#### THE UNIVERSITY OF MANITOBA

#### FLAVOUR CHARACTERISTICS OF FABABEANS

bу

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#### A THESIS

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

#### MASTER OF SCIENCE

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#### ABSTRACT

A series of tests was initiated to determine the flavour characteristics of fababean flour and its air classified fractions. Air classification of pinmilled fababeans (Vicia faba, minor) resulted in three fractions differentiated according to protein content. The high protein concentrate contained approximately 70% protein, the flour 31% protein, and the starch-rich fraction about 7% protein. Dried pea flavour and bitter aftertaste were considered the dominant flavour characteristics of raw fababean flour by a 12 member sensory panel. From the original 12 volunteers, seven panelists were selected to participate in the remaining series of tests. Selection was based on an individual's taste acuity, ability to discriminate flavours, and internal scoring consistency. Panelists were trained to judge the flavour intensity of fababean samples using a semi-structured rating scale. To investigate the effect of composition on flavour, dried pea and bitterness flavour intensity values (FIV) were defined for the concentrate, the flour, and the starch-rich fraction of the 1973 crop of fababeans (var. Ackerperle). Analysis of covariance showed that dried pea and bitterness were more intense in the concentrate than the flour or starch-rich fraction in decreasing order respectively. absolute threshold of the three fractions in water appeared

to be of the same magnitude. Fababean flour and concentrate samples were heat-treated to determine the effect on dried pea flavour and to determine the effect on lipoxygenase activity. As heat increased dried pea FIV decreased, however, the heat treatment induced strong, objectionable flavours. Lipoxygenase inactivation increased with an increase in temperature. Comparison of dried pea FIV in flour from two crop years showed that panelists judged year-old flour significantly stronger in dried pea flavour than flour from a new crop. Fababean flour and concentrate samples held under accelerated storage conditions did not show a flavour change.

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#### INTRODUCTION

International food agencies have emphasized the need to develop protein resources on a world wide basis. Legumes high in protein content offer the greatest opportunity in terms of additional protein reserves. Since 1930, and particularly within the last decade in North America, the soybean has become the major source of good quality vegetable protein. In this regard the soybean has become important as a food protein supplement and as the main ingredient in many new processed food products.

Evidence in the literature suggests that the soybean lacks greater acceptance because of its characteristic raw beany flavour. Marketed under the name "Superburger" a soybeef mixture has been available to consumers in Canada since the spring of 1973. The product has been criticized for its flavour (Vaisey & Tassos, 1974) and consumer attitude studies show that product acceptance is limited (Woolcott et al., 1974). However, soybean TVP has been used successfully in the school lunch program in the United States. Regulations permit replacement of up to 30% of the serving of meat with TVP (Brudnak, 1974). During the 1971-72 school year 23 million pounds of TVP (hydrated) was used. The amount doubled in the 1972-73 year and an estimated 50 million pounds was used in the past year (Adolphson & Horan, 1974). In the

baking industry alone 60 million pounds of soy flour and grits were used in 1970 (Wolf & Cowan, 1971). If current trends continue the rate of increase in food uses of soybean proteins will continue to grow. Growing conditions in Canada permit production of a limited amount of soybeans. However, soybean protein products designed to replace meat or to extend meat are available to the Canadian consumer.

Since 1970 attention has focused on the fababean as a potential protein crop for the Canadian prairies. Although the fababean is indigenous to the Mediterranean basin and Western Europe, its introduction to northern growing conditions appears favourable from multidisciplinary research carried out in Manitoba and Saskatchewan. Researchers have shown the fababean to be a good yielding crop, high in protein and in terms of nutritive value, a good quality protein. During preliminary work with fababean flour, wet batters and doughs exhibited undesirable odours which after baking appeared to be replaced by a bitter taste. Since flavour is so important to food acceptance, research on fababean flavour components was initiated.

The major objectives of the present study are as follows:

- To characterize in descriptive terms the predominant flavour characteristics in pinmilled air-classified fababeans.
- 2. To determine flavour intensity values (FIV) for the predominant flavour characteristics in pinmilled airclassified fababeans.
- 3. To determine thresholds of fababean fractions in water.
- 4. To investigate the effect of accelerated storage on

flavour intensity values and lipid composition of the fababean fractions.

5. To assess the effect of heat treatment on the FIV of the fababean fractions and in relation to lipoxygenase activity.

#### REVIEW OF LITERATURE

#### Soybean Flavour Characteristics

(a) Sensory evaluation of the raw flour, concentrate and isolate

The possible applications of a bland high protein product within the food industry are numerous. Extensive research on the soybean has been directed towards creating a product high in protein content and bland with respect to Research reported in the literature dealing specifically with the flavour of the raw soybean flour is limited. Moser et al. (1967) investigated the mechanics of various taste testing methods. To acquaint panelists with the raw beany flavour panelists tasted a small amount of raw dry soy flour. Adherence of the flour to the mouth necessitated a rinsing step. The panel decided that unsalted soda crackers, or water, or both was necessary to prevent flavour carry over between samples. Two other methods were tested. The gelation method used a 3% gel as a carrier for the raw flour sample. The slurry form was composed of 25% solids and 75% water. panel preferred the latter two methods over the dry flour as the wet methods facilitated tasting with respect to rinsing and mouth clearing between samples. A direct comparison between each method showed the dry and slurry form to be equally good and superior to the gelation method. Panelists' ability to detect soy flour in a soy/wheat blend when tasted

in the dry form was evaluated as part of the selection process. The panelists selected could detect raw soy flour at a concentration of one part soybean to 500 parts wheat flour.

Kalbrener et al. (1974) studied the flavour properties of commercially available soy flour, concentrates, and isolates. The sensory taste panel described their flavour as beany, bitter, chalky and astringent. Sample detection thresholds, that is, the concentration of sample where the panel could detect a flavour other than water, were determined for 4 soy samples. Since beany and bitter were considered the most objectionable flavours in soy products, these thresholds were determined as well. Table I contains the three flavour thresholds of the commercially available soybean products. The authors concluded that the objectionable flavour constituents were intense since they were detectable at very low concentrations. As well, the sensory data showed that a truly bland soy protein product was not commercially available.

#### (b) Factors influencing flavour

#### (i) steaming

Moser et al. (1967) hypothesized that the application of heat reduced the raw beany flavour of the soy flour. Therefore, the effect of steaming on flavour was determined in another series of tests. Panelists were instructed to rate the intensity of the overall flavour and describe the flavours present. The scoring system used was based on a 10 point scale with 10 representing the blandest flavour and 1

TABLE I

FLAVOUR THRESHOLDS OF SOYBEAN PRODUCTS
(KALBRENER et al., 1971)

	THRESHOLDS a			
SAMPLE	Sample detection %	Beany %	Bitter %	
Flour H	0.005	0.033	0.04	
Concentrate D	0.04	0.16	0.20	
Isolate E	0.06	1.25	3.00	
Isolate F	0.06	0.20	2.00	

 $<sup>^{\</sup>mathrm{a}}$  Percent sample in charcoal-filtered tap water.

<sup>&</sup>lt;sup>b</sup>Codes designated by the author.

the strongest. No steaming resulted in a low grade of 1.5 with beany, bitter and green the flavour descriptors. The flour steamed for 40 minutes received a grade of 6.1 which indicated a decrease in the raw beany flavour. The flavour descriptors for the 40 minute steamed sample were beany, nutty, bitter, toasted and sweet. From the panel scores it is evident that increased time of steaming decreased the intensity of the undesirable raw beany flavours.

#### (ii) enzyme activity

Mattick and Hand (1969) contend that the objectionable "green beanlike" flavour is not present in the whole soybean but develops after mechanical disruption of the bean tissue. The authors cite enzymatic action such as occurs in the horseradish and the onions as a probable factor in the development of this undesirable flavour. Using gas chromatography and mass spectroscopy the authors identified ethyl vinyl ketone as the major volatile contributing to the green bean odour and flavour.

The storage stability of full fat soy flour has represented a major problem to its greater use in human foods.

Mustakas et al. (1969) demonstrated that the inactivation of lipoxygenase in the whole bean by heat treatment prior to grinding resulted in a full fat flour free of rancid odours and flavours. Since lipid deterioration is cited as a major cause of off flavour development Rackis et al. (1970) extracted 99.8% of the oil from full fat soybean flakes and evalu-

ated the flavour of the major lipid components. The components had little flavour and attempts to identify the origin of the green beany flavours were not conclusive. The authors suggest that these flavour characteristics pre-exist in the whole bean since the low level of lipid oxidation that occurs contributes little to overall soybean flavour when dry processed. If the product was wet processed, lipid oxidation products could be expected to contribute significantly to the strong objectionable flavours since the rate of reaction is increased in the presence of water. The authors concluded that secondary extraction removed most of the objectionable flavours.

Lipoxygenase activity has been linked to the oxidation of unsaturated fats and the development of undesirable flavours. Rackis et al. (1972) used a trained sensory taste panel to define flavour intensity values using a scale of 1 to 3 where weak was assigned a score of 1 and 3 was strong, for the beany, bitter flavours in maturing soybeans. The panel tasted the whole raw bean in an attempt to ascertain at which stage during maturation the beany, bitter factors appeared. The possible relationship between beany and bitter flavour development and the lipid transformation which occurs in the maturing bean was not confirmed. The beany flavour intensity value did not correlate with lipoxygenase activity, however the data showed a positive correlation (r = 0.73) between lipoxygenase activity and the increase in bitterness flavour

intensity as the beans matured. Sessa et al. (1974) suggest that the bitter taste present in the soybean involves the oxidation of the phospholipid component. Autoxidation of soybean phosphatidylcholine and its subsequent storage resulted in the development of a bitter taste as judged by a sensory taste panel. Soybean lipoxygenase catalyzed the oxidation reaction. Kalbrener et al. (1974) prepared linoleic and linolenic hydroperoxides using soybean lipoxygenase. The authors used a trained sensory panel to evaluate flavours associated with the hydroperoxides. The sensory data shows that soybean lipoxygenase produces flavours similar to the flavour of the raw soybean flour. The hydroperoxides and their breakdown products had flavours similar to the grassy/beany type in raw soybeans. From the sensory data lipoxy-genase activity appears to influence flavour.

Although research has advanced complicated methods designed to eliminate the characteristic flavour, no process to date is economically feasible according to the major soybean producers (Maga, 1973).

#### Fababean Research

Research reported in the literature on fababeans grown on the Canadian prairies is limited. Crop yields of fababeans grown in Manitoba and Saskatchewan have been compared. Evans et al. (1972) reported that the mean test yield of several cultivars of fababeans was significantly higher than that of

the high-yielding wheat cultivar Glenlea. There was also a significant difference in yield within the same fababean cultivar grown in different locations. Bhatty (1974) analyzed twelve cultivars of fababeans for gross composition. Among the cultivars tested protein content varied from 26 to 35%, total carbohydrate ranged from 55 to 61%, with fat, fiber and ash contents remaining approximately the same. McEwen & Bushuk (1973) reported a method for dehulling the beans by dry milling using an Allis Chalmers mill. The result was a 75% recovery of flour with negligible quantities of hull.

The low fat content, about 1.3% (Presber, 1972) eliminates an initial fat extraction step, permitting direct dry milling of the dehulled beans. Air classification of the flour results in fababean fractions with different protein contents. Craig (1974) reported that dehulled beans ground in the pin mill yielded a flour with approximately 31% protein. Subsequent air classification of this flour yielded a concentrate fraction with about 70% protein and a starchrich fraction with 20% protein.

#### (a) Nutritive quality

Fababean proteins have a well-balanced amino acid composition except for low levels of methionine typical of most legumes. Relative to wheat the fababean has a higher lysine content, as shown in Table 2 (Evans et al, 1972). If added to wheat, the fababean proteins would improve the nutritional value of the wheat. A 9 percent replacement of wheat flour

TABLE 2

AMINO ACID COMPOSITION OF FABABEANS AND WHEAT IN PERCENT OF TOTAL PROTEIN (EVANS et al., 1972)

Amino acid	Horsebean (cult. Columba)	Hard red spring wheat (cult. Manitou)
Lysine	6.84	2
·		2.58
Histidine	2.78	2.39
Arginine	10.26	4.36
Aspartic acid	11.90	5.10
Threonine	3.85	2.94
Serine	5.30	4.91
Glutamic acid	18.30	36.40
Proline	4.61	12.09
Glycine	4.75	4.34
Alanine	4.56	3.64
Valine	4.78	4.43
Methionine	0.86	1.61
Isoleucine	4.25	3.83
Leucine	7.96	7.58
Tyrosine	3.31	2.54
Phenylalanine	4.53	5.55

by an equal weight of the high protein concentrate fraction increased total protein of a wheat bread (12 vs. 18% dry basis) and markedly improved protein quality. The average daily weight gains of rats fed this enriched bread as the sole source of protein were significantly higher than for those fed wheat flour bread (McDonald, 1974).

#### (b) Sensory quality of fababeans

Research cited up to this point has dealt primarily with the agronomic characteristics and nutritive value. Sensory quality has been evaluated in different ways. A consumer taste panel judged wheat bread enriched with the high protein fababean concentrate (9% replacement) inferior to commercial white bread. The bread enriched with fababean concentrate was criticized for its bland flavour, bitter after-taste, and its sticky or gummy texture. Mean hedonic scores were low for the enriched bread but within the "like" area of the scale. The 9-point scale ranged from "like extremely" given a score of 9, to "dislike extremely" with a score of 1. The dough exhibited undesirable odours and the sticky nature of the dough made handling difficult (McConnell et al., 1973).

Secondary processing of fababean fractions before inclusion in a food product improves flavour. A sheeted product can be made from a thick slurry of fababean concentrate and water. Craig (1974) points out that the improvement in flavour is a result of the wetting and drying involved. The fababean slurries are drum dried to produce the thin sheet-like

product which can be easily crumbled into flakes for incorporation into ground beef patties or wieners (Sumner et al., 1972). Vaisey & Tassos (1974) used the fababean concentrate flake to replace the soybean TVP in a superburger formulation (70% ground beef, 10% soybean TVP, 20% water). Inclusion of the fababean flake improved acceptability over those patties which contained raw fababean concentrate in the same proportion. Patties with the raw concentrate added were criticized for their cereal-like, bitter flavour. The mean hedonic scores (n = 90) for the superburger and the meat patties containing fababean flakes were not significantly different. Therefore processing the fababean concentrate prior to its addition in the meat pattie offered some improvement in flavour over the raw fababean concentrate.

#### (c) Lipid composition of fababeans

Table 3 contains data from the proximate analyses of fababean flour and its air classified fractions. Total fat is low and is associated quantitatively with protein. The fatty acid composition of fababean lipids is shown in Table 4. Eighty-four percent of the fat is unsaturated. Fifty-five percent of the total fat is present as linoleic acid which is particularly susceptible to oxidation.

Lipid degradation is a common cause of product deterioration. Unsaturated fats in particular have been linked to off flavour development. Presber (1972) cites the high level of unsaturated fats as the major cause of rancidity in the

TABLE 3

PROXIMATE ANALYSES OF AIR-CLASSIFIED FRACTIONS
OF PINMILLED FABABEAN FLOUR (g/100 g)
(VAISEY and McDONALD, 1974)

	•		
Fababean Fraction <sup>1</sup>	Moisture	Protein	Fat
Concentrate	4.80	68.90	2.50
Flour	5.16	31.96	1.14
Starch-rich	5.24	7.56	0.48

<sup>&</sup>lt;sup>1</sup>Blend of varieties, predominantly Ackerperle, 1973 crop.

TABLE 4

FATTY ACID COMPOSITION OF FABABEAN LIPIDS (1973 CROP) (VAISEY and McDONALD, 1974)

		% Methyl Esters
Fatty Acid		Canadian beans
Lauric	C12:0	trace
Myristic	C14:0	0.30
Palmitic	C16:0	13.43
Stearic	C18:0	1.92
Oleic	C18:1	24.16
Linoleic	C18:2	55.12
Linolenic	C18:3	4.73
Tota1		99.66
% Saturated		16%
% Unsaturated		84%

ground stored fababean. The author offered no supportive data and based his conclusion on an opinion survey of 4 processors in England.

Eskin and Henderson (1974a) confirmed the presence of lipoxygenase in fababeans. Lipoxygenase, in the presence of molecular oxygen, catalyzes the oxidation of cis, cis 1, 4-pentadiene systems such as linoleic acid to conjugated cis, trans hydroperoxides and their subsequent breakdown products. The authors have demonstrated that the lipoxygenase present in fababeans is specific towards linoleic and linolenic acids. Lipoxygenase present in the fababean appeared similar to lipoxygenase-2, a type present in soybeans with respect to isoelectric point, electrophoretic mobility, and molecular weight. Based on the similarities between fababean lipoxygenase and lipoxygenase-2 active in soybeans, Eskin and Henderson concluded that enzymic activity was a factor in the development of oxidative rancidity in ground fababeans.

As mentioned earlier, preliminary work with fababean flour indicates that it has a characteristic legume flavour similar to soybeans. Vaisey et al. (unpublished data) reported that raw fababean flour when tasted as a 5% (W/W) flour in water slurry had a green pea, or beany taste, slight sweetness, bitter aftertaste and astringency. Eskin and Henderson (1974b) have confirmed the presence of a lipoxygenase in fababeans which is active towards linoleic and linolenic acids. This enzyme has been associated with the development of off flavours in soy products (Mustakas et al., 1967) and

accordingly might be expected to contribute to similar problems in the fababean. Since high protein soy products are increasing in acceptance it is reasonable to assume that the fababean offers Canada the same potential in terms of a high quality vegetable protein if flavour problems can be solved.

#### METHODS AND MATERIALS

#### Preparation of Fababean Fractions

Fababean flour samples were obtained from the Prairie Regional Laboratories of the National Research Council in Saskatoon, Saskatchewan. Fababeans (Var. Ackerperle) were dehulled and pinmilled (Alpine Contraplex Wide Chamber Air Mill, Model A250CW) to yield a finely ground flour. classification (Alpine Milcroplex Spiral Air Classifier, Model 132MP) of this flour yielded two additional fababean fractions. The air stream separated the lighter protein fraction from the heavier starch molecules. Starting with 100 pounds of fababean flour, the first pass through the air classifier yielded 78 pounds of the starch-rich fraction containing 20% protein and 22 pounds of the high protein fraction containing 70% protein. Regrinding of the starch followed by a second pass through the air classifier yielded 59 pounds of the starch-rich fraction containing 4% protein and 19 pounds of the high protein fraction containing 65% protein. Therefore, this double pass system yielded 41 pounds of the high protein concentrate fraction containing 68% protein and 59 pounds of the starch-rich fraction containing 4% protein. Additional passes through this system yields flour, concentrate and starch with different protein contents.

Fababeans from the 1972 crop and the 1973 crop were pinmilled and air classified. The proximate analyses of the 1973 crop used for the taste panel evaluation is shown in Table 3.

#### Preparation of Slurries and Sensory Evaluation Technique

Fababean flour slurries consisted of fababean flour at different concentrations in glass distilled water (W/W). Flour was incorporated into a water slurry and mixed using a magnetic stirrer until all the solids were dispersed. The slurry was then filtered through Kim Wipe tissue paper for tasting. The high protein concentrate and the starch-rich fractions were prepared according to this same procedure. Slurries were presented to the taste panel in 3/4 oz. plastic creamers with foil lids which permitted tasting through a plastic straw without colour bias or odour loss. were coded using three digit random numbers. Coded samples were then randomized for presentation to the panel for each test series. Throughout the experiment an identified bland reference sample was presented to the panel along with the test samples. The control or bland reference was a 5% (W/W) wheat flour slurry which when filtered was similar in textural qualities to the fababean slurries.

#### Rinsing technique

Unsalted soda crackers and tap distilled water were used for mouth clearing and rinsing. Panelists chewed a small portion of an unsalted soda cracker, rinsed with water and rested 30 seconds before tasting the next sample.

#### Sensory Panel Selection

Twelve adult females volunteered to participate in a test series designed to select a taste panel for the sensory evaluation of fababean flour and its air classified fractions. Selection was based on an individual's taste acuity, ability to discriminate flavours, and internal scoring consistency.

#### Taste acuity

Solutions of the four basic tastes, sweet, sour, salty and bitter, were used to test individuals' taste acuity.

Table 5 contains the concentrations in percent of the 4 basic solutions. Panelists were asked to identify the basic taste associated with each coded sample solution.

#### Flavour discrimination

A series of tests was designed to assess the prospective panelists' ability to discriminate flavours. Fababean flour and wheat flour were combined in varying concentrations in a 5% total flour in water slurry (W/W). Each panelist was presented with 6 coded samples containing the various concentrations of flour in water and an identified reference sample of wheat flour (5% W/W) and water. Panelists were asked to state for each test sample whether it was identical to, or different from the reference sample. Table 6 contains the proportions of each flour used. The ballot used is shown in Figure 1. The detection threshold was taken as the concentration of fababean flour at which 50% of the panelists

TABLE 5

PERCENT CONCENTRATION OF THE 4 BASIC TASTES USED FOR PANEL SELECTION

Basic Taste	Compound Used	Percent	Concentration
Salty	Salt		0.200
Sour	Citric acid		0.070
Sweet	Sucrose		2.000
Bitter	Quinine sulphate		0.001

TABLE 6

## FABABEAN AND WHEAT FLOUR BLENDS USED IN THE PREPARATION OF SLURRIES FOR THE FLAVOUR DISCRIMINATION TEST IN THE PANEL SELECTION PROGRAM

### Flour source in 5% flour/water slurries (g/95g HOH)

Fababean	Wheat	
0.0	5.0	
0.2	4.8	
0.4	4.6	
0.6	4.4	
0.8	4.2	
1.0	4.0	

Name:			Date:_			
You have be	en given a r	eference sa	imple mar	ked <u>R</u> to	which	you
are to comp	are each sam	ple. Test	each sam	ple and s	how	
whether it	is identical	to or diff	ferent fr	om the re	ferenc	e
sample R.						
Sample Number						
Identical to <u>R</u>			·····		Paralle and the State and the	
Different from <u>R</u>						

Figure 1: Ballot used for flavour discrimination test in the panel selection program.

correctly identified the coded sample as different from the all-wheat control.

#### Dominant flavour characteristics in fababeans

Dominant flavour characteristics of fababean flour were defined by the twelve volunteers. Subjects were presented with a 5% fababean flour in water slurry (W/W) and were asked to describe in their own words the overall flavour of the sample. Subsequent agreement on the dominant flavour characteristics was reached by group discussion. The sample used for this test series was flour pinmilled from a composite of beans (variety Ackerperle) collected from 10 growing locations in Manitoba. The sensory taste panel decided that dried pea and bitterness were the predominant flavour characteristics of fababean flour.

#### Flavour intensity values

Panelists were trained to judge the flavour intensity of fababean flour samples from the 1972 crop year using a semi-structured rating scale. The ballot used is shown in Figure 2. Each panelist judged the degree of flavour difference between each test sample and the identified bland reference by marking the appropriate point on a line 10 cm. long. The score ranged from bland to strong with individual measurements ranging from 0 to 10 taken as a direct measure in centimeteres from the zero or bland end of the line. A flavour intensity value (FIV) is the mean score for all panelists for one sample for a specific flavour characteristic.

Name:	Date:	
	You have been given a BLAND reference sample. Taste the reference and then taste each of the other samples. Mark the intensity of the flavour characteristic (dried pea or bitterness) of each sample.	
CODE:	bland	strong
	bland	strong

Figure 2: Flavour intensity value ballot used throughout the entire experiment.

Flavour intensity values were defined for the dominant flavour characteristics dried pea and bitterness.

#### Scoring Consistency

Internal scoring consistency was measured according to the method devised by Kendall (1962). Flavour intensity values for each panelist were converted to ranks and the coefficient of concordance (W) was calculated.  $W = \frac{12S}{m^2(n^3-n)}$  where S is the sum of squares of the deviations; m refers to the number of rankings, and n is the number of replicates. The coefficient ranges from 0 which indicates poor agreement, to 1 which is perfect agreement. This score can be converted to a  $\mathbf{x}^2$ .  $\mathbf{x}^2 = m(n-1)W$  with n-1 degrees of freedom. The  $\mathbf{x}^2$  statistic was used as the test of significance for concordance. Those individuals who were consistent (significant at the 5% level) over the 6 replicates were invited to remain on the panel.

#### Calculated number of replicates

The standard error of a treatment mean is an index of the value or the amount of information in an experiment.

Accordingly, analysis of variance was calculated for each panelist selected, using the 1972 dried pea flavour intensity values over 6 replicates. The individual with the largest error mean square provided the basis on which the number of replications was calculated. The coefficient of variation measures the relative amount of variation in an experiment.

Since tasting is quite variable in nature a 30% coefficient

of variation was considered acceptable and useful as an estimate of the relative amount of variation in panelists' responses. For this experiment the standard error is the appropriate measure of variation. Using the following formula the number of replicates (n) was calculated based on the panelist with the greatest amount of error and a 30% coefficient of variation.

$$\frac{S\bar{x}}{\bar{x}} \text{ where } S\bar{x} = \frac{S}{\sqrt{n}}$$

The mean  $\bar{x}$  is that for a panelist and S is the square root of the mean square for error and n is the number of replications.

The number of replications is obtained by solving

$$\frac{S}{\bar{x}\sqrt{n}} = 0.3$$

Using actual experimental válues the number of replications was calculated as follows.

$$n = \frac{s^2}{\bar{x}^2(0.3)^2} \qquad n = \frac{5.9101}{(4.452)^2 (0.3)^2} = 3.313$$

Therefore the number of replicates calculated necessary for the coefficient of variation to approximate 30% is 4 in this experiment. With greater variation among panelists responses more replicates would be required. Throughout the experiment a series refers to 4 replications of the same test.

#### Panel Training

The initial selection period served as training for the 7 panelists selected from the 12 volunteers. The number of

panelists varied from five to seven throughout the entire study depending on their availability. The number of panelists participating in each test series is specifically noted in the results and discussion.

## Flavour Intensity Values of Fababean Fractions (1973 crop) Sample preparation

Flavour intensity values (FIV) for the predominant flavour characteristics dried pea and bitterness were defined for the concentrate, the flour and the starch-rich fraction of the 1973 crop of fababeans (var. Ackerperle). The sixmember trained panel tasted fababean slurries at concentrations varying from 0.35% to 1.75% of the fababean fraction in water (W/W). Panelists were presented with six test samples and an identified bland reference sample. This section consisted of six series of tests, a separate series for each flavour characteristic (dried pea and bitterness) and for each fababean fraction.

#### Statistical analysis

For each series, sources of variation were identified by the analysis of variance. Analysis of covariance which contains the elements of regression and analysis of variance was applied to the observations to determine the relationship between the concentration of the fababean sample in water and the corresponding FIV for dried pea and bitterness as judged by the panel. By applying regression analysis to the sensory data the nature of the relationship was determined. Using

simple linear regression, predicted dried pea FIV and bitterness FIV were calculated from the observed values. Analysis of covariance compared dried pea FIV between each fraction and bitterness FIV between each fraction. As well dried pea and bitterness FIV were compared for the same fraction.

#### Threshold of Fababean Fractions in Water

Threshold detection of fababean flour, concentrate and the starch-rich fraction in water were determined by the panel. Table 7 contains the solution concentrations of the fababean flour fractions. Solutions were prepared volumetrically from an initial concentration of 0.4% for each fababean fraction in water. The bland control or reference sample was a 0.4% (W/VOL) slurry of wheat flour in water prepared to simulate the texture of the fababean concentrations used. Panelists were instructed to taste the reference sample and then a test sample. Panelists indicated whether the test sample was identical to or different from the reference. A coded wheat control was included in the test samples as a check on panel performance. Simple linear regression analysis was used to calculate predicted threshold values from the observed values. The absolute threshold (detection) is defined as (ASTM, 1968) the minimum detectable level or concentration of a substance. The absolute threshold for each fababean fraction was determined graphically from the point at which 50% of the panel correctly identified the test sample as being different from the wheat reference.

TABLE 7

PERCENT (W/V) OF FABABEAN FRACTION IN WATER USED TO DETERMINE ABSOLUTE THRESHOLDS

(% (W/V)	Fababean Fraction in glass distilled water)	Reference wheat flour in water
	0.40	0.4% (W/VOL)
	0.20	
	0.10	
	0.05	
	0.00	

#### Heat Treatment of Fababean Flour and Concentrate

Sample preparation and presentation

To investigate the effect of heat treatment on flavour and enzyme activity fababean flour and concentrate from the 1973 crop were heat treated. Twenty gram samples of the flour and concentrate were incubated at different temperatures in pyrex petri dishes (lids on) for varying lengths of time.

Tables 8 and 9 contain the times and temperatures for both fractions. After the heat treatment samples were cooled uniformly, transferred to Scotchpak cellophane pouches (size 6" x 8") and held at -40°C until the taste testing. Duplicate samples were prepared for taste testing and chemical analysis.

#### Lipoxygenase assay

Lipoxygenase activity of all flour and concentrate samples was measured according to the method outlined by Eskin and Henderson (1974). All lipoxygenase assays measured oxygen uptake with a polargraphic oxygen electrode at 25°C using a YSI model 53 biological oxygen monitor. The linoleic substrate placed in the reaction chamber was oxygenated at 25°C for 1.25 minutes. Enzyme solution (10-30 ul) prepared from crude acetone powder was added and the oxygen uptake recorded. The specific activity is expressed at the mM of oxygen uptake per minute per mg. protein. At zero time all substrate solutions were 100% oxygen saturated. At 25°C, the concentration of oxygen in a 100% oxygen saturated aqueous solution is 1.26 mM (Yamaguchi et al., 1969). Protein present

TABLE 8

INCUBATION TIME AND TEMPERATURES USED TO INACTIVATE LIPOXYGENASE IN FABABEAN FLOUR

Temperature (°C)	Incubation T	Time	(minutes)
70	15, 3 15, 3		
90	15, 3	30,	60
130 140	15 <sup>*</sup> , 3	30,	-
150 160	15 <sup>*</sup> , 2		
Control	(No he	at)*	

 $<sup>^*</sup>$ Samples selected for taste panel evaluation.

TABLE 9

INCUBATION TIME AND TEMPERATURES USED TO INACTIVATE LIPOXYGENASE IN FABABEAN CONCENTRATE

Temperature (°C)	Incubation Time (minutes)
90	15 <sup>*</sup> , -
110	15, -
130	15*, 30
140	15, 30
150	15*, 30
160	15*, -
Control	(No heat)*

in sample solutions was determined by ultraviolet absorption.

A control sample which was not heat treated was included in each assay. Specific activity was subsequently expressed as percent inactivation.

#### Taste panel evaluation

Due to time limitations not all heated samples could be evaluated by the panel. Therefore, samples of flour and concentrate were selected which covered a range in enzyme inactivation from 0 to approximately 100% inactivation. The samples selected and the heat treatment required for both flour and concentrate is shown in Tables 8 and 9. The panel tasted fababean slurries at a constant concentration of 1.5% of the heat treated fababean flour in water and fababean concentrate in water. Panelists defined dried pea flavour intensity values for the flour and the concentrate in separate test series.

#### Statistical analysis

Analysis of variance identified sources of variation and a multiple range test identified samples judged significantly different with respect to dried pea flavour intensity.

#### Crop Year Comparison

Dried pea flavour intensity patterns were defined for dried pea flavour in flour from two crop years. Panelists were presented with 5% total flour in water slurries with increasing replacement of wheat flour by fababean flour in comparison to an all-wheat flour control. Dried pea FIV were

defined for flour held under ambient conditions for one year (1972 crop) and in another series dried pea FIV were defined for freshly milled flour from a new crop (1973 crop). The concentration of the fababean flour used is shown in Table 10.

#### Accelerated Storage Study

Sample preparation and presentation

An accelerated storage study was initiated to investigate changes in flavour under controlled storage conditions. One hundred gram samples were measured into Scotchpak pouches. Fababean flour and concentrate from the 1973 crop with 31 and 62% protein respectively were equilibrated to 65% relative humidity under controlled conditions. Additional flour and concentrate samples were equilibrated to the relative humidity or ambient humidity conditions of the laboratory. After equilibration the pouches were heat sealed and held at 38°C. ples were taken out each week for twelve weeks and held at  $-40^{\circ}$ C until the time of testing. At the time of equilibration of the samples to the relative humidities, moisture content was determined. Subsequently in the preparation of the slurries for panel evaluation the amount of sample used in a slurry was adjusted to account for the increased water content. Due to time limitations, samples were selected at the 2, 4, 6, 8 and 10 weekly intervals for taste panel evaluation. Panelists judged the dried pea FIV of the fababean fractions for the two relative humidity conditions.

TABLE 10

PROPORTIONS OF FABABEAN AND WHEAT FLOUR BLENDS
IN WATER SLURRIES USED IN CROP YEAR
COMPARISON SERIES

### 5% Flour/water Slurries (% W/W)

Fababean Flour	Wheat Flour	нон
0.0	5.0	95
0.3	4.7	95
0.7	4.3	95
1.0	4.0	95
1.3	3.7	95
1.7	3.3	95

Free fatty acid composition

Total lipid was extracted from flour and concentrate samples held under accelerated storage using an alcohol, ether method adapted from A.O.A.C. number 13033 (A.O.A.C., 1960). The free fatty acids in the flour and concentrate were separated from the other major lipid components using silica gel H on thin layer chromatographic plates. Plates were developed in a mixture of petroleum ether-diethyl etherglacial acetic acid 82:25:2 (V/V/V). The free fatty acids were methylated (Metcalfe et al., 1966) and then separated and quantitated by gas chromatography using heptadecanoic acid (C17:0) as an internal standard. Free fatty acids were measured on flour and concentrate fractions at week 0, 2, 10, and 12 of the samples that were equilibrated to 65% R.H. before storage at 38°C.

#### Additional Analysis

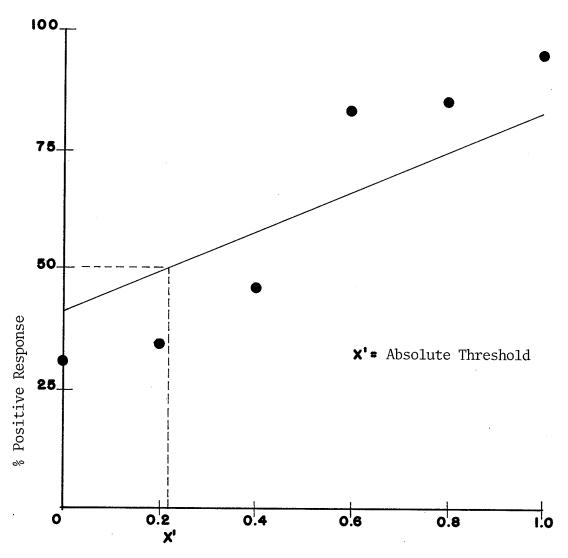
Components of fababean lipids in flour and concentrate fractions were separated by thin layer chromatography. Bands of phospholipid, free cholesterol, triglycerides and sterol esters were removed from the plate completely and added directly to an oxidizing dichromate reagent. The concentration of chromic ion resulting from the oxidation of the lipid component was measured by colorimetry on a Pye Unicorn SP600 spectrophotometer (U.S. Air Force School of Aviation Medicine Research Report #56). Lipid standards were used to calculate the amount of each lipid component in the sample.

#### RESULTS AND DISCUSSION

#### Sensory Panel Selection

Solutions of the four basic tastes which were presented to the panel were correctly identified by all 12 volunteers. The flavour discrimination series was designed to assess individuals' ability to detect fababean flour in a fababean/wheat flour blend. Regression analysis showed that there was a significant linear relationship between percent concentration of the fababean flour in the blend and a corresponding increase in panel response. Therefore predicted values were calculated based on the observed values. The absolute threshold was determined at the point at which 50% of the panel correctly identified the test sample as different from the control. The determination of the thresholds is illustrated in Figure 3.

In another series, the 12 volunteers described in their own words the flavour of the raw fababean flour. A 5% (W/W) fababean flour slurry was described as having a slightly sweet raw pea flavour which developed upon holding in the mouth into a dried pea flavour with a strong bitter taste and a lingering bitter aftertaste. The panelists agreed in a group discussion session that dried pea and bitterness were the dominant flavour characteristics of raw fababean flour.



% (W/VOL) concentration of fababean flour in a fababean/wheat flour blend in water.

Figure 3: Absolute threshold of fababean flour (1972 crop) in a fababean flour/wheat flour blend in water.

#### Flavour Intensity Values (FIV) for Fababean Fractions

To examine the effect of composition of fababean fractions on raw fababean flavour, dried pea FIV were defined for the concentrate, flour, and starch-rich fractions. six-member trained panel tasted fababean slurries at concentrations varying from 0.35% to 1.75% of the fababean fraction The relationship between concentration of each fraction and panel response is shown in Figure 4. The lines on the graph represent dried pea FIV predicted from the observed values. For each fraction there was a significant linear relationship between percent concentration of the fababean fraction and a corresponding increase in dried pea flavour intensity, as shown in Appendix A. Analysis of covariance showed a significant difference in dried pea FIV between each fraction (Appendix B). The significant difference between the slope of the concentrate lines and the other two illustrates that FIV for the concentrate increases at a greater rate. Although the slopes of the flour and starch-rich fraction appear the same, there was a significant difference in elevation between the two, and therefore the dried pea FIV was judged of least intensity in the starchrich fraction. From the sensory data it was evident that the panelists judged a dried pea flavour more intense in the concentrate than the flour or starch and in turn more intense in the flour than the starch-rich fraction.

The corresponding data for bitterness FIV for the three fababean fractions is shown in Figure 5. For each fraction

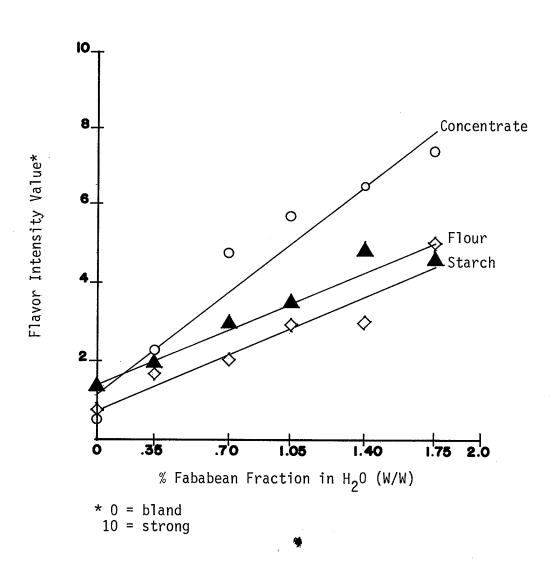


Figure 4: Dried Pea Flavor Intensity Values for Fababean Fractions

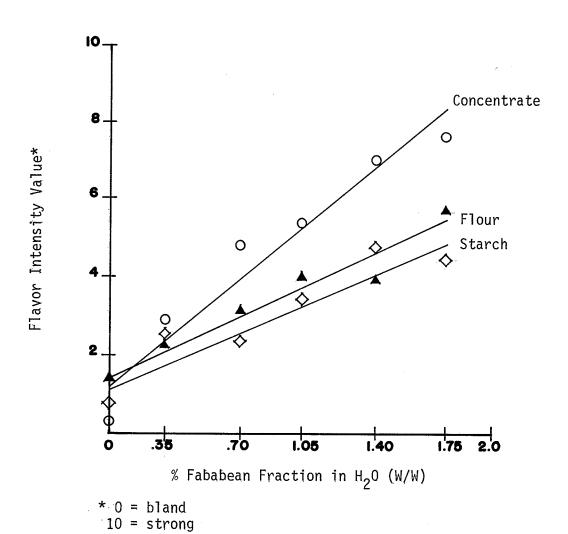


Figure 5:
Bitterness Flavor Intensity Values for Fababean Fractions

there was a significant linear relationship between percent concentration of the fababean fraction and a corresponding increase in bitterness FIV (Appendix C). Analysis of covariance showed a significant difference in bitterness FIV between each fraction (Appendix D). Bitterness was judged of greatest intensity in the concentrate and least intense in the starch-rich fraction.

It can be concluded from the sensory data that both dried pea and bitterness flavour characteristics were significantly stronger in the concentrate than in the flour or starch-rich fraction in decreasing order respectively. This order varies directly with both fat and protein content (Table 3).

Within each fraction there was not a significant difference between regression lines for the bitterness FIV and the dried pea FIV, suggesting that either the two flavour characteristics were of the same strength or the panel was not able to discriminate between the two in this test situation. A summary of the analysis of covariance applied to the sensory data is shown in Appendix E.

#### Thresholds of Fababean Fractions

Absolute thresholds for each fababean fraction in water were determined by the panel. The absolute threshold of each fraction in water was determined graphically from the point at which 50% of the panelists correctly identified the test sample as being different from the wheat reference.

Regression analysis was used to determine the line of best

fit for the observed values. There was a significant linear relationship between percent concentration of the fababean concentrate in water, and the percent starch-rich fraction in water. The lines on the graphs in Figures 6 and 7 represent predicted panel responses derived from the observed values. A corresponding relationship for concentration of fababean flour in water was not significant. Since no linear relationship was determined for the flour fraction, no predicted values were determined (Figure 8). The absolute thresholds of all 3 fractions appears to be of the same magnitude.

#### <u>Heat Treatment of Fababean Flour and Concentrate</u>

#### (a) effect on flavour

Dried pea FIV patterns of heat-treated fababean flour and concentrate samples were defined by the sensory panel. A summary of the analysis of variance and multiple range test applied to the sensory data is shown in Table 11. Panelists judged the heat-treated samples significantly different with respect to dried pea flavour. It is evident that as heat increases the dried pea FIV decreases. The dried pea FIV for both flour and concentrate are low at the 150 and 160°C levels, however these samples were far from bland. They were bland with respect to dried pea flavour but the heat treatment introduced novel flavours which were strong and highly objectionable. The relationship between temperature and dried pea FIV is shown in Figure 9. Contrary to

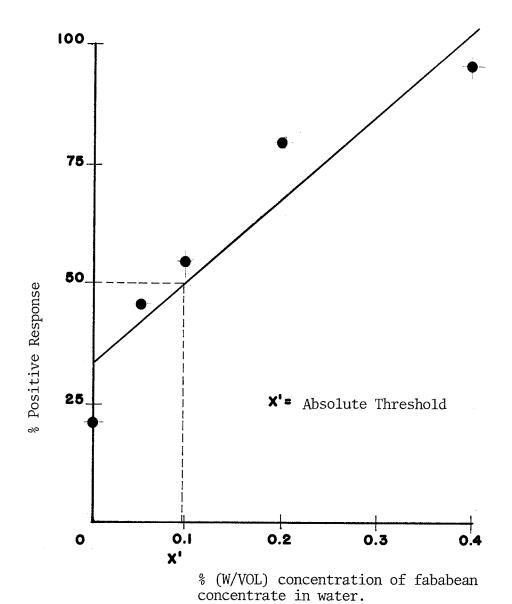
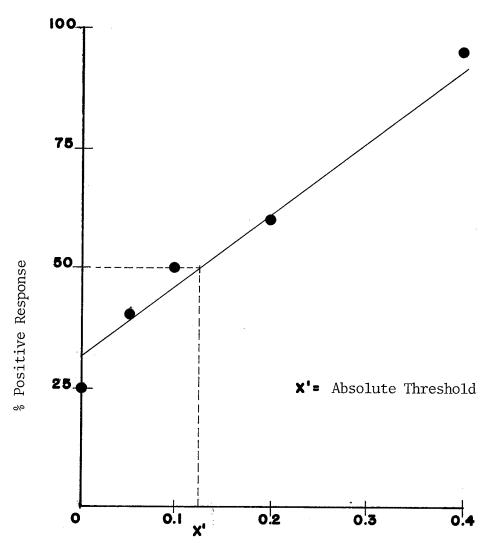


Figure 6: Concentration of fababean concentrate in water versus percent positive panel response used in absolute threshold determination.



 $\mbox{\%}$  (W/VOL) concentration of fababean starch-rich fraction in water.

Figure 7: Concentration of fababean starch-rich fraction in water versus percent positive panel response used in absolute threshold determination.

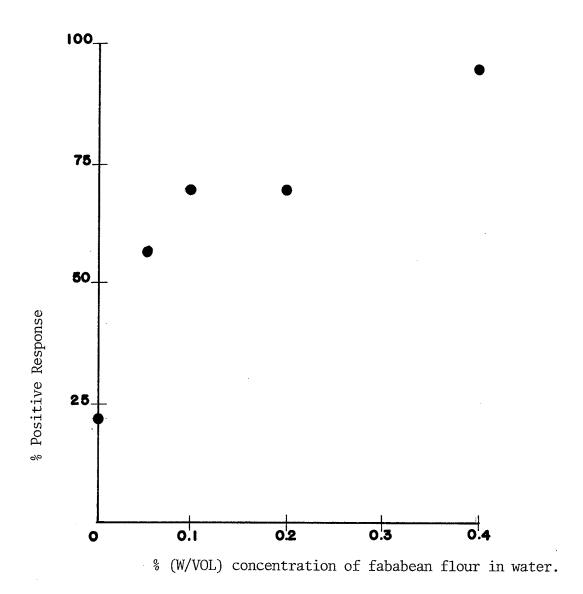


Figure 8: Concentration of fababean flour in water versus percent positive panel response.

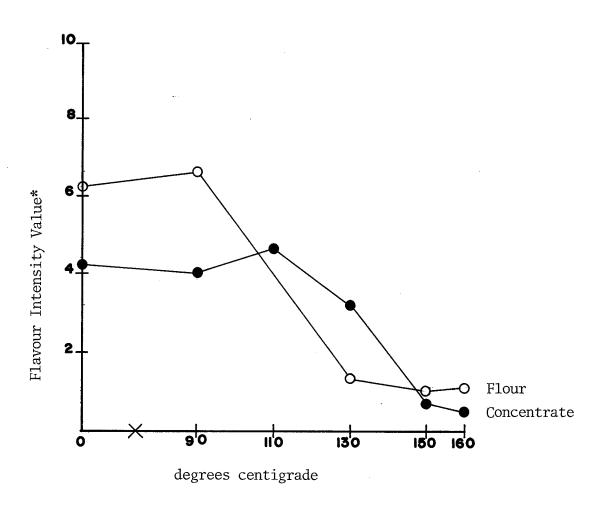
TABLE 11

DRIED PEA FIV IN FABABEAN FLOUR AND CONCENTRATE FRACTIONS WHICH WERE HEAT TREATED (15 minute incubation)

	FIV*		
Temperature	Flour	Concentrate	
o heat	6.2 <sup>a</sup>	4.2 <sup>c</sup>	
90°C	6.6 <sup>a</sup>	4.0 <sup>c</sup>	
10°C	-	4.6 <sup>c</sup>	
30°C	1.3 <sup>b</sup>	3.2 <sup>c</sup>	
50°C	1.0 <sup>b</sup>	0.7 <sup>d</sup>	
.60°C	1.1 <sup>b</sup>	0.5 <sup>d</sup>	

<sup>\*</sup>FIV Scale 0 = Bland; 10 = Strong

abcd Values in same column bearing different letters are significantly different (5% level).



\* 0 = Bland 10 = Strong

Figure 9: Relationship between temperature (incubation time 15 minutes) and dried pea flavour intensity values in fababean flour and concentrate fractions.

the dried pea and bitterness FIV series this graph reflects a lower dried pea FIV in the concentrate for the control (no heat) sample than for the corresponding flour sample. From the results of the analysis of covariance applied to the sensory data in the dried pea and bitterness FIV series, it was clear that the concentrate was judged significantly stronger in dried pea flavour than the flour fraction. Contrast error, a psychological error in judgement, may be partially responsible for the apparent discrepancy. Contrast error as described by Amerine (1965) can occur when a poor sample follows a good sample. The contrast appears greater than if the samples were judged separately. For the case of the concentrate fraction, the high intensity of the strong objectionable flavours present at the 150 and 160°C levels could have caused a decrease in the rating of the milder samples. Considering the strong nature of the heat-induced flavours, the rinsing step may not have prevented flavour carry-over in this series.

#### (b) effect on enzyme activity

Fababean flour and concentrate samples were heat-treated at different temperatures for varying lengths of time. Lipoxygenase activity in all samples was measured and subsequently expressed as percent inactivation. The results for the flour fraction is shown in Table 12. As temperature increased, the percent inactivation of lipoxygenase increased for each time of incubation. A temperature of 160°C was required to inactivate completely the enzyme in the flour

TABLE 12

LIPOXYGENASE INACTIVATION IN FABABEAN FLOUR AT DIFFERENT TEMPERATURES FOR THREE INCUBATION PERIODS

	15 Minute	Incubation	
Temperature	° C	Percent	Inactivation
Control (no	heat)		00.0
70			11.5
90			21.7
110			32.2
130			38.2
140			57.3
150			74.2
160			100.0
70 90 110 130 140	30 Minute	Incubation	21.4 27.8 29.4 86.5 100.0
	60 Minute	Incubation	
70			11.4
90			27.8
110			44.5
130			88.2

fraction. For the 30 minute time of incubation a temperature of  $140^{\circ}\text{C}$  was required to inactivate completely the sample. Complete inactivation was not achieved at the highest temperature tested in the 60 minute incubation. The corresponding data for the concentrate fraction is shown in Table 13. As with the flour samples, lipoxygenase inactivation increased with an increase in temperature. In contrast to the flour complete inactivation of lipoxygenase in the concentrate was not achieved at the  $160^{\circ}\text{C}$  level for the 15 minute time of incubation. Complete inactivation was achieved at the  $150^{\circ}\text{C}$  level for 30 minutes. Correspondingly, the enzyme in the flour was completely inactivated at the  $140^{\circ}\text{C}$  for 30 minutes reflecting perhaps a difference in volume. For the same weight of flour, the concentrate has a slightly larger volume.

Dry heat has been shown to effectively inactivate lipoxygenase in fababean flour and concentrate fractions. However, a suitable method would have to be developed to handle
samples on a larger scale. A fairly severe heat treatment
was necessary to achieve 100% inactivation of lipoxygenase
under the conditions of this experiment. Considering the
strong objectionable flavours which the heat treatment induced, a method such as spray drying, where a thin layer of
sample is rapidly heated, might be more applicable. Additional flavour studies are necessary to investigate further,
the effect of heat on the flavour of fababean fractions.

TABLE 13

# LIPOXYGENASE INACTIVATION IN FABABEAN CONCENTRATE AT DIFFERENT TEMPERATURES FOR THREE INCUBATION PERIODS

#### 15 Minute Incubation

Temperature <sup>O</sup> C	Percent Inactivation
Control (no heat)	00.0
90	1.3
110	4.1
130	19.5
140	49.3
150	78.5
160	95.0
30 Minute Incu	bation
130	94.2
140	95.9

100.0

150

#### Crop Year Comparison

Dried pea FIV for flour held under ambient conditions for one year (1972 crop) were compared to FIV for freshly milled flour from a new crop (1973). For both crop years there was a significant linear relationship between percent concentration of the fababean flour and dried pea FIV. relationship is illustrated in Figure 10. A summary of the analysis of covariance is shown in Appendix F. The data show that the panel judged the 1972 crop significantly stronger than the 1973 crop with respect to dried pea FIV. The difference in slopes indicates that the dried pea FIV for the 1972 crop increased at a significantly greater rate than the FIV for the 1973 crop (5% level). It can be concluded from the sensory data that year-old flour had a stronger dried pea flavour than did the freshly milled flour. This difference could be due to a number of factors. There could have been an inherent difference between the 2 crops. Since 1972 was the first crop pinmilled and air classified, milling was experimental in nature and extraneous material was evident in the samples and could have contributed to the flavour. A third factor could have been the fact that the flour was one year old and had been stored at ambient conditions. Lipoxygenase activity has been linked to the development of off flavours in ground-stored soybeans and accordingly might be expected to contribute to similar problems in the fababean which is known to be high in both linoleic and linolenic acids.

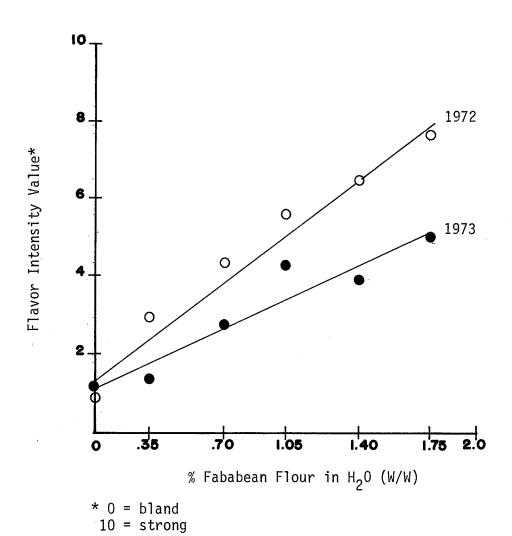


Figure 10: Dried Pea Flavor Intensity Values for Fababean Flour from Two Crop Years

#### Accelerated Storage Study

Fababean flour and concentrate samples were equilibrated to two humidity conditions, ambient conditions such as in the laboratory and under controlled conditions samples were equilibrated to 65% relative humidity. After equilibration samples were held at 38°C for varying lengths of time up to 12 weeks. Evidence in the literature suggests that fababean lipoxygenase acts specifically on linoleic and linolenic acids to produce off flavours in ground stored fababeans (Eskin & Henderson, 1974). Therefore, the accumulation of free fatty acids was monitored for the samples equilibrated to 65% relative humidity prior to incubation as shown in Table 14. Over the 12 weeks of accelerated storage conditions the total free fatty acid content of the flour and concentrate increased markedly suggesting the presence of an active lipase. It should be noted that the total free fatty acids present in the concentrate at each week measured are much greater than the total amount present in the flour over the same period reflecting higher initial fat content.

Changes in flavour were investigated in relation to the free fatty acid composition in the flour and concentrate fractions. Panelists used the flavour intensity value technique to judge dried pea flavour in the two fractions for both relative humidities. Although the amount of free fatty acids increased, the sensory data shown in Tables 15 and 16

TABLE 14

FREE FATTY ACID CONTENT OF FABABEAN FLOUR AND CONCENTRATE FRACTIONS EQUILIBRATED TO 65% RELATIVE HUMIDITY AND STORED AT 38°C (ug/gm sample)\*

		FLOU	JR		
Fatty Acid	S	Week 0	Week 2	Week 10	Week 12
Myristic Palmitic Stearic Oleic Linoleic Linolenic Eicosanoic	C14:0 C16:0 C18:0 C18:1 C18:2 C18:3 C20:0 C20:1	2.29 46.95 32.81 49.88 106.64 6.90 0.43	9.57 111.68 51.65 113.13 294.27 21.38	11.08 156.63 57.79 229.84 624.86 66.53 12.93	15.90 203.11 63.54 296.01 760.13 67.24 10.86 2.10
Total		245.67	596.88	1163.54	1417.82

#### CONCENTRATE

Fatty Acid	Week O	Week 2	Week 10	Week 12
C14:0	8.31	2.95	21.32	23.59
C16:0	103.71	184.17	355.76	396.66
C18:0	37.66	53.98	70.24	88.32
C18:1	144.72	314.68	595.92	664.92
C18:2	373.49	817.60	1569.96	1723.90
C18:3	35.67	83.67	165.11	187.12
C20:0	3.90	14.02	31.14	40.03
C20:1	1.29	4.14	9.95	16.09
Total	708.75	1473.72	2821.9	3140.62

<sup>\*</sup>Mean of two readings on duplicate samples.

TABLE 15

DRIED PEA FIV\* IN FABABEAN FRACTIONS EQUILIBRATED TO 65% RELATIVE HUMIDITY AND STORED AT 38°C

Weeks Stored (No.)	Flour	Concentrate
2	3.3	4.3
4	3.2	4.8
6	2.5	4.3
8	2.9	3.4
10	2.3	5.0

<sup>\*</sup>Flavour Intensity Scale 0 = Bland; 10 = Strong

TABLE 16

DRIED PEA FIV\* IN FABABEAN FRACTIONS EQUILIBRATED TO AMBIENT RELATIVE HUMIDITY CONDITIONS AND STORED AT 38°C

Weeks Stored (No.)	Flour	Concentrate
2	3.2	4.5
4	3.5	4.5
6	4.2	4.1
8	3.6	4.5
10	3.3	5.6

<sup>\*</sup>Flavour Intensity Scale 0 = Bland; 10 = Strong

did not show a flavour change under the accelerated storage conditions. Although the increase in free fatty acids did not result in a flavour change, their subsequent breakdown could lead to off flavour development.

The suggestion was made in the review of literature that unsaturated fats are a common cause of off flavour development in stored products. Since 84% of the total lipid present in fababeans is unsaturated it is likely, in view of the soybean research, that unsaturated fats could be a factor in the development of off flavours in fababeans. Lipid bands from the thin layer chromatographic analysis of fababean flour and concentrate were quantitated exclusive of the free fatty acids and are shown in Table 17. The majority of the lipid in both fractions is present in the form of phospholipid, indicating a potential source for lipid hydrolysis. Enzymatic hydrolysis of the phospholipid results in the generation of free fatty acids which, when oxidized, form hydroperoxides. Since lipoxygenase was shown to be active towards linoleic and linolenic acids it is reasonable that the breakdown products of these hydroperoxides could contribute to the flavour problems. The breakdown of the hydroperoxides formed from the oxidation of specific free fatty acids can result in a range of alcohols, aldehydes, ketones and lactones which have very low flavour thresholds. (Forss, Sessa et al., (1974) isolated soybean phosphatidycholine in defatted soybean flakes and demonstrated that the autoxidation of this compound and subsequent storage resulted

TABLE 17

COMPONENTS OF LIPIDS IN FABABEAN FLOUR AND CONCENTRATE FRACTIONS EXCLUSIVE OF FREE FATTY ACIDS 1

	F1our (%)	Concentrate (%)
Phospholipid	65.10	68.45
Cholesterol	1.50	3.40
Triglyceride	32.10	26.85
Sterol Esters	1.50	1.30

 $<sup>^{1}\</sup>mbox{Values}$  expressed as a percent of the total of these 4 components.

in the development of an intensely bitter flavour. The authors identified lipoxygenase as the reaction catalyst, however, further work is necessary to determine the relationship between enzymatically oxidized soybean phosphatiylcholine and the development of the bitter taste. Further investigation into the breakdown mechanism of the lipids in the fababean fractions is necessary.

#### SUMMARY AND CONCLUSIONS

A sensory panel considered dried pea and bitterness the dominant flavour characteristic of fababean flour. A trained sensory panel judged the fababean concentrate fraction stronger (significant at the 5% level) in both dried pea and bitterness flavour characteristics than the flour or starch-rich fractions in decreasing order respectively. Absolute thresholds determined for each fababean fraction in water approximated 0.1% (W/VOL).

Heat treatment of the fababean flour and concentrate fractions resulted in a significant decrease in dried pea flavour (5% level). However, the heat treatment induced novel flavours which were both strong and highly objectionable. Dry heat effectively inactivated lipoxygenase in fababean flour and concentrate samples. A fairly severe heat treatment (140-160°C) for 15 and 30 minutes was necessary to completely inactivate the enzyme. Lipoxygenase has been linked to development of rancid odors and flavours in soybeans (Mustakas et al., 1969). Eskin and Henderson (1974b) have demonstrated the similarity of the lipoxygenase present in fababeans to lipoxygenase-2, a type present in soybeans, and have determined its specificity towards linoleic and linolenic acids. Since 55% of the total fat in fababeans is present as linoleic acid, the breakdown products of the

free fatty acids could be partially responsible for flavour problems.

Although the sensory panel judged year-old flour significantly stronger than freshly milled flour with respect to dried pea flavour, no change in dried pea flavour was evident in fababean fractions after 12 weeks of accelerated storage (38°C). Under these accelerated storage conditions the amount of free fatty acids increased markedly. Their subsequent breakdown could lead to off flavour development.

The flavour characteristics of fababean fractions have been defined in this research. Further investigations are necessary to determine the origin of these flavours. If the flavour problems identified in this study can be solved, the fababean offers Canada a high protein crop suitable for human food usage.

#### BIBLIOGRAPHY

- Adolphson, L.C. and Horan, F.E. 1974. Textured vegetable protein products as meat extenders. Cereal Science Today 19:441-446.
- American Association of Analytical Chemistry. 1960. Methods of Analysis, 9th edition.
- Amerine, M.A., Pangborn, R.M., Roessler, E.B. 1965, Principles of Sensory Evaluation of Food. Academic Press, New York.
- A.S.T.M. 1968. Manual on Sensory Testing Methods. American Society for Testing and Materials, Philadelphia.
- Bhatty, R. S. 1974. Chemical composition of some fababean cultivars. Canadian J. Plant Science 54:413-421.
- Brudnak, J. A. 1974. Emerging meat alternatives. <u>Cereal</u> Science Today. 19:447-449.
- Craig, B. M. 1974. The industrial potential of fababeans.

  Proceedings of the National Fababean Conference.

  Winnipeg, Canada. February.
- Eskin, N.A.M., and Henderson, H.M. 1974a. Lipoxygenase in vicia faba minor. Phytochemistry 13: (in press).
- Eskin, N.A.M., and Henderson, H.M. 1974b. A study of lipoxygenase from small fababeans. Presented to the International Congress of Food Science and Technology. Madrid, Spain, September, 1974.
- Evans, L.E., Switzer, J.F., and Bushuk, W. 1972. Horsebeans
   A protein crop for Western Canada? Can. J. Plant
  Science 52:657-659.
- Forss, A. A. 1972. Odour and flavour compounds from lipids.  $\frac{\text{Progress in the Chemistry of Fats and Other Lipids.}}{13(4):181-258.}$
- Kalbrener, J.E., Eldridge, A.C., Moser, H.A., Wolf, W.J. 1971. Sensory evaluation of commercial soy flours, concentrates, and isolates. Cereal Chemistry 48:595-600.

- Kalbrener, J.E., Warner, K., and Eldridge, A.C. 1974. Flavors derived from linoleic and linolenic acid hydroperoxides. Cereal Chemistry 51:406-416.
- Kendall, M. G. 1962. Rank Correlation Methods. Hafner Publishing Co., New York.
- Maga, J. A. 1973. A review of flavour investigations associated with the soy products, raw soybeans, defatted flakes and flours, and isolates. J. Agr. Food Chemistry. 21:864-868.
- Mattick, L.R., and Hand, D.B. 1969. Identification of a volatile component in soybeans that contributes to the raw bean flavour. J. Agr. Food Chemistry 17:15-17.
- McConnell, L.M., Simmonds, D.H., and Bushuk, W., 1974. High protein bread from wheat fababean composite flours. Cereal Science Today. 19:517-521.
- McDonald, B. E. 1974. Fababeans and human nutrition. Presented to the National Fababean Conference, Winnipeg, Manitoba, February.
- McEwen, T.J., and Bushuk, W., 1973. Analysis of fababeans as a source of protein and starch. Presented at the 58th Annual meeting of the A.A.C.C. Paper #94.
- Metcalfe, L.D., Schmitz, A.A., Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. <u>Analytical Chemistry</u> 38:514-515.
- Moser, H.A., Evans, C.D., Campbell, R.E., Smith, A.K., and Cowan, J.C. 1967. Sensory evaluation of soy flour. Cereal Science Today 12:296, 299, 312.
- Mustakas, G.C., Albrecht, W.J., McGhee, J.E., Black, L.T., Bookwalter, G.N., and Griffin, E.L. 1969. Lipoxidase deactivation to improve stability, odor and flavour of full-fat soy flours. J. Am. Oil Chem. Soc. 46:623-626.
- Presber, A.A.W. 1972. An Inquiry into the Origin, Cultivation and Utilization of The Small Fababean (Horsebean) in Austria, The Federal Republic of Germany and England. Canada Grains Council, Winnipeg, Can.
- Rackis, J.J., Honig, D.H., Sessa, D.J., and Steggerda, F.R., 1970. Flavor and flatulence in soybean protein products. J. Agr. Food Chem. 18:977-982.

- Rackis, J.J., Honig, D.H., Sessa, D.J., Moser, H.A., 1972. Lipoxygenase and peroxidase activities of soybeans as related to the flavor profile during maturation. Cereal Chemistry 49:586-597.
- Sessa, D.J., Warner, K., and Honig, D.H. 1974. Soybean phosphatidylcholine develops bitter taste on autoxidation. J. of Food Science 39:69-72.
- Sumner, A.K., Lenan, L., Youngs, C.G. 1972. Texturizing Process for Vegetal Protein Concentrate. Can. Patents and Dev. Ltd. Case No. 4746.
- U.S. Air Force School of Aviation Medicine 1957. Research Report #56:113.
- Vaisey, M., and McDonald, B.E., 1974. Manitoba Research Council Report, August.
- Vaisey, M., McDonald, B.E., Eskin, N.A.M., and McDaniel, M. Unpublished data. University of Manitoba.
- Vaisey, M., and Tassos, L. 1974. Fababeans in human foods.
  Proceedings of the National Fababean Conference,
  Winnipeg, Canada, February.
- Wolf, W.J., and Cowan, J.C. 1971. Soybeans as a food source. C.R.C. Reviews in Food Technol. 2(1):81-158.
- Woolcott, D., Vaisey, M., and McDaniel, M. 1974. Consumer attitudes to a ground-beef soy mixture. Presented to the Annual Meeting of The Canadian Dietetic Assoc. Toronto, June, 1973.
- Yamaguchi, M., Henderson, H.M., Hwang, P.M., and Campbell, J.D. 1969. Effect of oxygen concentration on 0-diphenol oxidase activity. Anal. Biochem. 32:178.

APPENDIX A

#### SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF DRIED PEA FIV IN THE FABABEAN CONCENTRATE FRACTION

SOURCE	DF	SS	MS	F
Concentrations Linear Remainder	5 1 4	213.388 203.14 10.24		19.63** 79.35**
Panelists	5	51.610	10.320	
Error	25	54.390	2.180	
Total	35	319.388		

<sup>\*\*</sup>Significant at the 1% level of significance.

## SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF DRIED PEA FIV IN THE FABABEAN FLOUR FRACTION

SOURCE	DF	SS		MS	F
Concentrations Linear Remainder	5 1 4	66.252	62.580 3.675	13.250	5.68** 26.86**
Panelists	6	61.658		10.276	
Error	30	69.959		2.332	
Tota1	41	197.869			

<sup>\*\*</sup>Significant at the 1% level of significance.

### SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF DRIED PEA FIV IN THE FABABEAN STARCH-RICH FRACTION

SOURCE	DF	SS	MS	F
Concentrations Linear Remainder	5 1 4	67.049 62.390 4.659		11.46** 53.32**
Panelists	5	18.075	3.600	
Error	25	29.297	1.170	
Total	35	114.421		

<sup>\*\*</sup>Significant at the 1% level of significance.

APPENDIX B

### SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA FIV FOR FABABEAN CONCENTRATE AND THE STARCH-RICH FRACTIONS

SOURCE	DF	ХX	XY	YY	b (slope)	SSR	SSE	DF	MSE
Starch	5	2.8	5.396	11.169	1.920	10.398	0.771	4	0.193
Concentrate	5	2.8	9.742	35.599	3.480	33.895	1.705	4	0.426
Pooled							2.476	8	0.309
*Difference	s Amo	ng Regr	ession Coe	fficients			3.374	1	3.374
Common	10	5.6	15.138	46.769	2.700	40.920	5.849	9	0.850
*Difference	s Amo	ng Adju	sted Treat	ment Means			18.680	1	18.680
Total	11	5.6	15.138	65.452	-	40.920	24.530	10	_

<sup>\*</sup>Significant at the 5% level of significance.

### SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA FIV FOR FABABEAN FLOUR AND CONCENTRATE FRACTIONS

SOURCE	DF	XX	XY	YY	b (slope)	SSR	SSE	DF	MSE
					· · · · · · · · · · · · · · · · · · ·			·	
Flour	5	2.8	5.004	9.465	1.787	8.940	0.525	4	0.130
Concentrate	5	2.8	9.742	35.599	3.480	33.895	1.705	4	0.426
Pooled							2.230	8	0.279
*Difference	s Amo	ng Regr	ession Coe	fficients	-		4.000	1	4.000
Common	10	5.6	14.746	45.065	2.633	38.830	6.230	9	0.693
*Difference	s Amo	ng Adju	sted Treat	ment Means			4.958	1	4.960
Total	11	5.6	14.746	50.018	-	38.830	11.188	10	-

<sup>\*</sup>Significant at the 5% level of significance.

### SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA FIV FOR FABABEAN FLOUR AND STARCH-RICH FRACTIONS

SOURCE	DF	XX	XY	YY	b (slope)	SSR	SSE	DF	MSE
Flour	5	2.8	4.974	9.357	1.776	8.836	0.522	4	0.131
Starch	5	2.8	5.396	11.169	1.927	10.399	0.322	4	0.131
Pooled							1.292	8	0.162
*Differen	ces Amo	ng Regr	ession Coe	fficients			0.032	1	0.032
Common	10	5.6	10.370	20.527	1.852	19.203	1.324	9	0.147
*Differen	ces Bet	ween Ad	justed Tre	atment Mea	ns		1.463	1	1.463
Total	11	5.6	10.370	21.990	-	19.203	2.787	10	-

<sup>\*</sup>Significant at the 5% level of significance.

APPENDIX C

### SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF BITTERNESS FIV IN FABABEAN CONCENTRATE FRACTION

SOURCE	DF	SS		MS	F .
Concentrations Linear Remainder	5 1 4	221.479	209.258 12.221	44.296	25.469** 120.332**
Panelists	5	39.173		7.834	
Error	25	43.481		1.739	
Total	35	304.133			

<sup>\*\*</sup>Significant at the 1% level of significance.

#### SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF BITTERNESS FIV IN THE FABABEAN FLOUR FRACTION

SOURCE	DF	SS	MS	F
Concentrations Linear Remainder	5 1 4	80.677 75.790 0.097		10.62** 49.86**
Panelists	6	33.640	5.610	
Error	30	45.487	1.520	
Total	41	159.804		

<sup>\*\*</sup>Significant at the 1% level of significance.

## SUMMARY OF ANALYSIS OF VARIANCE AND REGRESSION ANALYSIS OF BITTERNESS FIV IN THE FABABEAN STARCH-RICH FRACTION

SOURCE	DF	SS		MS	F
Concentrations Linear Remainder	5 1 4	63.282	57.077 6.205	12.660	7.08** 31.92**
Panelists	5	30.149		6.030	
Error	25	35.766		1.788	
Total	35	129.197			

<sup>\*\*</sup>Significant at the 1% level of significance.

APPENDIX D

### SUMMARY OF ANALYSIS OF COVARIANCE OF BITTERNESS FIV FOR FABABEAN FLOUR AND CONCENTRATE FRACTIONS

SOURCE	DF	хх	XY	YY	b (slope)	SSR	SSE	DF	MSE
Flour	5	2.8	5.508	11.544	1.967	10.835	0.709	4	0.177
Concentrate		2.8	9.882	36.874	3.529	34.876	1.997	4	0.498
Pooled							2.706	8	0.338
*Difference	s Amoi	ng Regr	ession Coe	fficients			3.418	1	3.418
Common	10	5.6	15.390	48.419	2.748	42.295	6.124	9	0.680
*Difference	s Amor	ng Adju	sted Treat	ment Means			4.899	1	4.899
Total	11	5.6	15.390	53.321	_ ·	42.295	11.023	10	-

<sup>\*</sup>Significant at the 5% level of significance.

APPENDIX E

## SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA AND BITTERNESS FLAVOUR INTENSITY VALUES (FIV) FOR FABABEAN CONCENTRATE FRACTION

SOURCE	DF	ХХ	XY	YY	b (slope)	SSR	SSE	DF	MSE
Dried Pea FIV	5	2.8	9.742	35.600	3.479	33.895	1.705	4	0.426
Bitterness FIV	5	2.8	9.882	36.870	3.529	34.876	1.994	4	0.498
Pooled							3.695	8	0.512
Differences Amor	ng Reg	gressi	on Coeffic	ients	-		0.007	1	0.007
Common	10	5.6	19.624	72.470	3.500	68.768	3.702	9	0.410
Differences Amor	ng Ad	justed	Treatment	Means			0.075	1	0.075
Total	11	5.6	19.624	72.545	_	68.768	3.777	10	-

No significant differences were detected.

# SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA AND BITTERNESS FLAVOUR INTENSITY VALUES (FIV) FOR FABABEAN FLOUR FRACTION

SOURCE	DF	ХХ	XY	YY	b (slope)	SSR	SSE	DF	MSE
Dried Pea FIV	5	2.8	5.004	9.465	1.787	8.940	0.525	4	0.130
Bitterness FIV	5	2.8	5.508	11.544	1.967	10.835	0.709	4	0.177
Pooled							1.234	8	0.154
Differences Amor	ng Reg	gressio	on Coeffic	ients			0.042	1	0.042
Common	10	5.6	10.512	21.009	1.877	19.733	1.276	9	0.142
Differences Amor	ng Ad	justed	Treatment	Means			0.077	1	0.077
Total	11	5.6	10.512	21.086	<del>-</del>	19.733	1.350	10	-

No significant differences were detected.

## SUMMARY OF ANALYSIS OF COVARIANCE OF DRIED PEA AND BITTERNESS FLAVOUR INTENSITY VALUES (FIV) FOR THE FABABEAN STARCH-RICH FRACTION

SOURCE	DF	XX	ХҮ	YY	b (slope)	SSR	SSE	DF	MSE
Dried Pea FIV	5	2.8	5.396	11.169	1.920	10.398	0.771	4	0.193
Bitterness FIV	5.	2.8	5.156	10.528	1.840	9.494	1.034	4	0.259
Pooled							1.805	8	0.226
Differences Among Regression Coefficients							0.010	1	0.010
Common	10	5.6	10.552	21.697	1.880	19.883	1.815	9	0.202
Differences Among Adjusted Treatment Means							0.616	1	0.616
Total	11	5.6	10.552	22.314	-	19.883	2.431	10	-

No significant differences were detected.

APPENDIX F

# CROP COMPARISON SERIES: ANALYSIS OF COVARIANCE OF DRIED PEA FIV FOR THE FABABEAN FLOUR FRACTION FROM THE 1972 AND 1973 CROP YEARS

SOURCE	DF	ХХ	XY	YY	b (slope)	SSR	SSE	DF	MSE
1972	5	2.8	9.266	31.197	3.416	30.664	0 577	4	0 177
1973	5	2.8	5.716	12.770	2.040	11.669	0.533	4	0.133
Pooled	10				-,0,0	11.000	1.634	8	0.204
*Difference	2.251	1	2.251						
Common	10	5.6	14.982	43.967	2.675	40.082	3.885	9	0.432
*Differences Among Adjusted Treatment Means							7.285	1	7.285
Total	11	5.6	14.982	51.252	- -	40.082	11.170	10	-

<sup>\*</sup>Significant at the 5% level of significance.