′ nucleotide of each recognition sequence. The thin arrows inside the circle show the direction of transcription of the Ap^R and Tc^R genes. The thick arrow shows the direction of DNA replication.

TABLE-28 : Properties of some "Natural" plasmids used for cloning DNA.

Plasmid	Size (Mdal.)	Single sites for endonucleases	Marker for selection of transformants	Insertional inactivation
pSC 101	5.8	Eco R I	Tetracycline resistance.	-
		Xho-I, Hinc-II, Pvu-II		
		Hind-III, Sal-I	-	Tetracycline resistance
Col- E_1	4.2	Eco-R I	Immunity to colicin E1	Colicin E1 production
RSF 2124	7.4	Eco-R I	Ampicillin resistance	Colicin E1 production
		BAM HI	Ampicillin resistance	Colicin E1 production

(Cf: Old and Primrose, 1986)

TABLE-29 : Foreign proteins produced in micro-organisms.

	Protein	Application	Reference
1)	Interferans	Possible treatment of virus infections and cancer.	Houghton et al. (198
2)	Human growth hormone	Pitutary dwarfism	Goeddel et al. (1979a
3)	Insulin	Diabetes	Goeddel et al. (1979b
4)	Tissue plasminogen activator	Thrombosis	Pennica et al. (1983.
5)	β -endorphin	Analgesic	Shine et al. (1980)
6)	Interleukin-2	Cancer chemotherapy	Taniguchi et al. (19
7)	α_1 -entitrypsin	Emphysema	Bollen et al. (1983)
8)	Relaxin	Facilitate childbirth	Stewart et al. (1983.
9)	Chymosin	Cheese production	Emtage et al. (1983)
10)	Somatostatin	Growth regulation	Itakura et al. (1977.
11)	Poly aspartyl phenyl-alanine	Preparation of aspartame	Doel et al. (1980).

than mutations) after treatment with curing agents. Although plasmids are stably inherited and can replicate in synchrony with cell division, there is some small spontaneous loss of plasmid DNA presumably as a result of a defect in plasmid replication in small number of cells, which is infrequent. However, experimental conditions can sometimes be used which enhances the natural frequency of plasmid loss. Compounds which have been used so far as curing agents include acridine dyes, novobiocin, mitomycin-C, ethidium bromide, sodium dodecyl sulfate (SDS) etc. but the effectiveness of any individual compound vary greatly from plasmid to plasmid. Alternatively change in the physical conditions of growth such as elevated temperature or thymine starvation can also result in plasmid curing (Broda, 1979; Novick et al, 1980).

Acridine dyes are very effective in curing E. coli of the sex factor 'F' and 'R' factors (Hirota, 1960; Mitsuhashi et al, 1961; 1963) due to selective interference in the replication of the plasmid (Hon and Korn, 1969). Tomoeda and coworkers (1968) and Salisbury et al. (1972) showed that the detergent SDS was effective in eliminating 'F-lac' and the 'R' factor plasmids of E. coli. Antibiotic resistance plasmids in Enterobacteriaceae and Staphylococci were eliminated by treatment with ethidium bromide at the concentration of 1×10^{-6}M (Bounchaud et al, 1968). Curing with mitomycin-C was carried out in Pseudomonas putida of transmissible and degradative plasmids controlling camphor oxidation (Reinhold et al, 1973; Chakrabarty, 1974, 1982)

H_2 group of plasmids responsible for resistance to chloramphenicol kanamycin, streptomycin, tetracycline and to various coliphages were eliminated by treatment with novobiocin (Taylor and Levine, 1980). Novobiocin acts by its interaction with bacteria DNA gyrase which is required for the supercoiling of small bacterial plasmids.

The elimination of plasmids by mitogens is apparently not due to specific effects of drugs on the extrachromosomal DNA. DNA replication, which is necessary precondition for cell division, constitute a vulnerable target for the action of cytotoxic drugs shown in Fig.8. Acridine dyes and mitomycin, being flat molecules, intercalate between the stacked base pairs of DNA helix and interfere with replication of DNA. During intercalation, DNA helix (a) gets extended, (b) locally unwound due to the binding reaction and (c) the plane of the chromophore of the bound drug becomes parallel that of base pa irs; thereby interferes with the replication of DNA. (Waring, 1981)

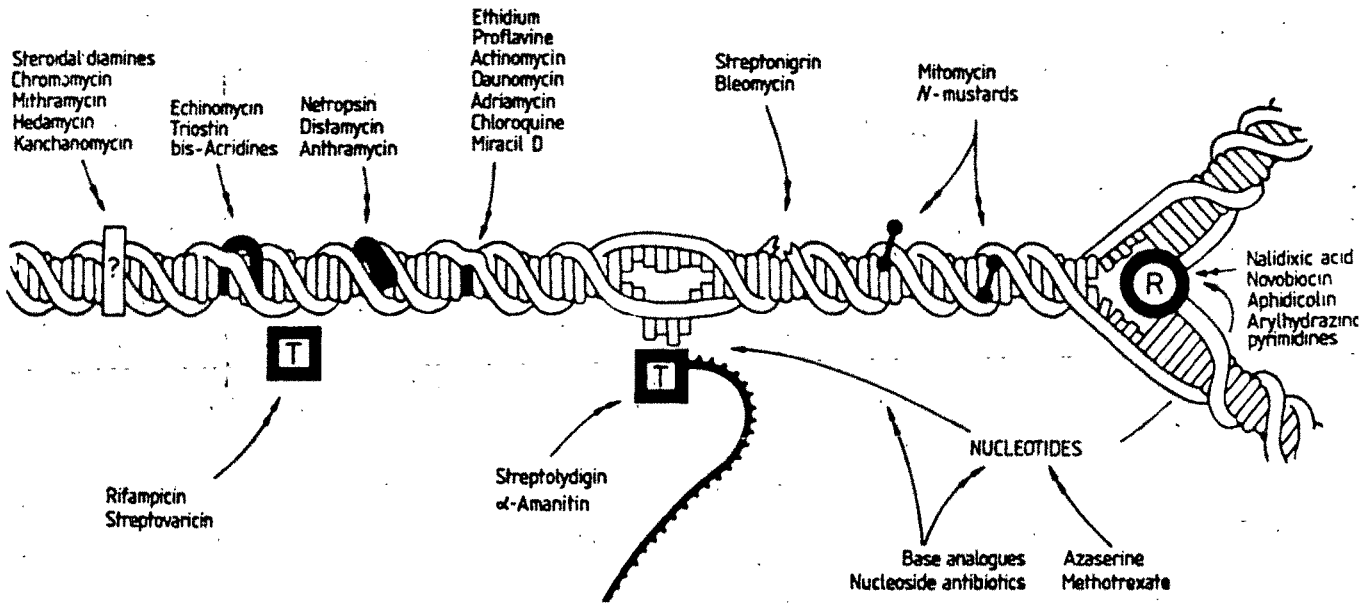


Figure 8 Sites for inhibition of nucleic acid synthesis by antibiotics and drugs. R represents the replicating enzyme complex, T the transcribing enzyme (RN polymerase). Actions of inhibitors are represented by double-headed arrows and are purely diagrammatic; it is not intended to imply that the sites of acti represented here account for the whole mode of action of any drug.

Ref: Waring, 1981.

Rationale of the present investigation :

Detailed studies were carried out in this laboratory to detect the type of micro-organisms involved in the fermentation of Indian foods such as 'idli' 'khaman', 'dhokla' and 'soyidli' (Ramakrishnan, 1979). The data on microflora identified in different Indian fermented foods by various investigators are presented in Table-30. Total and differential bacterial population in the batters of Indian fermented foods are given in Table-31, from which it can be concluded that L. mesenteroides and lactobacillus sp. are the dominant microflora in fermented batters. It was found that these micro-organisms originate from the grains used for preparing these fermented foods (Table-32). The chemical changes brought about by micro-organisms during fermentation are increase in free sugar, amino nitrogen and certain viramins as well as degradation of toxins and antinutritional compounds as described earlier. It was found that L. mesenteroides (HA) isolated from 'soyidli' batter can breakdown hemagglutinin due to the presence of hexosaminidase, mannosidase and protease (Rao and Ramakrishnan, 1977). When autoclaved soyidli batter was inoculated with a mixture of bacteria namely L. mesenteroides (HA), L. fermenti, L. delbrueckii and Bacillus sp., a complete hydrolysis of hemagglutinin was observed in addition to other chemical changes during fermentation (Rao, 1978).

TABLE-30 : Micro-organisms identified in Indian fermented foods.

Name of the food	Period of fermentation (h)	Bacteria	Fungi	Reference
Khaman	14	Lactobacillus sp., L. fermenti, L. lactis, Leuconostoc mesenteroides, Pediococcus acidilactici.	--	Rajalakshmi and Vanaja (1967),
Idli	14-16	L. delbrueckii, L. lactis, S. lactis, S. faecalis, P. cerevisiae, L. fermenti, L. mesenteroides.	Torulopsis sp., Odium lactis	Lewis and Johar(1953); Mukherjee et al.(1965) Batra and Milner (1974 Rajalakshmi and Vanaja (1967).
Soyidli	14-16	L. mesenteroides.	Yeast	Ramakrishnan et al. (1976)
Rabdi	14-16	P. acidilactici, Bacillus sp., Microbacterium flavum.	--	Cf. Ramakrishnan (1979
Fermented rice	14-16	S. faecalis, P. acidilactici, Bacillus sp.	--	Cf. Ramakrishnan (1979
Kenima	24-48	Rod shaped acid producing bacteria.	--	Batra and Milner (1976

TABLE-30 (Contd...)

Name of the food	Period of fermentation (h)	Bacteria	Fungi	Reference
Curd	12	Lactobacillus sp., S. lactis, S. faecalis,	Candida krusei, Trichosporon sp., Torulopsis sp.	Lakshminarayan et al. (1952); Batra and Milner (1974); Ramakrishnan (1976)

TABLE-31 : Bacterial flora of fermented batters.

Bacteria present	Soy-idli	Rice-black-gram idli	Khaman	Dhokla	Rabdi	Fermented Rice	Jalebi	Ambali
A. Total count (x 10^9/g)								
Unfermented	0.04	1.4	0.003	0.02	0.00003	0.0005	0.28	0.0009
Fermented	11.0	126	33	0.36	0.0021	0.326	2.2	4.0
B. Individual bacteria in fermented batter (x 10^9/g batter) :								
1. L. mesenteroides	3.2 (30)*	54 (42.8)	18 (54)	0.16 (44.4)	-	-	-	1.6 (40)
2. L. fermenti	1.6 (15)	18 (14.3)	9.54 (29)	0.16 (44.4)	-	-	0.6 (28.3)	1.6 (40)
3. L. delbrueckii	2.0 (19)	18 (14.3)	-	-	-	-	-	-
4. L. lactis	1.2 (12)	-	3.19 (9.7)	-	-	-	0.6 (28.3)	-
5. L. buchneri	0.8 (8)	-	-	-	-	-	0.32 (15.1)	-
6. S. faecalis	1.2 (12)	18 (14.3)	-	0.4 (11.1)	-	0.027 (8.3)	0.6 (28.3)	0.8 (20)
7. P. acidilactici	-	-	1.0 (3.2)	-	0.0003 (17)	0.027 (8.3)	-	-

TABLE-31 (Contd...)

Bacteria present	Soy-idli	Rice-Blac-grem idli	Khaman	Dhokla	Rabdi	Fermen-ted rice	Jalebi	Ambali
8. *Bacillus sp.*	-	-	1.0 (3.2)	-	0.0010 (50)	0.163 (50)	-	-
9. *M. flavum*	-	-	-	-	0.0007 (37.6)	0.109 (33.4)	-	-

* The figures in parentheses are percentages of total counts.

cf. Ramakrishna n (1979)

TABLE-32 : Microbial population of ingredients used for preparation of Indian fermented foods.

Bacterial flora present	Rice	Maize	Wheat	Maida	Suji	Ragi	Bengal-gram dhal	Bengal gram flour	Soy-dhal	Black-gram dhal
A. Total count ($X\ 10^4/g$).	2.25	2.75	2.55	37.5	1.5	4.5	0.19	11.1	29.5	1.27
B. Individual counts as '%' of total counts :										
1. *L. mesenteroides*	33.3	42.9	23.1	29.5	30.6	30	40	45	40	69.2
2. *L. fermenti*	16.6	21.4	-	-	-	10	20	10	-	-
3. *L. delbrueckii*	8.3	-	-	-	-	-	-	-	-	7.1
4. *L. lactis*	-	14.2	-	-	-	-	10	5	7.1	-
5. *S. faecalis*	8.3	6.9	30.6	23.2	38.6	10	-	15	20	7.1
6. *P. acidilactici*	16.5	-	30.6	23.2	15.3	30	10	15	20	-
7. *Bacillus sp.*	16.6	14.2	23.2	23.2	25.3	20	20	10	-	14.9

Cf. Ramakrishnan (1979)

These studies as well as changes found during fermentation, raised the question whether some of the micro-organisms present in fermented batter or stored grains can also breakdown flatulence producing oligosaccharides (present in almost all the legumes) and the neurotoxin (present in L. sativus).

As described earlier though L. sativus has got a high protein content and resembles other commonly used legumes in taste and nutritive value, it also contains antinutritional factors and neurotoxin. Preliminary studies carried out in this laboratory showed that fermentation of L. sativus removed oligosaccharides completely and neurotoxin to some extent. Therefore the objective of the present investigations was to study the mode of breakdown of oligosaccharides and neurotoxin (ODAP) by bacteria isolated from fermented foods or stored grains. Furthermore studies were also aimed to investigate whether bacteria can grow along with dominant microflora of fermentation and degrade these toxins. The present studies were therefore undertaken to study whether any bacteria involved in fermentation and those which are present in stored grains, can hydrolyse neurotoxin and flatulence producing oligosaccharides and if so, study the mode of hydrolysis of these compounds by the bacteria due to the elaboration of the enzymes.

Attempts were therefore made to :

1) search for and, if present, isolate raffinose and neurotoxin (ODAP) hydrolysing bacteria from different fermented foods and stored grains,

2) find out their ability to ferment L. sativus batter and degrade these toxins in the presence of other bacteria,

3) detect raffinose hydrolysing and ODAP hydrolysing enzymes namely ∝-galactosidase and ODAP hydrolase respectively in raffinose and ODAP hydrolysing bacteria,

4) purify and characterize ∝-galactosidase and invertase from a bacterial isolate,

5) screen the bacterial isolates which are capable of degrading antinutritional factors and toxins for the presence of plasmids and to study the genetic basis of raffinose degradation.

6) to raise antibodies against ∝-galactosidase of Bacillus sp. I for studies on immunological properties of the same.

These data are incorporated and discussed in this thesis.