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Studies on the low temperature infrared heat processing of soybeans and maize

Proefschrift

ter verkrijging van de graad van
Doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
Dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 19 juni 1985
des namiddags te 16.00 uur in de
aula van de Landbouwhogeschool
te Wageningen

Stellingen (Theorems)

1-The modified procedure for infrared radiation described in this thesis is versatile. Its applications can not be thought to be restricted to soya and maize.

2-The explanation for poor growth of rats caused by trypsin inhibitors in soybeans is controversial.

Liener, I.E., J.Amer.Oil Chem.Soc. 58(3), 406 (1981)

3-The results of research on phospholipase D presented in this thesis can help to clarify the following statement by List:

"Through some unknown enzymatic reaction(s), the natural phospholipids are presumably degraded to phosphatidic and lysophosphatidic acids."

G.R.List, Handbook of Soy Oil Processing and Utilization, American Soybean Association and American Oil Chemists' Society, p.355 (1980)

4-Refining of edible oil may make it less stable to oxidative rancidity by removing the natural antioxidants.

5-Peroxide Value, when used on its own, is not reliable for indicating the quality of edible oil and for making predictions of the storage stability.

6-Both under-and over-heating make full-fat soy flour less stable to oxidative rancidity than the adequately heated product.

This thesis, publications section, p.6

7-When applying heat to cereals and oilseeds, the effect on the functional properties of starch and proteins is often in opposite directions.

8-While the benefits of inactivating certain enzymes have been stressed in this thesis, some endogenous enzymes in foods do have beneficial consequences when active.

This thesis, p.13

9-Wrong kinds of food are often donated by developed countries to combat hunger in poor areas stricken by famine.

10-Although the benefits of dietary fibre are established, the possible problems associated with its excessive consumption are far less appreciated by the public.

Ian Macdonald, Fibre in Human Nutrition, ed by G.A.Spillers and R.J.Amen, Plenum Press, New York, p.263 (1976)

11- Considering the world's food situation it seems a questionable practice to use materials like soya and cereal germs in animal feeding.

M.Kouzeh Kanani
Studies on the low temperature infrared heat processing of soybeans and maize
Wageningen, 19 June 1985

A B S T R A C T

A modified process for the infrared heat processing of oilseeds and cereal grains at relatively low temperatures is put forward. The process which involves an additional holding step and potentials for saving energy was investigated on a pilot plant on the basis of which a design is proposed for industrial applications. The process was used in order to produce full-fat soy flour and maize germ with long shelf life and improved nutritive and organoleptic qualities. Antitrypsin factors, lipoxygenase and lipase could be inactivated with no damage to available lysine. Overheating not only caused damage to available lysine but also made the products more prone to rancidity possibly by causing destruction of natural antioxidants. The process caused protein solubility and dispersibility to fall and starch (in maize germ) to gelatinize. Water absorption of maize germ also increased. In soybeans, urease was found to be a good indicator of the extent of inactivation of antitrypsin factors, while lipoxygenase was found more heat sensitive than urease and antitrypsin factors. For evaluating storage stability, in addition to measuring peroxide value and % free fatty acids, sensory analysis was also carried out.

The process was further applied for treating soybeans prior to oil extraction. It was concluded that the quality of the crude oil obtained from the pretreated beans in terms of oxidation products, free fatty acids and nonhydratable phospholipids was such that the alkali treatment step in the refining process

could be circumvented. The improved quality of the crude oil was attributed to the inactivation of phospholipase D, lipoxygenase and lipase. The residual defatted flakes showed low levels of trypsin inhibitor activity and could be used directly as food or feed.

Finally, the involvement of phospholipase D in the hydrolysis of phospholipids and formation of nonhydratable phospholipids in soybeans was elucidated by radio(chemical) methods, as well as thin layer chromatography and densitometry. The presence of an active, soluble form of the enzyme with isoelectric point 4.8 was shown by isoelectric focusing.

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To my wife, Mahshid,

for her patience,

understanding and care

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A C K N O W L E D G E M E N T S

First and foremost, I would like to express my most sincere gratitude to Prof. Dr. W. Pilnik, Head of The Food Chemistry Division, for allowing me to carry out the present studies under his direction, and for his continued encouragement.

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I am particularly grateful to Ir. D.J. van Zuilichem who not only shared with me his vast industrial expertise and scientific knowledge, but also played an important role in my life in The Netherlands.

The progress of this work would not have been possible without the kind and continued cooperation of J.R. van Delden and W. Stolp. In this connection, I also acknowledge the assistance given to me by J. de Groot, J.L. Cozijnsen and H.J.A.R. Timmermans. H. Belling and I. van Kreuningen were great assets for the timely preparation and publication of my articles.

Finally, I wish to express my great appreciation to The Board of Directors of Codrico B.V., and in particular, to Mr. J.J. Laros, for his enthusiasm in my work and concern and support for my well being ; to Mr. F.W. Schmidt for sharing with me his extensive experience and knowledge of maize and its processing ; and to S.P.G. van Vlissingen, the Board's Secretary, who helped me greatly by turning the otherwise illegible manuscript into its present form.

LIST of ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOCS	American Oil Chemists' Society
BAPA	Benzoyl-DL-arginine-p-nitroanilide
FDNB	1-fluoro-2,4-dinitrobenzene
FFA	Free fatty acid(s)
GLC	Gas liquid chromatography
IR	Infrared
NHP	Nonhydratable phospholipids
NSI	Nitrogen solubility index
P	Phosphorus
PA	Phosphatidic acid
PC	Phosphatidylcholine
PDI	Protein dispersibility index
PE	Phosphatidylethanolamine
PER	Protein efficiency ratio
Ph-D	Phospholipase D
PV(pv)	Peroxide value
TI	Trypsin inhibitor(s)
TLC	Thin layer chromatography
TNBS	2,4,6-trinitrobenzenesulphonic acid
TUI	Trypsin units inhibited
WAI	Water absorption index

C O N T E N T S

PUBLICATIONS

1 - A modified procedure for low temperature infrared radiation of soybeans. Part 1) improvement of nutritive quality of full-fat flour. Lebensm.-Wiss.u.-Technol. 14, 242 (1981)

2 - A modified procedure for low temperature infrared radiation of soybeans. Part 2) inactivation of lipoxygenase and keeping quality of full-fat flour. Lebensm.-Wiss.u.-Technol. 15, 139 (1982)

3 - Infrared processing of soybeans, industrial design. Qual Plant Plant Foods Hum Nutr 33, 139 (1983)

4 - Infrared processing of maize germ. Lebensm.-Wiss.u.-Technol. 17, 237 (1984)

5 - A modified procedure for low temperature infrared radiation of soybeans. Part 3) pretreatment of whole beans in relation to oil quality and yield. Lebensm.-Wiss.u.-Technol. 17, 39 (1984)

6 - Involvement of phospholipase D in the hydrolysis of phospholipids in soybeans. Lebensm.-Wiss.u.-Technol. (accepted for publication)

SUMMARY and CONCLUSIONS

SUMMARY in DUTCH

AUTHOR'S CURRICULUM VITAE

INTRODUCTION

The rapid rise in energy costs in the seventies prompted much effort in the food industry to develop new processes and make modifications in the existing ones aimed at bringing down the energy consumption.

Industrial heat processing of oilseeds and cereals is widely carried out for improving the nutritive value by inactivating antinutritive factors, destroying enzymes and prolonging storage stability as well as enhancing the organoleptic quality (scheme 1).

The conventional infrared heat processing (micronization) of oilseeds and cereals involves heating the material rapidly to high temperatures (above 170-180^{o}C), and subsequently cooling. This method suffers from the disadvantage of using high temperatures requiring high energy input, with the risk of damaging heat-labile nutrients.

At the Food Technology Department of this University, we developed a modified infrared process involving an additional holding step in order to use the heat accumulated in the material. This enabled us to employ lower temperatures (110-130^{o}C) than in the conventional infrared process. An inherent advantage of this modification is the substantial reduction in energy consumption.

Soybeans and maize germ were treated with the modified process aimed at improving the nutritive quality and storage stability of full-fat soy flour and maize germ, as well as improving the quality of soy oil extracted from preheated beans. The potential applications of this process are indicated in schemes 2 and 3 relating to soy and maize processing, respectively.

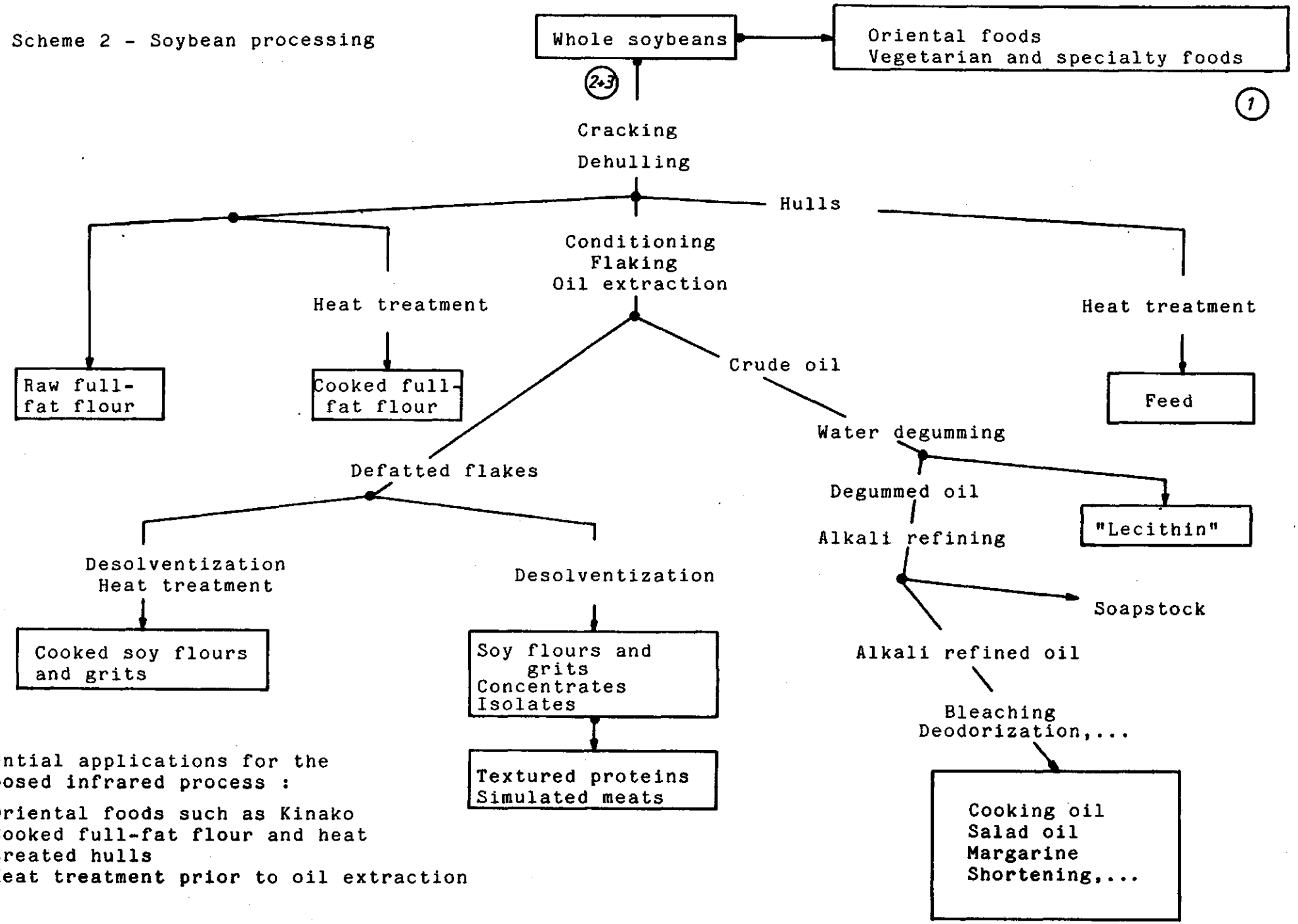
SCHEME 1 - Major factors and indicators involved in the heat processing of soybeans and maize and employed in the present studies :

Urease	- Indicator of inactivation of trypsin inhibitors.
Trypsin inhibitors	- Inhibit trypsin activity ; cause poor growth in animals ; mode of action controversial.
Protein dispersibility index (PDI)	- Indicator of extent of heat treatment.
Nitrogen solubility index (NSI)	- Indicator of extent of heat treatment.
Available lysine	- Indicator of overheating, level reduced by excessive heat.
Lipoxygenases	- Involved in oxidation of unsaturated lipids having one or more cis, cis-1, 4-pentadiene groups, thus causing off-flavours.
Peroxide value (PV, pv)	- Measure of oxidative deterioration of lipids.
Lipases	- Involved in hydrolysis of lipids and formation of free fatty acids.
% FFA (free fatty acids)	- Indicator of lipase activity.
Phospholipases	- Catalyze hydrolysis of phospholipids.

Phospholipase D	- Causes formation of nonhydratable phospholipids, thus alkali refining of oil necessitated.
Starch gelatinization	- An indicator of extent of heat processing in maize.
Water absorption index (WAI)	- Measure of degree of starch gelatinization, thus indicator of heat processing.

The results of the investigations have been published in six papers. Paper one and two describe the production of full-fat soy flour with improved nutritive quality and prolonged shelf life. Paper three presents the proposed continuous industrial design for the modified infrared process. The treatment of maize germ in connection with lipoxygenase, lipase, storage stability and nutritive quality is the subject of paper four. Paper five discusses how the modified method may be used for pretreating whole soybeans prior to oil extraction which results in improved oil quality and, most significantly, the elimination of the necessity for alkali refining. And finally, paper six is concerned with an investigation into the role of phospholipase D in the hydrolysis of phospholipids in soybeans bringing about the formation of nonhydratable phospholipids.

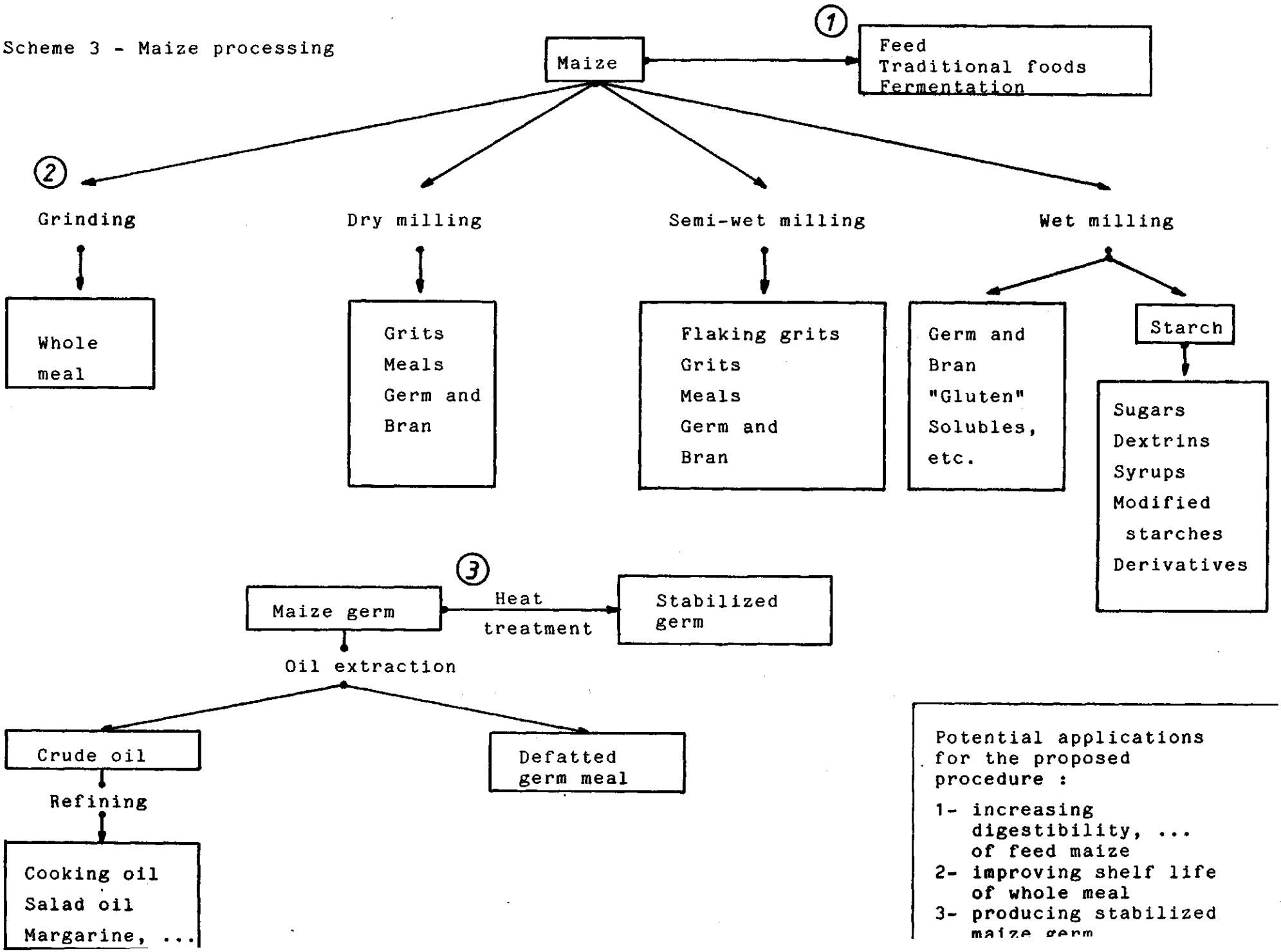
Scheme 2 - Soybean processing



Potential applications for the proposed infrared process :

1- Oriental foods such as Kinako
2- Cooked full-fat flour and heat treated hulls
3- Heat treatment prior to oil extraction

Scheme 3 - Maize processing



Potential applications for the proposed procedure :

1- increasing digestibility, ... of feed maize
2- improving shelf life of whole meal
3- producing stabilized maize germ

S O Y B E A N S

Although the soybean (Glycine max) originated in the Orient in the ancient times, it only became a crop of commercial and industrial importance after being introduced into the U.S. where it has been improved genetically and is grown extensively. The U.S. accounts for an average of 75% of the world's total production.

Composition and biologically active substances

Soybeans are legume seeds of different size and shape, and vary from yellow and green to brown and black in colour. The important commercial varieties are spherical and yellow. The seed consists of approximately 8% hull, 90% cotyledon and 2% hypocotyl and plumule (1). The structural features of the soybean seed are shown in fig. 1 and its proximate composition in table 1. It can be seen that the soybean is a protein and oil source. The remainder of the seed consists mainly of carbohydrates including various polysaccharides and oligosaccharides (stachyose, raffinose and sucrose). The composition of soybeans in terms of oil, protein and the pattern of amino acids suggests high nutritive value. However, it is well known that raw beans have a low protein efficiency ratio and do not support normal growth of rats. Biologically active substances, some with antinutritional activity, have been discovered in soybeans and extensively investigated ; the main ones are mentioned below (2, 3).

Trypsin inhibitors : these substances cause retarded growth in animals and at first the reason was thought to be simply poor protein digestion as a result of trypsin inhibition. Later, another mode of action was put forward. Raw soybeans and trypsin inhibitors cause hypertrophy of pancreas and increased secretion. Since pancreatic secretions are rich in S-containing amino acids, the increased secretory

Figure 1 - Structural features of the soybean seed

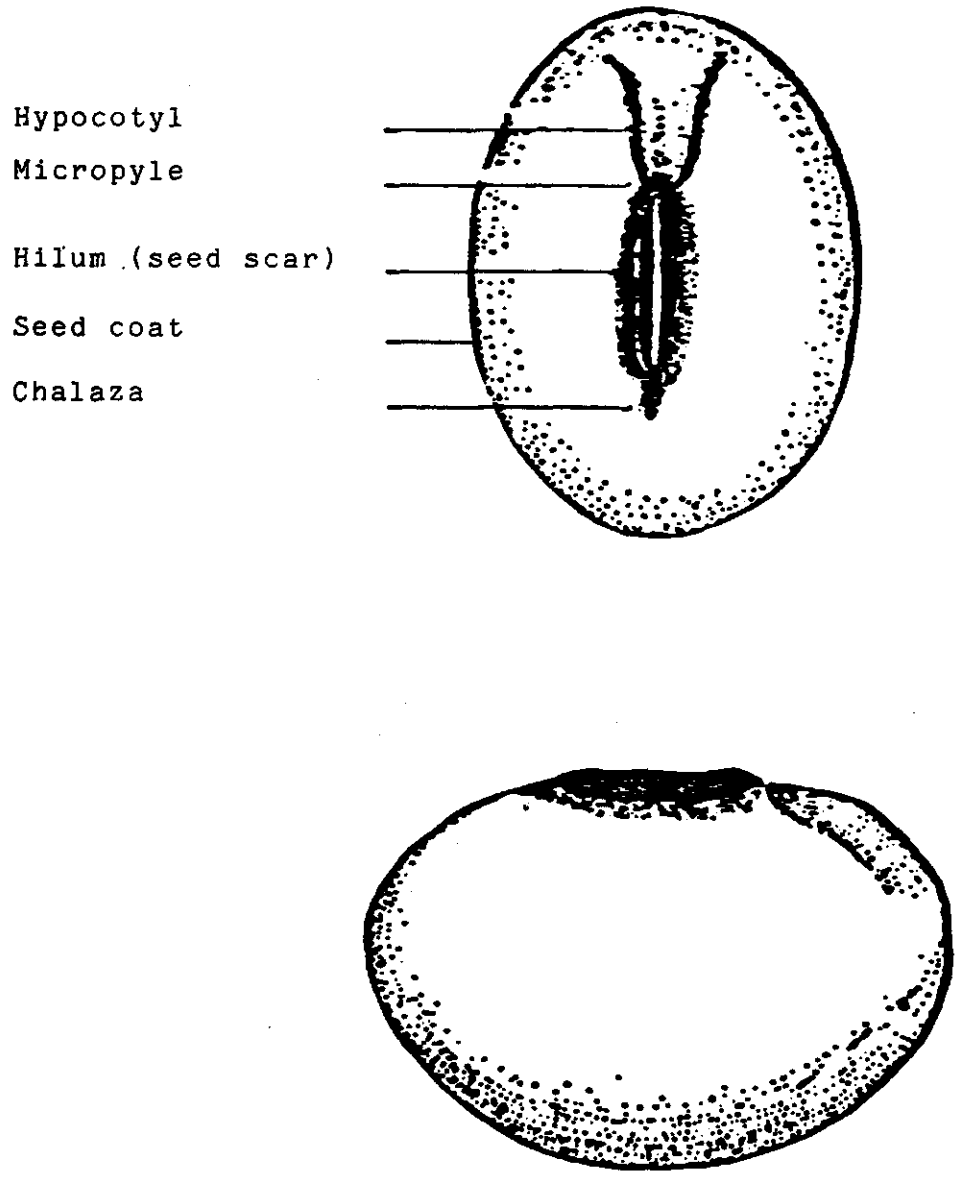


Table (1) - Composition of soybeans and its fractions

(%, dry basis)

	Protein (1)	Fat	Carbohydrates	Ash
Whole beans	40	21	34[2]	4.9
Cotyledon	43	23	29	5.0
Hull	8.8	1	86	4.3
Hypocotyl	41	11	43	4.4

(1) %N x 6.25

(2) includes (%) : cellulose, 4.0 ; hemicellulose, 15.0 ; stachyose, 3.8 ; raffinose, 1.1 ; sucrose, 5.0 ; other sugars, 5.1.

From reference 1

activity leads to a depletion of these amino acids and a drain on the body tissue, and as a result, retarded growth.

Hemagglutinins (lectins) : these substances have the property of binding carbohydrates and by interacting with the glycoproteins located on the surface of red blood cells, they cause agglutination of the cells in vitro.

Goitrogens : the enlargement of the thyroid gland in rats, chicks and possibly human infants as a result of consuming raw (or inadequately treated) soybeans has been attributed to goitrogens (reportedly oligopeptides) present in the beans.

Allergens : allergens cause such reactions as eczema and diarrhoea in sensitive persons.

Saponins : it appears that the soybean saponins are relatively innocuous to chicks and rats, and as a result, it has been suggested that they may be removed from the list of antinutritional factors in soybeans.

Antivitamin factors : antivitamin activity (vitamins D, E and B_{12}) has been reported in unheated soybeans.

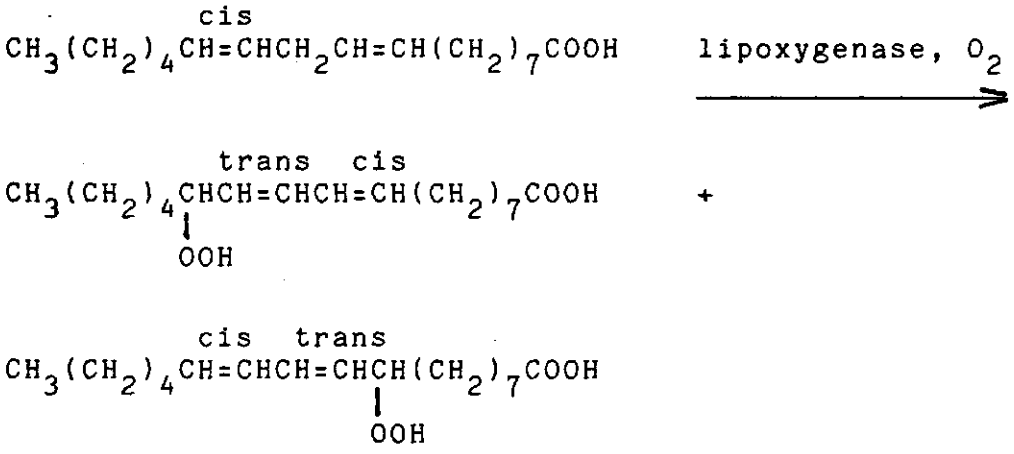
In addition to the above, several other important biologically active factors are present.

Flatulence factors : raffinose (trisaccharide) and stachyose (tetrasaccharide) present in soybeans can not be digested in the human gastrointestinal tract, but are rather fermented by microorganisms in the intestine resulting in flatulence.

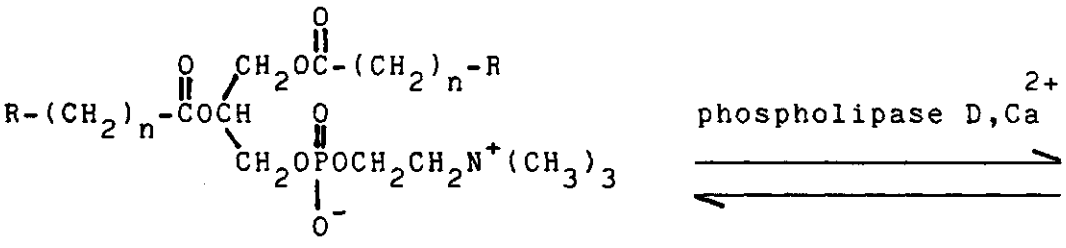
Amylases : α - and β -amylases are highly active in soybeans and if not inactivated, may adversely affect the quality of products when soy flour is used in bakery goods.

Lipases : the hydrolysis of oil leading to the formation of free fatty acids is caused by lipases.

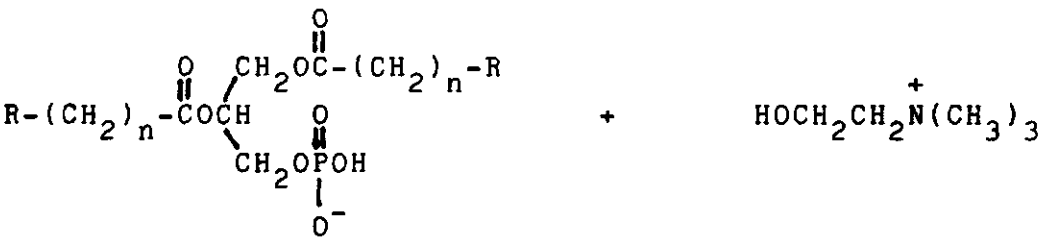
Lipoxygenases : this group of enzymes catalyze the oxidation of lipids containing a cis, cis-1, 4-pentadiene system. Hydroperoxides are formed which break down and give rise to secondary volatile products (ketones, alcohols, ...) responsible for off-flavours. Examples of substrates are linoleic and linolenic acids. With linoleic acid, the reaction would go as follows :



Phospholipases : these enzymes hydrolyze phospholipids ; in particular, phospholipase D causes the formation of nonhydratable phospholipids thus necessitating alkali refining of the crude oil which leads to oil losses and soapstock disposal problems.



Phosphatidylcholine



Phosphatidic acid choline

Urease : this enzyme breaks down urea into ammonia and carbon dioxide. This is undesirable when urea is used in animal feeds containing soy products with active urease.

Heat processing of soybeans

In order to inactivate the antinutritional factors and improve the nutritive value, soybeans must be heat processed. Heat processing also inactivates enzymes with possible deleterious effects. The inactivation of lipoxygenase and lipase prolongs the shelf life of fat containing soy products. Moreover, the bitter and beany flavour of beans is converted into a more acceptable flavour.

The main forms of heat treatment applied to soybeans are steaming (4) which is the conventional method used in industry, immersion cooking (5), dry heating or roasting (6), extrusion (7), dielectric heating (8), microwave processing (9) and infrared radiation (10).

Methods for controlling the extent of heat treatment

Urease activity : the enzyme urease found naturally in soybeans has the ability to release ammonia from urea thus increasing the pH of medium. The extent of inactivation of urease is normally taken as an indicator of the inactivation of trypsin inhibitors (and other antinutritional factors). A simple test (11) based on the pH increase when a suspension of soya and urea is incubated for 30 minutes at 37^{o}C, is widely used as a control measure. Values between 0.05 and 0.15 pH units indicate adequate heat processing (12).

Protein dispersibility index (PDI) and nitrogen solubility index (NSI) : the dispersibility and solubility of proteins decrease with heat treatment.

PDI (13) and NSI (14) are used very extensively as indicators of heat processing : low values (below 20) indicate adequate heat treatment (15). PDI and NSI are important industrial specifications for soy protein products.

Trypsin inhibitor activity : although not frequently used in the soy industry, the measurement of trypsin inhibition is regarded to be the ultimate chemical test to be performed to ascertain the inactivation of antinutritional factors. The method developed by Kakade et al (16) is preferred by many investigators. This method is based on the spectrophotometric determination of the amount of p-nitroaniline released as a result of the hydrolysis of a synthetic substrate, benzoyl-DL-arginine-p-nitroanilide (BAPA) by trypsin. The trypsin inhibitor activity of the (soy) sample is expressed as the number of units of trypsin inhibited under the conditions of the test.

Available lysine : the measurement of available lysine is used widely as an indicator of overheating during processing. Excessive heat causes a drop in the level of available lysine. Two methods are mostly used : Carpenter (17) and Kakade and Liener (18). These methods are based on the spectrophotometric measurement of the product of the reaction between lysine and an amino acid coupling reagent ; in Carpenter's method 1-fluoro-2, 4-dinitrobenzene (FDNB) is used and the dinitrophenylated lysine produced measured, whereas Kakade and Liener preferred to use the less dangerous 2, 4, 6-trinitrobenzenesulphonic acid (TNBS) and measure the trinitrophenylated lysine formed. In these methods, it is only the available lysine with its ε-amino group free that is involved in the reaction, while any (unavailable) lysine present with its ε-amino group already reacted can not take part in the reaction.

Utilization of whole soybeans and full-fat flour

Whole soybeans are an important food item in some regions such as East Asia. The soybean is the basis for a variety of oriental foods including tofu and kinako (Japan), soy milk (China) and tempeh (Indonesia). A description of these products and the processes involved in making them is not within the scope of this thesis but can be found in several publications (19, 20). In contrast to the above products, which have gained little popularity in the western world, full-fat soy flour has gained some importance in the west as a component of some foods. Being high in protein (40%) and oil (20%), full-fat soy flour has received much attention as an economical source of protein and energy. It is used for : enriching bread (21) and developing low cost, high quality food products for developing countries and famine relief programmes (22). Various other food uses are discussed by Wang et al (23). The functional properties of the flour are used advantageously in baked goods, confectionery, meat processing, soups, etc.. It may replace the more expensive ingredients such as eggs, milk, meat and related products (24, 25). Before using in food products, the antinutritional factors, enzymes and other deleterious factors are inactivated by heat processing using one of the forms discussed earlier.
Raw full-fat flour has a special use (bleaching) in breadmaking as a source of lipoxygenase the action of which on polyunsaturated fatty acids in the oil produces peroxides which in turn oxidize the carotenoid pigments into colourless products. This results in a whiter crumb in bread. The keeping quality and crumb softness are reportedly improved (26).

Soy protein products

The development of a number of edible products derived from

soybeans has increased the utilization of soybeans in the western world. Defatted flours and grits, protein concentrates, protein isolates and textured soy proteins are among such edible products. These are used in a variety of foods (27) as a) a source of protein in making low cost, high quality food products for relief and food aid programmes (28) and in feeding infants, children and other age groups under nutritional stress (29) and/or b) for their functional properties in improving the characteristics of a variety of food products such as baked goods (30), snacks (31) and meat products (32).

Defatted flours and grits : after oil extraction, the defatted flakes are desolventized by using one of several methods available. The method employed and the degree of heat treatment applied to remove the residual solvent have a profound effect on the properties of the flakes. If a well-cooked product with improved nutritive quality for consumption is required, then a severe enough heat treatment such as the desolventizer toaster process (33) is employed. If functional properties are sought, then the degree of heat treatment is mild resulting in products with a high PDI and good functional properties. Such products, however, contain antinutritional factors and need to be heat processed at some point before consumption. For the production of high PDI products, a process such as flash desolventization is most suitable (33). Flours and grits are produced by grinding the desolventized flakes. Material finer in size than 0.07-0.15 mm is classified as flour, while grits have a coarser granulation up to 0.85-1.7 mm. Table 2 shows the composition of defatted flour and grits.

Protein concentrates : for the production of concentrates (34), the soluble sugars, ash and other minor constituents of defatted flour or flakes are removed resulting in products with a minimum protein content of 70% (dry basis) by the trade standards. For removing the above components, the defatted meal is extracted with either aqueous alcohol

Table (2) - Composition of typical soybean protein products (%, as is basis)

	flours	Concentrates	Isolates
Protein[1]	50.0	70.0	96.0
Fat	1.0	1.0	0.1
Crude fibre	3.5	4.5	0.1
Ash	6.0	5.0	3.5
Carbohydrates	39.5	19.5	0.3

(1) % N x 6.25

From reference 27

or dilute acid or water (after heat treatment to insolubilize the protein). Acid extraction gives the highest PDI, while alcohol and heat treatment denature and insolubilize the protein resulting in low PDI's. The typical composition of soy protein concentrate is shown in table 2.

Protein isolates : for producing isolates (34), defatted flakes or meal are extracted with alkali. The major proteins are precipitated by adjusting the pH to 4.5. The resulting protein curd is neutralized and dried. The composition of a typical commercial soy protein isolate is presented in table 2.

Textured proteins and simulated meats : texture resembling that of meat can be imparted to soy protein. This may be done by several methods. One method is based on extrusion cooking (35) in which soy protein is first adjusted to proper moisture content and then extruded under appropriate temperature and pressure conditions. Flavour, colour and other additives are used to simulate different meats.

In another method referred to as soy fibre spinning (35), protein isolate is slurried in water and alkali or salt added. The resulting mass is forced through spinnerets into an acid, salt or hot water bath which immediately coagulates the protein and forms fibres 20-70 µm in diameter. The fibres are bound together with binders and a structure and texture similar to meat when rehydrated is obtained.

Functional Properties

In addition to being used as protein sources, the defatted soy flours, concentrates and isolates are used extensively in the food industry for their functional properties which are attributable in most cases to the protein fraction. The major functional properties of soy protein products include

(36) : emulsification and emulsion stabilization (meat products, bakery products, ...), fat absorption and reduction of cooking losses (various meat products), water absorption and retention (baked goods, ...), increasing viscosity and gelation (soups, ground meats, ...).

Conventional crude oil extraction

Most of the soybeans processed commercially are used for oil extraction with the residual meal being a valuable byproduct. Soy oil is primarily used for food purposes whereby such products as cooking oil, salad oil, shortening, margarine and mayonnaise are made (37). Other uses include pharmaceuticals and various other industrial uses (38). Table 3 shows the composition of soy oil.

The conventional extraction of crude soy oil involves the following steps (39) :

cleaning of the beans,

cracking through corrugated rolls into 6-8 pieces and removing the hulls by aspiration,

conditioning to 10-11% moisture at 60-80^{o}C to impart plasticity which is essential to good flaking,

flaking and solvent extraction using hexane.

Continuous extractors of the screw conveyor, basket or solvent rain type are normally used. The residual solvent in the flakes is removed by one of the methods referred to before.

Conventional refining of crude soy oil

The following steps are involved in the refining of crude oil.

Table (3) - Soy oil constituents[1] and fatty acid composition[2]

Triglycerides	95-97
Phospholipids	1.5-2.5
Unsaponifiables	1.6
Sterols	0.33
Tocopherols	0.15-0.21
Hydrocarbons	0.014
Free fatty acids	0.3-0.7
Iron (ppm)	1-3
Copper (ppm)	0.03-0.05
Saturated fatty acids	15.0
Palmitic	10.7
Stearic	3.9
Unsaturated fatty acids	80.7
Oleic	22.8
Linoleic	50.8
Linolenic	6.8

(1) in crude oil (%)

(2) refined oil (%, average)

From reference 38, chapter 2

Degumming (40) whereby the hydratable phospholipids are removed with either 1) addition of 1% water at 70°C, or 2) addition of 1% acetic anhydride with 1% water at 60°C. The phospholipids thus removed are referred to as soy lecithin and have numerous uses in food as well as other industrial processes (40, 41).

Alkali refining (42) whereby sodium hydroxide or sodium bicarbonate is added to the degummed oil at 60-70°C and the resulting "soapstock" separated by centrifugation, followed by washing with water or citric acid. In this step, fatty acids and the remaining phospholipids are removed from the oil.

Bleaching (43) with bleaching earths, activated clays, or activated carbon is done at 110°C to remove pigments, oxidation products and traces of phospholipids thus improving the colour and flavour.

Deodorization (44) with steam at temperatures of up to 250°C removes free fatty acids and various volatile compounds.

Finally, the use of metal inactivators such as phosphoric acid in the process, and antioxidants such as BHA and BHT in the finished oil, further improves and stabilizes the oil.

Heat treatment of soybeans prior to oil extraction

The conventional oil extraction suffers from major disadvantages. After cracking the beans, favourable conditions exist for enzymic activity. Lipoxygenase and lipase activity will result in oxidation products and free fatty acids, respectively, while phospholipase D brings about the hydrolysis of phospholipids into their nonhydratable forms (45, 46, 47). This necessitates alkali

treatment of the oil which results in high losses of neutral oil, and inferior quality of the fatty acids obtained, as well as soapstock disposal problems (48).

In order to overcome the above problems, heat treatment of beans prior to oil extraction has been proposed, and in some cases, industrially applied. The heat treatment inactivates the enzymes, thus minimizing their activity prior to and during oil extraction. This in turn makes it possible to circumvent the alkali treatment thus avoiding its problems. Steam heating of whole soybeans (49), steaming of soy flakes (45) and its industrial application (46, 47, 50) as well as infrared heat treatment of whole beans (51) have been described.

M A I Z E

Maize (U.S. : corn ; botanically : Zea mays Linnaeus), a plant belonging to the grass family, has been cultivated for more than 5.000 years. It was introduced into the U.S. from Mexico where it is thought to have originated. Much genetic improvement in the yield and other characteristics of maize has eversince been carried out and it is now the No. 1 commodity in U.S. agriculture which accounts for more than half of the world's total maize production. Although producing far less, Argentina and France are also involved in the export of maize. In Europe, West Germany and The Netherlands are among the main importers.

Maize is used worldwide as an ingredient and raw material in food, feed and various industrial processes. A large number of snacks, convenience foods, breakfast cereals, alcoholic beverages, etc. are entirely or partly based on maize. Maize oil, maize starch and derived sugars, syrups and dextrins find numerous applications in food products and various industries. In addition, the byproducts of maize milling are extensively used in animal feed formulations.

Composition (52)

The maize kernel consists of four major parts : endosperm, germ, bran and tip cap. The structural features of maize kernel are illustrated in fig. 2 and its composition in table 4.

Endosperm : maize endosperm is composed mainly of starch and consists of two regions : hard or vitreous, and soft or floury endosperm. Different types of maize vary in the hard to soft ratio. Plate maize (flint type) grown in Argentina

Figure 2 - Structural features of dent maize kernel

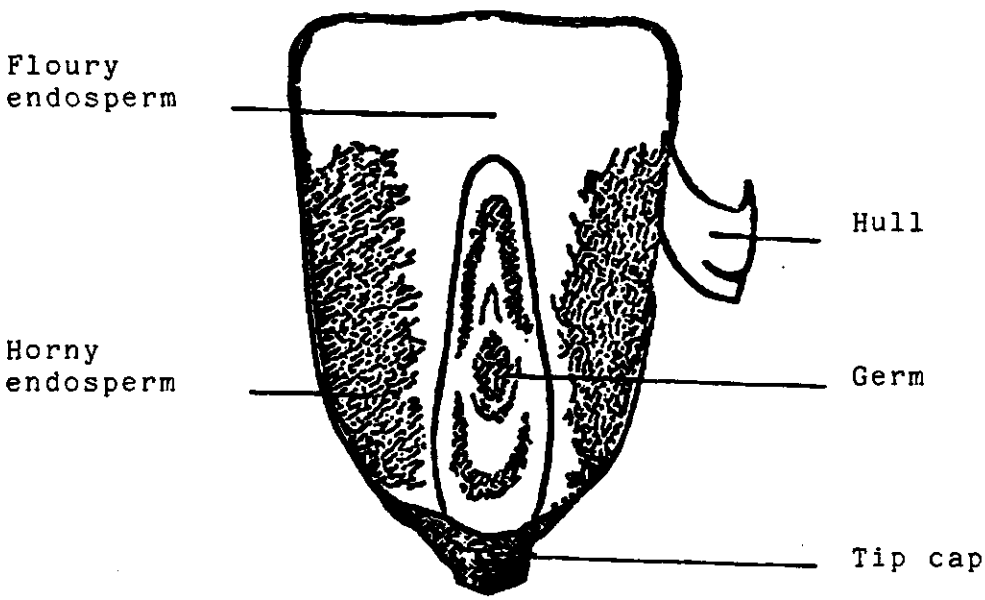


Table (4) - Average composition of whole dent maize and its main fractions (%, dry basis)

	Starch	Protein[1]	Lipid	Sugar	Ash
Whole kernel	71.5	10.3	4.8	2.0	1.4
Endosperm	86.4	9.4	0.8	0.6	0.3
Germ	8.2	18.8	34.5	10.8	10.1
Bran	7.3	3.7	1.0	0.3	0.8
Tip cap	5.3	9.1	3.8	1.6	1.6

(1) %N x 6.25

From reference 52

has a 6 : 1 ratio, U.S. yellow maize (dent type), 5 : 3, while certain floury types have almost no vitreous endosperm. The harder types are preferred in the semi-wet or dry milling of maize because of higher yields of prime products. Maize starch in the normal dent type contains about 73% amylopectin and 27% amylose, while a certain type developed and produced commercially (waxy maize) is almost devoid of amylose and contains 99-100% amylopectin. High amylose maize is also available (50-75% amylose content). The protein fraction in the endosperm of normal dent maize consists of 3.2% albumin (water soluble), 1.5% globulins (salt soluble), 47.2% zein and 35.1% glutelin (alkali soluble).

Germ : the maize germ comprising about 12% of the whole kernel contains most of the oil, sugars, minerals and vitamins of maize, as well as a high content of protein with much better quality than that of endosperm or whole kernel. Maize germ oil is a premium oil in the world market due to its nutritional and physical characteristics (53). The defatted or full-fat maize germ is used extensively as a valuable component of animal feed. The nutritional properties of maize germ in terms of oil, protein with PER of 2.5 (54), vitamins and minerals combined with price considerations, have directed much interest in recent years towards the utilization of maize germ in formulating and developing human food products. These uses include baked goods (55, 56) and meat products (57).

Bran (pericarp) and tip cap (the remaining point of attachment of the kernel to the cob) comprise relatively small fractions of the kernel (5 and 1%, respectively). These fractions are used traditionally in animal feed formulations, but given their high dietary fibre content (58), and the increasing awareness of the function of dietary fibre in alleviating human gastrointestinal problems (59), interest in the utilization of maize bran

in human food products has grown (60).

Maize milling

The objective in maize milling is to separate, as efficiently as possible, the different parts of the kernel referred to before. Whole maize meal, however, is produced without removing the germ and simply by grinding the whole kernel. This product is quite popular in some parts of the U.S., Latin America, Africa and elsewhere, but it might have storage problems, particularly under unfavourable conditions, due to its rather high fat content with lipase and lipoxygenase present. The three basic forms of maize milling are referred to as wet, semi-wet and dry milling.

Wet milling : this type of milling of maize is employed in order to produce starch. Other constituents of maize are considered only byproducts in this industry. The basic operations involved include (61) :

- cleaning of maize ;
- steeping (soaking) whereby maize is soaked in water containing SO_2 for periods of 24-48 hours to soften the kernel for grinding and aid in the removal of solubles ;
- coarse milling which brings about the separation of germ resulting in a pulpy material containing germs, hulls, starch and protein ;
- separation of germ which is lower in density by liquid cyclones ;
- further grinding by attrition or impact mills which leaves hulls large enough in size to be screened off ;
- separation of starch and protein from each other in high speed centrifuges and liquid cyclones making use of the higher specific gravity of starch compared to protein ; and,
- filtration and drying of starch in tunnel driers.

Starch is the main product of the wet milling of maize. It not only has uses in its natural form, but is also a raw material and starting point in the production of a very large number of products of immense importance to the food and various other industries (62, 63). In order to induce functional properties and make it useful for various industrial applications, structural changes in the starch granule or molecule are required. These changes can be brought about by one or more of the following : heat processing, action of enzymes, chemical derivatization, and so on.

Semi-wet and dry milling : the semi-wet milling of maize is used primarily for producing flaking grits, the raw material for corn flakes. The following steps are involved (64) :

- selection of suitable maize of vitreous type,
- cleaning and conditioning with added moisture and steam which facilitates separation of germ and bran,
- degerminating in an attrition cone mill,
- drying and cooling,
- classifying various fractions using sifters, aspirators, gravity tables and reduction rolls.

In addition to flaking grits, various other grits, meals, germ and bran are also obtained.

In the dry process (64), the tempering step is bypassed ; i-e, no moisture is added to the kernels. Most of the machinery and equipment are the same as those used in the semi-wet milling. However, instead of the attrition degerminator, an impact-type vertical machine is used. After thorough cleaning, the kernels are subjected to repeated impacts in the machine which cause the separation of germ and bran. The fine and coarse particles in the

machine are separated by aspiration and gravity and classified in the subsequent operations. Roll mills reduce further the size of endosperm pieces and particles, so that a variety of grits and meals are produced. This process is normally not employed for producing flaking grits.

The main products and byproducts of semi-wet and dry milling of maize include flaking grits for the production of corn flakes (65), brewers' grits used as a source of carbohydrates in beer production (66), snack grits used in the production of extruded snacks (67), maize meals of various granulations utilized in baked goods and snacks (68) and germ and bran which have been discussed before.

Maize oil

Maize oil, as referred to traditionally, denotes the oil obtained from the germ, since about 85% of the oil is contained in the germ. Maize germ oil became a premium oil in the world markets (53) after the role of essential fatty acids in human nutrition, and the correlation between the polyunsaturated fat content of the diet and reduced blood cholesterol levels were discovered. Maize germ oil is almost exclusively used as edible oil in such products as margarine (unhydrogenated fraction), cooking oil and salad oil. Table 5 shows the constituent fatty acids in maize germ oil.

The recovery of crude oil is done (69) by either solvent (hexane) extraction, or mechanical expelling (screw pressing) or a combination of both. The refining of crude oil (69) normally involves the same basic operations as those for soy oil including :

Table (5) - The constituent fatty acids in refined maize oil (%)

Palmitic	11.1
Stearic	2.0
Arachidic	0.2
Oleic	24.1
Linoleic	61.9
Linolenic	0.7

From reference 69

Degumming with 1-3% water, although this step is not always done ;

Alkali refining with sodium carbonate or sodium hydroxide ;

Bleaching with activated clays, earths, etc. ;

Deodorization by vacuum-steam processes.

Antioxidants such as BHA, BHT and propyl gallate are commercially used to extend the shelf life of finished oil.

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A Modified Procedure for Low Temperature Infrared Radiation of Soybeans

Part I: Improvement of Nutritive Quality of Full-Fat Flour

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A new infrared (IR) radiation procedure at low temperatures is described for the heat processing of soybeans. The procedure involves exposure of whole soybeans to IR radiation for approximately one minute. This results in a rapid rise of temperature to around 124°C. The beans are then held for 15 minutes at this temperature. Full-fat soy flour produced from these beans shows levels of urease and trypsin inhibitor activity which are as low as those of fully toasted flours and meets the accepted criteria for food and feed. At the same time, available lysine content is maintained. Other important indicators, nitrogen solubility and protein dispersibility indices are also reported.

Introduction

In many parts of the world, the soybean is an important component of human food and animal feed. It is used as whole beans, grits, flours, concentrates, isolates, etc.

Full-fat soy flour has received much attention as an economical and widely available source of protein and energy and is a good proposition for meeting the increasing requirement in different parts of the world (1). It contains 40% of high quality protein and 20% of valuable oil, high in essential fatty acids.

Full-fat soy flour can be used for enriching bread. According to one procedure, quantities of up to 24% (based on 100 kg of flour) can be incorporated (2). The flour constitutes the basis for high quality – low cost food formulations for developing countries (3), for dietary supplements for pre-school and school children, as well as famine-relief programmes. Other food uses in various countries have been published recently (4).

In addition to its use for enrichment purposes, full-fat soy flour has gained world-wide application in food processing industries for its desirable functional properties including bakery and confectionery, meat processing, baby foods, dry mixes, beverages, soups, sauces and a variety of health foods. In such products, full-fat flour can partially replace the more expensive and scarce ingredients such as eggs, milk, meat and related products (5, 6).

Heat treatment of soybeans is necessary for

a) destruction of trypsin inhibitors and other antinutritive factors in raw beans;

b) inactivation of the enzyme lipoxygenase in order to increase storage life (oxidative stability);

c) removal of the raw, bitter and beany flavour of raw beans.

Excessive heat, however, damages heat-sensitive amino acids and vitamins such as (available) lysine, cystine, methionine and thiamine. Oxidative stability is impaired due to the destruction of natural antioxidants present in soybeans. Furthermore, such a treatment results in poor colour and flavour. In addition to conventional processes such as steaming (7), other methods for heat treatment of soybeans have been applied including immersion cooking (8), dry heating or roasting (9), extrusion (10), dielectric heating (11), and microwave processing (12).

Infrared radiation

Following a development in animal feed preparations in the early seventies, the term "micronization" has often been used to refer to a continuous process of heat treatment of cereals, pulses, oilseeds, etc. which is based on IR radiation (13). The process involves exposing the material to IR radiation for a short period of time which results in a rapid rise of temperature and an increase of water vapour pressure in the product. However, in this paper we will use the term IR radiation or treatment.

Although some studies concerning the effect of this process on soybeans have been carried out (14, 15, 16), none of these have dealt with the production of full-fat flour and the investigation of its nutritive value and its oxidative stability. Moreover, the studies have employed short-time processes at high temperatures with immediate cooling of the beans afterwards.

A study on sorghum (17) showed a loss of up to 23% lysine due to the high temperatures employed in the process. Such high temperatures cause other undesirable changes like browning as well as poor flavour. Finally, a point of major importance overlooked in other studies was the large consumption of energy for heating the beans to such high temperatures.

VAN ZUILICHEM *et al.* (18, 19) redesigned a gas-heated micronizer plant to a HTST process for dehulling and decontaminating cocoa beans. They reported a modified procedure in order to use the residual heat of the hot material leaving the conveyor belt of the IR plant. After being subjected to IR radiation for a short period, the product was passed and held for a predetermined period in a well-insulated container. The residual heat would equilize by conductivity and diffusion and further act to achieve the processing objectives. This procedure offers a possibility for reducing energy

requirements. In order to overcome the problems associated with the application of the "micronization process" to soybeans, we adopted and used the modified procedure which enabled us to employ lower temperatures for producing full-fat soy flour. In this way, we were able to reduce the gas consumption for processing the beans by almost one half.
The present paper gives information on the heat treatment criteria, urease and trypsin inhibitor activity, nitrogen solubility and protein dispersibility indices as well as available lysine of the samples. The lipoxygenase activity and storage (oxidative) stability of the products over a one year period is currently under investigation and will be the subject of our next paper.

Experimental

Material
Soybeans of American Golden Yellow variety, Nr. 2, harvested 1979, were obtained from Cargill BV, Amsterdam.

Methods
– Preparation of full-fat soy flours: the ceramic burner plates above the belt of a pilot infrared machine (modified micronizer) were heated by a gas-air mixture producing infrared radiation **(Fig. 1)**. In this experiment, cleaned, whole soybeans (8.5% moisture) were spread in layers one bean thick and subjected to radiation on the running and vibrating conveyor belt of the plant at various residence time-temperature combinations. They were then transferred and held in thermos bottles with minimum loss of heat **(Tab. 1)**. Raw soybeans as well as treated samples, which were allowed to cool immediately, were used as control samples. Next, the beans were cracked through small rolls and dehulled by means of aspiration. Finally, each sample was ground to full-fat flour and kept in glass bottles at room temperature before being tested.
– Urease activity of the hexane defatted samples was determined according to AOCS Official Method Ba 9–58.
– Trypsin inhibitor activity of the hexane defatted samples was determined according to the method of KAKADE *et al.* (20), with minor modifications. Samples were extracted for 3 hours with 0.01 N NaOH. The absorbance of colour was measured at 410 nm using a Perkin-Elmer-Hitachi Model 139 spectrophotometer. Each increase in absorbance of 0.01 is arbitrarily defined as one trypsin unit. The trypsin inhibitor activity is expressed in terms of trypsin units inhibited (TUI) per mg sample.
– Nitrogen solubility index of the full-fat samples was measured by the AOCS Official Method Ba 11–65, revised 1969, corrected 1979:

$$\% \text{ NSI} = \frac{\% \text{ water soluble N} \times 100}{\% \text{ Total N}}$$

– Protein dispersibility index of the full-fat samples was measured following the AOCS Official Method Ba 10–65, revised 1978, corrected 1979:

$$\% \text{ PDI} = \frac{\% \text{ Water dispersible protein} \times 100}{\% \text{ Total protein}}$$

– Available lysine of the hexane defatted samples was essentially measured by the TNBS method of Kakade and Liener as modified by HALL *et al.* (21). The hydrolysis time was 1 hour which has been found sufficient according to our experience.
– Temperature measurements (13). Filling and emptying a thermos flask with hot beans leaving the IR conveyor belt until equilibrium, as determined by a thermometer.

Tab. 1 IR radiation time, temperature and holding time of whole soybeans with initial moisture of 8.5%

Sample	Residence time[1] (sec)	Temperature[2] (°C)	Holding time[3] (min)
Raw	—	—	—
1	80	133 ± 1	0
2	80	133 ± 1	15
3	80	133 ± 1	25
4	60	124 ± 1	0
5	60	124 ± 1	15
6	60	124 ± 1	25

[1]Residence time in sec of whole beans on the vibratory belt of IR plant (exposure to IR radiation)
[2]Final temp of beans immediately off IR belt measured by thermos flask technique
[3]Holding time in minutes of samples immediately off IR belt, transferred and held in thermos bottles.

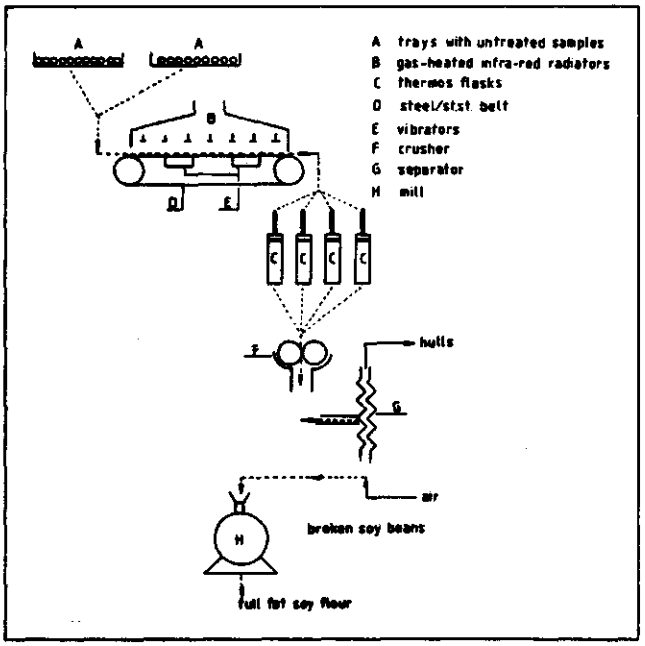

Fig. 1 Production of Full-fat Soy Flour Using IR Radiation

Results and Discussion

At the start of the investigation, a series of trials was made without operating the vibratory mechanism of the IR plant. A tendency for localized burning of the beans was noticed. All subsequent trials were made with vibrators in operation allowing the beans to be subjected to a uniform heating on the running conveyor belt. Six combinations of residence time (exposure to IR radiation) and the resulting temperature as well as holding time (in thermos bottles) were selected for this study **(Tab. 1)**.
Samples, 1, 2 and 3 were exposed to IR radiation for 80 sec giving a final thermos bottle temp of 133 ± 1 °C, and samples 4, 5 and 6 for 60 sec with a final temp of 124 ± 1 °C. Samples 1 and 4 were allowed to cool while other samples were immediately transferred to thermos bottles and kept for 15 and 25 min in respective cases. **Tab. 2** shows the moisture, protein and oil content of the corresponding full-fat flours produced from whole beans and **Tab. 3** presents the main heat treatment criteria.
It is generally considered that residual urease activity values of 0.05–0.15 pH increase indicate adequate heat treatment for destruction of antinutritive factors (22). The raw flour in the study showed a high activity of 2.2 and samples 1 and 4,

Tab. 2 Moisture, protein and oil content in percentage of full-fat flours produced from IR processed and dehulled soybeans

Sample	Moisture	Protein[1,2]	Oil[1]
Raw	8.5	41.3	20.7
1	7.8	40.7	21.0
2	7.3	41.0	21.3
3	7.0	41.8	20.9
4	8.0	40.8	20.7
5	7.6	41.4	21.0
6	7.7	41.1	21.4

[1]Dry-matter basis, [2] % N × 6.25

Tab. 3 Indicators of the adequacy of heat treatment of full-fat soy flours.

Sample	Urease (a)	TUI (b)	NSI (c)	PDI (d)	Available lysine (e)
Raw	2.20	72.0	83	91	2.3
1	0.40	18.8	25	30	2.2
2	0.05	6.8	10	18	2.1
3	0.00	5.9	10	15	1.9
4	0.50	26.1	31	42	2.4
5	0.05	8.2	13	20	2.3
6	0.05	7.1	10	18	2.2

a) Units of pH rise; b) Trypsin units inhibited per mg sample; c) Nitrogen solubility index (%); d) Protein dispersibility index (%); e) % of full-fat soy flour (dry matter)

which had been allowed to cool immediately after IR treatment gave corresponding figures of 0.4 and 0.5 indicating inadequate heat treatment. As for TI activity, adequately-heated flours show TUI values of below 10 with corresponding values for raw flours of 70–80 or higher (20). In our case, the raw flour as well as samples 1 and 4 showed high trypsin inhibitor figures in accordance with their high urease activity. The TUI figures for samples which had received an additional treatment in thermos bottles ranged from 5.9 to 8.2 signifying minimal activity.

Heat treatment causes denaturation of proteins with a subsequent decrease in their solubility and dispersibility in water. An index of 90% or higher for raw flours, and 20% or lower for fully heat-treated samples has been reported (23). PDI values are generally higher than NSI which is an inherent characteristic of the methods of determination. However, each method gives consistent and comparative results. The additional heat in the case of samples 2, 3, 5 and 6 reduced NSI and PDI indiees markedly to 20 or below while samples cooled immediately after IR treatment (1 and 4) showed higher values, 25 and 30, 31 and 42 respectively. These rather high figures suggest inadequacy of heat treatment (23).

Available lysine is generally regarded as an indicator of overheating since it is reduced by excessive heat. In a study with sorghum, a destruction of 15–23% lysine due to the high temperature was reported (17). Another investigation with micronized soybeans, however, showed 1.76% available lysine (on dry matter basis) in raw soybeans compared with 2.18% for micronized sample (14). We found about 15% loss in available lysine in the case of sample 3 which had received an additional 25 min heat treatment at 133°C in thermos bottles. This clearly indicated overheating. The values in other samples did not show any significant change and ranged from 2.1 to 2.4% compared with 2.3% for raw soybeans.

In conclusion, the above results suggest that samples 1 and 4 are underheated as shown by their high levels of urease and trypsin inhibitor activity while sample 3 is overheated because of the drop in its available lysine. The remaining samples, i.e., 2, 5 and 6 have received adequate heat treatment for the destruction of antinutritive factors without lowering available lysine. However, sample 5 is economically superior because it is processed at a lower temp (than no.2) and for a shorter time (than no.6). This flour also possesses an acceptable light-yellow colour. Thus among the various combinations investigated in this study, the procedure consisting of a residence time of 60 seconds (exposure to IR radiation) giving a final temperature of 124 ± 1°C followed by 15 minutes holding in thermos bottles resulted in a better product. The results of this study indicate that the modified IR treatment procedure has a potential as an economical heat treatment method of soybeans. It offers a possibility for reducing energy requirements and production costs, since comparatively lower temperatures are employed than in the conventional process. The effect on fuel gas consumption is remarkable since we were able to operate with only one half the gas quantity compared with the conventional procedure on IR machinery.

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A Modified Procedure for Low Temperature Infrared Radiation of Soybeans II. Inactivation of Lipoxygenase and Keeping Quality of Full-Fat Flour

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The processing conditions of IR treatment of soybeans for improving the shelf life of full-fat flour were investigated. Optimum conditions were found to be: exposure of beans to IR radiation for 1 min, followed by holding in an isothermal container for 15 min. Full-fat flour produced from these beans showed no lipoxygenase activity. Also, peroxides formation was negligible after one year of storage. A rapid polarographic procedure for lipoxygenase assay in flour suspension was worked out. In sensory evaluation, no significant difference in freshness could be detected between the well-treated flours stored for one year and a fresh commercial flour. However, raw and overheated samples developed rancid odours and high peroxide values upon storage. This indicates that if the optimum processing conditions are exceeded or not attained, quality deterioration takes place.

Introduction

In our previous paper (1), a modified procedure of low temperature infrared (IR) radiation was presented as an effective method for the production of adequately-processed full-fat soy flour. The procedure involved exposure of whole beans to a short period of IR radiation, immediately followed by a predetermined holding period in an isothermal container. The beans were subsequently dehulled and ground to full-fat flour. This procedure inactivated trypsin inhibitors without detectable damage to available lysine. The application will also result in up to 40% saving in the energy requirements for adequate processing.

The shelf life of full-fat soy flour depends mainly on its oxidative stability which is in turn influenced by the activity of lipoxygenase (linoleate: oxygen oxidoreductase, E C 1.13.11.12). It is well established that the enzyme catalyzes the oxidation of polyunsaturated fatty acids and their esters and glycerides containing one or more cis, cis-1,4-pentadiene groups such as linoleic, linolenic and arachidonic acids as well as their esters and glycerides. The principal primary products are conjugated and isomerized hydroperoxides which in turn readily break down into smaller volatile compounds responsible for the off-flavour of oxidized fat.

Lipoxygenase-active soy flour has an advantageous use in breadmaking where it is used for flour bleaching (2). However, when the full-fat soy flour is intended for storage, the high activity of the enzyme combined with the high concentration of natural substrates (mainly linoleic acid) makes it imperative to inactivate the enzyme. The deleterious effects which are caused by the enzyme include (2):

1. formation of volatile off-flavours,
2. destruction of the essential fatty acids as well as vit. E and β-carotene.

Several assay methods are available for determining lipoxygenase activity, of which the spectrophotometric measurement at 234 nm and the polarographic procedures are generally preferred (2, 3). The latter involves the use of a recording oxygen electrode which measures oxygen consumption during the course of the enzymic reaction. This method is suitable for both crude enzyme extracts and pure enzyme preparations. Since other oxygen absorbing systems may be present, a blank is required. The spectrophotometric procedure is only applicable to purified enzyme preparations. Polarography has been used for determining the lipoxygenase activity in a number of crops, e.g., lentils and lupins (4), peas (5, 6) and small faba beans (7). In these studies lengthy procedures for enzyme extraction and purification have been described. However, we used soy flour suspensions for enzyme assay without any need to purify the enzyme.

For investigating the storage properties of the full-fat flour produced as described before, we stored samples at room temperature for one year. Lipoxygenase activity and peroxide value were measured and sensory evaluation of flours was carried out after storage. Results are discussed in the present paper.

Experimental

Material

American Golden Yellow Soybeans (No.2), harvested in 1979, were obtained from Cargill B.V. Amsterdam. As described earlier (1), whole beans were treated with IR radiation and kept for various periods of time in isothermal containers, and then dehulled and ground to full-fat flour. Samples were stored in glass jars at room temperature for one year. For comparison purposes in sensory analysis, a freshly-produced sample of full-fat flour of trade quality was obtained from W.Ruitenberg CZn N.V., Amersfoort, Holland.

Polarographic assay of lipoxygenase (ref. 8).

Instrumentation. For monitoring the oxygen uptake, a Metrohm Herisau Polarecord model E 261 polarograph equip-

ped with a YSI 4004 Clark Oxygen Probe (Yellow Spring Instrument Co., Ohio, USA) was used. The latter comprises a platinium cathode, silver anode and KC1 solution held around the electrodes by a teflon membrane. The potential applied between the electrodes was kept constant at 0.8 volt. Water, from a waterbath, was pumped around the reaction vessel to maintain the reaction temperature constant at 30°C. For enzyme dispersion, a high speed homogenizer (Measuring and Scientific Equipment Ltd., England) operating at 14,000 rpm was used.

Preparation of reagents. Buffers (pH 6–11): Phosphate buffers (Sörenson, pH 6 and 7), and borax buffers (Palitzsch, pH 8–11) were made according to ref (9). To remove dissolved oxygen, buffers were purged with nitrogen gas. Oxygen-rich buffers were made by using air stream. At 30°C, the enriched solution contained 0.21 μ mole 0_2 per cm^3 solution as calculated from ref. (9).
Substrate solution: Substrate solution was prepared according to the procedure described by SURREY (10): linoleic acid (Fluka AG, Buchs SG, Switzerland) solution was made in various oxygen-free buffers of pH 6 to 11, and kept refrigerated under nitrogen before use. The solution was prepared fresh daily.

Enzyme dispersion. A suitable quantity of finely-ground soy flour of 200 B.S. mesh (0.075 mm) was added to 50 cm^3 of buffer corresponding to pH of assay and blended at 14,000 rpm for 30 sec, keeping the temperature at 4°C. This was repeated once more after a one-min interval to prevent any possible temperature rise. The homogeneous suspension obtained was used directly for lipoxygenase assay. The suspension was prepared daily and kept refrigerated while in use.

Assay procedure. The assay was started by pipetting 1.0 cm^3 of oxygen-rich buffer to the reaction vessel, followed by 0.2 cm^3 of the substrate solution. One min later 0.2 cm^3 of the properly-diluted flour suspension containing the enzyme was added and the rate of oxygen consumption recorded. Two blanks were made, one for the substrate (without the flour suspension) and the other for the enzyme (without substrate solution). The activity is expressed as μ moles of 0_2 consumed per min per g dry, full-fat flour.

Peroxide value (pv) and sensory evaluation.
The pv determination was made on the hexane-extracted oil of full-fat flours (11). In this method, the peroxides in the oil liberate iodine from potassium iodide; iodine is subsequently titrated with sodium thiosulphate. Sensory evaluation of rancidity in samples was conducted according to the forced-choice, paired comparison test (12). Using X and Y codes, 6 flour samples (**Tab.4**) were combined to form a set of 15 pairs. Thirty assessors participated and evaluated 10 pairs each, one pair at a time. Each pair was tested 20 times and a total number of 300 responses were obtained. The assessors were asked to indicate the sample with the more rancid odour in each pair. The samples consisted of 4 IR-treated flours, a raw control, and a sample of freshly-prepared full-fat flour obtained from a commercial supplier. The test was conducted at 11 a.m. and 2:30 p.m. in a sensory evaluation room with individual booths. A sample of rancid flour was available as reference to each assessor throughout the test.

Results and Discussion

Enzyme dispersion
Tab.1 shows the activity and stability of lipoxygenase in

Tab.1 Activity and stability of soybean lipoxygenase in 0.2% raw soy flour suspension compared with centrifuged extract
(pH9; incubated in ice; activity in μ moles O_2 consumed per min per g dry, full-fat flour)

time after preparation (hr)	activity in suspension	% loss of activity	activity in supernatant	% loss of activity
0	277	–	276	–
1	274	1	270	3
2	272	1	260	7
3	272	1	252	9
4	270	3	244	12
5	261	7	227	18
76*	154	36	66	69

*stored in refrigerator (6–8°C)

0.2% raw soy flour suspension compared with the clear supernatant obtained after centrifuging a portion of the flour suspension for 10 min at 5000 rpm. This comparison was made to find out if flour suspensions could be used for polarographic enzyme assay without further extraction and concentration steps.
When used immediately after preparation, both the whole flour suspension and the supernatant showed an activity of about 277 units, indicating that the extra step of centrifugation was superfluous. As far as stability is concerned, the enzyme in the whole flour suspension was found more stable. Three hours after preparation, the activity of the suspension had hardly changed, while it had dropped by 9% in the supernatant. After 76 hours of refrigerated storage (6–8°C), the suspension still retained 56% of its original lipoxygenase activity, whereas the activity in the supernatant was only 24%. So, we prefer to use the whole flour suspension for lipoxygenase assay in soy flour.

Optimum pH of lipoxygenase assay.
A series of 0.2% raw, full-fat soy flour suspensions were used for determining the effect of pH on the activity of

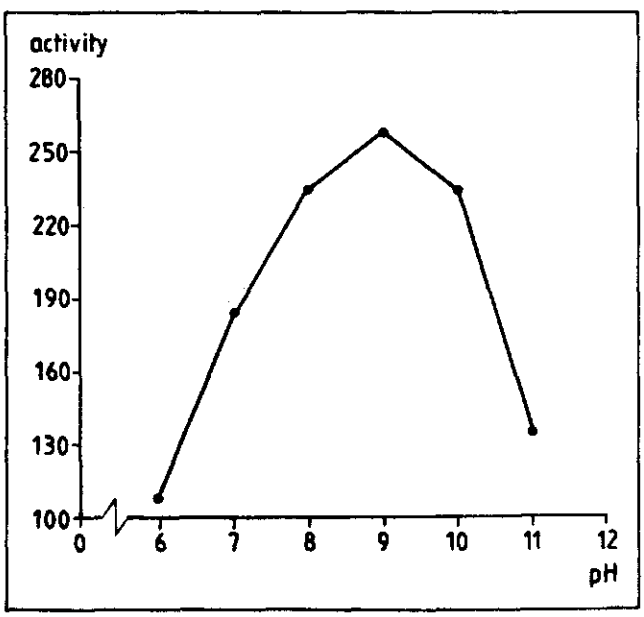

Fig. 1 pH activity profile of soybean lipoxygenase. (activity in μ moles 0_2 per min per g dry full-fat flour; at pH 6 and 7: phosphate buffer and at pH 8, 9, 10 and 11: borax buffer)

Tab. 2 IR radiation time and temperature, and lipoxygenase activity of full-fat soy flour produced according to modified IR procedure

	(a) IR radiation (sec)	(b) holding time (min)	(c) temperature (°C)	(d) lipoxygenase activity	(e) % residual activity
Raw	–	–	–	271	100
	20	0	82	192	71
	30	0	95	43	16
	35	0	104	28	11
	40	0	111	19	7
	45	0	115	11	4
	50	0	120	2	0.8
	60	0	124	1.5	0.5
	60	5	124	0.2	0.0
	60	10	124	0.1	0.0

a) Exposure of whole soybeans to IR radiation (seconds)
b) Holding time in isothermal containers immediately after IR radiation
c) Temperature of beans after IR radiation measured upon equilibrium in thermos flasks
d) Activity in μ moles O_2 per min per g dry flour
e) Residual activity after IR treatment as a percentage of activity in raw flour

Tab. 3 IR treatment, residual lipoxygenase activity, and peroxide values of full-fat soy flours produced according to modified IR procedure

Sample	(a) radiation (sec)	(b) holding time (min)	(c) temp. (°C)	(d) lipoxygenase activity	peroxide value	
					fresh (e)	stored (f)
A, raw	–	–	–	271	2.0	26
B	80	0	133	0.5	1.7	2.0
C	80	15	133	0.1	1.5	18
D	60	0	124	1.5	1.0	5.2
E	60	15	124	0.1	1.6	1.7

a through d: see footnote of table 2
e: peroxide value in mequiv/kg of hexane – extracted oil (freshly prepared)
f: peroxide value after 12 months of storage at room temp.

lipoxygenase. Investigations of the pH effect on lipoxygenase activity at acidic pH values is hampered by the relative insolubility of linoleic acid. Complete solubility will only be attained at alkaline pH (>8). The result of our study is shown in **Fig. 1**. The highest activities occurred in the pH range of 8–10. It is apparent that the optimum lies close to pH 9. Moreover the substrate is relatively insoluble at pH 8, and also the high alkalinity of pH 10 may cause erosion of the glassware. Therefore, we selected pH 9 for our subsequent enzyme assays.

Lipoxygenase assay of IR-treated flours.
Data on the residual lipoxygenase activity of the full-fat soy flours produced according to our modified IR treatment are presented in **Tab. 2**. It can be seen that up to 71% of the original activity was still present after 20 sec of IR radiation giving a temperature of 82°C. After 30, 35 and 40 sec of radiation, the activity decreased gradually while treatment for 50 and 60 sec resulted in more than 99% inactivation of the enzyme. It should be pointed out that the above samples were allowed to cool gradually after radiation and that the residual heat was still effective before the temperature finally dropped to room temperature. For instance, 5 min after the termination of IR radiation, the temperature of the beans receiving 50 and 60 sec of radiation was still above 70°C.

Peroxide value and sensory evaluation.
Tab. 3 shows the pv of full-fat flours freshly produced and also after 12 months of storage at room temperature. **Tab. 4** presents the results of sensory evaluation of flours in terms of rancidity development after storage. These samples were the same as previously reported in connection with their nutritive value (1), except sample F, which was freshly prepared and supplied by a commercial producer. The raw control sample showed a large increase in pv from 2.0 to 26 during storage. In sensory evaluation, this sample was found significantly more rancid compared to all samples except C. The lightly-treated sample (D) showed some increase in pv from 1 to over 5, indicating a slight oxidation due to the residual enzyme activity. In sensory evaluation, this sample was found significantly more rancid at the 5% level than sample E, but not compared with others. It can be expected, however, that the residual enzyme activity in sample D would have had more serious effects if the sample had been stored longer or under conditions more favourable to enzyme activity such as high moisture content or high relative humidity. In sensory analysis, the assessors were not able to detect a significant odour difference between sample F (fresh commercial flour) and the well-treated samples B and E. This indicates that B and E were comparable in freshness to the commercial flour. However, the overheated sample (C)

Tab. 4 Response of assessors in paired comparison test of full-fat soy flours for rancidity development

Pair	Sample (a)	A	B	C	D	E	F	significance (b)
AB		16	4					5
AC		14		6				n.s.
AD		16			4			5
AE		19				1		0.1
AF		18					2	0.1
BC			3	17				1
BD			11		9			n.s.
BE			12			8		n.s.
BF			9				11	n.s.
CD				15	5			5
CE				17		3		1
CF				17			3	1
DE					15	5		5
DF					12		8	n.s.
EF						10	10	n.s.
Total scores		83	39	72	45	27	34	

least significant difference = $z\sqrt{\frac{nt}{2}}$

+0.5 (Ref. 14)

n = no of judgements = 20
t = no of samples = 6
z = 1.96 (5% level)
= 2.56 (1% level)
LSD (5% level) = 15.8 (= 16)
(1% level) = 19.8 (= 20)

a) sample codes in table 3, except F in text
b) level of significance of difference in each pair (13)
n.s.: not significant

showed a high pv of 18 after storage and was assessed more rancid than all other samples except the raw flour (A). Possibly, in this case, autoxidation is involved due to the destruction of natural antioxidants and enhanced oxidation reactions caused by excessive heat.

Maillard reaction products were also expected to be present in sample C. The intermediate products formed in the early stages of the Maillard reaction are known to have antioxidative properties (15). However, it seems that these intermediates were no longer present during the long storage of this sample to provide protection against autoxidation. Moreover, a recent study has failed to show significant antioxidative action by the Maillard reaction products (16). Thus, overheating not only damages available lysine (1), but it may also bring about oxidative rancidity. It follows that, if optimal conditions of processing for improving the nutritive quality and storage stability of full-fat soy flour are not attained, or if they are exceeded, quality deterioration will take place. Also the results show that, although almost complete inactivation of lipoxygenase was achieved by 60 sec of IR radiation followed by a 5-min holding in an isothermal container, or by 80 sec of radiation with no holding, these conditions were not adequate for inactivating urease and trypsin inhibitors (1). This provides evidence that in soybeans, lipoxygenase is more heat-labile and more easily inactivated than urease and trypsin inhibitors. So, the minimum processing conditions are primarily determined by the requirements for inactivating urease and trypsin inhibitors.

In conclusion, a treatment involving IR radiation of whole beans for 1 min (124°C) followed by a holding period of about 15 min in an isothermal container constitutes the optimal conditions for the production of adequately-processed full-fat soy flour. The full-fat flour produced in this manner will have an improved nutritive quality due to the inactivation of antinutritive factors, as well as a prolonged shelf life because of lipoxygenase inactivation.

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Infrared processing of soybeans

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Key words: infrared radiation and holding, full-fat soy flour, shelf life, energy requirement, bread ingredient

Abstract. A procedure on laboratory and industrial scale is described for the production of full-fat soy flour of high nutritive quality and long shelf-life. The procedure involves infrared radiation of soybeans followed by holding in an insulated bin. Production costs are reduced due to the use of relatively low temperatures, elimination of the preconditioning step, and elimination of drying after processing. A preliminary test is also reported using the flour in Dutch, white bread.

Laboratory-scale processing

In our previous papers [9, 10] we reported a modified procedure (modification of the micronizing process [11] on laboratory scale for infrared (IR) heat processing of American Golden Yellow soybeans. As stated, the application resulted in a considerable reduction in energy requirements. The beans received 0–80 s IR radiation which raised the temperature of the beans from 8 to 125–133 °C. This was followed by an additional holding stage in a well-insulated container for 0–25 min. The beans were then cracked, dehulled and ground to full-fat flour.

The effectiveness of the process was investigated by determining the indicators commonly used in soybean heat processing, namely, urease [2], trypsin inhibitor activity [8], nitrogen solubility index [4] and protein dispersibility index [3]. Available lysine [6] was measured as an indicator of overheating. The residual lipoxygenase activity after processing was measured by an oxygen electrode connected to a recording polarograph [10]. Figure 1 shows the steady decrease in enzyme activity with radiation time. For investigating the oxidative stability and shelf-life of full-fat flours produced, samples were stored in glass jars at room temperature for one year. Peroxide values (PV) were determined on the extracted oil before and after storage [1]. The development of rancidity in the flours after storage was also investigated by a sensory test in which assessors judged the samples for rancid odour [10].

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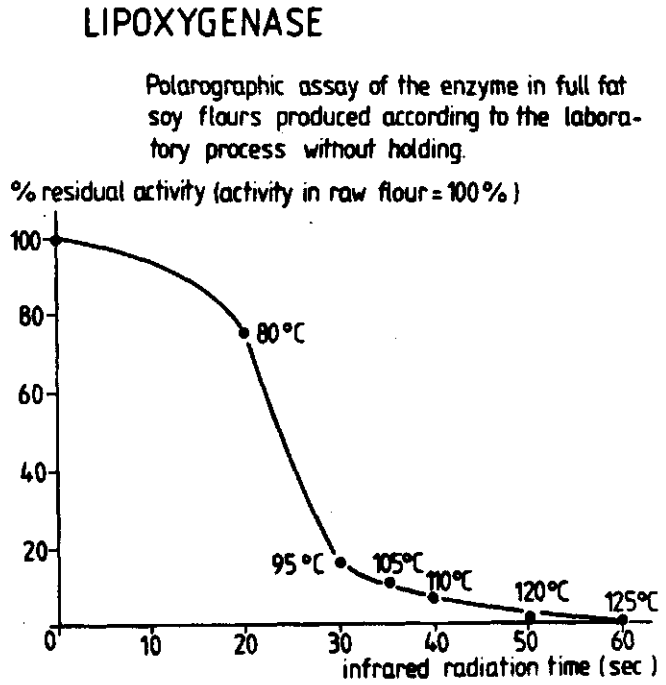

Figure 1. Inactivation of lipoxygenase in soybeans during IR radiation as a function of temperature of beans (after equilibrium in a thermos flask).

The urease test was found to be a good indicator for residual trypsin inhibitor activity (Table 1). Both activities were acceptably low in the samples exposed to IR radiation 60–80 s (125–133 °C) and held for about 15 min. Overheating caused a drop in the level of available lysine, high values for PV and rancidity after storage. An optimum procedure was found to be 60 s IR radiation (125°C) and 15 min holding. The sample thus produced had no detectable lipoxygenase activity and was judged fresh after one year of storage.

Utilization of the flour in breadmaking

After investigating the effectiveness of this procedure for inactivating trypsin inhibitors and lipoxygenase, it seemed necessary to evaluate the flour in food systems. An important application of soy flour is in breadmaking [5]. As a preliminary test, the optimally treated flour was used at 3 levels of 3, 6, and 12% (on flour weight basis) in the production of the typical Dutch, white bread. The 3 and 6% levels caused an increase in the loaf volume, while the 12% level had an adverse effect on the volume. The use of SSL (sodium stearoyl-2-lactylate) is reported to overcome this problem [7].

Table 1. Quality indicators of soy flours from beans with and without IR radiation

Treatment[a]	Trypsin inhibitor[b]	% Available lysine[c]	Peroxide value[d]	
			Before storage	Stored 1 yr
0/0 (raw)	72.0	2.3	2.0	26
60/0/125	26.1	2.4	1.0	5.2
80/0/133	18.8	2.2	1.7	2.0
60/15/125	8.2	2.3	1.6	1.7
60/25/125	7.1	2.2	n.m.	n.m.
80/15/133	6.8	2.1	1.5	18
80/25/133	5.9	1.9	n.m.	n.m.

[a] First no. refers to IR radiation time in sec; second no. refers to holding time in insulated container; third no. refers to the temperature (°C) of beans after equilib. in thermos bottle measured with a thermometer.
[b] Trypsin units inhibited per mg sample [5].
[c] % available lysine in full-fat flour [8].
[d] m equiv. per kg of hexane extracted oil [9].
n.m = not measured.

However, a paired comparison sensory test with 42 assessors did not show a difference in taste acceptability between the 3 levels. Further work on the functional properties of the flour is underway at this department.

Industrial design for the modified IR process

Figure 2 shows the industrial process diagram of the modified IR procedure. The beans flow continuously from storage and are cleaned in a cleaning unit Then, they drop in layers one bean thick on the endless conveyor belt of the plant via a hopper equipped with a flow regulatory mechanism. The belt travels through the IR unit which is provided with gas-heated infrared burners (wavelength 3–8 μm). The vibratory mechanism of the plant causes constant turning around of the beans in the radiation area and ensures uniform heating. The speed of the belt, and thus the residence time, can be varied to obtain any desired final temperature in the beans. The heated beans then move into a well-insulated holding bunker where they are held for a predetermined period. Next, the beans pass through a cooling unit and are cooled. For the production of dehulled, full-fat flour, the beans are cracked, dehulled, ground and packed.

In an industrial IR unit, the processing of 3 t/h of soybeans would require 40–45 m^3/h of natural gas.

Advantages

The advantages of the procedure is reduction of production costs:

– The combined IR radiation and holding procedure permits the use of relatively low temperatures for soybean processing.

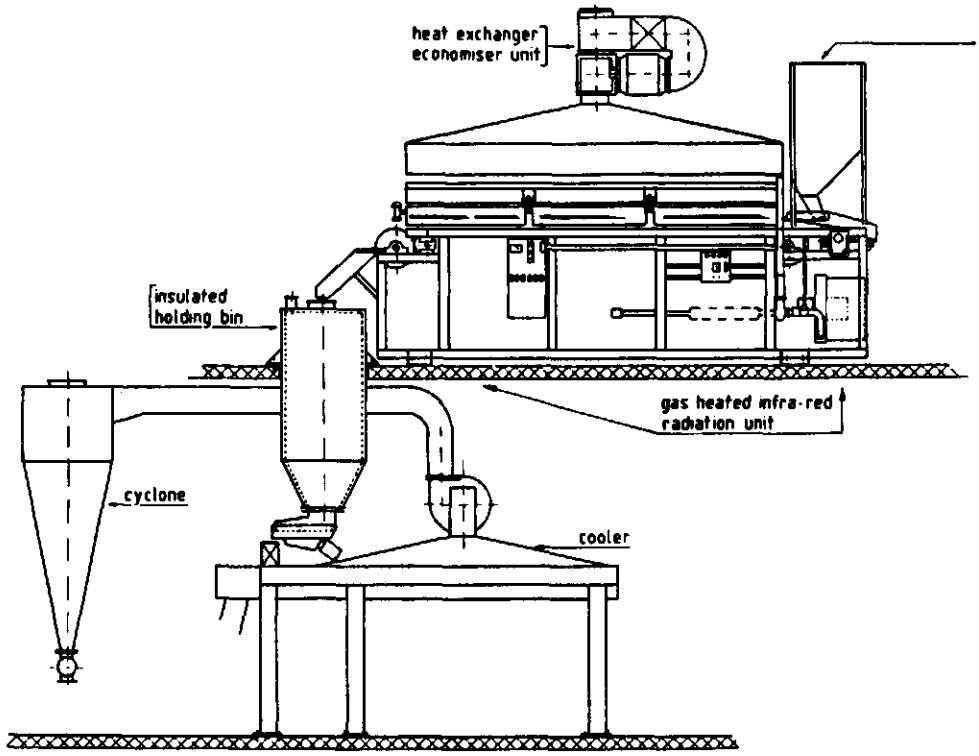

Figure 2. Diagram of the continuous industrial IR process as modified in our department.

- No preconditioning is required. The beans as received can be processed after cleaning.
- No drying after processing in contrast to steaming.

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Infrared Processing of Maize Germ

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Maize germ samples were treated with infrared heat at different temperatures. The degree of starch gelatinization and water absorption increased whereas protein dispersibility and enzyme activity decreased with higher infrared temperatures. Raw and treated samples were stored at room temperature and tested after 0, 6, 12, and 18 months of storage. The changes in %free fatty acids and peroxide values of oils even from the raw germ were slow up to 12 months. After 18 months, however, the raw and under-treated samples developed rather high %free fatty acids and peroxide values and were found rancid. It was concluded that infrared heat could be applied to prolong the safe storage time of maize germ.

Introduction

Maize (corn) germ being high in protein, fat, vitamins, and minerals is regarded as an excellent source of nutrients (1). Traditionally, however, most of the germ produced in maize milling is used for obtaining maize germ oil which is a premium oil on the world market (2) due to its desirable physical, chemical and nutritional aspects. The defatted meal is used for animal feed. The composition of maize germ **(Tab. 1)** is reported by INGLETT (3) and BLESSIN (4). The protein efficiency ratio (PER) of maize germ is reported by TSEN (1) to be 2.6 exceeding that of casein, 2.5 and soy, 2.3. Addition of 12% germ flour to bread increased the PER of the latter from 0.9 to 1.2 (1). Further information on the protein quality of maize germ is provided by its content of amino acids. Lysine which is the limiting amino acid in whole maize (as in other cereals) is concentrated in maize germ: 6.1 g/100 g protein, as compared to only 2.0 in endosperm (5).

Such information on the nutritive quality coupled with the availability of supplies and cost considerations, have directed much interest in recent years towards the utilization of maize germ in human food products (1, 6, 7). Maize germ flour is already available on the US market for food applications (8). It has been used in baked goods such as bread and cookies (5, 9, 10, 11, 12) and meat products (5, 9). The water absorption of dough increased from 67 to 72% when 12% of wheat flour was replaced by germ flour (5). Maize germ flour is used in the production of corn-soy-milk (CSM) which is supplied by the US food aid programme (13). It has also been investigated as a partial replacement for milk solids and eggs in foods. When replacing milk solids in cakes, volume is reported to increase (1). The functional properties of maize germ in terms of water and fat absorption and emulsification have been studied (14). It must be pointed out, however, that the germ contains several enzymes which may have deleterious effects on oil, and as a result, on maize germ quality. The main enzymes in this connection are reported to be lipase, lipoxygenase, and linoleate hydroperoxide isomerase (13, 15, 16). GARDNER *et al* (13) and GARDNER and INGLETT (15) used heated rolls at 99–204 °C to toast full-fat germ flakes and reported on the inactivation of the above enzymes. ANDRES (17) also reported on the stability of heat-treated germ. The possible advantages of heat treatment of maize germ include: (1) minimizing the activity of enzymes and thus prolonging shelf life, 2) improving the microbiological status, and 3) improving the flavour.

As a continuation of our previous studies (18, 19, 20) whereby shelf stable full-fat soy flour was produced, we investigated the effects of infrared (IR) heat treatment on physico-chemical properties and changes during storage of maize germ.

Tab. 1 Composition of maize germ, hand-dissected compared with milled stream, and partially purified fraction (in % dry matter).

component	hand-dissected[a]	milled stream[b]	purified fraction[b]
protein	18.8	16.1	17.1
fat	34.5	22.8	27.1
ash	10.1	6.2	7.4
starch	8.2	31.0	20.2
sugar	10.8	–	–

[a] data from Inglett (3). [b] germ fraction from dry maize milling industry as used in this study (see text for purification steps)

Material and Methods

Dry milled germ from US yellow maize was screened and aspirated to remove most of the hulls and endosperm pieces which are normally present in the milling operation. The final material contained approx. 80% germ as judged by its fat content (27.1%, dry matter). The moisture content was 9.1%.

Tab. 2 Various treatments of maize germ with IR

sample codes	radiation time[a]	temp[b]	holding time[c]	% water	
				initial	final
Raw	–	–	–	9.1	9.0
1	35	98	5	9.1	7.8
2	40	110	5	9.1	6.1
3	45	118	5	9.1	5.5

[a] exposure of samples to IR radiation in sec. [b] temp attained after radiation in °C. [c] holding time in min in insulated containers

IR treatment of germ: The IR equipment used has been described earlier (18, 20). Samples of purified germ were heated at predetermined temp, held, and allowed to cool (table 2). The heat-treated as well as raw samples were then stored in glass jars at room temp and tested after 0, 6, 12, and 18 months' storage. Temp of the material after IR treatment, protein dispersibility index (%PDI), available lysine of the hexane-defatted samples, and peroxide values (PV) were measured as described earlier (18, 19).

Free fatty acids (%FFA) was determined according to the AOCS Method Ca 5a 40.

Lipoxygenase activity was measured as described earlier (19). Crude enzyme extract was prepared and a pH of 6.9 used for assay as described by GARDNER (16).

Moisture, protein, fat, and ash were determined according to the methods of the American Assoc. of Cereal Chemists (AACC) respectively (21): 44–15A; 46–10; 30–25; 08–01.

Starch and starch gelatinization were measured using amyloglucosidase enzyme (22).

Water absorption index (WAI) was measured according to the method of Anderson et al (23) as modified by GARDNER *et al* (13).

Gas liquid chromatography (GLC) analysis of fatty acids: 0.5 g oil was dissolved in 3 ml water-free heptane, 2 ml 6% HCl in abs. methanol added and the mixture boiled under reflux for 1 hr. 2 µl of the cooled and neutralized solution was used in a Fractovap model C gas chromatograph (Carlo Erba, Italy) equipped with a F.I.D. The column temp was maintained at 190°C, and N_2 used as carrier gas. The column was packed with 15% CP, sil 84 on chromosorb, WHP 100–120 mesh.

Results and Discussion

Tab. 2 shows the various IR treatments applied to the maize germ samples. Radiation time varied from 35 to 45 sec with a final temp from 98 to 118°C. Radiation was followed by holding in insulated containers for 5 min in all cases. There was a drop in the moisture content from the initial value of 9.1% to 7.8–5.5%. Low moisture content in the product is preferred since it not only helps in the shelf stability of the product but it also imparts crispness to the whole germ which is desirable in some applications where nuts are normally used.

Tab. 3 Effect of various IR treatments on maize germ samples[a]

	Raw	IR heated		
		1	2	3
gelatinized starch (% of total)	–	31	64	82
water absorption index	3.3	3.5	4.2	4.9
protein dispersibility index	78	56	31	18
available lysine (g/100 g protein)	5.2	5.2	5.1	5.1
lipoxygenase activity[b]	5	0.5	0.3	n.d.[c]

[a] sample codes in table 2. [b] µ moles O_2 consumed/min/g dry full-fat germ. [c] n.d. = not detectable

Tab. 3 shows the effects of IR treatment on the germ samples. Starch gelatinization increased as temp rose. Up to 82% of the starch in sample 3 was gelatinized. WAI which is usually taken as a rapid indicator of starch gelatinization was also measured. It increased from 3.1 in the raw germ to 4.9 in sample 3 in correlation with the degree of gelatinization. The increase in water absorption is desirable in some food applications such as baked goods. The degree of heat treatment received by the sample can also be investigated by measuring the dispersibility of proteins expressed as %PDI which decreased considerably from 78 (raw sample) to 18 in sample 3. Available lysine is often regarded as an indicator of overheating. None of the samples showed any significant drop in the level of available lysine.

Tab. 4 FFA and PV of raw and IR-treated germ samples, stored 0, 6, 12, and 18 months at room temp

	FFA (% free fatty acids)				PV (mequiv/kg oil)			
storage (months)	0	6	12	18	0	6	12	18
samples								
raw	0.8	1.9	2.8	5.6	1.4	1.8	3.1	6.1
1	0.6	1.1	2.2	4.1	1.6	1.9	3.5	4.1
2	0.6	0.8	0.6	0.8	1.4	1.4	2.4	2.4
3	0.8	0.6	0.8	0.7	1.1	1.3	1.8	1.5

sample codes in table 2

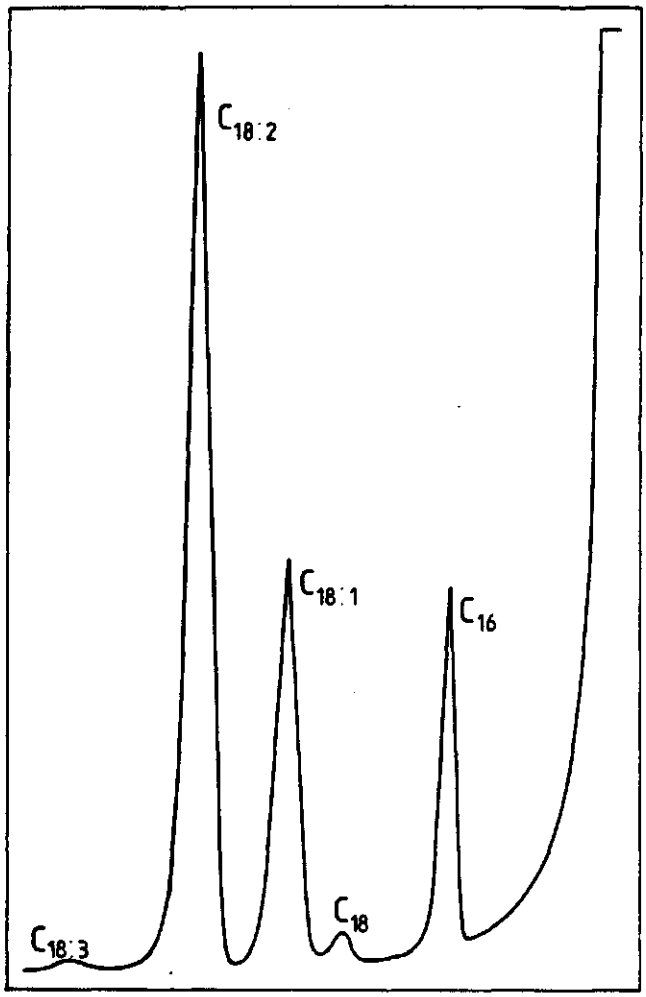

Fig. 1 Gas chromatogram of methylated fatty acids from maize germ sample 1
(sample codes in table 2).

The results of storage tests performed on the raw and IR-treated germ samples are presented in **Tab. 4.** FFA and PV of the samples were measured after 0, 6, 12 and 18 months of storage at room temp. It was only in the raw sample and sample 1 that some increase in FFA was noticed on storage. The increase was, however, only slight up to 6 months of storage and started to become significant after 12 months. In the raw sample, the FFA rose to 5.6% after 18 months from an initial figure of 0.8%. It is conceivable that these two samples would have developed much higher FFA if the moisture content and/or other storage conditions had been favourable for FFA development. Samples 2 and 3 did not show any increase in FFA indicating that the heat treatment in combination with the low moisture content (5.5–7.8%) had minimized lipolytic degradation.

The change in PV of the samples (even the raw germ) was found to be small up to 12 months of storage. It was only when the samples were tested after 18 months that the raw sample and sample 1 were found to have increased PV's and a rancid odour. Samples 2 and 3 still showed small PV's and retained their fresh odour.

The slow increase in PV of raw germ was in line with the rather low lipoxygenase activity in maize germ (15) which was also encountered in this study **(Tab. 3).** The lipoxygenase activity of maize germ used by us might have further been reduced during drying with heated air after harvest (maize is often dried with air heated to above 100°C with the temp of kernels at times exceeding 80°C). GARDNER (15) found up to 40% lipoxygenase inactivation in maize dried with air heated to 116°C and 20% at 88°C.

Moreover, maize germ contains considerable amounts of antioxidants such as tocopherols and phospholipids (24) which play an important role in preventing the oxidation of unsaturated fatty acids of the germ. GLC analysis showed only insignificant changes in the concentration and proportion of individual fatty acids in oil from the samples after 18 months. **Fig. 1** shows the gas chromatogram of methylated fatty acids in the rancid sample 1. Equal chromatograms for all the oils were attained with the following fatty acid contents: 24.5% oleic, 60.5% linoleic, and 0.7% linolenic. These figures compare well with the published data on maize germ oil (24).

The fact that the proportion and concentration of fatty acids were unchanged in all the samples (regardless of rancidity) shows that the oxidation of only minute amounts of $C_{18:2}$ and $C_{18:3}$ fatty acids was responsible for the development of rancid odour in the raw germ and sample 1.

It is evident from the results of this study that IR heat treatment can be used to prolong the safe storage time of maize germ. At the same time, starch is gelatinized improving the food value, raw cereal flavour of germ converted into a more desirable nut-like flavour, and crispness imparted to the product.

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A Modified Procedure for low Temperature Infrared Radiation of Soybeans

III-Pretreatment of whole Beans in Relation to Oil Quality and Yield

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The effects on oil quality and yield of infrared heat treatment of whole soybeans prior to oil extraction was investigated. Peroxide value, off-flavours, and % free fatty acids of the oils from pretreated beans were considerably lower than that from raw beans. At the same time, the crude, water-degummed oil after bleaching had a sufficiently low phospholipid level to meet the refiners' specification for deodorization. This procedure may be employed to circumvent alkali refining and also produce extracted flakes free from urease activity and so suitable for direct utilization as feed. Oil yield was reduced slightly.

Introduction

Traditionally, the manufacture of edible soya oil involves the following steps (1): cleaning of beans, cracking, dehulling, conditioning, flaking, solvent extraction, degumming, alkali refining, and physical refining (bleaching, deodorizing,...). After the cracking step and during the subsequent operations and time lapses normally occuring in the industrial extraction process, favourable conditions for enzymic activity exist. Lipoxygenase will catalyze the oxidation of polyunsaturated fatty acids giving rise to peroxides and secondary oxidation products responsible for off-flavours (2), while lipase will hydrolyze the oil forming free fatty acids (FFA). Furthermore, phospholipase D has been reported to bring about the hydrolysis of phospholipids naturally occuring in soybeans (3, 4, 5), causing the formation of phosphatidic and lysophosphatidic acids and their Mg and Ca salts (6).

Generally, phospholipids are removed from the oil by degumming and alkali refining to avoid the following problems (7): deposit formation on storage, oxidation and polymerization, high oil losses due to the emulsifying properties of phospholipids, and darkeninig of the oil colour in the deodorizer. Besides, there exists the possibility of fishy odour and flavour formation in refined oil on storage when residual phospholipids are present (8). Residual phospholipids also cause poor hydrogenation (9), and by acting as sequestering agents, transfer metal ions such as Fe and Cu from water into oil during caustic washing, causing lower oil stability (9).

In the refining of crude oil, natural phospholipids, i-e, lecithins and cephalins, are hydrated with water and removed by centrifugation (water degumming), whereas, the products of hydrolysis of phospholipids mentioned above are nonhydratable (6), and remain in the crude, water-degummed oil. These are referred to as nonhydratable phospholipids (NHP). The presence of NHP in the crude, water-degummed oil which can not be removed with water nececitates alkali refining which is a problematic process. The main disadvantages of alkali refinning have been outlined by GROTHUES (10):

1 numerous steps involved and a long time required,
2 high losses of neutral oil,
3 the disposal problem associated with soapstock,
4 inferior quality and colour of fatty acids obtained.

Oil refiners have specifications regarding crude, degummed oil which according to THOMAS (11) include: phospholipids content (max. of 160 or 200 ppm phosphorus), FFA (max. of 0.6 or 0.75%), oxidation products in terms of peroxide value (PV), and anisidine value (max. of 2.0). According to the trading rules of the National Soybean Processors Association, a discount is applied to degummed oil with a P-content over 200 ppm (12) and also when FFA is over 0.75% (13).

From the above, it follows that the inactivation of lipoxygenase, lipase, and phospholipase D prior to oil extraction would be very beneficial in minimizing the problem and may make it possible to circumvent alkali refining; i-e, the crude, degummed oil could be subjected to physical refining only. The main advantages of physical refining as outlined by GROTHUES (10) are:

1 shorter process time,
2 higher yields of neutral oil,
3 elimination of the soapstock disposal problem, and
4 that the fatty acids obtained can be marketed directly without further handling.

Heat treatment of beans prior to oil extraction in order to inactivate the enzymes mentioned above has been investigated and in some cases industrially realized. RICE *et al.* (14) steam-heated whole soybeans prior to oil extraction in order to inactivate lipoxygenase. They reported an increase in the oxidative stability of the oil and an improvement in the organoleptic blandness of the meal. ONG (3) reported the effects of steam treatment of soybean flakes on oil quality and yield. He concluded that it was possible to obtain water-degummed, crude oil with sufficiently low phosphorus for physical refining, thus avoiding the alkali process. He attributed this finding to the inactivation of phospholipase D, thus little or no formation of NHP prior to or during extraction. KOCK (4) and PENK (5) reported the industrial application of a process, referred to as the ALCON PROCESS,

whereby soybean flakes are steam-heated before oil extraction. By eliminating the enzyme activity, crude, degummed oils with low phosphatide content and a low anisidine number have been obtained. In all of these treatments, however, a lower yield of oil (up to 0.5%) compared to untreated beans has been reported. As a continuation of our research with infrared (IR) treatment of soybeans (15, 16), we carried out studies involving IR treatment of whole soybeans prior to oil extraction and its effects on the extracted oil in terms of yield, FFA, phosphorus, oxidative stability and off-flavours.

Experimental

Material

American Golden Yellow soybeans (no.2), harvested in 1979 and 1982.

Methods

IR treatment of soybeans. This was accomplished as described earlier (15). The beans were heated to the desired temp and held in an insulated container for predetermined periods before being allowed to cool. Preparation for oil extraction: raw and IR-treated beans were cracked, dehulled, conditioned with steam for 45 min at 75°C and flaked. The flakes were held at 30°C for 1 h to simulate time lapses in industry.

Oil extraction. The flakes were extracted with hexane at 69°C for 90 min in a soxhlet apparatus.

Degumming. 2% water was added to the crude oil. The mixture was stirred for 30 min at 70°C. The degummed oil was separated by centrifugation at 2700 RPM for 10 min.

Bleaching. 0.5 and 1% tonsil standard FF (Süd Chemie, Munich) was added to the crude, degummed oil at 70°C and the mixture stirred for 30 min in a water bath at 90°C, and finally filtered.

Phosphorus. The determination was carried out according to the AOCS Official Method Ca 12–55 and a factor of 31.7 was used to convert %P to %phospholipids (17).

Other methods. % FFA, PV, urease activity, Protein Dispersibility Index (%PDI) and lipoxygenase activity were measured as described in our previous papers (15, 16).

Results and Discussion

Analytical data on the beans and crude (undegummed) oils are presented in **Tab.1** and **2** and show that pretreatment of whole beans with IR radiation results in lower PV's in treated oils than in raw-extracted oils. Sensory tests at our department (18) have confirmed this: oils from the raw and inadequately-treated beans (90°C, no holding) had much more off-flavour than the oils from well-treated beans. A temperature of 104°C with a 5 min holding step was found adequate for this purpose. The lipoxygenase activity was reduced to 0.5% of that in raw beans which explains the much less off-flavour formed. This is in agreement with work reported by RICE *et al.* (14). However, the meal from these beans still had a relatively high urease activity of 0.45 (pH unit rise) and would need further heat treatment for inactivating trypsin inhibitors unless used for special purposes where a high PDI is the prime consideration. The oil from heat-treated beans also had lower % FFA than those from raw and inadequately-treated beans indicating lipase activity in the course of preparation and extraction. The fresher beans from 1982 had a lower % FFA than 1979 beans, an indication of FFA increase on storage. There was little dif-

Tab.1 Effect of IR treatment on urease, lipoxygenase and PDI of soybeans (104°C, 5 min holding)

	1979 beans		1982 beans	
	treated	raw	treated	raw
urease	0.45	2.15	0.35	2.10
lipoxygenase activity (a)	0.5	100	0.6	100
PDI (%)	55	91	51	88

(a) – % of activity compared to raw beans = 100

Tab.2 Comparison of FFA, PV and phospholipid content of crude oils from IR-treated and raw beans

	1979 beans		1982 beans	
	treated	raw	treated	raw
FFA (%)	0.5	0.8	0.3	0.6
PV (meq/kg oil)	0.9	3.0	0.6	2.8
Phosphorus (ppm)	765	790	540	575
Phospholipids (%)	2.42	2.50	1.71	1.82

Tab.3 Effect of water degumming on FFA, PV, and phospholipid content of oils from IR-treated and raw beans

	1979 beans		1982 beans	
	treated	raw	treated	raw
FFA (%)	0.4	0.8	0.2	0.5
PV (meq/kg oil)	0.9	3.2	0.6	2.8
Phosphorus (ppm)	48	230	24	185
Phospholipids (%)	0.15	0.72	0.07	0.59

Tab.4 Effect of bleaching on FFA, PV, and phospholipid content of water-degummed oils from IR-treated and raw beans

	1979 beans		1982 beans	
	treated	raw	treated	raw
FFA (%)[a]	0.4	0.7	0.2	0.5
PV (meq/kg oil)[a]	0.6	1.2	0.2	1.5
Phosphorus (ppm)[a]	18	85	7	63
Phosphorus (ppm)[b]	12	49	4	40

(a) – at 0.5 % bleaching earth, (b) – at 1.0% bleaching earth

Tab.5 Effect of different IR treatments on oil yield (% dry matter)

Treatment	% oil extracted
Raw	18.8
125°C/15 min holding	18.1
115°C/15 min holding	18.0
104°C/ 5 min holding	18.2

ference between PV of oils from 1982 and 1979 beans. A difference was noted between the P-content in crude, undegummed oils from 1979 and 1982 beans. The raw 1979 beans contained 790 ppm phosphorus while those of 1982 had a lower P-content of 575 ppm. This can be attributed to differences in variety and growing conditions.

Tab.3 shows the data on crude, degummed oils. There was a drastic decrease in P-content after water-degumming indicating the removal of hydratable phospholipids. Two points should be discussed at this stage. Firstly, the water-degummed oils from IR-treated beans had much lower P-contens (48 and 24 ppm) than those from raw beans (230 and 185 ppm). This indicates the formation of NHP during the course of preparation and extraction of raw beans attributable to the action of phospholipase D (3, 4, 5, 10). Secondly, the water-degummed oil from 1979 beans contained more NHP (48 ppm) than that from 1982 beans (24 ppm). Possibly, some NHP had been formed in beans on storage as has also been reported by KOCK (4). He assesed various shipments of beans for NHP and found higher figures in oils from bad quality beans and those with a bad storage history. Further, WIEDERMAN (19) discusses storage conditions leading to the formation of NHP. In this connection, the presence of splits and damaged beans is particularly important.

The proposed specification for P-content in crude, water-degummed oil for physical refining is 20 ppm (3) and the oil from the pretreated 1982 beans with 24 ppm phosphorus is very close to this figure, but that from 1979 beans does have a high P-content (48 ppm) and far exceeds the max limit. All the water-degummed oils from pretreated beans, do, however, meet the proposed specifications for FFA (max 0.75%) and PV (max of 2.0 meq/kg) for physical refining (3). In **Tab.4** are presented data on the degummed, bleached oils. Bleaching caused a sharp reduction in PV: from 3.2 and 2.8 to 1.2 and 1.5 meq/kg in raw oils and from 0.9 and 0.6 to 0.6 and 0.2 meq/kg in pretreated oils, respectively. WIEDERMAN (19) reports similar findings and points out that in addition to decolorizing, another effect of bleaching is the removal of peroxides and secondary oxidation products. At the same time, a marked drop in P-content was noted. When bleaching earth was used at 0.5% level, the P-content in oils from treated beans dropped to 24 and 7 (from 48 and 24 respectively) and at 1% level, the corresponding figures were reduced still further to 12 and 4 ppm. This should be compared with 49 and 40 ppm in oil from raw beans. The specification for the bleached oil suitable to enter the deodorizer for steam refining is max 5 ppm phosphorus (4). Thus, the bleached oil (1% bleaching earth) from pretreated 1982 beans with 4 ppm phosphorus meets this specification and is suitable for physical refining. By the way, the amount of earth used (1%) is still less than the 1.5% level recommended by GROTHUES (10).

With regard to oil yield, as it can be noted from **Tab.5**, pretreatment of beans with IR radiation caused a general reduction in the oil yield which is an important consideration. This can be overcome by a longer extraction time (5). If this extra oil is allowed to remain in the meal, it might pose additional oxidation problems on storage. The same tendency for slightly lower yields of oil has been reported with steam pretreatment (3, 5, 14). **Tab.5** also shows that the drop in oil yield in the case of IR-treated beans was irrespective of the treatment conditons. Thus, if conditions such as 125°C and 15 min holding are employed, as described by us before (15), flakes free from urease activity with feed levels of trypsin inhibitor activity will be obtained directly after extraction with no further drop in oil yield.

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Involvement of Phospholipase D in the Hydrolysis of Phospholipids in Soybeans

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Formation of free choline was investigated by (radio) chemical procedures as a measure of enzymic degradation of phospholipids in soybeans. The involvement of phospholipase D in the hydrolysis of phospholipids was ascertained by thin layer chromatographic separation and densitometric quantification which showed the sum of phosphatidyl choline, phosphatidylethanolamine and phosphatidic acid to be unchanged before and after hydrolysis. The pretreatment of whole beans with infrared radiation prior to oil extraction caused inactivation of the enzyme and resulted in formation of less choline and phosphatidic acid than in the corresponding raw soya sample. Isoelectric focusing of the enzyme extract showed the presence of an active phospholipase D with isoelectric point 4.8.

Introduction

Phospholipase D (Ph-D) has been reported to be involved in the hydrolysis of phospholipids (PL) naturally occuring in soybeans (1). This causes the formation of phosphatidic and lysophosphatidic acids (PA and LPA, respectively) which form the nonhydratable phospholipids (NHP) (2) remaining in the water degummed oil and possibly leading to poor flavour and other problems in the final oil (3). NHP is removed by alkali refining which causes heavy losses of oil and involves disposal problems and as a result, much interest exists in circumventing it (4).

In our previous paper (5), we reported on a procedure based on the infrared (IR) treatment of whole soybeans prior to oil extraction which resulted in substantially lower concentrations of PL determined as phosphorus (P) in the degummed oil permitting the alkali refining to be avoided.

It was postulated that the heat pretreatment had caused the inactivation of Ph-D reducing the amount of NHP formed during the preparation of beans and oil extraction. Several investigators have reported the existence of soluble and particulate forms of Ph-D. For instance, Heller *et al* (6) found two forms of Ph-D in peanuts: a highly active, soluble form in the dry, mature seeds, and other form associated with particulate fraction with only 5% activity of the dry seeds.

The main procedures for assaying the activity of Ph-D are classified as physical, chemical (colorimetric), and radiochemical methods (7, 8).

Physical methods involve using a labelled substrate and following the enzymic activity by measuring surface radioactivity or phase boundry potential. Drawbacks of such approaches have been discussed (7,8). Chemical methods are based on the measurement of either the products of the enzymic reaction, i-e, choline, ethanolamine, and PA or the disappearance of substrates, i-e, phosphatidylcholine (PC) or phosphatidylethanolamine (PE). The chemical determination of choline can be done by precipitation with KI_3 to yield choline periodide (9) or choline enneaiodide (10), and with reineckate salt to yield a complex measurable at 526 nm (11, 12). Ethanolamine is measured using the periodide or ninhydrin procedures (8, 13). The measurement of PA liberated by the enzymic action may be carried out by thin layer chromatography (TLC) followed by a staining procedure (14). This supplements the choline or ethanolamine determination for the assay of Ph-D activity, since the free bases may be produced by the combined action of phospholipases and phosphodiesterases (7, 8). The one-dimensional (15) and two-dimensional (16) TLC have been described for the separation, identification, and quantification of PL and the products of their hydrolysis by phospholipases. Radiochemical procedures are reported to be the quickest and most sensitive assay methods available (7, 8, 17). In these procedures, labeled substrates using ^{14}C, ^{32}P, or ^{3}H are employed and the formation of products and/or disappearance of substrates are determined. When the enzymic reaction is followed by chromatographic separation of the products as mentioned simultaneous determination of the activity of various phospholipases may be accomplished (17).

In this paper we report on the results of further research carried out in order to ascertain the involvement of Ph-D in the formation of NHP and throw light on its hydrolytic action, products of hydrolysis, and activity in relation to the extent of heat treatment. Isoelectric focusing was also carried out to investigate the type of Ph-D obtained in the extract.

Experimental

Materials

The following materials and reagents were used: US Yellow soybeans, grade 2 (Cargil, Amsterdam); egg lecithin

(Merck, Darmstadt); labelled lecithin, ^{14}C-L-α lecithin, 50–60 mCi/mmol (Amersham International, England); commercial cabbage phospholipase D (Boehringer, Mannheim); sodium dodecylsulphate (SDS; BDH Chemicals, Poole, England); dithiothreitol (DTT; Sigma Chemical Co., St. Louis); all other reagents and solvents were from Merck, Darmstadt.

Methods

IR pretreatment of soybeans and the procedure of oil extraction were carried out as described earlier (5).

Preparation of enzyme extract (6, 18): 10 g soybeans were soaked overnight and homogenized for 1 min at full speed in a Waring blender at 4°C in 100 ml of 0.05 M tris-HCL buffer (pH 7.4) containing 1mM EDTA, and 0.25 mM DTT. After letting stand at 4°C for 1 h, the homogenate was centrifuged at 14,000 g at 4°C and filtered through glass wool. Ammonium sulphate was added to a final concentration of 20% and centrifuged as before. The precipitate was then dissolved in the extraction buffer and dialized overnight with 100X volume of buffer.

Preparation of substrate (17): 1 ml of 50 mM carrier lecithin and 0.15 ml of 1.33 μ Ci/ml ^{14}C lecithin dissolved in ethanol were dried under N_2, resuspended and sonicated in 1 ml of 1 M $CaCl_2$, 2 ml of 0.4 M tris-acetate buffer (pH 5.7) with 13 mM SDS. Sonication was done for 10 min using Sonicor model SC-101-22. The substrate was used directly.

Radiochemical assay of enzymic activity (8, 17): 1.7 ml of enzyme extract was added to 0.3 ml of substrate, mixed and incubated for 10 min at 30°C. The reaction was terminated with 1 ml of 6% $HC10_4$, and the solution extracted 4 times with 5 ml of ether. The ether layer was removed after centrifugation and contained the remaining lecithin and PA produced, while the water layer contained the liberated choline. The layers were measured separately using a Packard Tricarb 300 Counter (United Technologies Packard) and the enzyme activity calculated.

Chemical determination of choline: The method of ACKWE and ERNST (10) was used with minor modifications. Hexane extracted flakes were ground and extracted with water overnight. After centrifugation, the supernatant was treated 3 times with 20 ml of ether, then 67% HNO_3 added (sufficient to give a final conc. of 20% HNO_3), and refluxed for 3 h to hydrolyze polymers such as proteins and carbohydrates. After neutralization with 33% NaOH, 10 ml of sample and 0.5 ml of KI_3 solution (157 g I_2 and 200 g KI in 1 l dist. water) were mixed under ice and the choline enneaiodide crystals collected quantitatively by centrifuging, decanting, and washing over an Allihn filter. The crystals were then dissolved in chloroform and titrated with thiosulphate using starch as indicator: 1 ml 0.01 N thiosulphate = 0.1335 mg choline.

TLC of PL (8): Precoated TLC plates (Kieselgel 60/Kiesel F254) were activated by placing them in a 60°C oven for 90 min. Using capillaries (Drummonds), 10 or 15 μl of the samples were applied and the plates developed twice in the same direction with chloroform: methanol: water, 70: 26: 4. After drying the plates, the molybdenum reagent (15, 16) was sprayed to stain the separated PL. The amount of individual PL was determined using a densitometer (Shimadzu Dual Wavelength, TLC-Scanner CS-910) with integrator.

The Biuret method for protein determination was used according to LAYNE (19) with bovine serum albumin as standard and corrected for EDTA absorbance.

Isoelectric focusing (IF) was done using a LKB 2117 multiphor and LKB 2103 power supply with Ampholine PAG gel (pH 3.5–9.5). The electrode solutions were 1 M NaOH (kathode) and 1M H_3PO_4 (anode). In addition to the enzyme extract, a standard solution containing proteins of known pI (Pharmacia protein standard) was also analyzed. The potential and current before and after IF were respectively 210 and 1080 V, and 50 and 28 mA. Pieces of filter paper (½ × 1 cm) were dipped into the protein solution and placed on the gel. IF was carried out 2 h after which the gel was cut into 2 halves. The protein bands on one half were made visible following the MORRISSEY *procedure (20) with modifications for removing the ampholytes: 1 h treatment with 30% methanol, 10% TCA, and 3.5% sulfosalicylic acid, 2 h with 30% methanol, and 12% TCA, followed by 24 h treatment with 40% methanol and 10% acetic acid. The pH gradient was determined with the pattern of the standard proteins. The other half was cut into ½ × 1 cm pieces and the pI of Ph-D measured by placing the pieces in 1 ml extraction buffer and analyzing accoraing to the radiochemical method for Ph-D assay.*

Results and Discussion

The activity of the enzyme was investigated by: the chemical determination of the amount of choline present in the soybean samples and formed during the oil extraction processes, the radiochemical method using egg lecithin and labelled lecithin as substrates, and TLC of the products of enzymic reaction on egg lecithin which could ascertain the involvement of Ph-D in the hydrolysis. Much preliminary work was done to select a suitable method for measuring choline in the soy samples. Among the methods tried out, that of ACKER and ERNST (10) was found satisfactory and adopted. Difficulties were encountered unless samples were first defatted. Foaming was also a problem specially with the raw sample due to the soluble proteins present. The presence of soluble proteins in the raw sample also caused difficulties in the separation of water and ether layers. When HNO_3 was used to hydrolyze proteins and carbohydrates, much turbidity occured again with the raw sample while mixtures from the pretreated samples remained clear. **Fig. 1** illustrates two important points. Firstly, under the conditions of the method used, the regression lines are parallel meaning that the extractibility of choline was independent of the degree of heat treatment. The proportion of choline recovered averaged about 60% in all the samples. So, the intercepts should be increased by 1.66 times in order to obtain the amount of choline present in the samples. Secondly, the amount of free

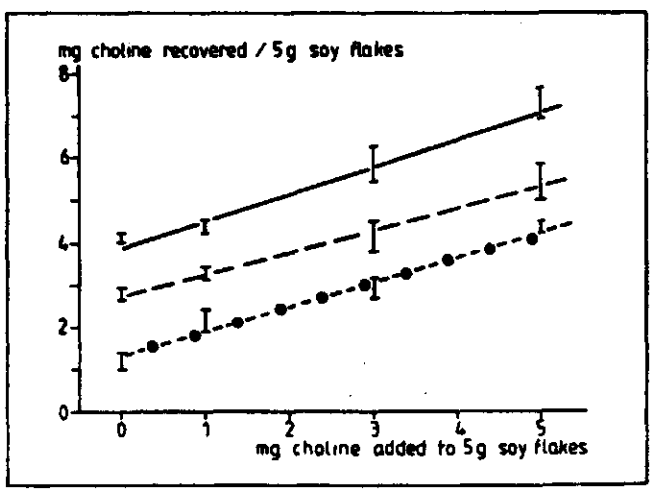

Fig. 1 Recovery of original and added choline from soy flakes

— raw flakes; - - - - infrared treated: 105°C/5 min holding; -.-.-. infrared treated: 115°C/15 min holding

choline present depended on the degree of heat pretreatment. The raw flakes after oil extraction contained 1300 ppm choline, more than three times in the samples which had received heat pretreatment for 15 min at 115°C (400 ppm). The sample with a pretreatment of 5 min at 105°C was intermediate (900 ppm). This shows that the phospholipase activity during the oil extraction processes had been higher in the raw beans with the formation of higher amounts of free choline than in the pretreated beans.

The phospholipase activity of the enzyme extracts was assayed radiochemically (**Tab. 1**). On a comparative basis, the IR pretreatment destroyed 56% (105°C/5 min) and 88% (115°C/15 min) of the phospholipase activity.

The action of Ph-D may not be the only cause of free choline formation, and therefore, the assay of Ph-D activity based solely on the determination of free choline may be misleading (7). The separation and quantification of PA by such methods as TLC gives a clear picture of the enzyme activity involved. **Fig. 2** shows the photograph of PL spots separated by TLC.

It can be seen that the egg lecithin sample showed two components to be present: PC (R_f = 0.45) and PE (R_f =0.80). The addition of commercial Ph-D produced a third spot, that of PA (R_f = 0.55) accompanied by a reduction in the amount of PC and PE. **Tab. 2** presents the densitometric determination of the concentration of PE, PC, and PA shown on **Fig. 2**. The observation that the sum of PE, PC, and PA before and after treating egg lecithin with the commercial enzyme and soy enzyme extract are similar (within the accuracy limit of the method) means that only Ph-D has acted on the substrate with PA as the only P-containing hydrolysis product. Since other phospholipases must be present in soybeans, it is clear that they were not extracted and so the extraction method appears to be suitable for Ph-D. From the result, it is also clear that there is a preferential hydrolysis of the PE by the

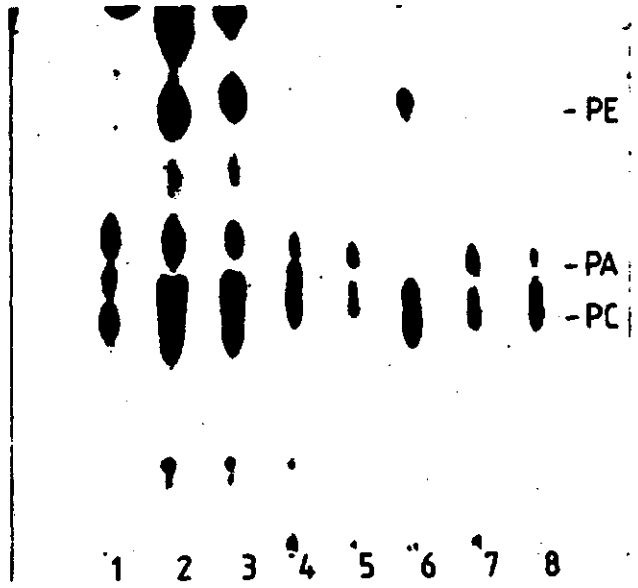

Fig. 2 Photograph of TLC plate showing phospholipids separated:
PE = phosphatidylethanolamine; PA = phosphatidic acid; PC = phosphatidylcholine

	µl applied
1–from raw soybeans	5
2–gum from pretreated beans: 105°C/5 min holding	5
3–gum from pretreated beans: 115°C/15 min holding	5
4–egg lecithin degraded by soybean enzyme extract	15
5–egg lecithin degraded by commercial cabbage enzyme	10
6–egg lecithin	15
7–egg lecithin degraded by commercial cabbage enzyme	15
8–egg lecithin degraded by soybean enzyme extract	10

Tab. 1 Phospholipase-D extracts from soybeans and their activity, influenced by heat treatment of the beans

sample	extracted protein (a)	units/mg protein (b)	% activity (c)
raw	2.3	3.8×10^{-3}	100
105°C/5 min	2.0	1.9×10^{-3}	44
115°C/15 min	1.6	0.7×10^{-3}	12

(a) g protein extracted/kg soaked beans; (b) µ moles choline liberated/min at pH 5.7 at 30°C; (c) comparative basis: activity in the raw beans = 100

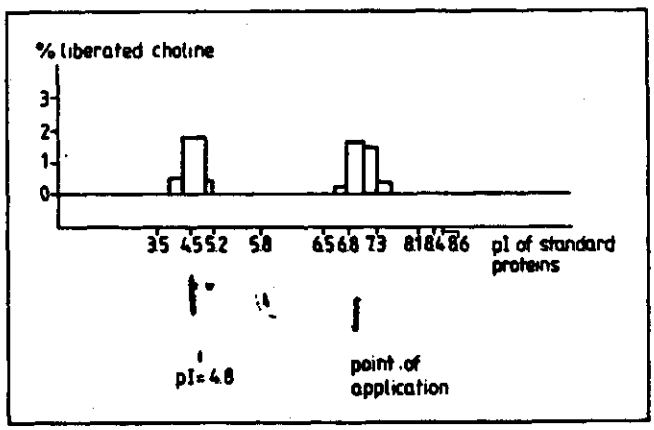

Fig. 3 Phospholipase activity at various pH values in the isoelectric focusing gel showing the isoelectric point of Ph-D

Tab. 2 Densitometric determination of the area before and after tratment of egg lecithin with commercial phospholipase-D and soybean enzyme extract (average counts from lanes 4–8 of Fig. 2)

	PE	PC	PA	Total
Substrate	10	49	–	59
commercial enzyme	6	27	18	51
soybean enzyme extract	3	46	12	61

Tab. 3 Concentration of individual phospholipids (%, i–e, g in gum from 100 g crude oil; calculated from Fig. 2 lanes 1–3 and 6)

sample	PC	PA	PE	PC+PA+PE	$\frac{PC + PE}{PA}$
raw	0.34	0.14	0.04	0.52	2.7
105°C/5 min	0.67	0.22	0.40	1.29	4.8
115°C/15 min	0.52	0.13	0.27	0.92	6.0

soy Ph-D, whereas the commercial enzyme from cabbage has hydrolyzed the same proportions of PC and PE indicating the existence of different types of PH-D in cabbage and soybeans.

Tab.3 shows the concentration of individual PL in the gums. It can be seen that whereas the ratio PC + PE to PA in the gum from the raw sample was 2.7, the ones of pretreated samples were higher: 4.8 and 6.0. The gum fractions from the latter contained more PC and less PA as a result of heat pretreatment. Moreover, the presence of PC has been reported to improve the removal of PA during water degumming (21). This was confirmed in our experiments and indicates that if the enzyme action is allowed to take place, more NHP will be formed, less PC will be available, and as a result, the capacity to remove PA will be reduced. The removal of higher amounts of NHP during water degumming in the case of pretreated samples also means that there will be less left behind in the degummed oil, making it easier to bypass the alkali refining step. Another point of interest is evident from **Tab.3** and our previous findings. We reported 1.71 and 0.07% PL respectively in the crude oil and degummed oil of pretreated 1982 beans (5) giving 1.64% PL in the gum. In the case of raw beans, the respective figures were 1.82, 0.59, and 1.23% PL. According to PRIVETT (22), PC, PE, and PA together account for 76% of the total PL of soybeans. From this we expect to have in the gum from the raw beans an equivalent of 1.03% and from the pretreated beans 1.25% of PL. The latter is in line with the 1.29% reported in **Tab.3**, but the results of the raw beans do not match at all. This can only be justified if one considers that during the preparation of raw beans and oil extraction, other phospholipases than Ph-D have also been active on PL. The enzymes produced hydrolysis products other than PA which were not determined in our present study.

Finally, isoelectric focusing was done to investigate the types of Ph-D present in the enzyme extract. The results are shown in **Fig.3** which indicate the presence of an active form of the enzyme with isoelectric point 4.8. Moreover, enzyme activity could also be measured at the point of application of the extract on the gel irrespective of the pH. Probably, some enzyme molecules had associated during the ammonium sulphate precipitation step and remained too large to migrate from the filter paper into the gel.

In conclusion, the results of this study indicate the involvement of Ph-D in the hydrolysis of PL and formation of NHP in the course of preparation of soybeans and subsequent oil extraction, and the beneficial effects of pretreatment of beans with IR heat.

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SUMMARY AND CONCLUSIONS

1.- A modified procedure for heat processing of cereals and oilseeds by infrared (IR) radiation has been developed and some of its applications described. In the conventional, continuous IR process (micronization, as it is called in industry), the material is rapidly heated to high temperatures (170-180°C and higher) and cooled immediately.

In the modified process, the material is continuously passed on the vibrating conveyor belt of the equipment under infrared burners for a short period of up to one minute with the temperature rising to 110-130°C. The heated material is subsequently held in an insulated bin where the accumulated heat can further act for achieving the processing objectives. The use of lower temperatures in the modified process than in the conventional method not only reduces energy consumption, but also minimizes the risk of damage to the heat-labile components of oilseeds and cereals (e.g. lysine) which may be caused in the conventional process.

The modified procedure was developed and investigated on a pilot plant scale on the basis of which a continuous design for industrial application has been proposed.

2.- The efficacy of the modified procedure for producing full-fat soy flour with improved nutritive value and long shelf life was studied. Antinutritional factors (trypsin inhibitors) were found to have been inactivated with no apparent damage to heat-sensitive

components as evidenced by the level of available lysine. Under the conditions studied, urease was found to be a good indicator of the extent of trypsin inhibitors inactivation. The dispersibility and solubility of proteins dropped as expected. For the assay of lipoxygenase, a modified polarographic method was developed and used to study the inactivation of the enzyme by IR treatment. Optimum pH, temperature and other conditions for the assay were also established. The activity in the well-treated beans dropped to insignificant levels in relation to shelf life. This was further confirmed by measuring peroxide value (PV) which showed negligible development of peroxides. A sensory test carried out after one year of storage showed no difference in the organoleptic properties of the stored sample with a freshly produced industrial flour. Under- and overheated samples showed extensive oxidative deterioration on storage. The oxidative deterioration in the overheated samples was possibly due to the destruction of natural antioxidants such as tocopherols at elevated temperatures. It was also established that under the conditions employed in the investigation, lipoxygenase was less heat-stable than urease and trypsin inhibitors. Therefore, any criteria for the adequacy of heat processing must be based on the inactivation of the latter. Finally, the well-treated full-fat flour was tested in white bread and judged organoleptically. The overall advantages of such a procedure in producing full-fat flour over steam processing which is the traditional method in industry include the elimination of a) drying after processing, which implies an inherent energy saving potential, and b) the inconvenience of using steam.

3.- The application of the process was further extended to the treatment of full-fat maize germ, a highly nutritious material which has recently received much attention as a food ingredient. Lipoxygenase and lipase were inactivated and the full-fat maize germ sample showed excellent storage stability for at least 18 months. The treatment caused the starch to become gelatinized, water absorption to increase, and protein dispersibility to drop, proportionally to the extent of heat treatment. Available lysine measurement was used as an indicator of overheating. The temporary stability of under-treated maize germ samples was attributed to the action of natural antioxidants (e.g. tocopherols) which are known to be abundant in maize germ and also to the low moisture levels in the products. Gas liquid chromatography of rancid maize germ oil showed that the oxidation of only minute amounts of $C_{18:2}$ and $C_{18:3}$ had been responsible for the development of rancid odour. It was concluded that the modified IR procedure could be employed to produce a shelf stable germ product with improved nutritive characteristics.

4.- Another potential industrial application for the process which was investigated relates to the treatment of soybeans prior to oil extraction. The interest in this area has intensified following the findings that in the course of conventional oil extraction from raw flakes, lipid converting enzymes, i-e, lipoxygenase, lipase and phospholipases become active and cause deterioration of oil. Phospholipase D has been implicated in the formation of nonhydratable phospholipids (NHP) which in turn necessitate the conventionally practiced alkali treatment of crude oil, a problematic step in oil refining, causing high

losses of oil and involving disposal problems. Oil from the pretreated beans showed lower free fatty acids (FFA) content and PV compared to the non-treated oils, an obvious advantage in oil refining. Moreover, substantially lower amounts of NHP were formed in the course of preparation and extraction, explicable on the basis of the inactivation of phospholipase D. Subsequently, in the refining of the extracted oil, it was found possible to circumvent the alkali step which may be regarded as an important achievement with useful industrial implications. Also, the residual defatted flakes showed acceptably low levels of trypsin inhibitor activity and could be used directly as food or feed. The significance of using good quality fresh beans for oil extraction with as little splits and damaged seeds as possible, was highlighted when it was found that the oil from the untreated 1979 beans used in the study and that from the untreated damaged and split seeds had considerably higher FFA and NHP contents than the oil from the sound untreated 1982 beans. The higher FFA and NHP quite possibly result from the action of lipase and phospholipase D on storage. The pretreatment of beans caused a slight drop in oil yield (from 18.8% to around 18.2%) almost irrespective of the extent of heat treatment. The lower yield might be remedied by a longer extraction time, but will anyway be compensated for by avoiding the losses occuring due to alkali refining.

5.- Finally, phospholipase D in soybeans was shown to cause the formation of phosphatidic acid from phosphatidylcholine and phosphatidylethanolamine. The chemical and radiochemical determination of choline liberated from phosphatidylcholine coupled with thin layer chromatographic separation and densitometric

quantification of phosphatidic acid, phosphatidylcholine, and phosphatidylethanolamine showed the significance of phospholipase D activity during oil extraction processes. As phosphatidic acid is nonhydratable, it tends to remain in the oil after water degumming and necessitates alkali refining. Isoelectric focusing showed an active soluble form of phospholipase D with pI 4.8 to be present in the enzyme extract.

In conclusion, it has been shown that the modified IR procedure developed and presented in this thesis may be used in order to produce shelf stable full-fat soy and maize germ flours with high nutritive value and improved organoleptic characteristics, and produce crude soy oil with low enough FFA, PV and NHP contents so that the alkali treatment step in the oil refining may be omitted altogether, and defatted meal with low enough trypsin inhibitor activity obtained directly.

An additional advantage of the modified process lies in its inherent potential for reducing energy consumption in comparison with the conventional IR process. A possible drawback of this process might be the relatively lower production rates compared to the conventional steaming process, making its adoption in plants with very large production rates rather problematic. The procedure could best be applied in the processing of materials at medium or small scales and for the production of specialty products.

SAMENVATTING EN CONCLUSIES

1.- Er is een gewijzigde werkwijze ontwikkeld voor de hitte-behandeling van granen en oliezaden door middel van infrarood (IR) bestraling en sommige van haar toepassingen zijn uitgewerkt. Met de bestaande IR werkwijze (micronizatie, zoals het genoemd wordt in de industrie) wordt het materiaal snel verhit tot hoge temperaturen (170-180°C en hoger) en weer snel afgekoeld.

In het gewijzigde proces wordt het materiaal onafgebroken gevoerd op een trillende transportband, die voor een korte periode (maximaal 1 minuut) infrarood branders passeert, waarbij de temperatuur van het materiaal oploopt tot 110-130°C. Het hete materiaal wordt vervolgens naar een geïsoleerde bak gevoerd, waarin de hitte verder kan inwerken om het gewenste resultaat te bereiken. Het gebruik van lagere temperaturen dan in de bestaande methode vermindert niet alleen het energieverbruik maar ook het risico van beschadiging van hitte-labiele componenten van oliezaden en granen (b.v. lysine).

De gewijzigde werkwijze werd ontwikkeld en onderzocht op een proefinstallatie, op basis waarvan een ontwerp is gemaakt voor een continu proces voor industrieèl gebruik.

2.- De doeltreffendheid van de gewijzigde werkwijze voór het produceren van volvet sojameel werd bestudeerd met betrekking tot verbeterde voedingswaarde en langere houdbaarheid. Anti-trypsinefactoren bleken

geïnactiveerd te worden zonder aanwijsbare schade voor hitte-gevoelige componenten, bijvoorbeeld het gehalte aan beschikbare lysine. Onder de bestudeerde omstandigheden bleek urease een goede indicator te zijn voor de omvang van de inactivering van anti-trypsinefactoren. De dispergeerbaarheid en de oplosbaarheid van de eiwitten daalden naar verwachting. Voor de analyse van lipoxygenase werd een gewijzigde polarografische methode ontwikkeld en gebruikt voor de bestudering van de inactivering van het enzym door de IR behandeling. Optimale pH, temperatuur en andere omstandigheden voor de assay werden eveneens onderzocht. Op de juiste manier behandelde bonen hadden een onbeduidende enzym activiteit met betrekking tot de houdbaarheid van het meel. Dit werd ook bevestigd door de bepaling van de peroxide waarde (PV), die tijdens bewaring een te verwaarlozen vorming van peroxides te zien gaf. Na één jaar bewaring werd een sensorische test uitgevoerd, die geen verschil liet zien in de organoleptische eigenschappen van het bewaarde meel en een vers bereid meel uit de handel. Onder- en oververhitte monsters vertoonden een fiks oxidatief bederf tijdens bewaring. Dit bederf was in de oververhitte monsters mogelijkerwijs te wijten aan de afbraak van natuurlijke anti-oxydanten zoals tocopherolen bij verhoogde temperaturen. Onder de omstandigheden van onze experimenten bleek lipoxygenase minder hittebestendig te zijn dan urease en de anti-trypsinefactoren. Daarom moeten alle criteria voor de geschiktheid van een hitte-behandeling gebaseerd zijn op de inactivering van de laatstgenoemde. Uiteindelijk werd het goed behandelde volvette meel getest in wit brood met een sensorische beoordeling. De belangrijkste voordelen van de gewijzigde werkwijze voor de productie van volvet meel ten opzichte van de gebruikelijke

stoom behandeling zijn a) het achterwege laten van het drogen na behandeling, wat een energiebesparing oplevert, en b) het ongerief van het gebruik van stoom.

3.- De toepassing van het proces werd verder uitgewerkt met de behandeling van volvette maiskiemen, een erg voedzaam materiaal waaraan pas nog veel aandacht is geschonken als voedselingrediënt. Lipoxygenase en lipase werden geïnactiveerd en het volvette maiskiemenmonster was uitstekend te bewaren gedurende 18 maanden. Door de behandeling verstijfselde het zetmeel, steeg de waterabsorptie en daalde de dispergeerbaarheid van eiwitten in de mate van hitte-behandeling. Bepaling van het beschikbare lysine werd gebruikt als een indicator voor oververhitting. De tijdelijke stabiliteit van de minder goed behandelde maiskiemenmonsters werd toegeschreven aan de werking van natuurlijke anti-oxydanten (b.v. tocopherolen), waarvan bekend is dat ze rijkelijk aanwezig zijn in maiskiemen, en tevens aan de lage vochtgehalten van de producten. Gaschromatografie van ranzige maiskiemolie toonde aan dat de oxidatie van slechts kleine hoeveelheden van $C_{18:2}$ en $C_{18:3}$ verantwoordelijk was geweest voor de ontwikkeling van ranzigheid. Er werd geconcludeerd dat de gewijzigde IR werkwijze gebruikt zou kunnen worden om een beter houdbaar product te maken met goede voedings-eigenschappen.

4.- Een andere mogelijke industriële toepassing van de gewijzigde werkwijze houdt verband met een hitte-behandeling van sojabonen die voorafgaat aan olie-extractie. De interesse op dit gebied is groter geworden vanwege de bevindingen dat gedurende

de gebruikelijke olie-extractie van onbewerkte flakes, allerlei enzymen zoals lipoxygenase, lipase en phospholipase, actief worden en verlies of bederf van olie veroorzaken. Phospholipase D is betrokken bij de vorming van "nonhydratable phospholipids (NHP)", dat de gebruikelijke loog behandeling van ruwe olie noodzakelijk maakt tijdens de raffinage, waardoor grote verliezen van olie en problemen met afval ontstaan. Olie van de vóórbehandelde bonen had een lager gehalte aan vrije vette zuren (FFA) en PV in vergelijking met de olie van onbehandelde bonen : een duidelijk voordeel voor de olie raffinage. Bovendien werden belangrijk kleinere hoeveelheden NHP gevormd tijdens de voorbewerking en extractie, hetgeen te verklaren is op basis van de inactivering van phospholipase D. Door de raffinage van de geëxtraheerde olie werd vervolgens gevonden dat de loog stap overgeslagen mag worden, hetgeen een belangrijk resultaat is met bruikbare industriële toepassingen. De overgebleven ontvette flakes hadden ook nog een acceptabel laag niveau in anti-trypsine activiteit en konden daardoor zonder meer gebruikt worden als voedsel of veevoer. De betekenis van het gebruik van een goede kwaliteit verse bonen voor de olie-extractie, d.w.z. zo min mogelijk halve en beschadigde bonen, kreeg bijzondere betekenis toen bleek dat de olie van de gebruikte slechte, onbehandelde bonen uit 1979, aanzienlijk hogere FFA en NHP gehalten had dan de olie van de goede onbehandelde bonen uit 1982. De hogere FFA en NHP zijn zeker mogelijk door de inwerking van lipase en phospholipase D tijdens bewaring. De vóórbehandeling van bonen veroorzaakte een lichte daling in de olie-opbrengst (van 18.8% naar ongeveer 18.2%) nagenoeg ongeacht de

omvang van hitte-behandeling. De lagere opbrengst zou verbeterd kunnen worden door een langere extractietijd, maar wordt in ieder geval gecompenseerd door het vermijden van de verliezen die optreden bij de loog stap tijdens raffinage.

5.- Tenslotte werd aangetoond dat phospholipase D in sojabonen de vorming veroorzaakte van fosfatidine zuur uit fosfatidylcholine en fosfatidylethanolamine. De chemische en radiochemische bepaling van choline, vrijgemaakt uit fosfatidylcholine, werd gekoppeld aan dunne laag chromatografie en densitometrie van fosfatidine zuur, fosfatidylcholine en fosfatidylethanolamine. Hieruit bleek de betekenis van de activiteit van de phospholipase D tijdens olie-extractie processen. Omdat fosfatidine zuur niet hydrateerbaar is, heeft het de neiging om bij de ontslijming in olie achter te blijven. Iso-electrische focusing toonde, in het enzym extract, de aanwezigheid aan van een actieve oplosbare vorm van phospholipase D met iso-electrisch punt 4,8.

Tenslotte is aangetoond dat de in dit onderzoek ontwikkelde en beschreven gewijzigde IR behandeling geschikt is om houdbare volvette soja- en maiskiemmelen met hoge voedingswaarde en verbeterde sensorische eigenschappen te produceren. Tevens kan ruwe soja-olie met een laag gehalte aan FFA, PV en NHP geproduceerd worden, zodat de stap van de loog behandeling in de olie raffinage helemaal achterwege gelaten kan worden, terwijl het ontvette meel een voldoende laag gehalte aan anti-trypsine activiteit heeft.

Een bijkomend voordeel van het gewijzigde proces in vergelijking met het bestaande proces is de mogelijkheid voor het verminderen van energieverbruik. Een mogelijk bezwaar van dit proces in vergelijking met het gebruikelijke stoomproces is het relatief lagere productiecijfer, hetgeen de toepassing in fabrieken met zeer hoge productiecijfers tamelijk problematisch maakt. De methode zou het best gebruikt kunnen worden voor de behandeling van grondstoffen op kleine of middelgrote schaal en voor de productie van specialiteitsproducten.

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