1991

The use and acceptability of lupin seed and sprout as a food ingredient

Pam Yates

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THE USE AND ACCEPTABILITY OF
LUPIN SEED AND SPROUT AS A
FOOD INGREDIENT

by

Pam Yates

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Award
of
Bachelor of Applied Science Honours
(Consumer Science)
at the
Department of Consumer Sciences,
Edith Cowan University
Submitted
December 1991
ABSTRACT

This study set out to determine whether germination improved the biochemical and sensory properties of lupins and whether an acceptable food could be made from the sprout.

The lupin seed, (*L. angustifolius* cv. *Gungurru*), was sprouted using a commercial sprouter for up to 6 days. The seeds were soaked for 24h, then germinated in the dark at 20°C. The sprouts were used in each part of the study: Biochemical Analysis and Sensory Evaluation, Food Product Development and Consumer Market Survey.

Many biochemical changes occurred especially in the soaking and early germination stages; including increases in protein (6%) and polysaccharides (36%) and a decrease in fat (18%) and antinutrients, alkaloids (67%) and oligosaccharides (98%).

An attempt was made to match the biochemical changes in the sprouts to the sensory changes. The sprouts were freeze-dried, milled then made into pellets. Minimal sensory changes could be detected. Biochemical changes could not be matched to sensory changes in the developing sprout.

A basic food product, a vegetable soup, was developed and evaluated by a taste panel. The soups made with younger
sprouts were preferred to soups made with older sprouts. Soup made with lupin kernel was preferred to soup made with the sprouts.

A market survey of 524 shoppers found that lupin soup was preferred over soybean soup when unmarked samples were tasted. Respondents were likely also to buy lupin soup when they knew it contained lupin. Respondents felt lupin would make a food product tastier, more nutritious and cheaper. A large number (85%) of respondents had heard of the name lupin, most felt the name lupin was positive (59%) and even more (71%) felt it was a positive name for a food product.
(iii)

DECLARATION

"I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published except where due reference is made in the text."
ACKNOWLEDGEMENTS

Foremost, I would like to thank my supervisors, Dr Frank Flanagan and Mr David Petterson for their guidance and support. I would also like to thank Dr David Allen, Dr David Harris and Chemistry Centre staff for their assistance and patience. I thank all the 2nd and 3rd year students of the Consumer Sciences Department for their invaluable help with sensory evaluation. I thank all the Consumer Science students who assisted in data collection at Midland Gate shopping centre.

I would also like to extend my thanks to the Grains Research and Development Corporation for funding my research.

Finally, I would like to thank Rohan and my family for their encouragement and support.
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1.0 INTRODUCTION

Legumes (members of the Leguminosae family) fix nitrogen in the soil; they can be divided arbitrarily into large and small seeded plants. Many of the large seeded legumes are edible and are often called pulses. At least two legumes, peas and lentils, date back to 9500-9000 BC (Williams, 1986, p. 1).

The nutritional characteristics of legumes are mostly favourable. They are rich in protein which is relatively high in lysine but low in sulphur-containing amino acids. However legumes can complement cereals in which lysine is the limiting amino acid, so that when legumes and cereals are eaten together they provide all the essential amino acids, providing a complete protein (Rogers, 1990, p. 301).

Legumes are also high in fibre and it is believed that the soluble fibre they contain may help control cholesterol levels in the blood (Rogers, 1990, p. 301).

Legumes are valuable sources of thiamine, niacin equivalent, and minerals such as iron, calcium, phosphorus, zinc and magnesium. The starch in legumes is absorbed slowly which gives rise to a slow, steady release of glucose (Rogers, 1990, p. 301). This is a benefit, particularly to diabetics.

Legumes are used throughout the world. They are particularly important in the diets of people in developing countries as a major protein source and are an integral
component of Mediterranean, Asian and Latin American cuisines. There are many different ways legumes can be used; in soups, dips, salads and as a meat or vegetable substitute.

Compared to meat, legumes are an inexpensive source of protein. They can be bought in cans or in the dry form; both forms can be stored for long periods. Once cooked legumes can be stored in the refrigerator for a short period or frozen for long periods (Ward McKenzie pamphlet).

It is preferable not to eat legumes raw as the seeds contain trypsin inhibitors, which interfere with protein digestion, and lectins, which can act as gastric irritants. These proteinaceous antinutrients in legumes are usually denatured during germination, cooking or canning.

Germination of legumes produces a sprout which can be eaten as a fresh vegetable or added to dishes such as stir-fries. It is believed that germination enhances the nutritional and flavour characteristics of legumes and lowers the content of antinutrients.

Soybeans are presently the most sought after vegetable protein source. In 1984 Americans consumed 832 million pounds of protein food made from soy (289 million pounds of soy milk, 175 million pounds of soy flour and grits and 149 million pounds of soy sauce), as well as 9803 million pounds of soy oil. Low technology consumer foods have the largest growth rates. The growth rates for low technology soyfoods
for a five year period up to February 1984 were:

- Tempeh 33%
- Tofu 15%
- Soy Sauce 14%
- Miso 10%

(Shurtleff & Aoyagi, 1985, p. 38).

Cerletti, Fumagalli & Venturin (1978, p. 1409) assert that many countries depend on imports for their supply of soy. As lupins have similar chemical and physical properties to soybean, they may have the potential to gain at least a small percentage of the billion dollar soy market. The world’s demand for protein is increasing and new sources of protein that are easily produced and economical are being continually sought. Lupin may well become a generally acceptable protein source for human consumption in many cuisines.

The lupin seed has a high content of protein and fibre and a low content of fat and antinutrients. Lupins have a high digestibility and a low allergenicity which potentially makes them an excellent nutrient source.

Lupins have been cultivated for centuries in various parts of the world for both animal and human consumption. The disadvantage of lupins in the past has been their alkaloid content which gave lupins a bitter taste and a degree of toxicity. Research on lupins in the 1920s and 1930s resulted in the development of a sweet lupin with a low alkaloid content. Since this development, further work has been conducted on lupins, leading to the production of
the first commercial sweet cultivar of *Lupinus angustifolius* (the narrow-leafed lupin) in 1967.

There are four main species of lupin: *L. albus* (the white flowered lupin), *L. angustifolius*, *L. mutabilis* (the pearl or Andean lupin) and *L. luteus* (the yellow flowered lupin). The most important species of lupin to Western Australia is *L. angustifolius*, as it is most suited to the climatic conditions.

Lupins have mainly been used for stock feed in Australia. Research into the use of lupin was concentrated in this area until about 1985. Since then many studies have been conducted into the use of lupins in human foods (Hung, Papalois, Nithianandan, Jiang & Versteeg, 1990; Kyle, Petterson & Evans, 1990). The National Health and Medical Research Council permitted the unrestricted use of lupins in human foods in 1988. Lupins have been used in Oriental foods such as tempeh, miso, fermented sauces, bean curd and vegetable milks; and have also been used in a number of Western foods such as biscuits, cakes, pasta, bread and as a snack food.

Western Australia is the world’s largest producer and exporter of lupins. If lupins were to be used in more foods for human consumption this would increase the return on lupins for Western Australia, as well as increase the food choices available for consumers.
1.1 PURPOSE OF THE STUDY

It is widely believed that germinating legume seeds increases their nutritional value and enhances their flavour, however there has been little published work on the germination of lupin seeds for human consumption.

The purpose of this study was to examine and compare the biochemical and sensory characteristics of lupin seed (L. angustifolius) and the developing lupin sprout, and then assess the suitability of the seed or sprout as an ingredient in foods for human consumption.

The research questions posed in this study ask: whether the germination of the lupin seed enhances its nutrient and sensory properties and whether consumers find lupin acceptable as an ingredient in food products for human consumption.
2.0 LITERATURE REVIEW

2.1 THE HISTORY OF LUPINS

Lupins (lupines) belong to the Genistae tribe of the Papilionoideae sub-family of the Leguminosae plant family; they are related to peas, beans and lentils. There are about 200 lupin species native to the Americas. These are predominantly perennials with the notable exception of *L. mutabilis* from South America. There are 12 species of Old World lupins centred around North Africa and the Mediterranean basin. These are all annuals.

For thousands of years lupins have been cultivated in various parts of the world for both animal feed and human consumption. It is believed that the use of lupins as a food dates back to 4000-3000 BC (Lee, 1986 p. 64), whereas the use of at least two other pulses (vegetable legumes), peas and lentils, date back to 9500-9000 BC (Williams, 1986, p. 1). The two species of lupins that are said to date back to 4000-3000 BC are *L. albus*, in the Mediterranean Basin, and *L. mutabilis*, in the highlands of South America (Gladstones, 1970, p. 123).

The modern expansion of lupin cultivation did not start until 1780 when lupin seeds were imported from Italy into Prussia by King Frederick who personally supervised experiments on their cultivation (Gladstones, 1970, p. 125). By the end of the 18th century *L. albus* had arrived in Germany and *L. luteus* and *L. angustifolius* in North West
Europe (Williams, 1986, p. 2). *L. cosentini*, the so-called 'Western Australian blue lupin,' was introduced into Western Australia by the middle of the 19th century (Gladstones, 1970, p. 125). It was not until the 1930s, that *L. angustifolius* was introduced into Australia, New Zealand and South Africa (Gladstones, 1970, p. 125; Williams, 1986, p.2).

The main disadvantage in using lupins for human consumption in the past has been their bitterness. Early varieties of lupins had an alkaloid content of 1.0-2.5% in the dry seed (Lee, 1986, p. 64). This meant that a long process of soaking and washing in running water was necessary to reduce the alkaloid content and to make the seed palatable and safe for consumption. Between 1928-1935, a German scientist, Dr. Reinhold von Sengbusch, developed a sweet lupin with alkaloid content of only 0.02% (Gladstones, 1970, p. 126; Lee, 1986, p. 64). By 1943 the remaining primitive features of lupins, pod shattering and impermeable seeds, had been overcome and this enabled the first true crop types of *L. luteus* and *L. albus* to be developed (Gladstones, 1990, p. 270). Further work in Western Australia by Dr. John Gladstones in the 1950s and 1960s, resulted in the development of the first commercial sweet cultivar of *L. angustifolius*. This cultivar has a white flower and seeds, which distinguish it from the higher alkaloid, wild, bitter lupins with the blue flower and dark seeds. In 1967 this cultivar, Uniwhite, was released (Nelson & Delane, 1990, p. 6). Since then over 10 cultivars (all white flowered) have been released for commercial production.
in Australia. At present there are 5 main cultivars grown commercially in Australia: Danja, Yorrel, Gungurru, Warrah and Geebung.

2.2 LUPINS IN WESTERN AUSTRALIA

In Western Australia lupins are grown south and west of the line from Northampton extending east to Southern Cross and south east to Esperance except for the very high rainfall zones in the lower south west corner of Western Australia (see Figure 1).

Figure 1. Lupin cultivation areas in Western Australia

(Nelson & Delane, 1990, p. 16)
Today, Western Australia is the world's largest producer and exporter of lupins (Table 1).

**Table 1**

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<td>1976/77</td>
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<td>0.05</td>
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<tr>
<td>1977/78</td>
<td>23.6</td>
<td>0.06</td>
</tr>
<tr>
<td>1978/79</td>
<td>23.8</td>
<td>6.3</td>
</tr>
<tr>
<td>1979/80</td>
<td>25.2</td>
<td></td>
</tr>
<tr>
<td>1980/81</td>
<td>48.2</td>
<td>13.4</td>
</tr>
<tr>
<td>1981/82</td>
<td>95.0</td>
<td>51.4</td>
</tr>
<tr>
<td>1982/83</td>
<td>179.6</td>
<td>82.7</td>
</tr>
<tr>
<td>1983/84</td>
<td>315.6</td>
<td>224.4</td>
</tr>
<tr>
<td>1984/85</td>
<td>500.5</td>
<td>356.6</td>
</tr>
<tr>
<td>1985/86</td>
<td>411.6</td>
<td>215.7</td>
</tr>
<tr>
<td>1986/87</td>
<td>684.6</td>
<td>424.1</td>
</tr>
<tr>
<td>1987/88</td>
<td>730.0</td>
<td>404.6</td>
</tr>
<tr>
<td>1988/89</td>
<td>789.0</td>
<td></td>
</tr>
</tbody>
</table>

(Nelson & Delane, 1990, p. 83)

Lupins are Western Australia's second largest crop, the first being wheat (see Table 2). The largest importers of lupins from Western Australia are Japan, Korea and the European Economic Community; minor importers include Middle Eastern and South-East Asian countries.

**Table 2**

<table>
<thead>
<tr>
<th>CROP</th>
<th>$M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>628</td>
</tr>
<tr>
<td>Lupins</td>
<td>127</td>
</tr>
<tr>
<td>Barley</td>
<td>81</td>
</tr>
<tr>
<td>Oats</td>
<td>58</td>
</tr>
</tbody>
</table>

(West Australian Yearbook, 1989)
The Grain Pool of Western Australia presently sells lupins on the international market for about $200 a tonne, which is approximately 75% of the price of soybean meal (B. Cox, personal communication, April, 1991). These prices are influenced by the international levels of supply and demand for vegetable protein.

The lupins produced in Western Australia are sold mainly for stock feed. Indonesia, which is the only country that imports significant quantities of lupins for use in human food, uses 5000-10000 tonnes of lupins a year for tempeh production (B. Cox, personal communication, April, 1991). If more food uses for lupins were to be developed, it should be possible to increase the return on lupins to growers, and to develop a processing industry thereby providing extra income for Western Australia.

2.3 THE COMPOSITION OF LUPIN

The lupin seed is made up of the seed coat or hull and the kernel (see Figure 2).

![Figure 2. Structure of lupin seed](image)

Physically lupins are similar to soybean. Table 3 compares the seed masses of *L. angustifolius* and soybean.
Table 3
Average Seed Mass and Percent of Seed Coat of Soybean and Lupin Seed (L. angustifolius).

<table>
<thead>
<tr>
<th></th>
<th>Av. mass of seed (g)</th>
<th>% seed coat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupin seed</td>
<td>0.17</td>
<td>22.4</td>
</tr>
<tr>
<td>Soybean</td>
<td>0.23</td>
<td>6.6</td>
</tr>
</tbody>
</table>

(Lee, 1986, p. 65)

Lupin has a lower seed mass than soybean but the proportion of seed coat is much greater than soybean.

2.3.1 NUTRIENT COMPOSITION

Lupins are comparable to soybean in terms of nutritional value and physical properties. Nutritionally, lupin is considered as valuable as soybean and superior to other legume species (Hill, 1986, p. 54). Table 4 shows the chemical composition of lupin seed in comparison to soybean. The composition of the lupin kernel and hull are also shown for comparison between the hull and kernel.

Table 4
Chemical Composition of Soybean and Lupin (L. angustifolius)

<table>
<thead>
<tr>
<th></th>
<th>SOYBEAN</th>
<th>LUPIN SEED</th>
<th>LUPIN KERNEL</th>
<th>LUPIN HULL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8.6</td>
<td>8.8</td>
<td>9.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.1</td>
<td>2.8</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>34.3</td>
<td>32.4</td>
<td>38.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Ether extractives (%)</td>
<td>18.7</td>
<td>5.5</td>
<td>7.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Crude Fibre (%)</td>
<td>3.8</td>
<td>15.0</td>
<td>2.2</td>
<td>50.9</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.22</td>
<td>0.23</td>
<td>0.13</td>
<td>0.61</td>
</tr>
<tr>
<td>Phosphorous (%)</td>
<td>0.48</td>
<td>0.31</td>
<td>0.39</td>
<td>0.04</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.28</td>
<td>0.16</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>1.82</td>
<td>0.82</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>na</td>
<td>0.23</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>14.0</td>
<td>5.0</td>
<td>5.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>87.0</td>
<td>70.0</td>
<td>140.0</td>
<td>42.7</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>54.0</td>
<td>16.0</td>
<td>26.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>45.0</td>
<td>34.0</td>
<td>38.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>

(Adapted from Harris & Jago, 1985)
Lupins are high in protein, most of which is contained in the kernel: dehulling lupins can significantly increase the total protein content, typically from 31 to 39%. The main protein constituents of lupins are globulins; albumins make up the rest (Cerletti, Duranti, & Restani, 1984, p. 472). Globulins and albumins are soluble in water and they are enzymic or metabolic protein (Saxelby, 1980, p. 70).

For protein quality sulphur-containing amino acids, methionine and cysteine, are the first limiting amino acids followed by threonine (Cerletti et al., 1984, p. 472), hence lupin protein is not "complete." Lupin needs to be combined with another food that is rich in sulphur-containing amino acids. Crosbie et al. (1988, p.1) state that supplementing a lupin-based diet with 0.2% methionine results in a "...protein efficiency ratio similar to that of casein." Dagnia (1990) found that supplementing a diet of lupin kernel or sprouts with 0.2% DL-methionine improved protein quality (1990, p. 60).

Lupins are also high in fibre. The seed coat (hull) contains the majority of the crude fibre content of lupin, thus dehulling would significantly reduce the fibre content. The soluble fibre in legumes is believed to help control cholesterol levels in the blood (Rogers, 1990, p. 301). There is also evidence that lupins have cholesterol-lowering properties. The thickened cell wall of lupins consists mostly of pectin-like material and small amounts of cellulose and hemicellulose. This type of fibre has been shown by some researchers to have cholesterol-lowering
properties. Evans, Cheung, & Cheetham, (1990) found after completing numerous experiments, that the serum cholesterol of rats decreased by 10% when they were fed lupins.

Lupins are low in fat, they contain 5.5% fat while soybeans contain 18.7% fat. The fat content of *L. angustifolius* is made up of 75% unsaturated fatty acids, of which a high proportion are linolenic and linoleic acids (Crosbie et al., 1988, p. 1). Though the lupin and soybean have similar protein content, there are major differences in their fibre and fat content.

2.3.2 ANTINUTRIENT COMPOSITION

It is believed that the presence of antinutrients, such as trypsin inhibitors, phytates, oligosaccharides and lectins, affect the utilisation of some nutrients in legumes. Petterson & Crosbie (1990, p. 266) state that the content of these antinutrients in lupins are lower than they are in soybean (see Table 5).

Table 5
Antinutrient Content of Lupin and Soybean

<table>
<thead>
<tr>
<th>ANTINUTRIENT</th>
<th>LUPIN SEED</th>
<th>SOYBEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate (%)</td>
<td>0.44</td>
<td>1.59</td>
</tr>
<tr>
<td>Trypsin inhibitor (mg/g)</td>
<td>0.18</td>
<td>17.90</td>
</tr>
<tr>
<td>Saponins (%)</td>
<td>1.40</td>
<td>1.90</td>
</tr>
<tr>
<td>Total phenolics (%)</td>
<td>0.29</td>
<td>0.57</td>
</tr>
<tr>
<td>Lectins</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>4.60</td>
<td>5.70</td>
</tr>
</tbody>
</table>

(Petterson & Crosbie, 1990, p. 266)

Williams (1989, p. 180) states that although lupins are low in most antinutrients they have unacceptably high levels of
oligosaccharides.

2.3.2.1 TRYPsin INHIBITOR Trypsin inhibitor, also called protease inhibitor, interferes with the action of trypsin, a pancreatic enzyme that digests polypeptides containing arginine and lysine (Chen, 1989, p. 343; Stare & McWilliams, 1984, p. 108). Lupin seeds contain approximately 0.18mg/g trypsin inhibitor. This is low compared to soybean 17.9mg/g (see Table 5).

2.3.2.2 PHYTIC ACID Phytic acid is found in varying amounts in all oilseeds, legumes and cereals (Altschul & Wilcke, 1985, p. 240). Phytic acid can reduce the bioavailability of minerals such as calcium and zinc, by binding to them (Pettersson et al., 1988, p. 2). The phytate content of lupin seed is approximately 0.44%, which is lower than that of soybean, 1.59% (see Table 5).

2.3.2.3 OLIGOSACCHARIDES Oligosaccharides, simple oligomers of raffinose in soybean and other legumes, have been identified with causing flatulence (Altschul & Wilcke, 1985, p. 233, Chen, 1989, p. 343). Altschul & Wilcke (1985, p. 233) found that these low molecular weight sugars (raffinose, stachyose and verbascose) are not broken down by normal enzymes. They pass into the hind gut where intestinal microorganisms ferment the sugars to release carbon dioxide and methane (i.e. leading to flatulence). Oligosaccharides are soluble in water and alcohol thus they can be removed by soaking (Altschul & Wilcke, 1985, p. 235). Lupin seeds contain approximately 4.6% oligosaccharides while soybeans
contain 5.7% (see Table 5).

2.3.2.4 SAPONINS Saponins are contained in all grain legumes. Some saponins have been associated with favourable properties such as reducing plasma cholesterol, whilst others produce adverse effects on the lining of the intestinal wall, increasing its permeability and possibly causing sensitivity reactions (Petterson, personal communication, 1991). Lupin seeds contain approximately 1.4% saponins, while soybeans contain 1.9% (see Table 5).

2.3.2.5 TANNINS Tannins are present in all grain legumes. They are believed to cause irritation in the digestive tract, lowered energy conversion from food, the excretion of high levels of nitrogen in the faeces and cancer (Petterson, personal communication, 1991). Tannins are also reported to bind and reduce the availability of iron.

2.3.2.6 ALKALOIDS Alkaloids are toxic compounds that give lupins a bitter taste. Modern sweet, or low alkaloid cultivars of *L. angustifolius* contain less than 200mg/kg of alkaloids. Processing lupin may further decrease the level of alkaloids present.

In summary, lupins appear to have some favourable nutritional properties such as a high protein and fibre content. However, there are concerns about their antinutrient content. Germination is believed to decrease the antinutrient content of legumes and thus increase their nutritional quality.
2.4 GERMINATION

The Chinese have been sprouting mung beans (*Vigna radiata*) and soybeans (*Glycine max*) for centuries. In the second quarter of the 19th century it was discovered that germination increases the nutritional value of such beans as mung and soybean (Rudra, cited by Lee & Karunanithy, 1990, p. 438). Lee & Karunanithy (1990) also found this true for *Phaseolus* beans as did Sattar, Durrani, Mahmood, Ahmad & Khan (1989) for mung beans. However others have found that germination does not improve the nutritional value of all legumes. For example, Chang & Harrold (1988, p. 783) found that germinating navy and pinto beans (*Phaseolus vulgaris*) for 6 days did not improve their nutritional quality.

The evidence about germination appears to be equivocal. Further investigation is needed into the effects of germination of other legumes.

2.4.1 THE GERMINATION PROCESS

For the germination process to take place the dry seed must be moistened. During germination the moistened seed coat ruptures due to the expanding embryo and forming sprout (Whaley, 1964, p. 423). *L. angustifolius* is 'soft seeded' and thus the seed germinates readily when exposed to moisture (Nelson & Delane, 1990. p. 7).

In order for germination to take place, certain environmental conditions are necessary: adequate water (changed regularly to prevent microbial growth) and suitable
temperature (warm, room temperature). Legumes can be germinated in dark or light conditions. Germinating in the dark will produce sprouts of similar appearance to mung bean sprouts. If seeds are germinated in the light there will be chlorophyll production through photosynthesis resulting in a green or 'salad' sprout.

The conditions under which legumes are germinated have a large effect on the biochemical changes which occur during germination. For the optimal biochemical changes to occur the correct germination conditions must be found.

Sattar et al. (1989) found that when soaking mung beans at a temperature of 55°C, phytate and trypsin inhibitor activity decreased more than when soaked at a lower temperature, 27°C. Trypsin inhibitor activity and phytate content was also decreased with increasing length of soaking the mung beans. Soaking temperature and time had little effect on the protein content (1989, p. 111).

Sattar et al. (1989, p. 114) found that the biosynthesis of protein was greater at ambient conditions (20-35°C) than at low temperatures (20°C).

Sprouting can be done at home. The advantage of being able to sprout at home is that the seeds, which can be stored dry for long periods, are sprouted when required to produce fresh vegetables.
2.5 EFFECTS OF GERMINATION ON CHEMICAL AND NUTRITIONAL VALUE OF LEGUMES

When a seed is germinated a number of biochemical reactions occur. Certain materials are broken down (catabolism), others are transported from cotyledon to the growing parts, and new materials are synthesized (anabolism) from broken down products.


Lee (1986, p. 66) found when sprouting lupin and soybean under the same conditions that lupin absorbs more water, more quickly than the soybean even though the seed coat of lupin is thicker and has a larger fibre content. Dehulling further accelerates the soaking rate. Lee (1986, p. 68) also claimed that lupin sprouts grew faster than soybean sprouts. After three days of germination, the dry weight of the lupin sprouts had increased by 30%, while the soybean sprouts had only increased by 10%.

Yu et al. (1985) reported similar results. The lupin sprouts were superior to soybean sprouts in terms of physical appearance and eating qualities.

The results from the biochemical studies of lupin sprouts by El-Habbal & Attia (1989) and Dagnia (1990) will be discussed in more detail in the following sections.
Because there has been little work completed on the effects of germination on the chemical composition of lupin, work on other legumes were reviewed as they may relate to lupins (Table 6 and Table 7).

2.5.1 PROTEIN

Many researchers have found that when germinating legumes the protein content increased, however it is difficult to know whether these were real increases or as a result of losses of other soluble components, or from metabolic processes.

Sattar et al. (1989) found that protein content of mung beans increased due to germination, however they state, "The marginal increase in total protein is not in fact a real one but merely the result of dissolution of starch content into the soaking medium" (1989, p. 114). Lupins contain very little starch so it is unlikely that any increase in protein in germinated lupin sprouts would be the result of dissolution of starch content into the soaking medium. There are however low concentrations of other soluble components which may dissolve into the soaking medium.

Pawer & Ingle (1988, p. 7) found that after germinating moth beans for 72 hours there was a small increase in crude protein which they attributed to "...changes resulting from the uptake of water during germination."

Hsu, Leung, Finney & Morad (1980, p. 89) reported little change in the protein content of peas, lentil and faba beans after 4 days of germination.
Lee & Karunanithy (1990, p. 438) claim that the protein content of *Phaseolus* beans greatly increased after 5 days germination.

Dagnia (1990, p. 38) noted an increase in protein after germinating *L. angustifolius* for 6 days.

The evidence about whether or not there is any increase in protein is confusing due to the way results can be reported. When results are reported on a dry basis they may show an increase in protein when in fact protein may have decreased on a per seed basis, as the seeds dry weight may have decreased due to a loss in soluble compounds and metabolic processes. There can be no net increase in protein until the developing seedling can absorb nitrogen from the growth medium. For a cost-benefit analysis results need to be calculated on a per seed basis taking into account the loss of dry matter.

2.5.2 AMINO ACIDS

The amino acid content of raw legumes appears to change when they are germinated, however the sulphur-containing amino acids methionine and cysteine remain unchanged.

Sattar et al. (1989, p. 117) assert that most amino acids in mung beans including lysine, phenylalanine and isoleucine, increased during germination, however the sulphur-containing amino acids did not change. Sattar et al. (1989) stated however that an increase in amino acid may be due to the increased protein content. The increases in amino
Table 6
Summary of the Findings in the Effect of Germination on the Nutrient Content of Legumes

<table>
<thead>
<tr>
<th>LEGUME</th>
<th>PROTEIN</th>
<th>AMINO ACIDS</th>
<th>LIPIDS</th>
<th>VITAMIN C</th>
<th>RIBOFLAVIN</th>
<th>NIACIN</th>
<th>THIAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>+1</td>
<td>+1</td>
<td>+1</td>
<td>+10</td>
<td>+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moth beans</td>
<td>+2</td>
<td>=2</td>
<td>+2</td>
<td>+2</td>
<td>-10</td>
<td>+10</td>
<td></td>
</tr>
<tr>
<td>Peas</td>
<td>+2</td>
<td>=2</td>
<td>+2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentils</td>
<td>+2</td>
<td>=2</td>
<td>+2</td>
<td>+10</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba beans</td>
<td>+2</td>
<td>=2</td>
<td>+2</td>
<td>+10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaselous</td>
<td>+3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupin</td>
<td>+4</td>
<td>-3</td>
<td>+4</td>
<td>-5</td>
<td>+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mung bean</td>
<td>+6</td>
<td>+6</td>
<td>+6</td>
<td>+10</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navy bean</td>
<td>+7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinto</td>
<td>+7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>-9</td>
<td>+7</td>
<td>-9</td>
<td>10</td>
<td>+10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7
Summary of the Findings on the Effect of Germination on the Mineral and Antinutrient Content of Legumes

<table>
<thead>
<tr>
<th>MINERALS</th>
<th>ANTINUTRIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.I</td>
</tr>
<tr>
<td>LEGUME</td>
<td></td>
</tr>
<tr>
<td>Phaselous</td>
<td>+3</td>
</tr>
<tr>
<td>Lupin</td>
<td>+10</td>
</tr>
<tr>
<td>Mung bean</td>
<td>-10</td>
</tr>
<tr>
<td>Navy bean</td>
<td>-11</td>
</tr>
<tr>
<td>Chickpea</td>
<td>-11</td>
</tr>
<tr>
<td>Cowpea</td>
<td>-11</td>
</tr>
<tr>
<td>Greengram</td>
<td>-11</td>
</tr>
</tbody>
</table>

KEY:
+ Increase
- Decrease
= No Change

REFERENCES: The numbers in the table correspond to the references. eg. (1) Pawer et al. (1988) found protein and lipids content of moth beans increased when germinated.

acids were "...not significant when data was expressed on a total protein basis" (1989, p. 117). Chang & Harrold (1988, p. 786) found similar results when germinating navy beans and pinto beans. They found that germinating navy beans resulted in changes in glycine, alanine and cysteine and the germination of pinto beans also resulted in changes to the alanine and cysteine. There was no change in the total sulphur-containing amino acids, methionine and cysteine.

Hsu et al. (1980, p. 89) noted little change in the amino acid content of peas, lentils and faba beans after 4 days of germination.

Dagnia (1990, p. 39) found, following the germination of *L. angustifolius* for 6 days, that most amino acids decreased including sulphur-containing amino acids. Aspartic acid and valine were the only ones that showed an increase.

The evidence above suggests some changes occur to the amino acid profile of raw legumes during germination, however sulphur-containing amino acid generally do not change. These changes will be possibly due to the storage protein profiles differing from those newly synthesised metabolic and structural protein.

2.5.3 PROTEIN DIGESTIBILITY

The prominent feature of lupins and other legumes is their high protein content, however the utilisation of this protein is often low due to poor protein digestibility.
Germination is believed to increase the protein digestibility of legumes.

Chang & Harrold (1988) found that after germinating navy beans for 6 days the in vitro protein digestibility was significantly increased (1988, p. 785); however the digestibility of bean proteins was still poor, possibly due to high trypsin inhibitor activity (Chang & Harrold, 1988, p. 781, 785). This was also supported by Chang and Satterlee 1981, p. 1372).

El-Hag, Haard & Morse (1978) found that the germination of red kidney beans for 10 days increased the digestive coefficient of both raw and cooked red kidney beans. "A combination of sprouting and heat treatment resulted in beans with excellent digestibility coefficient (84.4%)" (1978, p. 1274). They also found trypsin inhibitor activity decreased by approximately 50%, suggesting that there is an inverse relationship between digestion coefficient and trypsin inhibitor activity; an observation supported by Chang & Satterlee (1981, p. 1372).

There is a general consensus that germination increases the protein digestibility of raw legumes; much of this increase is probably related to a decrease in trypsin inhibitor activity.
2.5.4 LIPIDS

Pawer & Ingle (1988) reported that the crude fat content of moth beans increased after 3 days germination (1988, p. 8), whereas Hamilton & Vanderstoep (1979) found that the fat content of alfalfa sprouts decreased after 72 and 120 hours germination (1979, p. 443).

El-Habbal & Attia (1987, p. 960) claim there was no significant increase in the fat content of lupins (cv. Giza 1) after 7 days germination.

Dagnia (1990, p. 42) asserted that the fat content of *L. angustifolius* decreased following 6 days germination.

It is unclear whether the fat content of raw legumes increases or decreases after germination. A decrease might be expected if the fat were a major source of energy and an increase due to the loss of other solubles.

2.5.5 VITAMINS

2.5.5.1 Ascorbic Acid Hsu et al. (1980, p. 89) noted a marked increase in ascorbic acid content of dry peas, lentils and faba beans after 4 days of germination. Sattar et al. (1989) found that ascorbic acid was not detectable in ungerminated mung bean seeds however, after germination for 48 hours, ascorbic acid increased markedly to a maximum at 20°C. The increase was greatest at 48 hours, thereafter it decreased, however it was still greater than the initial ascorbic acid content in the ungerminated seed (1989, p. 115). Kylen & McCready (1975, p. 1008) reported similar results for mung beans, lentils and alfalfa sprouts after 7
days germination.

These studies clearly show that ascorbic acid content of raw legumes increases during germination.

2.5.5.2 Riboflavin Hsu et al. (1980, p. 89) stated that riboflavin increased twofold after 4 days of germination in dry peas, while riboflavin in faba beans only increased slightly. Sattar et al. (1989) claim that riboflavin in mung beans reached a maximum after 48 hours of germination, and thereafter decreased, but still contained more than before germinated (1989, p. 115). Kylen & McCreedy (1975) found riboflavin in lentils remained the same after germination, while riboflavin in alfalfa sprouts showed a threefold increase and soybean and mung beans a twofold increase.

The evidence above suggests riboflavin increases in most legumes during germination.

2.5.5.3 OTHER VITAMINS Sattar et al. (1989) found that the thiamine content of mung beans increased when germinated, reaching a maximum at days 2 and 3. "The biosynthesis of riboflavin and thiamine was faster at ambient than low temperatures..." (Sattar et al., 1989, p. 115).

Kylen & McCreedy (1975, p. 1008) noted that the niacin content of alfalfa, mung bean and soybeans increased after 7 days germination.

It appears from the above evidence that the vitamin content of raw legumes increases with germination.
2.5.6 MINERALS

Ganesh & Kumar (1978, p. 78) found that when germinating chickpeas, cowpeas and greengram for 24 and 72 hours, magnesium and calcium decreased. Lee & Karunanithy (1990, p. 443) reported that the germination of Phaseolus beans decreased the magnesium, iron and potassium content but the calcium content increased.

Kylen & McCreedy (1975) did not find the same decrease in minerals when germinating alfalfa, mung beans, soybeans and lentils. The mineral content remained the same except for calcium which increased slightly. Kylen & McCreedy (1975, p. 1008) claim the increases in calcium may be due to the calcium in the tap water.

There is conflicting evidence as to whether germination causes the mineral content of raw legumes to decrease.

2.5.7 ANTINUTRIENTS

It appears that antinutrients contained in raw legumes can be destroyed or at least reduced by processing treatments such as heat and germination.

Chang & Harrold (1988, p. 784) found that after germinating 'navy' beans for 6 days, lectin activity decreased but trypsin inhibitor activity did not. Gupta & Wagle (1980) report that germination of Phaseolus mungoreous resulted in trypsin inhibitor activity decreasing after 9 hours then increasing up to 72 hours, then decreasing again after 9 days. The initial decrease of trypsin inhibitor activity
during sprouting may be due to "...part of the activity leaching out during the early stages of sprouting. However, during the onset of germination, trypsin inhibitor increased slowly due to the transformation of the dormant state of seed to the vigorous metabolic state" (1980. p. 395).

Ganesh & Kumar (1987, p. 85) found when germinating cowpeas, chickpeas and greengram, phytate content decreased in both uncooked and cooked legumes. Sattar et al. (1989, p. 114) claim that when soaking mung beans, the phytate content decreased, more so at 55°C than at 29°C. Lee & Karunanithy (1990, p. 444) stated that the decrease in phytic acid meant losses in divalent minerals, iron, calcium and zinc, were less as they became bioavailable. Dagnia (1990, p. 45) noted a decrease in phytic acid after germinating *L. angustifolius* for 6 days.

Gupta & Wagle (1980, p. 395) found when germinating *Phaseolus* mungoreous for 9 days, the content of oligosaccharides, raffinose and stachyose, reduced.

Trugo, Farah, Trugo, Sierra & Camacho (1990, p. 2) noted a sharp decrease in the oligosaccharide content of *L. angustifolius* and *L. albus* following 3 days germination. Dagnia (1990, p. 44) found similar results after germinating *L. angustifolius* for 6 days.

Dagnia (1990) reported that germinating lupins for 6 days lowered the alkaloid content by 78% (1990, p. 37).
Although there is conflicting evidence as to the sort of changes that occurred to the protein, amino acid, and fat content of raw legumes during germination, the results show that biochemical changes do occur. There is clear evidence that vitamin content of raw legumes increases during germination and that antinutrient content decreases.

Previous research on germination took little account of which stage of germination (soaking, early and later germination stages) the biochemical changes occur. One aim of this study was to determine what biochemical changes occurred at each stage of germination of lupins.

2.6 EFFECT OF GERMINATION ON THE SENSORY PROPERTIES OF LEGUMES

It is believed that germination not only improves the chemical properties of legumes, but also their sensory qualities. There has been however, very little work completed on the sensory evaluation of lupin sprouts.

Lee (1986, p. 68) found that the colour, freshness and crispness of lupin sprout are excellent (1986, p. 68).

Yu et al. (1985, p. 177) found similar results when germinating lupins. Yu et al. (1985, p. 177) found that lupin sprouts had superior eating qualities compared to soybean sprouts and that lupins sprouts have a higher moisture content than soybean. Yu et al. (1985, p. 177) assert that lupin sprouts have the advantage of having a low lectins and trypsin inhibitor content, thus they can be
eaten raw as salad vegetables, while soybean sprouts cannot.

Zweck (1988, p. 23) completed a sensory evaluation of lupin and soybean sprouts, and reported that consumers found lupin sprouts to be more acceptable than soybean sprouts.

Although there are only a small number of known studies have been completed on the sensory properties of lupin sprouts, all reports appear to suggest that germination increases the eating quality of lupins, and that lupin sprouts are more acceptable than soybean sprouts.

2.7 RELATIONSHIP BETWEEN THE BIOCHEMICAL AND SENSORY CHANGES THAT OCCUR DURING GERMINATION

While there are reports about the types of biochemical and physical changes which occur during the germination process (see section 2.5), there is a notable absence of similar evidence regarding any sensory changes occurring in the same period. There is no evidence which relates the known biochemical changes which occur during germination with any changes in sensory properties.

As such data would be of obvious importance, one aim of this study was to attempt to match the biochemical and sensory changes that occur in the developing lupin sprout.
2.8 THE USE OF LUPINS IN FOODS FOR HUMAN CONSUMPTION

Lupins have been consumed by humans for thousands of years in Mediterranean countries and in the highlands of South America (Lee, 1986, p. 64). In pre-Colombian cultures in South America, *L. mutabilis* was mixed with potato, maize and quinoa to provide energy and protein (Lopez de Romana et al., cited by Petterson, 1985, p. 27). Lupins were also used for snacks, salads and mixed into mashed potato and soups in the Andean highlands (Gross & von Baer, cited by Petterson, 1985, p. 27). Italians, for centuries, have used *L. albus* for snack foods (Petterson & Crosbie, 1990, p. 268) and to make pasta.

It is estimated that less than 1% of the population is allergic to lupins. Fewer than 20 of 3000 people that consumed lupin seed or derived products have reported any adverse reactions (Petterson, personal communication, 1991). This is very low considering 4-6% of the population are allergic to milk, 2-3% to soybean and 3-12% to other legumes (Petterson & Crosbie, 1990, p. 268). This may offer several advantages to potential consumers of foods containing these ingredients.

In Australia the use of lupins in foods for human consumption has been minimal until 1988. In the early 1970s the National Health and Medical Research Council (NHMRC) approved the addition of up to 10% lupin in foodstuffs. Subsequently various submissions were made relating to the safety of low alkaloid lupins and in 1988 the NHMRC approved
the unrestricted use of lupins in foods for human consumption provided they have an alkaloid content of less than 200mg/kg (Crosbie, Petterson & Wilkinson, 1988, p. 1).

There is not a lot of lupins being consumed by people at present; a few hundred tonnes of hulls for fibre in bread and a few tonnes for incidentals, including farmers chewing lupins whilst feeding their sheep.

Currently, the United Kingdom Ministry for Agriculture Fisheries and Foods is reviewing the use of lupin under their Novel Food guidelines. The Japanese Department of Health, Education and Welfare is also reviewing lupins for human consumption.

The use of lupins in many different types of food is being continually explored. Research and sensory evaluation into the use of lupins in foods shows that it has great potential in both Oriental and Western foods (Kyle et al., 1991, p. 3).

2.8.1 THE USE OF LUPINS IN ORIENTAL FOODS

In Asia, soybeans are used in a variety of products, such as fermented sauces, bean curd and vegetable milk. Due to the similar physical and chemical properties of lupins and soybeans, extensive food product development work has been started into using lupins as a substitute for soybeans in Oriental foods. Asians, being used to the beany/nutty flavour of soybean, may find the blander taste of lupins
unacceptable. Caucasians, being less familiar with the taste of soybeans, may find the introduction of lupins more to their liking (Petterson & Crosbie, 1990, p. 266-267).

FERMENTED SAUCE: A fermented sauce is made by fermenting a legume and cereal grain with a fungus, usually Aspergillus oryzae, for 6 - 18 months. The type and proportion of cereal grain used can be varied as can the strain of fungus used. A blend of 30% lupin seeds and 70% soybean made an acceptable alternative to traditional soy sauce in terms of colour, taste and flavour (Hung et al., 1990). Lee (1986, p. 70) prepared a Korean style sauce by fermenting lupins; it was less acceptable than soy sauce but it was acceptable overall.

BEAN CURD: Bean curd can be made by coagulating the protein of a milk with calcium sulphate, magnesium chloride or glucono-delta-lactone. The curd may be left to set with a soft gel-like consistency (silken tofu) or pressed to form a compact product. The curd can be used in salads or soups. The blending of 30% of lupin milk with 70% soy milk enabled the production of a bean curd with no loss of yield or texture. A fried lupin/soy curd has favourable characteristics such as a golden colour, good texture and sponginess (Hung et al., 1990). Current work in progress (Mok, 1991) indicates that a silken tofu containing lupin is not acceptable to Caucasian consumers in its present form.
MISO: Miso is a paste-like product used as an ingredient in soups and sauces. It is made by fermenting a legume and cereal, usually rice, in the presence of salt (Cunha & Beirao da Costa, 1990, p. 2; Kyle et al., 1991, p. 4). Cunha & Beirao da Costa (1990, p. 32) found that lupin miso was similar in chemical properties to soybean miso but had more acceptable sensory characteristics.

TEMPEH: Tempeh is an Indonesian food that is traditionally made by fermenting dehulled cooked soybeans with a mould (*Rhizopus oligosporus*) to form a compact cake. Agosin, Diaz, Aravena & Yanez (1989, p. 104) found that the sensory characteristics of deep-fried lupin showed potential.

VEGETABLE MILK: Lee (1986, p. 73) successfully produced a lupin milk from lupin protein concentrate. The milk was not gritty and had a sensory quality comparable to a commercial soybean milk.

Vegetable milks are not just being developed in Asia but also in other countries. In Chile, an investigation found the use of lupins in milk substitutes for children was very beneficial for nutritional reasons, due to the high protein content, and for economic reasons, as it provided potential savings (Ivanovic, cited by Hill, 1986, p. 54).

Camacho, Vasquez, Leiva & Vargas (1988, p. 238) produced a milk from *L. albus* that was found to be less acceptable than cow's milk. The quality of lupin milk was
still considered good enough to justify expanding the program. A commercial scale milk product is now used in the Chilean National Food Program for school children (A.J. Evans, personal communications, 1991).

Creagh (1988, p. 38) found no statistical difference in the sensory characteristics of lupin milk and 'So-good' soy milk.

Petterson, Kyle and Evans, used a commercial pilot plant to produce milk from *L. angustifolius* kernels. Problems were encountered with pH stability in the process and with flavour and dispersibility of the final product. This is the subject of continuing investigations. (D. Petterson, personal communication, 1991).

The evidence above suggests that lupin, at least in small amounts, can be successfully incorporated into a number of Asian food products.

2.8.2. THE USE OF LUPINS IN WESTERN FOODS

In many Western foods wheat flour is one of the major ingredients. Research and sensory evaluation has been conducted into the possibility of incorporating some lupin flour into wheat flour based products such as bread, pasta, cakes and biscuits. Other products, such as milk substitutes have also been made using lupin.
BREAD: Research indicated that 10-12% of *L. albus* or *L. mutabilis* flour can be added to wheat flour before there is a reduction in the quality of the bread in comparison to conventionally made bread. Studies indicate that bread containing lupin flour stayed fresh longer than does conventional bread (Campos & El-Das, cited by Hill, 1986, p. 53). Bergl and Smith (1989) found that the addition of 1% to 4% *L. angustifolius* flour was acceptable in bread.

BISCUITS: The addition of 25% *L. mutabilis* flour to biscuits has been successful (Reynso et al., cited by Hill, 1986, p. 53). A combination of 50% *L. angustifolius* flour and 50% rice flour produced a successful biscuit in terms of taste and appearance (Dagnia, 1990).

CAKES: The addition of 20% *L. mutabilis* flour to cakes was found to be successful (Reyes & Gross, cited by Hill, 1986, p. 53).

PASTA: Studies conducted by Lucisano and Pompei (1984, p. 91) found that the addition of 30% *L. albus* flour to pasta was successful. This was supported by Yates (1990, p. 60). Hung et al. (1990) found that the addition of 25% lupin flour was acceptable. The colour, taste and texture of 15-25% lupin flour pasta was rated higher than the 100% wheat flour pasta.
SNACK FOODS: Lupin pops are made by soaking lupins in flavoured brine and then roasting them. Results from sensory evaluation tests showed that they were chewy and nutty (Schweers, 1989, p. 39) *L. albus* has been used for centuries as a snack food by the Italians; they cook the lupins then soak them in brine (Petterson & Crosbie, 1990, p. 268).

It appears from the research above that in small amounts lupin can be incorporated into Western food products successfully.

2.9 ATTITUDBINAL ACCEPTABILITY OF LUPIN AS A FOOD INGREDIENT

While there has been a number of studies completed on the use of lupins as a food ingredient since its approval for human consumption in 1988, there are still no foods on the commercial market that contain lupin. Although the studies completed suggest that a small amount of lupin is acceptable in many Asian and Western foods, the lack of products on the commercial market may reflect negative attitudes towards the use of lupin in foods for human consumption. The name lupin is associated with animal feed by many consumers and manufacturers. Manufacturers may be unwilling to produce foods containing lupin because of the perceived negative attitudes of consumers.

In a pilot study on consumers' attitudes toward lupins as a food for human consumption, Zweck (1988, p. 21) found that only 57% of respondents felt the name lupin was
positive and fewer (49%) felt lupin was a positive name for a food ingredient. Most of the those consumers for whom the name lupin was negative, associated lupins with animal feed.

More work needs to be done on consumer attitudes towards the name lupin. One aim of this study was to examine consumer attitudes toward the name lupin and its acceptability as a food ingredient for human consumption.
2.10 RATIONALE

Much of the research conducted into the use of lupins as a food for human consumption has been focused on the lupin seed. These studies indicate that lupins are highly nutritious, being high in protein and fibre, and low in fat and antinutrients. However, there is limited information on biochemical and sensory changes that occur following germination of the lupin seed. The studies which have been completed (Dagnia, 1990; El-Habbal & Attia; 1987; Lee, 1986; Yu et al., 1985) on the germinated lupin sprouts indicate that germination improves the biochemical properties of lupins and enhances their eating qualities. Therefore, it appears that the biochemical and sensory qualities of the sprouted lupins are at least as good as that of the seed, and that there may be some relationship between the biochemical and sensory changes in the developing lupin sprout. One aim of this study was investigate the biochemical and sensory changes of the developing sprouts and to establish the relationship, if any, between the biochemical and sensory changes in the developing lupin sprout. It was expected that germination would improve the biochemical and sensory characteristics of the lupin seed.

Research on the use of lupin as a food ingredient for human consumption (Agosin et al., 1989; Bergl and Smith, 1989; Hung et al., 1990; Lee, 1986), has shown that a small amount of lupin is acceptable in many Asian and Western foods, however there are still very few food products on the
commercial market that contain lupin. It appears that consumer attitudes toward the name lupin may be negative due to its traditional association with animal feed. Another aim of this study was to identify consumer attitudes towards the use of lupin in human food by developing a food product containing lupin for consumers to sample. It was expected that consumers would find the developed lupin food product acceptable. This may not of course reflect the attitudes of food manufacturers and processors who may feel quite differently about the acceptability of the food product developed in this project.

2.11 OBJECTIVES

In order to test the research hypothesis the research program was designed with the following objectives:

1. To determine and assess the biochemical changes of developing *L. angustifolius* sprouts up to day 6.

2. To assess the sensory changes in developing *L. angustifolius* sprouts up to day 6.

3. To determine the relationship, if any, between the biochemical and sensory properties of lupin sprouts.

4. To develop a food product using lupin sprout as an ingredient.

5. To investigate consumer acceptance of the developed lupin product.

6. To investigate the attitudes of consumers towards the use of lupins as a food for human consumption.
3.0 PREPARATION OF LUPIN SPROUTS

To achieve the specified objectives, this study was divided into three parts, Biochemical Analysis and Sensory Evaluation, Food Product Development and Consumer Research Study. The seeds and sprouts were used for each part of the study. The lupin seeds were germinated for up to 6 days and then frozen.

The lupin seeds, (L. angustifolius cv. Gungurru) provided by the Grain Pool of Western Australia, were sprouted using an automatic commercial sprouting system, a Micro Malter (Union Maltings, W.A.). The Micro Malter is a machine which has baskets attached to a drum that can be rotated within a confined, controlled environment. This enables the germinating seeds to be 'freed' from one another. The Micro Malter was only available for two weeks so all the germination for these experiments had to be done in one batch. As discussed later, this restriction on the access to the Micro Malter, had important implications for this study.

The sprouting procedures used were adapted from previous sprouting studies (Dagnia, 1991; Lee, 1986; Yu et al., 1985). The seeds were soaked for 24 hours in tap water, then germinated in the dark at 20°C and turned 4 times every 3 hours. The seeds were rinsed for 30 minutes every 7 and a half hours with tap water. Sprouts were removed after soaking for 24h (Day 0), then after germination for 24h (Day 1), 48h (Day 2), 72h (Day 3), 96h (Day 4) and 164h (Day 6).
The number of batches which could be sprouted was limited by the number (6) of baskets available in the Micro Malter. Sprouts were aged to day 6 so that data could be compared to similar work completed by Dagnia (1990) on day 6 sprouts.

Once removed, the sprouts were frozen using a domestic freezer and stored at -18°C until required. Each batch of sprouts were randomly divided into three so that the sprouts used at each part of the study came from the same batch.

Moisture content was determined by weighing the fresh and freeze-dried samples. The samples then milled and all assays were subsequently conducted on the dry material so that results could be reported on a dry basis. This enabled figures to be directly compared to other data in the literature.

4.0 PART 1: BIOCHEMICAL ANALYSIS OF AND SENSORY EVALUATION OF THE LUPIN KERNEL AND SPROUTS

This part of the study involved conducting biochemical assays and sensory evaluation on the lupin kernel and developing lupin sprouts.

4.1 BIOCHEMICAL ANALYSIS

Biochemical assays were conducted on the lupin kernel and the dehulled lupin sprouts aged day 0, 1, 2, 3, 4, and 6, and for comparative purposes the day 0, 3 and 6 sprouts with the hull were also analysed. Assays procedures used were those used and recommended by the Chemistry Centre (see appendix A for analytical procedures).
The assays included:

- Protein (by Leco nitrogen analyser)
- Fats and fatty acid profile (by Gas Chromatography)
- Ash (Oven dry method)
- Crude Fibre (Gravimetric method)
- Carbohydrates (by difference, proximate analysis)
- Sugars (Anthrone method - suitable for relative values within a single legume species)
- Polysaccharides (Anthrone method on residue from sugar analysis)
- Minerals (Acid digestion the analysis by ICP-AES)
- Trypsin inhibitor (Spectrophotometric method)
- Phytate (Anion-Exchange method)
- Oligosaccharides (High pressure liquid chromatography method)
- Alkaloids (Gas Chromatography)
- Tannins (Folin-Denis method for polyphenolics)
- Gross Energy (Bomb calorimetry, completed by J. Hooper, W.A.D.A)

The assays were completed at the Chemistry Centre (Western Australia), except where indicated, using standard methods of analysis. The student researcher was involved in all the analysis under the supervision of the Chemistry Centre staff, who are highly qualified and experienced in the field of chemical analysis. Appropriate validity and reliability checks were employed when conducting all assays.

4.1.1 DATA ANALYSIS

The findings from the biochemical analysis were reported in a comprehensive written format and graphs and tables were used where necessary to summarise the data.
4.1.2 RESULTS OF BIOCHEMICAL ANALYSIS

Various biochemical assays were conducted on the lupin kernel and sprouts, both with and without the hull, up to day 6 of germination. A number of biochemical changes occurred over the 6 days of sprouting (Table 8-13).

### Table 8
Chemical Composition of Lupin Kernel and Sprouts Without the Hull (g kg⁻¹ dry basis)

<table>
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<tr>
<th></th>
<th>soaked</th>
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<th>DAY 4</th>
<th>DAY 6</th>
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<td>24h</td>
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### Table 9
Mineral Content of Lupin Kernel and Sprouts Without the Hull

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<th>DAY 1</th>
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<tr>
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</table>
4.1.2 RESULTS OF BIOCHEMICAL ANALYSIS

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Table 8
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<table>
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<th></th>
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<td>28</td>
<td>29</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>417</td>
<td>378</td>
<td>399</td>
<td>394</td>
<td>398</td>
<td>399</td>
</tr>
<tr>
<td>Sugars</td>
<td>106</td>
<td>101</td>
<td>88</td>
<td>81</td>
<td>81</td>
<td>86</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>97</td>
<td>102</td>
<td>115</td>
<td>124</td>
<td>133</td>
<td>134</td>
</tr>
<tr>
<td>Energy (MJ/kg)</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 9
Mineral Content of Lupin Kernel and Sprouts Without the Hull

<table>
<thead>
<tr>
<th></th>
<th>soaked KERNEL</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.7</td>
<td>1.0</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.4</td>
<td>1.7</td>
<td>1.6</td>
<td>1.7</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Sulphur</td>
<td>2.5</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.7</td>
<td>3.9</td>
<td>3.8</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.1</td>
<td>7.2</td>
<td>8.3</td>
<td>8.2</td>
<td>8.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>g kg⁻¹ dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>4.4</td>
</tr>
<tr>
<td>Manganese</td>
<td>33</td>
</tr>
<tr>
<td>Iron</td>
<td>42</td>
</tr>
<tr>
<td>Zinc</td>
<td>38</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.01</td>
</tr>
<tr>
<td>Lead</td>
<td>0.07</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Table 10
Antinutrient Content of Lupin Kernel and Sprouts Without the Hull (g kg\(^{-1}\) dry basis)

<table>
<thead>
<tr>
<th></th>
<th>soaked</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligosaccharides</td>
<td>65</td>
<td>57</td>
<td>6</td>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Phytate</td>
<td>5.1</td>
<td>5.1</td>
<td>5.4</td>
<td>5.3</td>
<td>4.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Tannins</td>
<td>2.1</td>
<td>2.2</td>
<td>2.4</td>
<td>2.6</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Trypsin Inhibitor</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>0.09</td>
<td>0.09</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 11
Proximate Analysis of Lupin Kernel and Lupin Sprouts With the Hull (g kg\(^{-1}\) dry basis)

<table>
<thead>
<tr>
<th></th>
<th>soaked</th>
<th>DAY 1</th>
<th>DAY 3</th>
<th>DAY 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>635</td>
<td>623</td>
<td>584</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>374</td>
<td>374</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>73</td>
<td>61</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>149</td>
<td>148</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>29</td>
<td>29</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>375</td>
<td>388</td>
<td>395</td>
<td></td>
</tr>
<tr>
<td>Sugars</td>
<td>79</td>
<td>63</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>98</td>
<td>111</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

Table 12
Mineral Content of Lupin Kernel and Sprouts With the Hull

<table>
<thead>
<tr>
<th></th>
<th>g kg(^{-1}) dry basis</th>
<th>mg kg(^{-1}) dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>2.1</td>
<td>21</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.8</td>
<td>18</td>
</tr>
<tr>
<td>Sulphur</td>
<td>2.1</td>
<td>21</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.2</td>
<td>32</td>
</tr>
<tr>
<td>Potassium</td>
<td>6.9</td>
<td>69</td>
</tr>
<tr>
<td>Copper</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Manganese</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Iron</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Zinc</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Lead</td>
<td>1.88</td>
<td>188</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.68</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 13
Antinutrient Content of Lupin Kernel and Sprouts
With the Hull (g kg⁻¹ dry basis)

<table>
<thead>
<tr>
<th></th>
<th>soaked 24h</th>
<th>DAY 3</th>
<th>DAY 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligosaccharides</td>
<td>46</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td>Phytate</td>
<td>49</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td>Tannins</td>
<td>2.1</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Trypsin Inhibitor</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

4.1.3 DISCUSSION

The protein content of the lupin sprouts without the hull increased by 6% after 6 days of sprouting. The protein content of the sprouts with the hull did not change notably. This increase in protein may not be real, but due to the loss of materials, such as fat and carbohydrates (see Figure 3). These results are similar to those found by Dagnia (1990) after germinating lupins for 6 days.

---

Figure 3. Changes in protein during germination
The fat content of the lupin sprouts with the hull increased during the soaking and early germination stages, then decreased considerably in the later stages of germination. The initial increase is likely to be due to the loss of other materials. The fat content of the day 6 sprout without the hull decreased by 18% while the fat content of the day 6 sprout with the hull decreased by 26% (see Figure 4). It is likely that the decrease in fat content is due to fat being broken down by lipase to be used for energy in respiration (Mayer, 1963, p. 113-114). These results support those found by Dagnia (1990) after germinating lupins for 6 days.

![Fat content change during germination](image)

**Figure 4.** Changes in fat during germination
### Table 14
**Fatty Acid Profile of Lupin Kernel and Sprouts Without the Hull**

<table>
<thead>
<tr>
<th>FATTY ACID</th>
<th>soaked DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KERNEL 24h</td>
</tr>
<tr>
<td>Myristic</td>
<td>0.2</td>
</tr>
<tr>
<td>Palmitic</td>
<td>11.4</td>
</tr>
<tr>
<td>Stearic</td>
<td>7.4</td>
</tr>
<tr>
<td>Oleic</td>
<td>34.5</td>
</tr>
<tr>
<td>Elaidic</td>
<td>0.8</td>
</tr>
<tr>
<td>Linoleic</td>
<td>37.1</td>
</tr>
<tr>
<td>Linolenic</td>
<td>3.7</td>
</tr>
<tr>
<td>Arachidic</td>
<td>1.0</td>
</tr>
<tr>
<td>Eicosenoic</td>
<td>0.3</td>
</tr>
<tr>
<td>Behenic</td>
<td>2.2</td>
</tr>
<tr>
<td>Lignoceric</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The most abundant fatty acids in the lupin sprouts (with and without the hull) are linoleic, which is polyunsaturated, and oleic, which is unsaturated (see Table 13 and 14 and Figure 5). There were few other changes in fatty acids content. There was a decrease in oleic acid
and an increase in linolenic acid, which is a polyunsaturated fatty acid. It is likely that the decrease in oleic acid is due to it being used for energy. The change in oil quality and by-products from the breakdown of fatty acids may affect the flavour of the sprouts.

Figure 5. Changes in fatty acids during germination (without the hull)
Fibre content of the lupin sprouts without the hull increased by 67% after the soaking stage (day 0). This increase may be due to a loss of soluble material. The fibre content of the lupin sprouts with the hull was, as expected, much higher than the sprouts without the hull, however was not affected by germination (see Figure 6).

Changes were also noted in the proportion of the various constituent sugars that make up the cell wall. For example the content of galactose (the major sugar present) fell by 32%, mannose increased by 83%, uronic acid increased by 41% and glucose increased by 29%. Other sugars varied by less than 10% from the original kernel. These relative changes suggest that the cell wall material was broken down and converted from storage carbohydrates to structural carbohydrates, and used as a source of energy.

![Graph showing changes in fibre content during germination.](image)

**Figure 6.** Changes in fibre during germination
The carbohydrate content of the lupin sprouts with and without the hull decreased during germination. There was a sharp decrease in the soaking stage. It is likely that carbohydrates were broken down for energy (see Figure 7).
The content of low molecular weight saccharides in the lupin sprout decreased during the soaking and early germination stages. These sugars are likely to have been used as an energy source for metabolism. The sugar content of the sprout then increased in the later stages of germination as the complex carbohydrates may have been broken down to form a pool of simple sugars for growth of the sprout (see Figure 8).

![Graph showing changes in sugars during germination](image)

**Figure 8.** Changes in sugars during germination
Polysaccharides increased considerably in both the sprout with and without the hull following 6 days germination, 36% and 17% respectively (see Figure 9). This increase again may be due to a loss of other nutrients. It is likely that polysaccharides are not metabolised as quickly as other nutrients such as fat. It would seem unlikely that further synthesis of cell wall material would occur at this stage.

*Figure 9.* Changes in polysaccharides during germination
The gross energy value of the germinating sprouts did not change notably (see Figure 10).

**Figure 10.** Changes in energy during germination.
Ash content changed slightly during germination indicating some electrolyte changes. There were no significant changes in the calcium, magnesium, phosphorus, copper, manganese, zinc and iron content of sprouts with or without the hull during germination. Potassium was the only mineral that decreased following germination of the sprouts, without the hull, possibly due to leaching into the soaking medium (see Figure 11).

Figure 11. Changes in mineral content during germination (without the hull)
Sprouting made very little difference to the cadmium or lead content of lupin sprouts with or without the hull. Nickel content increased immediately after germination commenced but decreased by day 6 in sprouts with and without the hull (see Figure 12).

Figure 12. Changes in cadmium, nickel and lead during germination (without the hull)
Some antinutrients decreased as a result of germination (see Figure 13).

Alkaloids, which are bitter toxic compounds, decreased considerably following 6 day germination (67% in the sprout without the hull and 57% in the sprout with the hull). The low levels of alkaloid in the sprouts should make the bitterness of lupins less detectable. These results are similar to those of Dagnia (1990, p. 37) who found a 78% decrease after 6 days of germination.

Oligosaccharides are simple oligomers of raffinose which ferment in the hind gut to cause flatulence. The oligosaccharide content of lupins decreased by 98% (with and without the hull) during germination. Oligosaccharides are likely to have been used as an energy source for the growing sprout. It is unlikely that the low oligosaccharide content in the day 2 to day 6 sprouts would cause flatulence, thus sprouting would be beneficial. These results are similar to those of Dagnia (1990) and Trugo et al. (1990). Dagnia (1990, p. 37) found that the oligosaccharide content of lupin sprouts decreased by 95% after 6 days of germination. Trugo et al. (1990, p. 44) noted a sharp decrease in oligosaccharide content of lupin after 3 days of germination.
Figure 12. Changes in antinutrients during germination.

% OF RELATIVE CHANGE (baseline 100)

- ALKALOIDS
- OLIGOSACCH
- TANNINS
- TRYSIN I#
- PHYTATE

* Oligosaccharides
# Trypsin Inhibitor
The tannin content of lupin increased after germination. This is not a favourable result as tannins are believed to cause irritation in the digestive tract, lowered energy conversion from food, increased excretion of nitrogen in the faeces and cancer (Petterson, personal communication, 1991).

There was no change in the trypsin inhibitor due to germination.

Phytate content did not change notably during germination. These results differ from Dagnia (1990) who showed a 66% decrease in phytate after 6 days germination.
The moisture content of lupin sprouts increased sharply in the soaking stage then decreased slowly during germination (see Micro Malter figures on Figure 14).

This was an unexpected result. Moisture content should have increased throughout germination due to the continued uptake of water as found by Dagnia (1990), Lee (1986), & Yu et al., (1985). Figure 15 shows the differences in the
Fresh Sprout figures (see appendix B for domestic sprouting conditions) and Micro Malter sprout.

![Graph showing comparison of moisture content of sprouts germinated in the micro malter machine and those sprouted domestically (fresh)](image)

**Figure 15.** Comparison of moisture content of sprout germinated in the micro malter machine and those sprouted domestically (fresh).

It appears that the sprouts suffered moisture stress as a result of the sprouting conditions. Seeds probably needed to be soaked for longer periods and more frequently.
Biochemical changes may have been different if other sprouting conditions were used.

While the moisture stress in the sprouts was discovered at a relatively early stage of the study, as stated earlier, the Micro Malter was not available for use again until 1992. Therefore another batch of sprouts could not be germinated. Sprouting by other methods could not be done under controlled methods nor would they produce adequate amounts of sprouts for the whole study. Therefore it was decided to continue the experiment using the moisture stressed sprouts, but with a recognition that this may have an effect on the results of the research.

This problem could be overcome with more experience with the use of the Micro Malter as a means of germinating legume seeds. The Micro Malter is presently used mainly for sprouting barley for malting. Different conditions have to be developed for its use with germinating lupins. Another alternative may be to use a commercial sprouter plant that already germinates legumes such as mung beans and alfalfa.
4.1.4 SUMMARY

There appears to be distinct evidence of moisture stress in the developing sprout. This would have a marked effect on the biochemical changes which occurred during the later stages of germination in particular. It does appear that the experimental conditions chosen for germination using the Micro Malter were inappropriate and this problem had a major and negative influence on the quality and physical characteristics of the older sprouts.

Even so there were a large number of biochemical changes that occurred during the germination of lupin seeds, especially in the soaking and early stages of germination. The major changes in nutrients included a decrease in fat and sugars, and an increase in fibre and polysaccharides.

The most favourable changes that occurred during germination were the decreases in the antinutrients, oligosaccharides and alkaloids. The decrease in oligosaccharides would be expected to reduce the likelihood of lupins causing flatulence, and the decrease in alkaloids would be expected to reduce the likelihood that any bitter taste would be detected.

The results were reported on a dry basis, so the sprouts could be compared to the kernel and other reports on germinated legumes. If a cost benefit analysis was made results would need to be reported on per seed basis taking into account any loss of dry matter that may have occurred with soaking and sprouting.
It could be expected that the large biochemical changes would have affected the sensory characteristics of the lupin seed especially during the soaking (day 0) and early stages of germination. A sensory evaluation was undertaken to see if any relationship could be shown between the biochemical and sensory changes in the developing sprout. The next stage of the study involved an attempt to identify any relationships between biochemical and sensory characteristics of the developing lupin sprout.
4.2 SENSORY EVALUATION OF THE LUPIN SPROUT

This part of the study involved the evaluation of dehulled lupin sprouts aged day 0, 1, 2, 3, 4 and 6, in terms of their sensory characteristics.

4.2.1 SAMPLE PRESENTATION

The various lupin sprouts (see Plate 1) change in physical appearance at different stages of development. As such difference would make it impossible to control for psychological biases (based on expectations relating to size, shape, colour and so on), it was decided that the physical differences had to be removed. This was achieved by freeze drying the sprouts and then milling them into a fine powder.

The powder was then compressed into pellets about 5mm high. The pellets were identical in terms of size, shape, colour texture, particle size and moisture level (Plate 2). Any differences found between the pelletised sprouts at different stages of development should be based on sensory rather than physical characteristics.
Plate 1. Lupin sprouts aged day 0, 2, 4, and 6

Plate 2. Different aged lupin sprouts and kernel made into pellets
4.2.2 SUBJECTS

A sensory evaluation panel composed of a small number of respondents (20-25) studying in the area of foods, was trained to evaluate the sprouts.

4.2.3 TEST INSTRUMENT

A questionnaire (see below) was designed to evaluate the lupin sprouts in terms of their sensory attributes. Undifferentiated scales were used which do not provide an explicit escape clause. The undifferentiated scales were made up of a 50-point scale with anchors at each end. Some attributes were evaluated by hedonic measurement (that is assessment of pleasure) while other attributes were evaluated using quantitative measurements which measure the strength or intensity of a particular characteristic (eg. bitterness) (Flanagan, 1991).

Pilot testing of the questionnaire was conducted in order to ensure that the appropriate attributes and end anchors were chosen for the evaluation of this type of product.
4.2.4 QUESTIONNAIRE

INSTRUCTIONS:
Please rate each sample by placing the appropriate symbol (0, V, +) in the desired position along the scales below.

FLAVOUR
very unpleasant
I.... .... .... .... .... .... .... .... .... .... .... .... I

SWEETNESS
Nil
I.... .... .... .... .... .... .... .... .... .... .... .... I

SALTINESS
Nil
I.... .... .... .... .... .... .... .... .... .... .... .... I

BEANINESS
Nil
I.... .... .... .... .... .... .... .... .... .... .... .... I

UNUSUAL FLAVOURS
Nil
I.... .... .... .... .... .... .... .... .... .... .... .... I

OVERALL ACCEPTABILITY
totally unacceptable
totally acceptable
I.... .... .... .... .... .... .... .... .... .... .... .... I
4.2.5 DESIGN

As a total of six samples were to be evaluated, a linked experimental method was used with no more than three products being evaluated at a time. In order to minimise response bias, samples were coded using symbols (ϕ, v, +) to control for that source of psychological response bias. Samples were placed into 30mL cups and placed on a white plate divided into three pre-coded sections. The cups were placed on the section of the plate with the corresponding code (see Figure 16). Spoons were supplied for ease of tasting.

Water was provided for respondents to drink between samples to help neutralise their palate. The respondents were not given any information about the samples in order to reduce any biases due to expectations.

Figure 16. Plate presentation

4.2.6 EXPERIMENTAL VARIABLES

Age of the sprouts: Day 0, 1, 2, 3, 4, or 6.

4.2.7 CONTROLLED VARIABLES

Sprouting Methods: the same sprouting methods were used for all the seeds (see section 3.0 Preparation of lupin sprouts).
Serving Temperature: all the sprouts were served at room temperature.

Dehulling: all sprouts were dehulled.

4.2.8 DATA ANALYSIS

Results from the questionnaire responses were analysed statistically using the Minitab statistical package. Descriptive statistics such as means, standard deviations and frequencies were calculated. One-way Analysis of Variance (ANOVA) was used to identify differences within each set of data. If significant differences were detected by the ANOVA F-test, the Rodgers Range Test was used to determine whether any significant differences existed between the pairs of samples (see appendix C).

The findings are reported in a comprehensive written format with the use of figures and tables to summarise the data.
4.2.9 RESULTS OF SENSORY EVALUATION OF LUPIN KERNEL AND SPROUTS

TRIAL A: SOAKING AND EARLY GERMINATION OF LUPIN SPROUTS

As reported above, a large number of biochemical changes occurred in the sprout during the soaking stage. The most notable biochemical changes were the decrease in the antinutrients, oligosaccharides and alkaloids. It was expected that sensory changes would occur in the sprout during the soaking. For example changes in alkaloid content may reduce the bitter taste of lupin sprouts and therefore the day 1 sample should be less bitter than the other samples. Trial A aimed to evaluate the sensory characteristics of the kernel, day 0 and day 1 sprout.

VARIABLES:
+ Kernel
v Day 0 (soaked for 24h) without the hull
φ Day 1 sprout without the hull

Table 16
Results of Sensory Evaluation: Trial A (average response)

<table>
<thead>
<tr>
<th></th>
<th>Kernel</th>
<th>Day 0</th>
<th>Day 1</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>v</td>
<td>φ</td>
<td>+/v</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>12</td>
<td>11</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>30</td>
<td>26</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil -very unusual)</td>
<td>29</td>
<td>29</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

COMMENTS:

The results from trial A showed that there was no significant difference in the sensory properties of the kernel,
day 0 and day 1 sprouts. This result was unexpected. A number of biochemical changes had occurred in this time but these did not appear to have affected the sensory properties of the lupin sprouts. It may be possible that the sensory changes were too subtle to be detected, or that the sensory methods used were not sensitive enough to detect the changes in biochemical properties.

TRIAL B: LATER GERMINATION STAGE OF LUPIN SPROUTS

A large number of biochemical changes occurred during the early germination stage. It was expected that the sensory changes would have also occurred during the early germination stage. Trial B aimed to evaluate the sensory properties of the day 1, day 2 and day 3 sprouts. It was expected that the older sprout (day 3) would be more acceptable than the day 1 sprout due to the lower content of alkaloids.

VARIABLES:
\( \phi \) Day 1 sprout without the hull
\( v \) Day 2 sprout without the hull
\( + \) Day 3 sprout without the hull

**Table 17**
Results of Sensory Evaluation: Trial B (average response)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \phi )</td>
<td>( v )</td>
<td>( + )</td>
<td>( \phi/v )</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>11</td>
<td>6</td>
<td>6</td>
<td>*</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>29</td>
<td>29</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>36</td>
<td>39</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>*</td>
</tr>
</tbody>
</table>

* Significant Difference \( (\alpha: 0.05) \)
COMMENTS:

The younger day 1 sprout was perceived as being more pleasant in flavour and overall more acceptable than the older day 2 and day 3 sprouts. There were no significant differences between the day 2 and 3 sprouts. These results were also unexpected. It was expected that the older sprouts would have been more acceptable as a result of the decrease in bitter alkaloids. As stated earlier, there was clear evidence of moisture stress problems at the later stages of germination. This may have produced the unexpected results found in this experiment.

TRIAL C: FINAL STAGES OF GERMINATION

Trial C aims to evaluate the sensory changes that occurred in the final stages of germination. It was expected that the older (day 6) sprout would be more acceptable than the younger sprouts (day 3 and day 4) as it should be less bitter, due to the decrease in alkaloids.

VARIABLES:
+ Day 3 sprout without the hull
φ Day 4 sprout without the hull
v Day 6 sprout without the hull
Table 18
Results of Sensory Evaluation: Trial C (average response)

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>16</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>15</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>12</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>32</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>29</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>14</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

COMMENTS:

The results from trial C show that there were no significant sensory differences between the day 3, day 4 and day 6 sprouts. These results were unexpected. It was expected that the older sprout (day 6) would have been more acceptable due to the decrease in alkaloids. The results suggest that the attempt to match sensory and biochemical changes was not successful and that further work on developing both sprouting and sensory methods is necessary before any firm conclusions can be drawn.

TRIAL D: AGE AND MOISTURE STRESS EFFECTS

It was felt that the age differences in the sprouts tested in trials A, B, and C, were not large enough to show sensory changes and that a larger age difference maybe necessary to detect the changes. Trial D aimed to compare the largest age difference, the ungerminated kernel with the oldest sprout, day 6. A large number of biochemical changes occurred during these 6 days of
germination. It was therefore expected that sensory changes would have also occurred during this time.

Secondly it was felt that the moisture stress which occurred in the sprouts during germination as a result of the germination conditions, may have caused the failure to detect any systematic sensory changes. Trail D also aimed to compare the day 6 sprout germinated in the Micro Malter with the day 6 sprout germinated under domestic conditions in order to determine the effects of moisture stress on the sensory characteristics of the sprout.

**VARIABLES:**

+ Kernel

\* Day 6 sprout without the hull (Micro Malter)

\# Day 6 sprout without the hull (Fresh)

<table>
<thead>
<tr>
<th>Table 12</th>
<th>Results of Sensory Evaluation: Trial D (average response)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kernel</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>19</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>16</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>12</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>29</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>25</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>20</td>
</tr>
</tbody>
</table>

* Significant Difference ($\alpha=0.05$)

**COMMENTS:**

The results from trial D indicate that there was no significant difference between the kernel and day 6 Micro Malter sprout. This result was unexpected due to the large number of biochemical changes that occurred in this time. Again it appears
if sensory changes did occur during germination they were not detectable using these methods and procedures.

The fresh day 6 sprout was not perceived as being as pleasant in flavour or as acceptable as the day 6 sprout germinated in the Micro Malter machine. The fresh day 6 sprout was also perceived as having more unusual flavours. It is difficult to explain these results.

From these results, it seems that the failure to be able to match sensory and biochemical results is likely to be due to the problems of moisture stress and also to the inadequacies of the sensory evaluation techniques used in this part of the study.

4.2.10 SUMMARY

There were few sensory differences that could be detected in the lupin sprouts of different ages. This was an unexpected result as a large number of biochemical changes had occurred during the 6 days of germination, including decreases in fat, oligosaccharides, and alkaloids, and an increase in polysaccharides. It was expected that sensory changes would have been detected as a result of the biochemical changes in the sprouts.

It is possible that the moisture stress that occurred in the sprout as a result of the sprouting conditions, may have reduced the ability to detect any sensory changes. It is also possible that the sensory changes in the sprout were just not
detectable by the taste panel, or that a larger, more trained panel was necessary to detect the sensory changes.

The sensory techniques used, particularly the use of the pellets, may have not been suitable for this type of experiment, although the use of pellets as a way of controlling biases was found to be successful by Barlow (1991) who found significant difference when evaluating muesli. It is possibly that a paste would have been more suitable for evaluating lupin sprouts as many respondents found the lupin pellets to be an unpleasant way of evaluating this type of product.

More work is needed on the sensory techniques and the use of lupin pellets. There also needs to be more work done on developing optimum germination conditions for lupin sprouts.

While the sensory evaluation techniques used were found to be unsuccessful for analysing the effects of biochemical changes, this does not mean that they cannot be used as an experimental tool for the development of a food product. The next stage of this study involved the use of sensory evaluation techniques for the purpose of developing a food product incorporating lupin.
5.0 PART 2: FOOD PRODUCT DEVELOPMENT

This part of the study involved the development of a basic food product and the evaluation of its sensory characteristics. Originally a lupin milk was chosen to be developed, but this proved to be unsuccessful (see appendix D). Preliminary trials indicated a soup would be a more appropriate way of incorporating lupins into a standard food product (see appendix E).

The soup market in Australia is worth millions of dollars. The value of the soup imported into Australia was $5.35m for the 1990/1991 financial year (I. Wilkinson, personal communication, 1991).

5.1 FOOD PRODUCT DEVELOPMENT

A lupin soup recipe was selected from a previous study (Smith, 1990) which successfully incorporated lupin kernel into a soup. The basic recipe (Smith, 1990) was adapted to suit the use of lupin sprouts rather than kernel, and then modified until a suitable soup was produced. Only one variable was altered for each experiment so that the effects of each treatment modification could be analysed. Details of the methods used for evaluation can be found below.

5.2 SUBJECTS

A sensory evaluation panel composed of a small number of respondents (20-25) studying in the area of foods. The respondents were trained to evaluate the soup products.
5.3 TEST INSTRUMENT

A questionnaire was designed to evaluate the soup product in terms of its sensory attributes. Undifferentiated scales were used which do not provide an explicit escape clause. The undifferentiated scales were made up of a 50 point scale with anchors at each end. Some attributes were evaluated by hedonic measurement (that is assessment of pleasure) while other attributes were evaluated using quantitative measurements which measure strength or intensity of a particular characteristic.

Pilot testing of the questionnaire was conducted to ensure that the appropriate attributes and end anchors were chosen for the evaluation of this type of product.
5.4 QUESTIONNAIRE

INSTRUCTIONS:
Please rate each sample by placing the appropriate symbol ($\Phi, V, +$) in the desired position along the scales below.

APPEARANCE

very unpleasant 
I.... .... .... .... .... .... .... .... .... .... .... ....I

very pleasant
I.... .... .... .... .... .... .... .... .... .... .... ....I

FLAVOUR PLEASANTNESS

very unpleasant
I.... .... .... .... .... .... .... .... .... .... .... ....I

very pleasant
I.... .... .... .... .... .... .... .... .... .... .... ....I

SWEETNESS

Nil
I.... .... .... .... .... .... .... .... .... .... .... ....I

very sweet
I.... .... .... .... .... .... .... .... .... .... .... ....I

SALTINESS

Nil
I.... .... .... .... .... .... .... .... .... .... .... ....I

very salty
I.... .... .... .... .... .... .... .... .... .... .... ....I

BEANINESS

Nil
I.... .... .... .... .... .... .... .... .... .... .... ....I

very beany
I.... .... .... .... .... .... .... .... .... .... .... ....I

UNUSUAL FLAVOURS

Nil
I.... .... .... .... .... .... .... .... .... .... .... ....I

very strong
I.... .... .... .... .... .... .... .... .... .... .... ....I

MOUTH TEXTURE

very unpleasant
I.... .... .... .... .... .... .... .... .... .... .... ....I

very pleasant
I.... .... .... .... .... .... .... .... .... .... .... ....I

TEXTURE BALANCE

far too thin
I.... .... .... .... .... .... .... .... .... .... .... ....I

far too thick
I.... .... .... .... .... .... .... .... .... .... .... ....I

OVERALL ACCEPTABILITY

totally unacceptable
I.... .... .... .... .... .... .... .... .... .... .... ....I

totally acceptable
I.... .... .... .... .... .... .... .... .... .... .... ....I

THANK YOU FOR YOUR CO-OPERATION
5.5 DESIGN

No more than three samples were tested at a time. Samples were coded using symbols (Φ, v, +) in order to control for these sources of psychological response bias. Samples were placed in 30mL cups and placed on a white plate divided into three pre-coded sections. This controlled for order effects. The cups were placed on the section of the plate with the corresponding code (Figure 4). Spoons were supplied for ease of tasting.

Water was provided for respondents to drink between samples to neutralise their palate. The respondents were not be given any information about the samples in order to reduce biases due to expectations.

![Plate presentation](image)

**Figure 4.** Plate presentation

5.6 EXPERIMENTAL VARIABLES

The experimental variables included:

- Cooking time.
- Presence of the hull: whether the hull was removed prior to cooking, cooked in the soup, or cooked in the soup and then removed by straining.
- Soup ingredients: type and amount of vegetables, stock and water used in the soup base.
Age of the sprouts: whether sprouts used were germinated for day 0, 1, 2, 3, 4 or 6 days.
Effects of freezing: whether the sample was freshly sprouted or sprouted then frozen.

5.6.1 CONTROLLED VARIABLES
Sprouting Methods: the same sprouting methods were used for all the seeds.
Serving Temperature: all the soups were heated and served at the same temperature.

5.7 DATA ANALYSIS
Results from the questionnaires were statistically analysed using Minitab statistical package. Descriptive statistics such as means, standard deviations and frequencies were calculated. An Analysis of Variance (ANOVA) was used to identify differences within each set of data. If significant differences were detected by the ANOVA F-test, the Rodgers Range Test was used to determine whether any significant differences existed between the pairs of the samples.

The findings were reported with the use of figures and tables to summarise the data (see appendix F for calculations).

Based on the results from the sensory evaluation, the most suitable soup product was selected for further development in part 3.
5.8 FOOD PRODUCT DEVELOPMENT TRIALS

TRIAL A: COOKING TIME AND STRAINING

AIMS:
1. To compare the effects of 40min and 30min cooking times (ϕ vs +).
2. To compare the effects of straining at 40 minutes (+ vs v).

BASIC INGREDIENTS:
100g chopped carrot 60g chopped celery
80g diced onion 1L water
3 chicken stock cubes

VARIATIONS:
ϕ 60g Day 2 sprouts with hull - cooked for 30 mins
+ 60g Day 2 sprouts with hull - cooked for 40 mins
v 60g Day 2 sprouts with hull - cooked for 40 mins (strained)

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 20
Results of Product Development: Trial A (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Day 2 30min</th>
<th>Day 2 40min</th>
<th>Day 2 40mins strain</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>31</td>
<td>31</td>
<td>32</td>
<td>ϕ/+</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>25</td>
<td>31</td>
<td>29</td>
<td>ϕ/v</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>18</td>
<td>19</td>
<td>21</td>
<td>+/v</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>20</td>
<td>19</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>28</td>
<td>23</td>
<td>20</td>
<td>*</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>22</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>26</td>
<td>30</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>24</td>
<td>23</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>26</td>
<td>29</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (α=0.05)

COMMENTS:
There was little difference between the three soups overall. 
Cooking Time: If anything, the longer (40 minutes) cooking time resulted in a slight reduction in beaniness and unusual flavours. 
Straining: The main effect of straining the soup cooked for 40 minutes was, as expected, a much thinner textured soup. There were no other differences.

RECOMMENDATION:
Increase the cooking time to 45 minutes to see if the longer cooking time improves the texture, thus eliminating the need to strain the soup.
TRIAL B: COOKING TIME AND STRAINING

AIMS:
1. To compare the effects of 40min and 45min cooking times (v vs +).
2. To compare the effects of straining at 45 minutes (+ vs φ).

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L water
3 chicken stock cubes

VARIATIONS:
v 60g Day 2 sprouts with hull - cooked for 40 mins
+ 60g Day 2 sprouts with hull - cooked for 45 mins
φ 60g Day 2 sprouts with hull - cooked for 45 mins - strained

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 21
Results of Product Development: Trial B (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Day 2 40min</th>
<th>Day 2 45min</th>
<th>Day 2 45mins strain</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=21</td>
<td>v</td>
<td>+</td>
<td>φ</td>
</tr>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>30</td>
<td>32</td>
<td>36</td>
<td>+/φ</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>26</td>
<td>26</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>23</td>
<td>22</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>23</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>27</td>
<td>25</td>
<td>17</td>
<td>+/φ</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>25</td>
<td>24</td>
<td>20</td>
<td>*</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>28</td>
<td>27</td>
<td>36</td>
<td>*</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>23</td>
<td>26</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>26</td>
<td>25</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (α = 0.05)

COMMENTS
There was little or no difference between the three soups overall.
Cooking Time: There were no differences perceived in the soups cooked for different lengths of time.
Straining: As expected, straining resulted in a more pleasant textured soup with a less beany flavour.

RECOMMENDATION:
Further increase the cooking time to 50 minutes, to see if longer cooking time improves the texture of the soup.
TRIAL C: COOKING TIME AND STRAINING

AIMS:
1. To compare the effects of cooking time 40min and 50min (+ vs 40).
2. To compare the effects of straining at 50 minutes (40 vs v).

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L water
3 chicken stock cubes

VARIATIONS:
+ 60g Day 2 sprouts with hull - cooked for 40 minutes
40 60g Day 2 sprouts with hull - cooked for 50 minutes
v 60g Day 2 sprouts with hull - cooked for 50 mins & strained

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 22
Results of Product Development: Trial C (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Day 2 40min</th>
<th>Day 2 50min</th>
<th>Day 2 50min strain</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>31</td>
<td>26</td>
<td>35</td>
<td>+/40</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>31</td>
<td>27</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>24</td>
<td>22</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>20</td>
<td>23</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>28</td>
<td>31</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>23</td>
<td>24</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>28</td>
<td>24</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>25</td>
<td>28</td>
<td>21</td>
<td>*</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>27</td>
<td>25</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (α = 0.05)

COMMENTS:
There was little difference between the three soups overall.

Cooking Time: The longer cooking time had the effect of thickening the soup. This may be due to loss of water through evaporation.

Straining: Straining improved mouth texture and appearance of the soup. Straining also produced a thinner textured soup with a less beany taste.

RECOMMENDATION:
Cook the soup for only 40 minutes. Do not strain the soup, it is not justified. Do not strain the soup as it increases cost and preparation time and reduces fibre content.
TRIAL D: SALT LEVEL (Sodium Chloride)

AIM:
To determine the effects of using 1, 2 and 3 continental chicken stock cubes ($\phi, +, v$).

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L water

VARIATIONS:
$\phi$ 60g Day 1 continental chicken stock cube (800mg sodium).
$+$ 60g Day 2 continental chicken stock cubes (1600mg sodium).
$v$ 60g Day 3 continental chicken stock cubes (2400mg sodium).

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 23
Results of Product Development: Trial D (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Day 2 1cube</th>
<th>Day 2 2cube</th>
<th>Day 2 3cube</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=18</td>
<td>$\phi$</td>
<td>$+$</td>
<td>$v$</td>
<td>$\phi/+$ $\phi/v$ $+/v$</td>
</tr>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>21</td>
<td>33</td>
<td>33</td>
<td>*</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>16</td>
<td>26</td>
<td>31</td>
<td>*</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>21</td>
<td>28</td>
<td>26</td>
<td>*</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>15</td>
<td>20</td>
<td>23</td>
<td>*</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>26</td>
<td>25</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>28</td>
<td>24</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>19</td>
<td>28</td>
<td>27</td>
<td>*</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>22</td>
<td>25</td>
<td>26</td>
<td>*</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>16</td>
<td>27</td>
<td>30</td>
<td>*</td>
</tr>
</tbody>
</table>

* Significant difference ($\alpha=0.05$)

COMMENTS:
One continental stock cube was perceived as being less acceptable than 2 and 3 continental chicken stock cubes.
Stock cubes: The more stock cubes the better the appearance, flavour, texture and acceptability of the soup.

RECOMMENDATION:
Use 2 continental stock cubes instead of 3, or try substituting continental stock cubes with ultra chicken stock cubes which are low in salt (sodium chloride).
TRIAL E SALT LEVEL (Sodium Chloride)

AIMS:
1. To compare 3 continental chicken stock cubes (2400mg sodium) with 1 ultra chicken stock cube (195mg sodium, no animal content, MSG or artificial flavours) and 1 & 1/2 ultra stock cubes (292.5mg sodium), (φ, v, +).
2. To ascertain whether the substitution of continental chicken cubes with Ultra stock cubes will improve the nutritional content of the soup.

BASIC INGREDIENTS:
100g chopped carrot 80g diced onion
60g chopped celery 1L water

VARIATIONS:
φ 60g Day 2 sprouts with hull - 3 continental stock cubes
v 60g Day 2 sprouts with hull - 1 & 1/2 Ultra stock cubes
+ 60g Day 2 sprouts with hull - 1 Ultra stock cube (amount recommended by manufacture)

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 24
Results of Product Development: Trial E (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>N=21</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3cube</td>
</tr>
<tr>
<td></td>
<td></td>
<td>φ</td>
</tr>
<tr>
<td><strong>APPEARANCE</strong> (pleasantness)</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>28</td>
<td>27</td>
</tr>
</tbody>
</table>

* Significant difference (α=0.05)

COMMENTS
There was little difference between the three samples overall.
Saltiness: The marked reduction in sodium (v & +) did not effect the saltiness detected.
Nutrition: The substitution of continental chicken stock cubes with ultra stock cubes reduced the sodium content. The use of ultra cubes also meant the soup contained no animal content, monosodium glutamate or artificial ingredients.

RECOMMENDATIONS
Use one ultra chicken stock cube instead of continental cubes for nutritional and flavour reasons.
TRIAL F: ADDITIONAL INGREDIENTS

AIMS:
1. To determine the effects of adding lupin sprouts (v vs +).
2. To determine the effects of adding vegetables to the lupin soup (ϕ vs +).

BASIC INGREDIENTS:
1L water

VEGETABLES
100g chopped carrot
80g diced onion
60g chopped celery

VARIATIONS:
ϕ 1 ultra cube and 60g day 2 sprouts (no vegetables)
+ 1 ultra cube and vegetables (no sprouts)
v 1 ultra cube, 60g day 2 sprouts and vegetables

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 25
Results of Product Development: Trial F (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>N=18</th>
<th>stock sprou no ve 0</th>
<th>stock sprou veg +</th>
<th>stock sprou veg v</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td></td>
<td>19</td>
<td>26</td>
<td>27</td>
<td>*</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td></td>
<td>16</td>
<td>31</td>
<td>25</td>
<td>*</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td></td>
<td>21</td>
<td>27</td>
<td>23</td>
<td>*</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td></td>
<td>28</td>
<td>24</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td></td>
<td>20</td>
<td>18</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td></td>
<td>26</td>
<td>22</td>
<td>25</td>
<td>*</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td></td>
<td>25</td>
<td>28</td>
<td>22</td>
<td>*</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td></td>
<td>12</td>
<td>20</td>
<td>27</td>
<td>*</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td></td>
<td>17</td>
<td>28</td>
<td>24</td>
<td>*</td>
</tr>
</tbody>
</table>

* Significant difference (α=0.05)

COMMENTS:
Overall the stock and vegetable soup and the stock, vegetable and sprouts soup were perceived as having a better appearance, a more pleasant flavour and higher overall acceptability than the stock and sprout soup.

Sprouts: Sprouts added a beany flavour and texture to the soup.
Vegetables: The vegetables enhance the appearance, flavour and texture of the soup, but did not mask the flavour of the sprouts.

RECOMMENDATION:
The use of vegetables is necessary for an acceptable soup, thus continue to use stock, sprouts and vegetables in the soup.
TRIAL G: AGE OF SPROUTS

AIM:
To compare soups made with different aged sprouts (\(\phi\), \(v\), \(+\)).

BASIC INGREDIENTS:
- 100g chopped carrot
- 80g diced onion
- 60g chopped celery
- 1L water
- 1 ultra chicken stock cube

VARIATIONS:
\(\phi\) 60g Day 0 sprouts (soaked for 24 hours)
\(v\) 60g Day 2 sprouts
\(+\) 60g Day 6 sprouts

METHOD:
1. Pressure cook the soup for required time, then puree.

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 6</th>
<th>(\phi/v)</th>
<th>(\phi/+)</th>
<th>(+/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>31</td>
<td>28</td>
<td>25</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWEETNESS (nil – very sweet)</td>
<td>29</td>
<td>26</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil – very salty)</td>
<td>23</td>
<td>21</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil – very beany)</td>
<td>22</td>
<td>25</td>
<td>30</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>20</td>
<td>25</td>
<td>28</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>30</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>24</td>
<td>24</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>34</td>
<td>27</td>
<td>23</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\alpha = 0.05\)

COMMENTS:
Overall the soup made with the younger sprouts (day 0) was preferred over the soup made with the older sprouts (day 6). The soup made with the youngest sprout (day 0) was perceived as having a more pleasant flavour. The soup made with the oldest sprout (day 6) was perceived as having a beanier flavour than the soups made with the day 0 and day 2 sprouts.

RECOMMENDATION:
Use a linked experimental method to compare the kernel and the sprouts at the youngest ages, day 0 and day 1.
TRIAL H: AGE OF SPROUTS

AIM:
To determine if there are any perceived differences between soups made with different aged sprouts: kernel, day 0 and day 1 (+, v, o).

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L water
1 ultra chicken stock cube

VARIATIONS:
+ 24g Kernel
v 60g Day 0 sprouts
o 60g Day 1 sprouts

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 27
Results of Product Development: Trial H (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Kernel</th>
<th>Day 0</th>
<th>Day 1</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>v</td>
<td>φ</td>
<td>+/- v</td>
</tr>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>36</td>
<td>32</td>
<td>21</td>
<td>*</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>34</td>
<td>33</td>
<td>24</td>
<td>*</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>26</td>
<td>24</td>
<td>23</td>
<td>*</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>22</td>
<td>23</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>19</td>
<td>21</td>
<td>26</td>
<td>*</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>32</td>
<td>30</td>
<td>24</td>
<td>*</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>27</td>
<td>22</td>
<td>19</td>
<td>*</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>34</td>
<td>31</td>
<td>25</td>
<td>*</td>
</tr>
</tbody>
</table>

* Significant difference (α = 0.05)

COMMENTS:
There was little difference overall between soups made with the kernel and the day 0 sprouts. The soup made with the day 1 sprouts was perceived as having a less pleasant appearance, flavour, mouth texture and overall acceptability than the soups made with the kernel and day 0 sprouts.

RECOMMENDATION:
Lupin sprouts are not highly acceptable as an ingredient in a soup. The lupin kernel soup is more acceptable. If lupin sprouts are to be used in a soup, day 0 sprouts (the kernel soaked for 24h) are most acceptable.
TRIAL I: FRESH VS FROZEN

AIMS:
1. To determine if moisture stress effected the taste of the soup by comparing sprouts germinated in the Micro Malter and those sprouted domestically, fresh (φ vs v/+).
2. To determine effects of freezing on the soup, by comparing fresh and frozen sprouts. (+ vs v).

BASIC INGREDIENTS:
100g chopped carrot 60g chopped celery
80g diced onion 1L water
1 ultra chicken stock cube

VARIATIONS:
φ 60g Day 6 sprouts - frozen micro malter
v 60g Day 6 sprouts - frozen home
+ 60g Day 6 sprouts - fresh home

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 28
Results of Product Development: Trial I (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>N=20</th>
<th>Day 6 Micro Malt φ</th>
<th>Day 6 Fresh v</th>
<th>Day 6 Fresh +</th>
<th>TABLE OF CONTRASTS φ/v</th>
<th>φ/+</th>
<th>v/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td>25</td>
<td>31</td>
<td>34</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td>27</td>
<td>32</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td>25</td>
<td>28</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td>22</td>
<td>20</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td>33</td>
<td>27</td>
<td>32</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td>28</td>
<td>20</td>
<td>22</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td>23</td>
<td>30</td>
<td>25</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td>25</td>
<td>24</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td>21</td>
<td>30</td>
<td>26</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant Difference (α=0.05)

COMMENTS:
Sprouting Method: The sprouts germinated domestically were perceived as having a more pleasant flavour and mouth texture than the sprouts germinated in the micro malter. The fresh sprouts were also perceived as being less beany than the micro malter sprouts.

Fresh vs Frozen: There was no significant difference in the soups made with the fresh day 6 sprouts or the frozen day 6 sprout. Freezing does not appear to effect the characteristics of the soup.

RECOMMENDATION:
To compare a soup made using freshly germinated sprouts with a soup made using lupin kernel.
TRIAL J: KERNEL VS SPROUT

AIM:
To compare the lupin kernel with the day 6 domestically germinated sprout.

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L water
1 ultra chicken stock cube

VARIATIONS:
ϕ 25g kernel
v 60g Day 6 sprouts - frozen home

METHOD:
1. Pressure cook the soup for required time, then puree.

Table 29
Results of Product Development: Trial J (Average response)

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>Kernel</th>
<th>Day 6 Fresh Frozen</th>
<th>TABLE OF CONTRASTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=15</td>
<td>φ</td>
<td>v</td>
</tr>
<tr>
<td>APPEARANCE (pleasantness)</td>
<td></td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>FLAVOUR PLEASANTNESS</td>
<td></td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>SWEETNESS (nil - very sweet)</td>
<td></td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>SALTINESS (nil - very salty)</td>
<td></td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>BEANINESS (nil - very beany)</td>
<td></td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>UNUSUAL FLAV (nil-very unusual)</td>
<td></td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>MOUTH TEXTURE (pleasantness)</td>
<td></td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>TEXTURE (far too thin-thick)</td>
<td></td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>OVERALL ACCEPTABILITY</td>
<td></td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

COMMENTS:
There was little difference between the kernel and the day 6 lupin sprout. The only significant difference was in texture. The lupin kernel soup was thicker than the lupin sprout soup.

RECOMMENDATION:
To use the lupin kernel for the final soup recipe as it is cheaper and less times consuming to use the kernel. This may not be the case if the problems experienced with sprouting can be overcome. There is however no access to the Micro Malter this year to germinate more seeds.
5.9 RESULTS

PRESENCE OF THE HULL

The various trials indicated that the presence of the hull (fibrous seed coat) appeared to add some flavour to the soup. The taste panel found the grainy texture that the hull gave to the soup is acceptable. Leaving the hull on the sprouts and not straining the cooked soup is also more economical and less time consuming, therefore it was decided to cook the sprout with the hull on and not strain the soup.

COOKING TIME

A number of trials that involved cooking the lupin soup for different lengths of time indicated that the soup needed to be cooked for 40 minutes. Cooking the soup for less time did not soften the hull enough, and cooking for longer made no difference in terms of eating quality and therefore was not justified.

INGREDIENTS

The minimal basic (vegetable) ingredients added to the soup were necessary to make an acceptable soup as consumers are used to vegetable based soups with a number of ingredients. Sprouts and stock alone did not make an acceptable soup.

Sodium level could be reduced by using one Ultra low salt chicken stock cube, which contains only 195mg sodium
instead of 3 continental chicken stock cubes containing 2400mg of sodium. The Ultra stock cubes also have no monosodium glutamate, no animal content, and no artificial flavours or colours. The reduction of stock cubes also reduced the possibility of masking the taste of lupins. The use of one Ultra stock cube was recommended.

AGE OF THE SPROUT

Soups made with the younger lupin sprouts were preferred over the soups made with the older lupin sprouts. It was expected that the older sprout would make a more acceptable sprout, as the older sprouts should be sweeter and less bitter due to the lower alkaloid content. This unexpected result may be due to the moisture stress that occurred during the sprouting process.

FRESH VS FROZEN SPROUT

A trial comparing the soup made with fresh and frozen sprouts suggested there was no difference in the soups; thus freezing did not affect the flavour properties of the sprouts and it is acceptable to freeze the sprouts prior to making the soup.

KERNEL VS SPROUT

The trials indicated that the lupin kernel soup was preferred to soups made with the sprouts, therefore the lupin kernel was chosen to be used in the final soup recipe.
MICRO MALTER SPROUT VS DOMESTICALLY GERMINATED SPROUTS

Due to the possibility that the sprouts germinated in the Micro Malter may have suffered moisture stress, a soup trial was completed so that Micro Malter sprouts could be compared with those sprouts germinated domestically. The results indicated that the soup made with the domestically germinated sprouts was more acceptable than the soup made with the Micro Malter sprouts. It therefore appears that the moisture stress that occurred in the Micro Malter sprouts may have adversely effected the taste of the sprouts.

The soup made with the domestically germinated sprouts when compared to the lupin kernel soup was found to be at least as acceptable.

5.10 SUMMARY

After considering all the results from the soup trials, the recipe (shown below) was chosen as the final soup. It was felt that the lupin kernel made an acceptable soup and that it would be suitable for consumers to sample. The final soup recipe was used in part 3 for the Consumer Research Study.

Soybean soup was chosen as the most suitable comparison for lupin soup due to soybeans similar chemical, physical and sensory characteristics. In marketing terms, lupin is also seen to be an alternative to soybean as a source of vegetable protein.
5.11 LUPIN SOUP RECIPE

100g carrot
80g onion
60g celery
25g LUPIN KERNEL
1 L water
1 Ultra chicken stock cube

1. Place all ingredients into a pressure cooker and bring up to pressure, then decrease the temperature to the lowest setting and cook for 40 minutes.
2. When cooked puree until smooth.

5.12 SOYBEAN SOUP RECIPE

100g carrot
80g onion
60g celery
25g SOYBEAN KERNEL
1 L water
1 Ultra chicken stock cube

1. Place all ingredients into a pressure cooker and bring up to pressure, then decrease the temperature to the lowest setting and cook for 40 minutes.
2. When cooked puree until smooth.
6.0 PART 3: CONSUMER RESEARCH STUDY

This study involved further development of the lupin soup as produced in part 2. The developed product was used in conjunction with an attitudinal study to investigate consumers acceptance of the soup and their attitudes towards the use of lupin as a food ingredient.

6.1 LOCATION

The Midland Gate shopping centre was chosen as the location for surveying as they were conducting a Grains Expo which attracted a number of shoppers. A display was set up to attract shoppers to the soup tasting section of the Expo (see appendix G).

6.2 SUBJECTS

The subjects were a non-probability based sample of shoppers from Midland Gate shopping centre. This shopping centre services a mixed population of socio-economic groups, including a large proportion of shoppers from rural areas.

6.3 TEST INSTRUMENT

A questionnaire was designed to provide sensory and attitudinal data on the use of lupin as a human food.

The instrument was pilot-tested and assessed by an expert in the area of scaling and measurement.
6.4 MARKET SURVEY QUESTIONNAIRE

Number ____________________________
Age (est) ____________________________
Gender ____________________________

Where were you raised? City / Country ____________

1. Would you please try these two soups and tell me which one you prefer?

2. If I told you that this soup contained soy (lupin) beans, how likely would you be to buy it?

<table>
<thead>
<tr>
<th></th>
<th>Sample +</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample v</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Would not buy at all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely would buy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Many foods on the market contain added ingredients such as soy and lupin beans. How do you feel about these food containing soy or lupin beans?

<table>
<thead>
<tr>
<th></th>
<th>Much less likely to try</th>
<th>Much more likely to try</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SOYBEAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LUPIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAMBURGER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOUP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PASTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. In your opinion, what is the effect of adding soy or lupin to a food product in terms of... (card)

<table>
<thead>
<tr>
<th></th>
<th>Much worse</th>
<th>Much better</th>
</tr>
</thead>
<tbody>
<tr>
<td>TASTE:</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NUTRITION:</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>COST:</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

5. Did you know that lupins are high in protein and fibre, low in fat and have cholesterol lowering properties? YES/NO

6. To what extent would you be likely to buy foods containing lupins now that you know more about their health value?

<table>
<thead>
<tr>
<th></th>
<th>No change</th>
<th>Much more likely to buy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

7. Had you heard of lupins before today? YES / NO / NOT SURE
   If yes, do you remember where you first heard of them?
   Comments ____________________________________________

8. What do you think of the name lupin? POS / NEG / NONE
   Comments ____________________________________________

9. Is lupin a good name for a food product? POS / NEG / NONE
   Comments ____________________________________________

97
6.5 DESIGN

The study involved asking respondents to sample the developed soup product (without being informed of its ingredients) then requesting them to respond to a series of questions asked by the interviewer. A number of interviewers were employed to conduct the interviews.

6.6 DATA ANALYSIS

Quantitative data were statistically analysed using the Minitab statistical package.

Qualitative data were pre-coded where possible prior to conducting the interviews to allow responses to be directly comparable.

The results were reported in a standard written format. Tables and graphs were used where necessary.
6.7 RESULTS OF THE MIDLAND GATE MARKET SURVEY

6.7.1 DEMOGRAPHIC DETAILS

There were 524 respondents sampled from Midland Gate shopping centre. The respondents sampled were mostly in the older age bracket (see Figure 18). The average age of respondents was 47 years.

![Age distribution of respondents](image)
More females (65%) than males (35%) were interviewed, this is likely to be because more females do the household weekday shopping than males. There was no marked difference between male and female responses to any of the questions asked. A relatively even distribution of respondents raised in the city (46%) and country (49%) were represented in the sample (see Figure 19). There was also no marked difference between responses of those respondents raised in the city and country to any of the question asked.

![Figure 19. Demographic details of respondents](image-url)
6.7.2 PREFERENCE TEST FOR LUPIN AND SOYBEAN SOUP

In question one, respondents were asked to try the two unidentified soups marked + and v, and state which soup they preferred. If respondents asked about the soups they were informed that they were vegetable based soups with some legumes added and that there was no animal content.

![Bar graph showing soup preference](image)

**Figure 20.** Soup preference

**RESULTS**

The lupin soup (58%) was markedly preferred over the soybean soup (35%). It appears that respondents found the taste of lupin soup acceptable, even though they were unfamiliar with the taste of lupins (see Figure 20). It
seems that in terms of sensory characteristics alone, the addition of lupin kernel actually is superior to the effects of adding the same amount of soybean kernel.

6.7.3 LIKELIHOOD OF BUYING THE SOUPS

In question two the respondents were then told that the soup marked + contained lupins and the sample marked v contained soybeans. Respondents were then asked to indicate if they would be likely to buy either of the soups if they were on the market at a reasonable price. Responses were indicated by rating the soups on a scale of 1 (would not buy at all) to 9 (definitely would buy).

SAMPLE + LUPIN SOUP

WOULD NOT BUY AT ALL

1 2 3 4 5

DEFINITELY WOULD BUY

6 7* 8 9

SAMPLE v SOYBEAN SOUP

WOULD NOT BUY AT ALL

1 2 3 4 5

DEFINITELY WOULD BUY

6 *7 8 9

Figure 21. Likelihood of buying lupin and soybean soup (* Mean score)

RESULTS

The average response for the lupin soup was 7.1 and 6.8 for the soybean soup (see Figure 21). The results were very similar for both soups. The results were on the high end of the scale indicating that consumers would be likely to buy either of the soups if they were on the market at a reasonable price. It appears that respondents found the
lupin soup to be at least as acceptable as the soybean soup even when told the soup contained lupin.

6.7.4 ACCEPTANCE OF OTHER FOODS THAT MAY CONTAIN SOYBEAN OR LUPIN

Question three involved asking respondents if they would try hamburgers, milk, soup and pasta, if they knew they contained soybean or lupin as an ingredient. Hamburgers and milk are foods already on the market which frequently contain soybean, while soup and pasta are foods that do not generally contain soybean. Respondents were asked to rate their responses on a scale of 1 (would not try at all) to 9 (definitely would try).

Figure 22. Acceptance of other foods containing lupin and soybean
RESULTS

Respondents rated hamburgers, pasta and soup on the higher end of the scale (see Figure 22). The soup (lupin 7.3 and soybean 7.0) and the pasta (lupin 6.7 and soybean 6.7) were the foods that respondents would most likely try. They were less likely to try the hamburgers (lupin 5.9 and soybean 5.9) or the milks (lupin 5.1 and soybean 4.9).

All the foods containing lupin were rated similarly to the foods containing soybean. This indicates that lupin is at least as acceptable as soybean as a food ingredient. The respondents attitudes towards lupin appears to indicate that they found lupin acceptable in the developed lupin soup when they tried it, and therefore they feel it would be acceptable in other developed food products, especially pasta. This suggests that lupin could be incorporated in other foods as a known ingredient and that there is no need to disguise its presence.

6.7.5 THE EFFECT OF LUPIN ON TASTE, NUTRITION AND COST

Question four involved asking respondents what they felt the effect of adding lupin to foods would have on the taste, nutrition and cost of a food product.

It was expected that respondents would feel that lupins would make a food product taste worse as they are unfamiliar with the use of lupin in foods for human consumption. In terms of nutrition, it was expected that respondents would feel that lupins will make a product more nutritious as legumes and grains are generally associated with good
health. It was also expected that respondents would feel that lupin will make a food product more expensive (much worse) as consumers usually perceive a new food product as adding cost to a food item.

Respondents were asked to rate their answers on a scale of 1 (much worse) to 9 (much better).

**Figure 23. Effect of lupin on taste, nutrition and cost (\*Mean score)**
RESULTS

The responses were very positive in that most respondents rated lupin highly on all questions. The average response for the effect of lupin on taste was 6.8, on nutrition 7.3 and on cost 6 (see Figure 23). It appears that respondents felt that adding lupin to a food product would make it tastier, more nutritious and cost slightly less. These results indicate that the addition of lupin to foods is likely to be seen as positive by consumers.

6.7.6 KNOWLEDGE OF NUTRITIONAL PROPERTIES OF LUPIN

Question five involved asking respondents if they were aware of the health properties of lupins: that lupins are high in protein and fibre and low in fat and have cholesterol-lowering properties. It was expected that very few, if any, respondents would be aware of the health value of lupins, especially because very little published work has been completed on the cholesterol-lowering properties of lupins (Evans et al., 1990).

RESULTS

Results showed that 41% of respondents said that they were aware of the nutritional properties of lupins. This result is questionable. It is likely that some respondents were aware of some of the nutritional properties of lupins due to their associations with lupins on the farms, however
very few, if any respondents would have been aware of the cholesterol-lowering properties. It is also possible that some respondents just answered yes to the question to appear knowledgeable.

6.7.7 EFFECT OF HEALTH VALUE ON ATTITUDES TOWARD LUPINS

In question six, respondents were asked if the health value of lupins, as stated in question five, would influence them when buying a food that had lupin as an ingredient. It was expected that an awareness of the health value of lupins would increase the likelihood of respondents buying a food product which contained lupin, due to the increasing consumer awareness of the importance of good nutrition.

The respondents were asked to rate their answers on a scale on 1 (no change) to 9 (more likely to buy).

No Change 1 2 3 4 5 Much more likely to buy 6 7 * 8 9

Figure 24. Effect of lupins nutritional properties on consumers' buying behaviour (* Mean score)
RESULTS

The average response 7.6, was on the high end on the scale (see Figure 24). It appears that the stated nutritional properties of lupin would certainly encourage consumers to buy a product containing lupin, and that the health value of lupins could be used as a marketing tool when promoting lupins.

6.7.8 AWARENESS OF THE NAME LUPIN

Question seven asked respondents if they had heard of lupins before, and if so where. It is expected that a lot of West Australians would have heard of lupins as they are a significant primary product in Western Australia being second only to wheat in terms of acreage and crop value.

![Bar Chart](image)

**Figure 25.** Whether respondents had heard of lupin and their attitude towards the name lupin
Table 30
Where Respondents had Heard of the Name Lupin

<table>
<thead>
<tr>
<th>WHERE THEY HEARD OF LUPINS</th>
<th>NO. OF RESPONDENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seen growing on Farms</td>
<td>138</td>
</tr>
<tr>
<td>As Animal Feed</td>
<td>57</td>
</tr>
<tr>
<td>Lupins Grower</td>
<td>47</td>
</tr>
<tr>
<td>Promotion Activities</td>
<td>33</td>
</tr>
<tr>
<td>Seen the Flower</td>
<td>21</td>
</tr>
<tr>
<td>Grow at Home</td>
<td>14</td>
</tr>
<tr>
<td>Plant puts nitrogen into soil</td>
<td>14</td>
</tr>
<tr>
<td>Country Women's Association</td>
<td>9</td>
</tr>
<tr>
<td>Other Countries (Italy, N.Z., Africa)</td>
<td>9</td>
</tr>
<tr>
<td>Weed</td>
<td>1</td>
</tr>
</tbody>
</table>

RESULTS

The majority of respondents (85%) had heard of lupins before. Most had seen lupins growing in the country or been involved in growing them (see Table 30 and Figure 25).

6.7.9 ATTITUDES TOWARD THE NAME LUPIN

Respondents were asked two questions (eight and nine) about the name lupin. Firstly they were asked if they thought the name was positive or negative and secondly, asked if they thought the name lupin was positive or negative for a food product. It was expected that many respondents may feel the name lupin is negative due to its traditional association with animal feed.

Respondents were also asked to suggest a suitable alternative name for lupins if possible.
Table 31
Why Respondents Felt the Name Lupin was Positive or Negative

<table>
<thead>
<tr>
<th>NEGATIVE</th>
<th>Number</th>
<th>POSITIVE</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Feed</td>
<td>26</td>
<td>Good for animals</td>
<td>16</td>
</tr>
<tr>
<td>Weed</td>
<td>5</td>
<td>Common name</td>
<td>4</td>
</tr>
<tr>
<td>Doesn't sound like a food</td>
<td>4</td>
<td>Good for soil</td>
<td>3</td>
</tr>
<tr>
<td>Flower not a food</td>
<td>4</td>
<td>Easy to remember</td>
<td>2</td>
</tr>
<tr>
<td>Disease</td>
<td>3</td>
<td>Beautiful plant</td>
<td>1</td>
</tr>
<tr>
<td>Not use to it</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City folk wouldn't like</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sounds Asian</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sounds French</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sounds like a bug</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

The name lupin was seen as slightly more positive (59%) than negative (19%), with many respondents neutral. This result supported findings by Zweck (1988, p. 21) who also found that 59% of respondents thought the name lupin was positive.

Those respondents who did comment on why they thought it was negative, said so because of its association with animal feed. Those that thought it was positive believed if lupins were good enough for animals, they were good enough for humans too; or if an animal will eat them they must be good.

The name lupin was seen as more positive for a food product (71%) than negative (22%). This is a very favourable result and appears to indicate that the name lupin is a suitable name for a food ingredient. This result differs from Zweck (1988, p. 21) who found that only 49% of respondents thought the name lupin was positive for a food
product. This suggests that consumers attitudes towards lupin may have changed as lupins have become more well known.

Very few respondents suggested an alternative name for lupin. Table 32 shows those name which were suggested and some comments on what the name should be like.

<table>
<thead>
<tr>
<th>Table 32: Alternative Names For Lupin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipul (lupin backwards)</td>
</tr>
<tr>
<td>Loopy</td>
</tr>
<tr>
<td>Lupini</td>
</tr>
<tr>
<td>Lupino</td>
</tr>
<tr>
<td>Lupea</td>
</tr>
<tr>
<td>Lupinosis</td>
</tr>
<tr>
<td>Ground beans</td>
</tr>
</tbody>
</table>

**COMMENTS:**
- Pea name
- Healthy name
- Name with lupin in it
- Something catchy
6.8 SUMMARY

The results of the Midland Gate Market Survey were very positive. Respondents indicated a marked preference for the lupin soup and that they would be just as likely to buy the lupin soup if it was on the market as they would a soybean soup. This is a very favourable result as respondents would not be as familiar with the taste of lupin, yet they did not appear to find it unacceptable.

Respondents indicated that they would not only try the lupin soup, but that they would at least be as likely to try other foods such as hamburgers, milk and pasta if they contained lupin, as they would if they contained soybean.

It was also generally felt that the effect of lupin as a food ingredient would be to make the food product taste better, more nutritious and cost less.

Some respondents indicated they were aware of the health value of lupins: that they are high in protein and fibre, low in fat and have cholesterol-lowering properties. Although this result is questionable (as few people are aware of lupins cholesterol-lowering properties) the results indicate if respondents were aware of lupins health value they would be more likely to buy a food product that contained lupin.

Most respondents had heard of lupins before, mainly by seeing them growing on farms or from being involved in
growing lupins. Respondents were reasonably evenly weighted in terms of whether they thought the name lupin had positive or negative connotations. Those respondents who did comment on why they felt the name lupin was negative stated it was because of the association of lupin to animal feed. However, the association of lupin to animals was also seen by some respondents as positive. They felt that if animals would eat lupins and if lupins were good for animals then lupins must be suitable for humans.

The name lupin was perceived as being more positive as a name for a food ingredient. It seems that there is no real risk in making known the inclusion of lupin in any food product.

It could be concluded that it would be acceptable to use the name lupin for a food ingredient used for human consumption as respondents’ attitudes toward lupin are generally positive. It also appears that it would be acceptable to state that a food product contained lupin, as respondents felt incorporating lupin would improve the taste, nutritional value and lower the cost of a food product. When promoting lupins the health value of lupins could be used as a positive marketing strategy.
7.0 CONCLUSIONS

The results of this study show that an acceptable food can be made using lupin kernel as a significant ingredient.

The lupin kernel soup which was developed through the use of sensory evaluation techniques was judged to be, in sensory terms, more acceptable than a vegetable soup incorporating a similar amount of soybean kernel.

The preference for the lupin soup also applied when the consumers were told of the presence of lupin in the soup. The results of the Consumer Research component of the study, clearly suggest that many consumers felt that the incorporation of lupin would be likely to make a food tastier and more nutritious.

A marketing message which stated that lupin had certain potential health benefits such as cholesterol-lowering properties, was found to be likely to have a positive effect on buying potential.

Generally the attitudes of consumers were positive towards the use of lupin as a food ingredient with little evidence of consumer resistance. The known association of lupin with animal food did not have any discernible negative effects on consumer attitudes. These results applied to both city and country raised consumers and were independent of the gender of respondents.

As well as showing that a satisfactory food can be developed using lupin as an ingredient, the study also
7.0 CONCLUSIONS

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A marketing message which stated that lupin had certain potential health benefits such as cholesterol-lowering properties, was found to be likely to have a positive effect on buying potential.

Generally the attitudes of consumers were positive towards the use of lupin as a food ingredient with little evidence of consumer resistance. The known association of lupin with animal food did not have any discernible negative effects on consumer attitudes. These results applied to both city and country raised consumers and were independent of the gender of respondents.

As well as showing that a satisfactory food can be developed using lupin as an ingredient, the study also
included a detailed analysis of the biochemical changes which occurred during the germination of lupin sprouts.

It was clear from the results that there were marked biochemical changes taking place during the 6 days of germination.

The early stages of germination, including the initial soaking of the kernel, produced a number of changes which indicated increases in protein and polysaccharides and a reduction in fat and the antinutrients, oligosaccharides and alkaloids.

Evidence relating to biochemical changes occurring during the later stages of germination was less clear due to technical problems experienced with the use of automatic sprouting equipment. The commercial Micro Malter apparatus is usually used for the sprouting of barley and a lack of experience with its use for lupin resulted in the production of a sprout which was affected by moisture stress.

This is believed to have altered the quality of the developed sprout and therefore influenced the results of both the biochemical assays and an attempt to conduct a sensory analysis of the effects of the biochemical changes which take place during germination.

It would be seen from these findings that there is a considerable value in conducting biochemical assays on good quality sprouts which have been germinated under commercial growing conditions rather than by using more laboratory centred techniques. This would assist in transferring data
gathered directly to the process of commercial food production.

Similarly there seems to be virtue in persevering with the attempt to identify the sensory effects of the biochemical changes taking place during germination. There does not appear to have been a serious attempt to do this previously and if successful this approach would add greatly to the pool of scientific data about the effects of germinating legumes. It could also help developing food products using both lupin kernel and lupin sprout.

The results of this study would appear to add to existing knowledge in the area and could well lead to the development of foods suitable for incorporation both in Australia and in off-shore markets.
8.0 REFERENCES


APPENDIX A: ANALYTICAL PROCEDURES
METHOD SHEET
Plant and Fertilizer Chemistry Section
Agricultural Chemistry Laboratory
Chemistry Centre (WA)

PRESSTUFFS AND PLANT MATERIAL - DETERMINATION OF MOISTURE
ROUTINE OVEN DRYING METHOD

1. SCOPE

This method is suitable for the routine determination of moisture in animal feedstuffs and plant material. It also describes the calculation of a dry basis factor (DBF) for use in converting analytical results from an "as received" or "as analysed" basis to a dry basis.

It is not suitable for export grains (Ref. 1) or gypsum. If used for silage, volatile substances other than water may be lost.

Further information on the limitations of moisture determination by oven drying is given in Ref. 2.

2. PRINCIPLE

Heating at 105°C, with gravimetric determination of weight loss.

3. APPARATUS

3.1 Analytical balance, readability 0.1 mg
3.2 Weighing dishes, aluminium, 50mm diameter, with tightly fitting lid
3.3 Mechanical convection oven
3.4 Desiccator, with freshly activated desiccant (Ref. 3)

4. SAMPLE PREPARATION

Moisture levels can change during storage and milling (Ref. 2). Special precautions must be taken if the moisture content of the original (unmilled) material is required (see for example Ref. 1). In most cases, the preparation procedure is determined by the requirements of the other analyses being done.
5 PROCEDURE

5.1 Dry the dishes with their lids beneath at 105 °C for at least 1 hour (Notes: 1, 2 and 3). Remove and place in a desiccator until cool (about 30-45 minutes).

5.2 Weigh the dried dish and lid (W1), and immediately add about 2g of sample (Note 4) and re-weigh (W2). Shake the dish gently to spread the contents within the dish, and dry with the lids beneath at 105 °C for 15 - 20 hours (Note 5).

5.3 Remove the dishes, cover immediately with the lids, and place in a desiccator. Weigh the dishes (W3) after they have cooled to room temperature (about 30-45 minutes).

6. CALCULATION AND REPORTING

% Moisture, as received

\[
\frac{\text{wt dish} \& \text{wet sample}(W2) - \text{wt dish} \& \text{dry sample}(W3)}{\text{wt dish} \& \text{wet sample}(W2) - \text{wt dish}(W1)} \times 100
\]

Report to 1 decimal point.

Dry basis factor (dbf)

\[
\frac{\text{wt dish} \& \text{dry sample}(W3) - \text{wt dish}(W1)}{\text{wt dish} \& \text{wet sample}(W2) - \text{wt dish}(W1)}
\]

or

\[
100 - \frac{\% \text{ moisture}}{100}
\]

Other analytical results may be converted from an "as received" or "as analysed" basis to a dry basis (db) as follows:

% or ppm dry basis = % or ppm as received / dbf.
7. NOTES

7.1 Hot dishes can be conveniently handled with light cotton gloves. For accurate work, gloves or tongs/tweezers should be used when transferring containers to and from the balance.

7.2 If ash is also required, the moisture determination may be done in crucibles prior to ashing.

7.3 Use of open containers such as glass beakers results in lower moisture values, as some water is re-absorbed during cooling in the desiccator. If crucibles are used, keep the cooling period to a minimum, or cover the crucibles with lids during the cooling.

7.4 If larger dishes are used, sample weights of 5-10g may be taken, weighed to the nearest mg.

7.5 Check that the vent at the top of the oven is fully open, and that the thermometer bulb protrudes at least 3cm into the main body of the oven.

8. REFERENCES - ACL METHOD AND INFORMATION SHEETS

8.1 Grain shipment samples

8.2 Moisture determination (MOISTINF.INF)

8.3 Desiccators and desiccants (DESICC.INF)
Preparation of colour solutions.

7.5 Transfer solutions from 7.2 and 7.4 to 15 ml centrifuge tubes and centrifuge at 3000 rpm for 15 minutes. Transfer 1 ml of extracts and appropriate standards into 30 ml tubes. The standard blank is also the sample or reagent blank; see 1.4.1.6.

7.6 Add 10 ml reagent 4.2 rapidly to all tubes before immediately stopping and shaking.

7.7 Place all tubes simultaneously in the boiling water bath.

7.8 After 12 minutes, remove all tubes simultaneously from the bath and place in cold water. Cool for 15 minutes.

7.9 Remove the batch from the sink and dry each tube. Briefly remove each stopper to release trapped liquid, then shake each tube.

Spectrophotometry (NOTE 5.3)

7.10 The coloured solutions are viscous and the following technique must be used with the flow-through cell on the SP6.

7.11 Set the zero on DI water.

7.12 Flush one cycle of Reagent 4.9 through the cell.

7.13 Starting with the ethanolic standards blank, insert the probe into the solution and leave it there while the flow-through cell is sent through repeated cycles of 'uptake' and 'empty'. Upon the third or fourth uptake it should be found that the displayed reading is stable and not more than 0.05 different from that shown after the previous uptake.

7.14 Flush one cycle of Reagent 4.9 through the cell.

7.15 Repeat the above steps for each ethanolic standard, then each ethanolic sample solution, then each ethanolic standard again.

Read the perchloric acid standards and samples in the same way.

8. CALCULATION AND REPORTING FORMAT

8.1 Average 'start' and 'end' absorbances for each set of standards.

8.2 Calculate percent glucose, or equivalent, in sample extracts using linear regression, r-squared for standards must be at least 0.999.

8.3 Calculate the standards % in solution. Record the percent errors. The Mean of Unsigned Errors (MUE) should be less than 3%.

8.4 Calculate % sugars or polysaccharides in samples as below:

% sugars as glucose = % in soln. x V1 / W1

% polysaccharides as glucose = % in soln. x V2 / W1

% polysaccharides as starch = % in soln. x V2 x 0.9 / W1 (NOTE 0.4)
Reporting

6. HEAD REF lists these reporting formats:

Sugars = % sugars extracted in hot 80% ethanol, calculated as glucose
Polysac = % polysaccharides, calculated as glucose
Polysac = % polysaccharides, calculated as starch

6. Report results to one decimal place, reporting limit 0.1%.

NOTES

1. 72% H2SO4 gives a more controllable reaction than conc. 42504,
   with less heat generated on addition to the sample.

2. Fructose gives a more intense colour than glucose after less time
   in hot water, but by the end of incubation this has faded to the
   same intensity as the glucose complex. Previous hydrolysis of
   polysaccharides is not necessary as they give the same final
   colour intensity directly as if they were first hydrolysed and
   then determined. Other reducing agents and small amounts of
   protein or fat do not interfere with the reaction (Ref. 10.1).

3. Read solutions within 3 - 4 hours of development. Aim for
   absorbances greater than 0.2, to reduce errors (Ref. 10.3).

4. Starch comprises long chains of dehydrated monosaccharide units
   (M.W. 162). The M.W. of glucose is 180. Hence the factor of 0.9

REFERENCES

10.1 Fairbairn N.J. (1963) Chem. & Ind. 38


   Circular 14, Dept. Agric., South Australia.


10.8 Carrez Reagents.
METHOD SHEET
Plant and Fertilizer Chemistry Section
Agricultural Chemistry Laboratory
Chemistry Centre (WA)

PLANT MATERIAL - NITROGEN - LECO FP-428 ANALYSER METHOD

SCOPE
Any material which will combust in oxygen at 950C.

The method is not suitable for samples low in nitrogen because the small amount of a 30% nitrogen, entrained with samples causes a significant error.

2 PRINCIPLE

3 SAFETY
1 No hazardous chemicals are used Lecosorb and Anhydride should not be allowed to contact the skin A burn hazard exists when changing the crucible and cleaning the side-arm
2 Reference 8 describes the safe way to use gas cylinders

4 EQUIPMENT
4.1 Leco FP-428 analyser
4.2 Oxidata 320 printer
4.3 Helium, oxygen, compressed air (Note 7.1)
4.4 Rack for samples
4.5 Tinfoil
4.6 Pellet press

5 AGENTS
5.1 Glucose, AR, for use as a blank
5.2 Ethylene diamine tetra-acetic acid (EDTA), AR, as a nitrogen standard
6 PROCEDURE

6 1 Log in using the SUPERVISER icon
6 2 Print the counter levels using the SPECIAL icon
6 3 Switch the gas control from STANDBY to ANALYSE
6 4 Allow furnace to reach operating temperature, then print AMBIENT MONITOR to check that the thermal conductivity cell voltage is in the range +0.4/- 0.0V
6 5 Go to LOGIN and run blanks with no tin foil until steady readings are obtained
6 6 Go to SYSTEM UPDATE STANDARD BLANK and enter 0.00 for the blank value. Select values from the answer stack

PROCESS RESULTS

6 7 Run 0.25g EDTA. Calculate the STANDARD CALIBRATION from the SYSTEM UPDATE screen. Enter 9.585 as the theoretical percent N. Select the EDTA result from the answer stack. Press PROCESS RESULTS to display the calibration factor.

6 8 Run a sample of 0.25g EDTA to check the blank and standard calibrations. The result should be in the range 9.5 - 9.9.

6 9 Weighing files are prepared using SETUP. The reference file is LECOSWE.REF. Most samples can be palletised. Handle pallets with care. EDTA (099) is not palletised. Pallets must be longer than the diameter of the sliding block entry case.

6 10 Using the LOGIN screen, enter the weighing file name, and a default weight (0?3). Continue pressing the ENTER button until all entries have been made. The actual weights of EDTA (099) are entered to check the run while in progress.

6 11 Line up the carousel and press the ANALYZE button.

7 PROCESSING OF RESULTS

7 1 Go to STD CALIBRATION and select the three EDTA results from the answer stack. Process, and recalculate the results.

7 2 Run UTRANS from a convenient terminal. On the LECO press SYSTEM OUTPUT, STACK TRANSMIT, RECORD FORMAT, and select results from the stack. Press PROCESS RESULTS. When transmission is complete, one more result from the stack must be sent as a 'finish' signal. On terminal check that no. of records transmitted is shown

7 3 Run SETUP to produce a loading file. The calculation file is LECOSWE and the reference loading file is LECOGLS REF.

7 4 Run BREED to process and record the results. There are 52 entries in a standard run. The procedural standards are
3 SHUTTING DOWN.

2 Switch the gas control from ANALYSE to STANDBY

A Press LOGOFF on the main screen

--- 1 ---

NOTES:
1. Use LECURBRT CAL for Tungsten reported as Boron in an.
2. Use LECWHER CAL for wheat reported as Boron in db.

ALTERNATIVE DATA TRANSFER-DISK.

If no deletions are required from the answer stack, go to
SYSTEM OUTPUT, DATABASE TRANSFER, DATA BASE, and enter the
weighting file no. then SHORT. Follow the instructions.
If deletions are required from the answer stack, go to
DATABASE TRANSFER, select results from the stack and press
PROCESS RESULTS. Follow the instructions.
See separate sheet for transferring results to POP-11.

9 1 GASES

9.1.1 HELIUM High purity. Gas Code No. 220

9.1.2 OXYGEN. Industrial grade is satisfactory. A blank must
be done when a cylinder is changed.

10. MAINTENANCE

10.1 The glass wool filter is changed when it is discoloured for
half its length. Use only about 4 gram of well-teased glass
wool, and ensure that it expands to fill the whole filter
after insertion.

10.2 The Loading head does not require much attention. A weekly
clean is sufficient. This can be done when the loading
head has been removed for other reasons. Clean and
grease the two seals on the piston, and wipe all surfaces.

10.3 If the combustion pressure exceeds 7 psi, the system should
be cleaned. Remove the Loading head and the side-arm plug,
and check the side-arm for blockage.
Remove the glass wool filter and disconnect the teflon tube
between furnace and filter at the furnace end. Force hot water
from a wash bottle into the teflon tube and out the filter
holder. Do not allow water to go into the instrument.
Reassemble and check combustion pressure.
A leak check should be run after any cleaning or dismantling.

11 STOPPING DURING ANALYSIS

Do not use the STOP icon since the current analysis may be
lost.
Use the LOGIN screen to enter HALT then press OUT OF SEQUENCE
The instrument will complete the current analysis and stop.

REFERENCES

i) Section information sheet 32.2.200]6ACPYL TXT

ii) Instruction manual, EP-428 Nitrogen determinator. System 601-
FATTY ACIDS IN OILS AND FEEDING STUFFS USING AN INTERNAL STANDARD.

SUMMARY

The fatty acid glycerides are extracted with petroleum ether and quantified using an internal standard and the HP5880A Cap. GLC.

APPLICATION

This method is used when the full fatty acid composition is not required or where percentage of a particular fatty acid, e.g. linoleic acid, in an oil or feeding stuff is required.

REAGENTS.

1. Caustic methanol, 0.5M. Dissolve 1g of NaOH in 50mL of MeOH.
2. Boron trifluoride in methanol, approx. 14% m/v. Available commercially (see SAFETY NOTE).
3. Petroleum ether, redistilled. B.P. 40-60°C.
4. Saturated sodium chloride solution.
5. Standard fatty acid solutions,
   (i) required fatty acid in redistilled petroleum ether (~4 mg/mL)
   (ii) internal standard in redistilled petroleum ether (~2 mg/mL)

Stopper standard solutions tightly and store in freezer.

PRECIURE

1. Preparation of Calibration Standards

Two calibration standards are required. The first standard is injected into the GC and the GC is calibrated to this standard. The second standard is then injected and the results presented should reflect (within a few percent) the calibration amounts in the calibration table.

A suitable standard for analysis of linoleic acid is 2mL C17:0 (2 mg/mL Stock Solution) plus 4mL C18:2 (4 mg/mL). The two calibration standards are esterified in an identical manner to the samples.

2. Sample Preparation

2.1. Oils

Accurately weigh about 100mg of oil into a 50mL round bottomed flask, add one or two pieces of pumice and 2mL of internal standard. Remove the pet. ether on a steam bath, blowing out the last traces with a puffer, and proceed to esterification immediately.

2.2. Feeding Stuffs

Extract 5g of sample for 16 hrs with petroleum ether. Reduce the volume of the extract on a steam bath and transfer quantitatively to a 25mL volumetric flask. Pipette a 2mL aliquot of oil solution
into a 50mL round bottomed flask, add one or two pieces of pumice and 2mL of internal standard. Remove the pet. ether on a steam bath, blowing out the last traces with a puffer, and proceed to esterification immediately.

3. Esterification

Reflux contents of the flask with 4mL of caustic methanol solution (Reagent 1) on boiling water bath for 10 min. (or until all oil is saponified).

Add 5mL of boron trifluoride in methanol (Reagent 2) through the top of the condenser, keeping reaction mixture under reflux for a further 3 min.

Add 5mL of petroleum ether (Reagent 3) through top of the condenser and reflux for a further 2 min.

Cool flask, still attached to a condenser, for 1-2 min. in cold water, then disconnect from condenser and add saturated NaCl solution to float solvent layer up into the neck of the flask.

Using a pasteur pipette transfer the fatty acid methyl esters in solvent to a stoppered or screw-cap tube containing anhydrous Na₂SO₄, shake well and leave stand until clear.

Transfer to a 1.5mL vial suitable for use on the HP7672A automatic sampler.

4. GC Analysis.

4.1. Setting up the 5880A GC

4.1.1. An oxygen scrubber is installed in the helium line between the helium cylinder and the moisture trap at the rear of the GC. This is required because the capillary column used, a carbowax 20M column, is degraded rapidly by oxygen.

4.1.2. A 12.5 metre by 0.2mm carbowax 20M fused silica capillary column is used.

4.1.3. The maximum column operating temperature is 220°C. The oven temp is left at 80°C when not in operation.

4.2. Operating Conditions for the 5880A GC (Note 1)

4.2.1. Linoleic analysis

<table>
<thead>
<tr>
<th>Init. Oven Temp.</th>
<th>150°C</th>
<th>Chart speed</th>
<th>0.1 cm/min</th>
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<td>Attenuation</td>
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<tr>
<td>Program Rate</td>
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<td>Threshold</td>
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<tr>
<td>Final Oven Temp.</td>
<td>180°C</td>
<td>Run Table:</td>
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<tr>
<td>Final Oven Time</td>
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<td>RT 0.01 Intg off</td>
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</tr>
<tr>
<td>Injector Temp.</td>
<td>250°C</td>
<td>RT 1.00 Intg on</td>
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</tr>
<tr>
<td>Detector Temp.</td>
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<tr>
<td>Split Ratio</td>
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<td></td>
</tr>
<tr>
<td>Column Flow</td>
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<tr>
<td>Injection Volume</td>
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4.2.2. Erucic analysis

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<tr>
<td>Chart speed</td>
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<td>Run Table: Helium</td>
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<td>RT 0.01 Intg off</td>
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</tr>
<tr>
<td>RT 1.00 Intg on</td>
<td></td>
</tr>
</tbody>
</table>

4.3. Calibration of the 5880A GC

Refer to 5880A Operating Instruction, Volume 5, Integration and Methods Section X, The ISTD Method and the ISTD % Variation. Prudent use of these facilities allow for the result to be printed with the trace as either percent or a weight hence no calculations are necessary.

NOTES

1. GLC conditions may be varied to obtain optimum separation and clearance.

2. It is preferable to use an acid not naturally present in the samples and having a retention time close to that of the fatty acid being determined, usually a saturated acid for increased stability e.g. C17:0 or C21:0 for linoleic acid determination. C21:0, C23:0 or C24:0 for erucic acid determination. Small but significant quantities of the chosen I.S. naturally present should be subtracted as a 'blank'.

3. Weights and volumes taken may be varied to suit the type of sample and the fatty acid being determined.

Oleic acid, C18.1. I.S. C17.0. Suitable ratio (C17.0:C18.1) for sunflower seed is 1:5, to conserve the fatty acid standard.

Linoleic acid, C18.2. I.S. C17.0 or C21.0. Suitable ratios (C17.0:C18.2; C21.0:C18.2) for poultry feeds have been found to be 2:1, 1:1, 1:2. Sunflower seed may range in linoleic acid content from 30% to 70% m/m of oil and ratios are adjusted accordingly. For high linoleic acid levels (60-70%), a ratio of about 1:10 is preferable to conserve the fatty acid standard.

Erucic acid, C22.1. I.S. C21.0, C23.0, C24.0. Rapeseed oil may contain up to 50% of the total fatty acids as erucic acid. Ratios are adjusted as for linoleic acid (above) and, if C24.0 naturally as I.S., it may be necessary to determine the level naturally present and subtract it as a blank. Low erucic acid rapeseed oils (<2% erucic acid) are a special case and should be determined by the method for Erucic Acid in Rapeseed Oil by GLC using an Internal Standard.
SAFETY

Boron trifluoride and its preparations are hazardous and toxic, and the vapour is highly irritating to the lungs. Work must be carried out in a fume cupboard. For contact with the skin or eyes wash with copious quantities of water for at least 10 minutes.

REFERENCES

1. H. Conacher (1975), JAOAC, 58, p 488
2. D. Firestone and W. Horwitz (1979), JAOAC, 62, p 709
FEEDING STUFFS AND PLANT MATERIAL - DETERMINATION OF CRUDE FIBRE

1. SCOPE

Crude fibre is not a specific plant constituent but is defined by the analytical conditions used.

The method is suitable for all plant materials and feeding stuffs. After milling under prescribed conditions, samples must contain less than 3% fat.

2. PRINCIPLE

Extraction of plant material with hot dilute sulphuric acid, then with hot dilute sodium hydroxide. Drying and weighing of the residue.

3. SAFETY

Reagent | Dangerous properties | Safety equipment and practices
---|---|---
Sulphuric acid | Heat and fumes when 98% H2SO4 mixed with water Corrosive | Wear safety glasses and rubber gloves when preparing and handling Prepare in a fume cupboard
Sodium hydroxide | Heat and fumes when solid NaOH dissolved in water Attacks human skin | Same as for Sulphuric acid
Acetone | Inflammable Toxic by inhalation Eye irritant Skin irritant | Do not use near flames or possible sources of sparks Use only with adequate ventilation Wear rubber gloves when using

4. REAGENTS

4.1 Sulphuric acid, 10% w/v. Carefully add, with stirring, 200g (109 mL) concentrated (98%) H2SO4 to 1 litre of water. Cool and make to 2 litres.

4.2 Sodium hydroxide, 10% w/v. Dissolve 200 g NaOH in water in a pyrex beaker. Make to 2 litres.

4.3 Acetone (technical grade, Mallinckrodt) for speeding up final washing and drying.
5. APPARATUS

5.1 400 ml beakers.

5.2 Sintered glass filter crucibles, pore size 100-120 um, e.g. Pyrex Grade 1.

5.3 Stainless steel or aluminium kettle, 2 litre capacity.

5.4 Filtration system and vacuum pump.

5.5 Hotplate with temperature controller set to 250deg.

5.6 Rapid dispenser calibrated to deliver 25 mL to a precision of +/- 0.25 mL.

6. SAMPLE PREPARATION

6.1 The result depends on particle size. Samples must be milled to pass 1mm mesh in a Wiley mill, whose cutting action produces less fines than crossbeater mills, for instance C & N.

6.2 The sample material must contain less than 3% fat. If it contains more the fat must be extracted by standing for 30 minutes in X4 solvent (Ref. 6), then decanting and rinsing.

7. PROCEDURE

7.1 Weigh accurately about 1.5g sample into a 400 ml beaker. Add 175 mL boiling water, making sure to disperse the material.

7.2 Add 25 mL Reagent 1 and boil GENTLY for 30 minutes, maintaining constant volume by adding boiling water.

7.3 While 7.2 is in progress fit a filter crucible to a Buchner funnel. Just before it is time to filter, pour in about 1 cm of boiling water and let stand, to heat the crucible.

7.4 After 30 minutes, remove beakers from the hotplate and allow to settle for about 1 minute. Then pour the acid mixture into the filter crucible.
7.5 Wash the beaker and the material on the filter with 3 lots of boiling water, allowing complete drainage between washings. Wash once with Reagent 4.3 from a wash bottle, to reduce remaining fats to a minimum. Then turn off the suction and add a little cold water to keep the material moist during the time taken to complete the batch.

7.6 Add 175 mL boiling water to the beaker, then 25 mL reagent 2. Place the crucible on its side in the beaker so that it is covered by the liquid.

7.7 Place on the hotplate and boil gently for 30 minutes, ensuring that the material is dispersed throughout the liquid. Maintain the volume by addition of boiling water.

7.8 Remove the crucible with the aid of tongs and hold it over the beaker while rinsing any fibres from the base and sides.

7.9 Fit the crucible to the Buchner funnel and filter and wash as before.

7.10 Dry the crucible at 105°C for 2 hours. Cool in a desiccator and weigh \( W_1 \).

7.11 Ash the crucible contents by heating to 500°C (NO HIGHER! NOTE 9.2) for 30 minutes. Cool in a desiccator and weigh \( W_2 \). \( W_3 \)

7.12 Brush the ash out of the crucible and weigh it again. This will be the tare weight for the crucible \( W_3 \).

8. CALCULATION AND REPORTING FORMAT (NOTE 9.3)

8.1 % Crude Fibre = \( \frac{(W_1 - W_2)}{W_{\text{sample}}} \times 100 \)

8.2 % Fibre Ash = \( \frac{(W_2 - W_3)}{W_{\text{sample}}} \times 100 \)

8.3 If results are required on a dry basis, the figure from 8.1 must be divided by a dry basis factor calculated from the % H2O in the sample. Refer to the method sheet for H2O determination.

8.4 The fibre ash result is not reported. A figure greater than 1% may indicate incomplete washing after the final filtration, or a high level of entrained soil in the sample. In the latter case, add a comment to the report.

8.5 Report results to one decimal place.
9. NOTES

9.1 Lupin seed, cereal grains and mixed feeds filter more slowly than pastures and tops.

9.2 Borosilicate glass must not be heated above the strain temperature of 515°C.

9.3 The Epson desktop computer contains a program for this, accessed by the command GOTO 4000.

10. REFERENCES

10.1 Divisional file 7.7.7


10.6 Plants Section method sheet: "Feeding Stuffss and Plant Material - Determination of Fat".

--oOo--
DETERMINATION OF ASH IN FEEDS AND PLANT MATERIAL

SUMMARY

Ash in feeds represents the nutritionally valuable mineral content; however, it may also contain significant amounts of silica or soil. It is determined under specified conditions of time and temperature to prevent loss of some elements.

REFERENCE

Torma L, JAOAC 1977, 60, 842.

APPARATUS

Silica or porcelain basins.

Electric muffle furnace.

PROCEDURE

Prepare the basins by heating at 550°C for 1 hour and cool in a desiccator. Weight 2g sample which has been ground to pass a 1mm screen into a tared basin and place it in a cool furnace. Increase the temperature to 550°C with the door slightly open and hold at this temperature for 3 hours with the door closed. Transfer the basin to a desiccator, cool and weigh.

CALCULATION

\[
\text{% ash as received} = \frac{\text{wt basin & ash} - \text{wt basin}}{\text{wt sample}} \times 100
\]

Note: Levels of ash up to 30 per cent may be found in some plants, e.g. saltbush.
PLANT MATERIAL AND FEEDSTUFFS - ACID DIGESTION -
FOR ANALYSIS BY ICP-AES

1. SCOPE

This method describes the acid digestion of plant material to produce a solution suitable for the analysis of P, K, Ca, Mg, S, Al, Cu, Fe, Mn, and Zn (Note 8.1). Some loss of boron occurs during digestion. Smaller sample weights must be used for samples containing more than 1.5% K or 1.2% Ca (Note 8.2).

The procedure is applicable to plant material and animal feedstuffs with an oil or fat content of less than about 10% (Note 8.3).

2. PRINCIPLE

Destruction of organic matter by heating with nitric and perchloric acids. Evaporation of nitric acid, and addition of water.

3. SAFETY

3.1 Use a perchloric acid standard fume cupboard (Ref. 10.1) and consult Ref. 9.1 before use.

3.2 Use safety glasses and gloves when handling concentrated acids and when inspecting the progress of the digestion. The work area must include a safety shower which is checked regularly.

3.3 Perchloric acid is an extremely powerful oxidising agent when hot, and may react explosively with organic matter such as fat which is only oxidised slowly by nitric acid. Consult Ref. 9.2 before use.

4. REAGENTS AND MATERIALS

4.1 Nitric acid, HNO₃, 70%w/w, 1.42g/mL, b.p. 120 °C (Note 8.4)
4.2 Perchloric acid, HClO₄, 70%w/w, 1.67g/mL, b.p. 203 °C
4.3 Nitric/perchloric acid mixture, 2+1. Carefully mix 2 volumes of nitric acid with 1 volume of perchloric acid.
4.4 Kerosene, domestic grade, highly refined, suitable for lamps
4.5 Plastic film wrap
5. APPARATUS

5.1 Heating block with windshield, racks and temperature controller to 250 °C, e.g. Windrift Instruments Model HP 2 (Note 8.5).

5.2 Test tubes, rimless, borosilicate glass, 150 x 18 mm, e.g. Crown Corning 1622/13 (re-usable) or Kimble culture tubes (disposable) (Note 8.6).

5.3 Oxford dispenser, 10 mL, with extended tip (Ref. 9.6).

5.4 Oxford dispenser, 30 mL (Ref. 9.6).

5.5 Pasteur pipettes and PVC bulbs.

5.6 Test tube racks and foam/board mixing system.

6. SAMPLE PREPARATION

Use a well-mixed sample that has been milled through a 1 mm screen (Ref. 9.3).

7. PROCEDURE

Take care to avoid contamination at all stages. Eliminate sources of copper and zinc from the work area. Rubber gloves contain zinc and should be covered with plastic gloves if used.

7.1 Weigh to the nearest 0.001 g between 0.85 and 0.95 g of sample into a test tube (Note 8.7). Include controls, replicates and blanks in each batch, with a blank as the first tube (Ref. 9.4, 9.5).

7.2 Add about 4 drops of kerosene (Note 8.8) from a Pasteur pipette to each tube and immediately add 6 mL of nitric/perchloric acid mixture (Ref. 9.6). Use the acid to rinse down material adhering to the walls of the tube. Cover with plastic film and pre-digest by standing overnight in the fume cupboard.

7.3 Transfer the tubes to the digestion block. Shake or vortex if material has risen up the tube, and heat at 70 °C for about 30 minutes. Some frothy materials may require an initial temperature of 50 °C, or a longer pre-digestion (Note 8.9). If only a few tubes are frothing, add a little nitric acid with a Pasteur pipette.

7.4 Increase the temperature to 90 °C for about 30 minutes, and then 110 °C for a further 30 minutes until brown fumes are no longer evolved. Increase the temperature to about 140 °C for 1-2 hours, until only a small amount of nitric acid is left, i.e. digest volume is about 2.5 mL. If the temperature during this stage is too high, the digests may bump (Note 8.10).

7.5 Increase the temperature to 160 °C to evaporate the remaining nitric acid. Straw coloured fumes are formed as the perchloric acid attacks any refractory organic material. Hold this temperature for about 30 minutes, until the digests are colourless. The blanks at this stage still contain nitric acid. Increase the temperature to 180 °C for 15 minutes, then 200 °C for 15 minutes, and then check that only perchloric acid remains in the blanks.

7.6 Set the temperature to 250 °C, and 15 minutes later reset it to 150 °C. Strong white perchloric fumes will be evolved near the end of the heating period. During the cooling stage, remove the racks from around the tubes, wash them and allow to drain.
After cooling for 20-30 minutes, remove each tube from the block and use the dispenser to add a known volume of water between 21 and 22mL (Note 8.11). Cover the tubes with 2 layers of plastic film, place the foam and board on top of the tubes, and mix by inverting at least 5 times. Store away from draughts, and minimise evaporation by placing foam or soft cloth over the covered tubes, and then placing a heavy flat object on top.

The volume of perchloric acid remaining after digestion is about 1.3mL, giving a solution concentration of about 5% acid. The final volume of solution = volume of water added + 1.3 mL (Note 8.12).

NOTES

This digestion procedure leaves a residue of silica, and also may not give total recovery of iron and aluminium (Ref. 10.2).

Potassium perchlorate may remain insoluble in the final solution at levels above 600-700 ug K/mL. Calcium remains soluble, but the ICP-AES calibration uses a top standard of 400ug Ca/mL. Samples containing high levels of Ca can be diluted using 5% HClO4 solution (see also Note 8.6).

Samples containing high levels of fat should be ashed, or alternatively, the material should be fat-extracted before acid digestion.

Use only high quality analytical reagent grade acids. Different brands of acid, and different batches of the same brand may contain varying levels of trace metals. It was once necessary to redistil all nitric acid (NEVER attempt to distil perchloric acid). At present, Mallinckrodt nitric and Merck perchloric acids are used directly.

The racks have been designed to rest on the windshield and enclose the upper part of the test tubes in a "hot box". This speeds up the evaporation of the nitric acid. Keep the holes in the block clean, to maintain good contact and heat transfer to the tubes.

This sized tube fits the racks for the Gilson sampler, thus avoiding a solution transfer step. The use of disposable tubes avoids the extra task of washing and drying re-usable tubes, with the risk of contamination. Re-usable tubes must be boiled with nitric acid before first use, and rinsed only with deionised water after use, as tap water can cause trace contamination. The disposable tubes are trace-clean as purchased, and do not require acid washing before use. Cost per tube, 11 cents (1988).

0.9g of sample is suitable for most cereal tops and grains. For pastures, use 0.4g as they generally have higher Ca and Cu levels. If levels are unknown in other sample types, analyse a few samples before starting the whole worksheet. Up to 2.5% Ca can be determined directly by using only 0.4g sample. Alternatively, up to 1g of heterogeneous materials such as high Ca feedstuffs can be digested in a similar manner using 25mm tubes in a larger block, with 9mL of 2+1 acid mixture, and made to volume with 2 x 22mL of water.
8.8 Kerosene acts as a surfactant to reduce frothing. Without it, no more than about 0.4g can be conveniently digested in this sized tube. The overnight pre-digestion is not necessary for most types of samples if kerosene is used, but is routinely included.

8.9 The actual times and temperatures for each stage of the digestion can only be established by experience. When first attempting this procedure, or when using new digestion blocks or controllers, monitor the progress of the digestion carefully. If a sample froths over the top of the tube, it must be repeated.

8.10 Inspect the rack and block surfaces for any signs of spitting if the digestion has been left unattended. Repeat samples that have bumped or have been contaminated by others bumping, or the whole batch if necessary. If a digest is interrupted before all the nitric acid is evaporated, e.g. stopped overnight, samples are more likely to bump when reheated.

8.11 If the digestion has not cooled sufficiently, tubes may spit when water is added. If they are too cold, potassium perchlorate may precipitate and not re-dissolve. If this occurs, heat the block to 150°C. After adding water to the digests (see 7.7) return the tubes to the block for 5-10 minutes and then remix thoroughly. The evaporation of water is negligible.

8.12 The weights and volumes used give about a 25-fold dilution of the plant material. These conditions were chosen to give a low detection limit for copper, and to provide enough solution to read twice on the ICP-AES if required.

9. REFERENCES - ACL METHOD SHEETS

9.1 Fume cupboards - information and operation
9.2 Notes on perchloric acid and its handling in analytical work
9.3 Sample preparation - methods and instructions
9.4 Weighing samples - a guide
9.5 Computer - sample weighing files - preparation using SETUP
9.6 Dispensers - use and maintenance

10. REFERENCES - OTHER SOURCES

10.1 Australian Standard 2243.8 Fume Cupboards
10.2 National Bureau of Standards, Certificate of Analysis for SRM 1571 Orchard Leaves
DETERMINATION OF INDIVIDUAL AND TOTAL QUINOLIZIDINE ALKALOIDS IN LUPINE SEED AND LUPIN FEEDSTUFFS

SUMMARY

There are three main forms of alkaloids in lupins and these are (a) alkaloid esters (b) free a'alkaloids (c) alkaloid N-oxide derivatives. The free alkaloid fraction is the major component and is reported as total alkaloid. The fine ground sample is extracted with trichloroacetic acid and the alkaloids are extracted with dichloromethane. The free alkaloids are determined by capillary gas chromatography on an OV-1 flexible capillary column using n-icosane as an internal standard.

APPLICATION

The method can be applied to lupin seeds, lupin flour and feedstuffs derived from lupin grain.

APPARATUS

Hewlett Packard 5890A or 5890A Capillary Gas Chromatography fitted with a 7673A Autosampler

Roller shaker

Rotary evaporator

Centrifuge and 50 mL centrifuge tubes

Glass separatory funnels 100 mL capacity

Glass vials with Septa 3 mL capacity

Screw capped plastic specimen jars 70 mL capacity

150 mL flat-bottomed flasks and stoppers
REAGENTS

1. 25 percent trichloroacetic acid
   Dissolve 500g of trichloroacetic acid in de-ionized water
   and make to 2 litres.

2. 5 percent trichloroacetic acid
   Dilute 400 mL of 25 percent trichloroacetic acid to 2 litres

3. Dichloromethane - re-distilled or chromat grade

4. 10 M Sodium Hydroxide
   Dissolve 400 g of NaOH in distilled water and make to 1 litre

5. Ethyl acetate - chromat grade

6. Trimethylol allylimidazole (TSIM)

7. 1 mg/mL n-icosane internal standard
   Dissolve 100 mg n-icosane in dry chromat grade ethyl acetate

8. Alkaloid Standard Solution (approximately 5 mg/mL)
   Dissolve approximately 60 mg of a mixed analysed alkaloid
   standard in 10 mL of dry chromat grade ethylacetate/ dichloromethane (1:1)

PROCEDURE

Extraction of Alkaloids from Lupinseed

Weigh 2 g of sample, ground to pass a 1 mm screen (note 1),
into a 70 mL specimen jar. Add 30 mL of 5 percent trichloroacetic
acid solution and cap. Extract on roller shaker overnight.

Pour approximately 20 mL of lupinseed slurry into a 30 mL
centrifuge tube and centrifuge (3200 RPM) for 30 minutes (note 2).
Pour 25 mL of dichloromethane into a 100 mL separatory funnel
and then pipette 15 mL of the centrifuged alkaloid extract into
the separatory funnel (note 3). Add 4.5 mL of 10 M NaOH and
carefully swirl the separatory funnel to mix the acid and base.
This makes the aqueous phase strongly basic (around 3 M).
Extract the basic aqueous layer with dichloromethane, repeat the extraction with another 25 mL of dichloromethane. Run these two extracts into a 50 mL flat bottomed flask (note 3) and remove the dichloromethane by rotary evaporation.

Add 100 µL of internal standard (n-pentadecane 1 mg/mL) to the flat bottomed flask. The third and final dichloromethane extract is run into the flask (note 3), add 15 mL of methanol to the dichloromethane and remove the solvent mixture by rotary evaporation (leave flask on rotary evaporator for 5 to 6 minutes) until completely dry (note 4). Dissolve the free alkaloids in 1.7 mL of dry ethyl acetate and transfer to a vial (able to fit in the H27673 autosampler). Add 150 µL of TSM (Trimethylsilylimidazole) (note 5), seal and shake vigorously.

N-oxides

The N-oxide alkaloids remain in the basic aqueous medium. The amounts of these in the lupin grain of locally cultivated varieties of lupin has been shown to be unimportant.

3. GC Analysis of Alkaloids

3.1 HP 5880A and 5890A Capillary GC operating conditions (note 6)

(a) Oven Temp Profile

| Initial value | 150 °C |
| Initial time  | 30 min |
| Program rate  | 12.00 °C/min |
| Final value   | 270 °C |
| Final time    | 5.00 min (note 6) |
| Equilibrating time | 10 min |

(b) Injector and detector conditions

| Flame ionisation detector temp | 300 °C |
| Injector temp                  | 250 °C |
| Column flow                    | 1.0 mL/min of Helium |
| Split ratio                    | 11:1 |
(c) Integration and recording parameters

- Chart speed: 0.5 cm/min
- Attenuation: 2 to power of 0
- % Offset: 10.0
- Peak width: 2.04
- Peak threshold: 0

(d) Calibration parameters

- Multiplier: 1
- Rm window: 3%
- Non-ref window: 3%
- Local RF: 0
- Sample amount: 0 (note 7)
- Internal standard amount: 100 micrograms

3.2 Calibration of GC

Prepare two calibration standards from the stock alkaloid solution (reagent A) as follows:

- 100 microlitres n-eicosane (1 mg/ml) internal standard
- 100 microlitres of mixed alkaloid standard (approx 5.0 mg/ml)

Percentage of four alkaloids in the standard mixture:

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angustifoline</td>
<td>6.9 percent</td>
</tr>
<tr>
<td>Alpha-isolumanine</td>
<td>0.6 percent</td>
</tr>
<tr>
<td>Lupanine</td>
<td>45.6 percent</td>
</tr>
<tr>
<td>13-hydroxy-lupanine</td>
<td>40.4 percent</td>
</tr>
</tbody>
</table>

Mixed alkaloid standard has been analysed against pure individual alkaloid standards.

3.3 Sample Analysis

Inject one microlitre of the samples prepared in vials into the GC. With the sample amount set at $1 \times 10$ to the power of 6 micrograms in the sample table, the readout on the GC terminal will list the percentage of each alkaloid in the sample as received. For dry basis data, the appropriate factor can be typed in the sample table under the heading multiplier (note 7).
REPORTING

Report the individual alkaloids for 

3.5.7. only. Report the result 
to three significant figures as 

Total alkaloids percent as received

NOTES

1. A tenor Cyclone Sample Mill has been found to give a 
uniform finely ground sample.

2. Do not fill centrifuge tubes to top as some acid solution 
spills into centrifuge bowls.

3. If acid solution is added first to separatory funnel and 
made basic there is some creaming of the basic mixture 
around the stopcock. No aqueous basic layer must be 
carried over into the 150 ml flat-bottomed flask with 
the dichloromethane. There is loss of internal standard 
and alkaloids when the sample is injected into the GC.

4. Sweet varieties usually have values in the range 0.002 
to 0.2 percent alkaloid. Samples with alkaloid content 
greater than 0.2 percent must be diluted to 5 ml with 
chromat grade ethyl acetate and 100 microliters taken 
for analysis. This dilution prevents the problem of column 
overload.

5. Gloves must be worn when handling TBIM as the toxicity 
of the reagent is unknown. The silylation reaction must 
be carried out in the fume hood.

6. The GC conditions given in this method are for a 25 metre 
column. For a 25 metre column the oven temperature final time 
is set at 10 minutes.

This laboratory uses Hewlett Packard columns for the lupin 
alkaloid analysis.

7. The sample amount is the amount (in micrograms) in the 
vial with 100 micrograms of eicosane. With the sample 
amount set to zero then the amount listed in the GC 
readout will be in micrograms instead of percentage. 
Sample amount should be set to zero for the initial 
calibration using standard 1 and also for the check on 
calibration with standard 2.
REFERENCES

1. Back, A B. The Alkaloids in Lupin of Agricultural Interest to Western Australia, Part 3 (Final), 1976-79 Investigations, p71


3. Priddis, C R. Lab Book 950/104-140.

4. Harris, D.J. Lab Book 1004/27-42

5. Harris, D.J. and Wilson, P.E. A Rapid Manual Method of Lupin Alkaloid Analysis. 5th International Lupin Conference, Poznan, Poland, 1988

23 February 1983.
SCOPES
The term 'tannin' refers to all polyphenolic compounds. The method is suitable for all cereal and legume seeds and pods.

2. PRINCIPLE
Extraction of tannin by boiling the sample in water. Filtration or centrifuging of extract. Colour development with Folin-Denis reagent at a high pH. Measurement of absorbance at 760 nm against tannic acid calibration standards.

3. SAFETY
Concentrated sodium carbonate solution is caustic and protective gloves should be worn when handling it.

4. REAGENTS
4.1 Folin-Denis Reagent

(a) Stock: To 750 ml water add in this order:

100g sodium tungstate, Na2WO4.2H2O
20g phosphomolybdic acid, H3P04(MoO3)12.xH2O
50 ml orthophosphoric acid, H3P04

Simmer for two hours in a beaker under a watchglass, maintaining the volume. The solution will become darker and greener. Cool and dilute to 1 litre with water. This reagent is not specific for tannic acid; it will react with any phenolic compound.

(b) Working: Dilute 1 part of stock with 4 parts of water in a bottle equipped with a dispenser (e.g. OPTIFIX) set to 25 ml.

4.2 Sodium carbonate saturated solution. To each 100 ml water add 35g Na2CO3, dissolve at 70-80deg and let cool. Pour into a bottle containing a little solid Na2CO3 10H2O. A layer of crystalline solid will form, in equilibrium with saturated solution.

Before each day's use, shake the bottle well and allow solids to settle before decanting supernatant into a 5 ml dispenser.

4.3 Tannic Acid Standard Solution. Prepare fresh daily.

Dissolve 100 mg tannic acid in 1 litre water

6. APPARATUS
400 ml or 600 ml beakers
100 ml polypropylene or heavy glass centrifuge tubes.
PROCEDURE

20 samples is a convenient batch. Weigh accurately about 2g sample into a beaker. Add 300 ml water and boil for 2 hours, maintaining the volume (NOTE 1). Cool and make to volume in 500 ml flasks.

Centrifuge at 4000 rpm for 20 minutes. Decant enough supernatant carefully into other containers.

Transfer 50.0 ml (sorghum, lupins) 25.0 ml (most other legumes) or 5.0 or 10.0 ml (canoh pods) of extract to 100ml volumetric flasks. If less than 50 ml, make up the difference with water and mix (NOTE 2).

STANDARDS. Pipette 2.4, 8.8, 19 ml of 4.3 into 100 ml flasks. Make to 50 ml with water. For a blank, add 50 ml water to a 100 ml flask.

Add 25 ml 4.1 (b) to each flask and IMMEDIATELY mix (NOTE 9.2)

Add 5 ml 4.2 and IMMEDIATELY mix

Make to the 100 ml mark and IMMEDIATELY mix

After at least 30 minutes (NOTE 9.3), read absorbances quickly at 760 nm. Read standards at the beginning and end and average them.

CALCULATION AND REPORTING FORMAT

8.1 Use the power (log-log) regression program on the EPSON HX20 (GOTO 6000). R-squared should be at least 0.999. Calculate sample tannin percent using a volume factor equal to:

\[
\text{sample tannin percent} = \frac{500 \times (\text{100/ml aliquot})}{\text{dry basis factor}}
\]

i.e. 1111.11 for 50ml aliquot, dbf 0.9.

8.2 Report results as: Tannin (total) as tannic acid.

NOTES

9.1 The extraction is empirical, so it is important to maintain the volume. Different sample/water ratios extract varying amounts of tannin, because tannins condense with the protein in the sample. Although there is an excess of protein, an equilibrium is probably set up during the extraction, which changes with the sample/water ratio, thus changing the extracted tannins.

9.2 Rapid uniform dispersal of sample extract and colour reagents in colour flasks, before colour development begins, is essential to avoid variations in colour strength and hue, which will give rise to poor standard curves and unrepeatable sample results.

9.3 Some solutions might turn cloudy during color development. If so, centrifuge them for 10 minutes at 3000 rpm before reading.

REFERENCES

Anion-Exchange Method for Determination of Phytate in Foods: Collaborative Study

BARBARA F. HARLAND and DONALD OBERLEAS
Howard University, School of Human Ecology, Washington, DC 20059

Collaborators: R. Ellis; J. Gelroth; D. Gordon; K. Phillips; G. Ranhotra; B. G. Shah; B. Stoecker; K. D. Trick; J. Zymonas

Phytate, a naturally occurring organic compound found in plant seeds, roots, and tubers, was determined in a collaborative study using a modified anion-exchange method. Seven samples (peanut flour, oats, rice, isolated soybean protein, a vegetarian diet composite, wheat bran, and whole wheat bread), supplied as blind duplicate samples, were analyzed in triplicate by 7 collaborators. Phytate concentrations in the samples ranged from 2.38 to 46.70 mg/g. Relative standard deviations (RSD = CV) for repeatability ranged from 2.5 to 10.1%, and for reproducibility, from 4.5 to 11.0%. The method has been adopted official first action.

Consumption of plant proteins has increased as a result of developments in food technology and an expanding need for less expensive sources of protein. The nutrient contribution of these plant foods must be assessed to ensure that the substituted plant-derived foods provide nutrients equivalent to those in the animal protein foods they replace. In addition, the nutrient concentrations must be bioavailable. Certain plant components, especially phytate, decrease the bioavailability of a number of minerals (1–3). Phytate (myo-inositol 1,2,3,5,6-hexakis(hexahydrogen phosphate) (4)), a naturally occurring organic compound found in plant seeds, roots, and tubers, by binding minerals necessary as cofactors, interferes with several essential metabolic processes, namely the utilization of protein (5, 6). A simple, inexpensive, reliable method for measuring phytate in foods is necessary to gain a better understanding of its significance in biological systems.

Anion-exchange resins (Bio-Rad Laboratories) were used to collect and concentrate phytate in foods. A simple modification suggested by Ellis and Morris (9, 10) to improve sensitivity at low concentrations of phytate. This modified method was collaboratively studied.

Collaborative Study

An ion-exchange method was tested by 7 collaborators on foods that were supplied as blind duplicates. Food samples were whole-wheat bread, A and D; and oats, G and K; peanut flour, F and J; uncooked, and rice, H and L; isolated soybean protein, B and C; a vegetarian diet composite, M and O; and Waldron wheat bran, I. The whole-wheat bread, oats, peanut flour, and ingredients for the vegetarian diet were purchased in the Washington, DC, area. The bread was broken into small pieces, freeze-dried, and blended to a uniform mixture. The quick-cooking oats were blended to a fine powder. Peanut flour was used as purchased. The rice (enriched, polished) was ground in a Wiley mill to a uniform powder. The isolated soy protein (Soy Assay Protein, Cat. No. 160480) was purchased from Teklad, Madison, WI. The vegetarian diet was prepared according to the recipe in ref. 11, freeze-dried, and blended to a uniform powder. The Waldron wheat bran was purchased from USDA Spring and Durham Wheat Quality Laboratory, North Dakota State University, Fargo, ND. 58105.

After the foods were prepared, they were packaged in 12 g portions and placed in mailing pouches. In addition to the samples, each collaborator received:

1. 100 mL P standard solution (80 µg/mL).
2. 20 g AGI-X4 100–200 mesh anion-exchange resin, all from the same lot.
3. A10 mL solution of sodium phytate containing approximately 180 µg P/mL to be used to check uniformity in the digestion procedure. Sodium phytate, 1.0806 g (Sigma Chemical Co., 97% pure, 15% water, Lot No. 118C-0069, Cat. No. P-5756), was dissolved in 50 mL water. This solution (12.5 mL) was placed in a 250 mL volumetric flask and diluted to volume with water.
4. A sample of American Association of Cereal Chemists wheat bran to use as an internal standard. The concentration of this standard is 3 ± 0.2% phytate.
5. A list of AOAC General Instructions to Collaborators.
6. A copy of the method, a data record sheet, and instructions to analyze each of the 14 samples in triplicate.
7. A return postcard so that study directors would know that the samples had arrived promptly and were in acceptable condition.

The statistical analysis of the data was performed according to Youden and Steiner (12).

Phytate in Foods

Apparatus

Anion-Exchange Method

First Action

Phytate is extd from duplicate samples of dried foods using dil. HCl. Ext is mixed with EDTA/NaOH soln and placed on an ion-exchange column. Phytate is eluted with 0.7M NaCl soln and wet-digested with mixt. of concd HNO3/H2SO4 to release P, which is measured colorimetrically. Amt of phytate in original sample is calcd as hexaphosphate equiv.

14.B01

Principle

Glass barrel columns.—0.7 × 15 cm, equipped with valve (Econo-columns, Bio-Rad Laboratories, or equiv.).

Apparatus

(a) Glass barrel columns.—0.7 × 15 cm, equipped with valve (Econo-columns, Bio-Rad Laboratories, or equiv.).
(b) Anion exchange resin.—AGI-X4, 100–200 mesh, chloride form (Bio-Rad Laboratories). Check resin (according to method below) by measuring recovery of purified Na phytate.
(c) Micro-Kjeldahl flasks.—100 mL, or 25 × 200 mm digestion tubes.
Table 1. Collaborative results for determination of phytate (mg phytate/g sample) in blind duplicate samples

<table>
<thead>
<tr>
<th></th>
<th>Bread, whole wheat</th>
<th>Oats, ground</th>
<th>Peanut flour</th>
<th>Rice, uncooked, ground</th>
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<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
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<td>3.76</td>
<td>3.75</td>
<td>10.51</td>
<td>10.89</td>
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</table>

Av. ± SD (n = 42)
- Soy, isolated
- Vegetarian diet
- Wheat bran, Waldron

<table>
<thead>
<tr>
<th></th>
<th>Soy, isolated</th>
<th>Vegetarian diet</th>
<th>Wheat bran, Waldron</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.9 ± 0.31</td>
<td>11.0 ± 0.56</td>
<td>16.2 ± 1.04</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>2.7 ± 0.15</td>
</tr>
</tbody>
</table>

### Preparation of Phosphate Standard

Adjust spectrophotometer to 640 nm and equilibrate for 15 min.

Pipe 1.0, 3.0, and 50 mL P std soln into 50 mL vol. flasks. Add ca 20 mL H2O. Mix thoroughly. Add 2 mL molybdate soln. Mix well. Add 1 mL sulfonic acid soln. Mix well. Dil. to vol. with H2O. Mix well. Wait 15 min and read in spectrophotometer at 640 nm. Calculate a typical std curve.

### Determination

Accurately weigh ca. 20.000 g sample and place in 125 mL erlenmeyer. Add 40 mL 2.4% HCl (20 mL of 2.4% HCl/g sample). Cover flask and shake vigorously 3 h at room temp.
Meanwhile, prep columns. Add 3 mL H2O to empty mounted column and then pour H2O slurry of 0.5 g resin into column. After resin bed has formed, wash column with 15 mL of 0.7 M NaCl.

Wash column with 15 mL H2O.

Remove sample from shaker and filter with vac thru Whatman No. 1 paper. Sample ext is stable at least 1 week if refrigerated.

- Prep blank by mixing 1 mL 2.4% HCl with 1 mL Na2EDTA-sodium reagent. Dil. to 15 mL with H2O and pour mixed onto column.

- Elute sample into 25 mL g-s graduate. Add 1.0 mL NaOH precipitate reagent. Dil. to 25 mL with H2O, Mix and quant.

- Add to column; discard eluate. Elute with 15 mL H2O; discard eluate. Elute with 15 mL 0.1M NaCl; discard eluate. Elute with 15 mL 0.7 M NaCl; collect this 0.7M fraction in digestion vessel. Add 0.7 M NaCl: discard eluate. Elute with 15 mL H2O; discard eluate. Elute with 15 mL of 0.7 M NaCl. Wash column with 15 mL 0.7 M NaCl: discard eluate. Elute with 15 mL H2O; discard eluate. Elute with 3 mL H2O to elute column, pour 15 mL H2O thru column. After turning if necessary to dissolve salt. Continue heating flask on low temp. 10 min. Let soln cool. Transf soln to total vol flask. Add 2.0 mL molv督办 soln; mix well. Add 1.0 mL sodium acid reagent; mix well. Dil. to vol. Mix well, let stand 15 min and read A at 640 nm. Calc. phytate concn in food or feed:

\[
\text{Phytate (mg sample)} = \text{mean } K' \times A \times 20(0.282 \times 1000)
\]

Phytorb: mean absorbance: mean \( K' = \text{std } P (\mu g/A/n} \text{studs); phytate x p}

Results and Discussion

Results of 18 analyses (results were received from only 6 collaborators) of the sodium phytate solution, which was sent to collaborators for checking uniformity of digestion procedures, showed a mean of 177.2 µg P/mL, with a standard deviation (SD) of 4.88. Results of 10 analyses of the AACC Wheat Bran supplied as an internal standard showed a mean concentration of 3.11 ± 0.13% (SD).

Table 1 shows collaborators’ results for determination of phytate content of the plant foods samples, which ranged from 0.00 to 46.70 mg/g in Waldron wheat bran. Results of individual determinations are shown as well as the overall means and standard deviation for each food.

Table 2 shows the range of values reported by all laboratories for each sample, and the repeatability and reproducibility relative standard deviations (RSDs; RSD = CV). The highest reproducibility RSDs occurred in the determination of phytate in Waldron wheat bran (9.9 and 11.0%). The RSDs were lowest in oats (4.5% and 5.8%). All sample analyses resulted in acceptable RSDs with the exception of those on Waldron wheat bran, which contained the highest amount of phytate (41.4%, duplicate samples E and I). The Waldron wheat bran was selected especially because the authors wanted to test a product at the uppermost level of naturally occurring phytate. Most foods commonly contain between 0 and 3% phytate. An extra dilution between digestion and color development may be necessary for those samples containing large concentrations of phytate.

Table 3 shows the combined means for each of the analyzed foods and the rank of each laboratory for each food analyzed.

The anion-exchange method used for determination of phytate in this collaborative study meets the criteria of rapidity, simplicity, reproducibility, and accuracy. It is sensitive at the lower limits of detection and otherwise compares favorably with the iron precipitation method, which has been used for determination of phytate for approximately 50 years (7).

Table 2 - Ranges and relative standard deviations for collaborative results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Range, mg/g</th>
<th>RSD, %</th>
<th>Repeat. Reprod.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Bread, whole wheat</td>
<td>3.34-4.70</td>
<td>8.2</td>
<td>9.0</td>
</tr>
<tr>
<td>B Bread, whole wheat</td>
<td>3.55-4.64</td>
<td>2.8</td>
<td>7.5</td>
</tr>
<tr>
<td>C Oats, ground</td>
<td>9.99-11.69</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>D Oats, ground</td>
<td>9.24-12.32</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td>E Peanut flour</td>
<td>15.58-19.41</td>
<td>3.5</td>
<td>6.5</td>
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<tr>
<td>F Peanut flour</td>
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<td>5.4</td>
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<td>6.1</td>
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<td>6.9</td>
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<td>K Wheat bran, Waldron</td>
<td>34.24-46.70</td>
<td>3.7</td>
<td>9.9</td>
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<td>L Wheat bran, Waldron</td>
<td>35.62-46.70</td>
<td>10.1</td>
<td>11.0</td>
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Table 3 - Yoden rank-sum test: average found (mg phytate/g sample) and rank for each collaborator

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>No of foods</td>
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</tr>
</tbody>
</table>
Recommendation

It is recommended that the anion exchange method for determination of phytate in foods and feeds be adopted official first action.

Acknowledgments

The authors are grateful to the collaborators who conducted the phytate determinations:

Rex Ellis, U.S. Department of Agriculture (USDA), Beltsville, MD

Dennis Gordon, University of Missouri, Columbia, MO

Kathryn Phillips, Ralston-Purina Co., St. Louis, MO

Gur Ranhotra and Janette Gelroth, American Institute of Baking, Manhattan, KS

B. G. Shah and K. D. Trick, Health and Welfare Canada, Ottawa, Ontario, Canada

Barbara Stoecker, Texas Tech University, Lubbock, TX

Joseph Zymonas, Quaker Oats Co., Barrington, IL

Special acknowledgment goes to Richard Albert, Food and Drug Administration, Washington, DC, who provided assistance with the computerized statistical analyses of the data.

The Vitamin and Mineral Laboratory, Human Nutrition Center of the USDA, Beltsville, MD, deserves special thanks for acting as consultant for this study, and for assisting in the preparation, packaging, and mailing of the samples to the collaborators.

References


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Food Research Division, Health Protection Branch
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April 27-30, 1987 • Skyline Hotel, Ottawa, Ontario, Canada
CEREAL GRAINS AND LEGUME SEEDS - OLIGOSACCHARIDES - HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) METHOD.

1. SCOPE

The sum of Stachyose and Raffinose is reported as Oligosaccharides. This method is suitable for all cereal grains and legume seeds from which fat and oil have been extracted.

2. PRINCIPLE

Extraction of material with dilute ethanol. Removal of ethanol by evaporation. Making to volume with water. Filtration or clarification. Injection to Dextropak column with water solvent.

3. SAFETY

Methanol should be handled only in a fumehood while wearing plastic gloves.

4. REAGENTS AND MATERIALS

4.1 Ethanol:water 70:30 v/v.

4.2 Methanol, CHROMAR grade.

4.3 Double-distilled Millipore-filtered water.

4.4 Carrez reagent 1. See Cho.DRF.

4.5 Carrez reagent 2. 30g ZnSO4.7H2O / 100mL water.

4.6 Centrifuge tubes, plastic or glass, 100mL.

4.7 Centrifuge tubes, plastic, 15mL.

4.8 30mL capped glass vials.

4.9 250mL round flasks.
5. STANDARDS.

Stachyose and Raffinose, 500, 1000, 2000, 4000 ug/mL. Weigh 400mg of each into a 100mL flask and make to volume; 4000ug/mL. Take 50.0mL of this into a 100mL flask and make to volume; 2000ug/mL. Take 50.0mL of this into a 100mL flask and make to volume; 1000ug/mL. Take 50.0mL of this into A 100ML flask and make to volume; 500ug/mL.

6. APPARATUS

6.1 Rotary evaporators.
6.2 Water bath at 65deg.
6.3 HPLC unit with dextropak column and microprocessor.

7. SAMPLE PREPARATION

7.1 Mill samples to pass a 0.5mm screen.
7.2 Quantitatively extract fat from samples (Ref. 11.1).

8. PROCEDURE

8.1 Weigh about lg sample into 100 mL centrifuge tubes. Add 50mL Reagent 4.1. Vortex-mix. Extract at 65deg for 30 minutes. Centrifuge at 3500 rpm for 15 minutes. Decant into round flasks.

8.2 Repeat 8.1 twice to collect about 150 mL of extract.

8.3 Evaporate the extract almost to dryness at 40deg under partial vacuum (ROTAVAP). NOTE 10.1.

8.4 Transfer the residue from 8.3 to a 25mL volumetric flask to which 0.4mL Carrez reagent 1 has been added. Mix. Add 0.4 mL Carrez reagent 2. Mix. Make to the mark and transfer to a 25mL capped vial. Rinse the volumetric flask to use for the next sample.

8.5 Centrifuge the solutions from 8.4 for 10 minutes at 4000 rpm. Transfer them to HPLC vials. Put these in numbered positions on the carousel with standards at each end, and in the middle, of the run.

8.6 Turn on the compressed air for the WISP HPLC system. The pressure gauge should read at least 200.

8.7 Turn on the system BEFORE turning on the computer that drives it. Turn on the computer and follow instructions as they appear. Select WATERS EXPERT SYSTEMS. Screen is red for system 2 for oligosaccharides. The screen is blue for System 1.

8.8. Select QUICK SET. Select LOAD METHOD. Method is OLIGO.

8.9 Transfer the solvent uptake tube from methanol to double-distilled water. Select QUICKSET CONTROL. Set flow rate to 1mL/minute.

8.10 EXIT. Select DESCRIPTION. DO. EXIT. Enter standards and samples against corresponding vial numbers. EXIT.

8.11 Select BASELINE MONITOR and let the system flow until the baseline is straight and level at about zero mV. OPTICAL ZERO on the refractometer adjusts this (NOTE 10.2).
8.12 Run a set of standards to find the retention times for stachyose and raffinose. These depend on ambient temperature and column history. Select CALIBRATION to change the times if necessary.

8.13 Select RUN METHOD. 'Sample name' names the whole batch. Select 1 injection, 50 microlitres, 15 minutes run time. Select the vials to be sampled. EXIT begins the run. Results will be printed.

PROCESSING OF DATA

8.14. Sometimes during a run the baseline will drop below the bottom of the chart range. Select REPORT METHOD to change the range. Select LC REPROCESS, then PRINT REPORT.

8.15 Often the baseline will be distorted and boundaries between peaks will be incorrectly drawn. Select LC REPROCESS, then SCANNER. Enter the sample name and vial number.

8.16 Find where the baseline should be for the peaks required and press INSERT HERE. Find the start of a peak and press SELECT. Find the end of the peak and press FIND. When satisfied, EXIT. Select PRINT REPORT. Enter the next vial number for reprocessing.

9. CALCULATING AND REPORTING FORMAT

9.1 When satisfied with figures for peak areas, use the Epson HX20 (GOTO 6000) to calculate a power (log-log) regression for standards, and to calculate sample % oligosaccharides on a dry or 'as received' basis, taking into account the fat extracted from the samples and the water of crystallisation (see WCF) in solid stachyose and raffinose.

% stachyose or raffinose in sample

\[
\frac{\text{ppm in extract} \times 25 \times \text{WCF} \times ((100-\%\text{fat})/100)}{\text{sample wt.} \times 10000 \times \text{dry basis factor}}
\]

WCF 0.9137 for stachyose and 0.8485 for raffinose.

The program computes the fat factor from the percent fat (as analysed or as received) figure entered.

10 NOTES

10.1 Some extracts may froth suddenly, causing sample loss. Insert a plastic stopper with a ~5mm hole, in the neck of the evaporator. This allows free passage of vapour while resisting froth surges.

10.2 It might be necessary to change the reference solvent in the refractometer. Set the flow to 0.2 mL/minute. Place a flask under the cell outlet and open valve C. Increase flow to 5mL/minute. Allow 2 minutes draining. Set flow 0.2 mL/minute. Close valve C. Set flow to 1mL/minute as before.

11 REFERENCES

11.1 Method sheet [120,205] FATSOXTEC.MET.
APPENDIX B: DOMESTIC GERMINATION CONDITIONS

The lupin seeds were soaked for 24h in tap water. The seeds were placed on sprouting trays, then germinated for the required time under ambient conditions. Sprouts were rinsed daily in tap water.
APPENDIX C: SENSORY EVALUATION CALCULATIONS

RODGERS RANGE TEST

Reject Ho if \((M_i - M_j) > \sqrt{v_i \times MSW \times F(1-\alpha)^v \times c_{ij}}\)

\(v = \) number of groups subtract 1
\(MSW = \) analysis of variance (error term)
\(N = \) number of respondents
\(F(1-\alpha)^v + F_{\alpha}, 100 = 2.62 (\alpha = 0.05)\)

TRIAL B:

FLAVOUR PLEASANTNESS

\(\sqrt{2 \times 35.9 \times 2.62 \times 2/18}\)

= 4.6

UNUSUAL FLAVOURS

\(\sqrt{2 \times 39.4 \times 2.62 \times 2/18}\)

= 4.8
APPENDIX D: PRELIMINARY MILK TRIALS

Trial 1: LUPIN MILK

SAMPLE:  
1. Gungurru sprout - with hull  
2. Gungurru sprout - with hull  
3. Gungurru sprout - without hull  
4. Gungurru sprout - without hull  
5. Gungurru sprout - with hull  
6. Gungurru sprout - with hull  
7. Gungurru sprout - without hull  
8. Gungurru sprout - without hull

METHOD:  
1. Soak 20g of samples 1-4 in 100ml 1% NaHCO₃ for 1 hour, drain.  
2. To all samples add 100ml of 1% NaHCO₃.  
3. Homogenise.  
4. Heat to 85°C for 5 minutes.  
5. Measure pH, then adjust with NaOH dilute to pH 8.00.  
6. Centrifuge at 500rpm 10C 10mins.  
7. Decant.  
8. Measure pH adjust with HCL to pH 7.00.

SENSORY RATING:

SCALE: 1 = low  -  10 = high

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CONCLUSION:  

All milks were a light green colour which was not pleasant to look at. The milks were very watery. They were not pleasant to drink. They were very metallic, they need to be less metallic/salty. Most had a good texture and were not floury. Milks were not bitter.

OVERNIGHT: milks became beanier, but remained stable, good emulsion.

RECOMMENDATION: Adjust the pH to pH 8.00 before heating to stabilise the protein. Compare milks made with seeds to milk made with the sprouts.
Trial 2: LUPIN MILK


METHOD:
1. Soak samples 1-4 in 100ml 1% NaHCO₃ for 4 hours, then drain.
2. Add 100ml of 1% NaHCO₃.
3. Homogenise.
4. Measure pH, then adjust with NaOH dilute to pH 8.00.
5. Heat to 90°C for 5 minutes.
6. Centrifuge at 1000rpm 10 C 10mins.
7. Decant.
8. Measure pH adjust with HCL to pH 7.00.

SENSORY RATING:

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CONCLUSION:

The appearance of the UH splits milk was very pleasant. Danja milk was also a good yellow colour. Both looked very creamy. Gungurru was less pleasant as it was a yellowy-green colour and very watery looking.

The UH splits were very salty - too metallic. The other samples were also salty but not as great as the UH splits.

The samples were slightly beany. The milk made with sprouts had the greatest beany-grassy smell.

OVERNIGHT: The milk made from sprouts was very beany. The other milks had no smell. Milk remained stable - good emulsion. Sprouts looked very watery and an unpleasant green colour.
Trial 3: LUPIN MILK

SAMPLE: 1. UH Splits [Soak 20g for 4 hours in 100ml 1% NaHCO₃]
2. Kiev mutant seeds
3. Gungurru seeds

METHOD:
1. Soak samples 1-3 in 100ml 1% NaHCO₃ for 4 hours, then drain.
2. Add 100ml of 1% NaHCO₃.
3. Homogenise.
4. Measure pH, then adjust with NaOH dilute to pH 8.00.
5. Heat to 90°C for 5 minutes.
6. Centrifuge at 1000rpm 10°C 10mins.
7. Decant.
8. Measure pH adjust with HCL to pH 7.00.

SENSORY RATING:

SCALE: 1 = low - 10 = high

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CONCLUSION:

All samples were a pleasant yellow colour. Kiev mutant milk was the deepest shade of yellow. All looked creamy - would make a good base. UH splits milk had a very beany smell. Kiev mutant milk had the least smell. Gungurru had a slightly beany smell.

OVERNIGHT: UH splits had quite a beany smell, but keiv mutant and gungurru had only a slight beany smell. All had a good emulsion.

AFTER 4 DAYS: UH splits and gungurru milks went off. The kiev mutant was very beany. All milks had settled slightly.

RECOMMENDATION: Compare sprouts germinated in the malting machine to sprouts germinated in a plastic sprouter.
**Trial 4: LUPIN MILK**

**SAMPLE:**
1. Kiev mutant  
2. Gungurru seeds  
3. UH splits  
4. Kiev mutant seeds  
5. Gungurru seeds  
6. UH splits - soaked for 2 hours in tap water  
7. Gungurru sprouts - 1 day (malting machine) Frozen  
8. Gungurru sprouts - 1 day (malting machine) 3 weeks

**METHOD:**
1. Drain all samples.  
2. Add 100ml of 1% NaHCO₃.  
3. Homogenise.  
4. Measure pH, then adjust with NaOH dilute to pH 8.00.  
5. Heat to 90°C for 5 minutes.  
6. Centrifuge at 1000rpm 10°C 10mins.  
7. Decant.  
8. Measure pH adjust with HCL to pH 7.00.

**SENSORY RATING:**

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**CONCLUSION:**
All milks were a pleasant yellow colour, they looked very creamy. The sprouts (7/8) were a slightly lighter yellow. Sample 5 - one day old gungurru sprout was pleasant to drink it tasted the best and was sweet. Sample 4, Kiev mutant one day old sprouts, were also pleasant to taste. All had a good texture and were not floury. Milks were not bitter.

**OVERNIGHT:** all remained stable - good emulsion. Sample 4, 6 and 7 had a beany smell. Sample 5, one day old gungurru sprout, had no beany smell.

**RECOMMENDATION:** Compare milks made with one day old sprouts to two day old sprouts. Compare fresh and frozen sprouts. Compare sprout germinated in the malting machine to sprout germinated in a plastic sprouting dish.
Trial 5: LUPIN MILK

SAMPLE:  
1. Gungurru fresh two days old sprouts (home)  
2. Gungurru fresh two days old sprouts (home) - 30g  
3. Gungurru fresh two day old sprouts (malting machine)  
4. Gungurru fresh two day old sprouts (malting machine)  
5. Gungurru frozen* one day old sprouts (malting mach)  
6. UH splits - soaked for 2 hour in tap water  
   * Sprouts had been frozen for 3 weeks.

METHOD: as for trial 4

SENSORY RATING:  
SCALE: 1 = low - 10 = high

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Second set of ratings were taken when the milk was a day old.  
* not drinkable, had gone off.

CONCLUSION:  
Appearance: The fresh two day sprouts (1 & 2) were a pale yellow colour. The frozen day two day sprouts (5) were also a pale yellow with a slight green tint. The fresh two day sprouts, (3 & 4) germinated in the malting machine, were a deeper yellow colour. The UH splits (6) milk was a very creamy yellow colour.

Smell: Fresh day two sprouts germinated at home (1 & 2) were quite beany. Fresh day two sprouts germinated in the malting machine (3 & 4) were slightly beany. The frozen, two day old sprout (5) was not beany. The Uh splits milk (6) had a metallic smell.

Best / most pleasant: The fresh two day old sprouts germinated at home (2) and the frozen two day old sprouts germinated in the malting machine (5) were the most pleasant to taste. The frozen sprouts had only been frozen for 3 weeks. They produced a better milk than milks made in previous trials with frozen sprouts. This may be because they had only been frozen a short period of time, while other sprouts used previously had been frozen for 3 months. The fresh two day old sprouts germinated at home (2) was very sweet.

The fresh two day old sprouts germinated at home (1) and the UH splits milks (6) were very metallic, however when their pH was remeasured it had risen from 7 to a pH of 7.3.

OVERNIGHT:  
Emulsion: all had a good emulsion and stability.  
Smell: 1 - beany, 2 slightly beany, 3, 4, 5, 6 - not beany

RECOMMENDATION: On the same day, compare sprouts that are a day and 2 days old germinated at home and in the malting machine.
Trial 6: LUPIN MILK

SAMPLE:  1. Gungurru sprouts, one day sprout (home)
         2. Gungurru sprouts, two days sprout (home) - no hull
         3. Kiev mutant sprouts, one day sprout (home)
         4. Kiev mutant sprouts, two day sprout (home) - no hull
         5. Frozen (3 days) 2 days sprouts - kilned
         6. UH splits soaked for 2 hours

METHOD:
1. To 20g lupins add 100ml of 1% NaHCO₃.
2. Homogenise.
3. Measure pH, then adjust with NaOH dilute to pH 8.00.
4. Heat to 90 °C for 5 minutes.
5. Centrifuge at 2000rpm 10 °C 10mins.
6. Decant.
7. Measure pH adjust with HCL to pH 7.00.

SENSORY RATING: assessed by a semi-trained panel

SCALE: 1 = low - 10 = high

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CONCLUSION:

Appearance: Samples 2, 3 & 4 were a pleasant yellow colour. Sample 1 & 6 was less pleasant and sample 5 was had an unpleasant appearance.

Best / most pleasant: The most pleasant samples were 3 and 4. Sample 2 was also acceptable.

RECOMMENDATION:
Make a milk using sprouts that have been kilned (malted).
Trial 7: LUPIN MILK

SAMPLE:  
1. Frozen (3mths) sprouts - kilned  
2. Frozen (3mths) sprouts - kilned  
3. Frozen (3mths) sprouts  
4. Frozen (1mth) sprouts - day one  
5. Frozen (1mth) sprouts - two days  
6. Frozen (3 days) sprouts - kilned

METHOD:  
* Increased quantity to 40g sprouts and 200ml 1% NaHCO₃  
1. To 40g lupins add 100ml of 1% NaHCO₃.  
2. Homogenise.  
3. Measure pH, then adjust with NaOH dilute to pH 8.00.  
4. Heat to 90°C for 5 minutes.  
5. Centrifuge at 1000rpm 10°C 10mins.  
6. Decant.  
7. Measure pH adjust with HCL to pH 7.00.

SENSORY RATING:

SCALE: 1 = low  - 10 = high

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CONCLUSION:  
Appearance:  
Samples 1 and 2 were a very dark, unpleasant green.  
Samples 3 and 5 were also a dark olive green. Sample 4 and 6 were a pleasant yellow colour.  
Smell:  
Samples 1 and 2 had a strong beany smell. All of the other samples had pleasant beany smells.  
   Best / most pleasant: Sample number 6, day 2 sprouts that had been kilned made the most pleasant tasting milk.  
The samples were general quite metallic, they tasted like chemicals - artificial.

OVERNIGHT:  
Emulsion: all had good emulsion, stable  
Smell: very slight beany smells.  
Colour: all samples had become darker.

RECOMMENDATION:  
Alter method to help remove metallic taste, at step 3, adjust the pH to 7.5. Eliminate step 7 - final adjustment of the pH. Make a soup from the sprouts.
Trial 8: LUPIN MILK

SAMPLE:
1. Frozen (3mths) sprouts - kilned
2. Frozen (3mths) sprouts - kilned
3. Frozen (3mths) sprouts
4. Frozen (1mth) sprouts - day one
5. Frozen (1mth) sprouts - two days
6. Frozen (3 days) sprouts - kilned

METHOD:
1. To 40g lupins add 100ml of 1% NaHCO₃.
2. Homogenise.
3. Measure pH, then adjust with NaOH dilute to pH 7.50
4. Heat to 90°C for 5 minutes.
5. Centrifuge at 1000rpm 10°C 10mins.
6. Decant.

SENSORY RATING:

SCALE: 1 = low - 10 = high

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<td>13</td>
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</tbody>
</table>

CONCLUSION:

Appearance: Samples 2, 3 & 6 were a pleasant yellow colour. Sample 1 was a darker yellow. Samples 4 and 5 were an unpleasant green colour.

Smell: Sample 1 & 6 had a beany smell. Samples 2, 3, 4 and 5 all had pleasant beany smells.

Best / most pleasant: The most pleasant samples were 1 and 6. Sample 5 was also acceptable. Sample 1, 5 and 6 were all sweet, the other samples were not as sweet. Sample 3 was very bland and unpleasant to taste, thus this suggests that sprouts that have been frozen for 3 months are not suitable for making a milk. Samples were less metallic than previous experiments, as pH was only adjusted to pH 7.50 not pH 8.00 in step 3.

RECOMMENDATION:
That continue with this method were pH is adjusted to pH 7.5 in step 3. Examine effects of freezing conditions (length and exposure to light) on milks, especially colour and taste.
Trial 9: LUPIN SOUP

INGREDIENTS:

SOUP ONE
60g lupin day two sprouts
100g chopped carrot
80g diced onion
60g chopped celery
750ml stock

SOUP TWO
60g kilned lupin sprouts
100g chopped carrot
80g diced onion
60g chopped celery
750ml stock

METHOD:

1. Place sprouts, vegetables and stock in a pressure cooker and cook for 25 minutes.
2. When soup is cooked, puree until smooth.

SENSORY RATINGS: scale 1 - 50

<table>
<thead>
<tr>
<th>SOUP</th>
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<tr>
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<tr>
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<tr>
<td>Chemical taste</td>
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<tr>
<td>Overall Acceptability</td>
<td>29</td>
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</tbody>
</table>

CONCLUSION:

Both soups were found to be more acceptable than the lupin milks. The kilned sprout soup was not as pleasant as the day two sprout soup.

RECOMMENDATION:
Further experiments need to be conducted on the use of lupin as an ingredient in soups.
Trial 10: LUPIN MILK

SAMPLE: 1. Gungurru seeds, soaked for 24h in water.
2. Gungurru sprouts, one day sprout (home)
3. Gungurru sprouts, two days sprout (home)
4. Kiev mutant seeds, soaked for 24h in water.
5. Kiev mutant sprouts, one day sprout (home)
6. Kiev mutant sprouts, two day sprout (home)

METHOD:
1. To 40g lupins add 100ml of 1% NaHCO₃.
2. Homogenise.
3. Measure pH, then adjust with NaOH dilute to pH 7.50.
4. Heat to 90 C for 5 minutes.
5. Centrifuge at 2000rpm 10 C 10mins.
6. Decant.

SENSORY RATING: revised table.

SCALE: 1 = low - 10 = high

<table>
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<tr>
<th>SAMPLE</th>
<th>1</th>
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<th>3</th>
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</tr>
</tbody>
</table>

CONCLUSION:

Appearance: All samples were a pleasant yellow colour.

Sample 4, 5 and 6 could not be tasted as the kiev mutant seeds went off. Sample one could not be completed due to equipment failure.

The samples all had nutty, grassy tastes, like chlorophyll. After taste from milks was unpleasant.

No samples were acceptable to drink.

RECOMMENDATION:
Lupin soup would be a better product to develop.
Trial 11: LUPIN MILK

SAMPLE:
1. Gungurru seeds, soaked for 24h in water.
2. Gungurru sprouts, one day sprout (home)
3. Gungurru sprouts, two days sprout (home)
4. Kiev mutant seeds, soaked for 24h in water.
5. Kiev mutant sprouts, one day sprout (home)
6. Kiev mutant sprouts, two day sprout (home)

METHOD:
1. To 40g lupins add 100ml of 1% NaHCO₃.
2. Homogenise.
3. Measure pH, then adjust with NaOH dilute to pH 7.50.
4. Heat to 90°C for 5 minutes.
5. Centrifuge at 2000rpm 10°C 10mins.
6. Decant.

SENSORY RATING: revised table.

SCALE: 1 = low - 10 = high

<table>
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<tr>
<th>SAMPLE</th>
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<td></td>
</tr>
</tbody>
</table>

CONCLUSION:

Appearance: All samples were a pleasant yellow colour.

Sample 4, 5 and 6 could not be tasted as the kiev mutant seeds went off.

The sample all had nutty, grassy tastes, like chlorophyll. After taste from milks was unpleasant.

No sample were acceptable to drink.

RECOMMENDATION:
Lupin soup would be a better product to develop.
SUMMARY OF MILK TRIALS

A series of experiments were conducted into the use of Lupinus angustifolius (cv. Gungurru) sprouts to produce a lupin milk.

From the experiments it was concluded that:

- milks made from both the lupin sprouts and seeds had a pleasant yellow colour, however milks made from sprouts that had been frozen for 3 months were an unpleasant green colour.

- milks made from the lupin sprouts were sweeter and more pleasant to taste than milks made from lupin seeds.

- lupin sprouts frozen for 3 months produced an unpleasant, watery milk, however sprouts kilned then frozen for 3 months, were sweet and quite pleasant to drink - but the milk was a dark green colour.

- milks made from kilned lupin sprouts were a sweeter, more pleasant flavour than milks made from sprouts that had not been kilned.

- milks made from two day lupin sprouts were a sweeter, more pleasant taste than one day sprouts.

- difficult to achieve the same results, even when experiment controlled. Results are not consistent.

- soup is a better product in terms of taste than milk.

CONCLUSIONS OF LUPIN MILK TRIALS

Analysis of preliminary lupin milk trials indicates that lupin milk is not a viable product as it is unpleasant to drink. Although some lupin milks were more pleasant to drink than others, consistent results were difficult to obtain even under controlled laboratory conditions. Milks containing the same types and quantities of ingredients, and made using the same method, tasted very different from each other.

Two types of lupin soups were made during the milk trials. A trained sensory tasting panel perceived the lupin soups to be a more acceptable product than the lupin milks. It was concluded from the sensory evaluation that the lupin soup would be a better direction to concentrate than the lupin milks as the taste characteristics of lupin, such as beaniness and nuttiness, are acceptable in a soup but not in a milk. Other ingredients added to soups would also help to complement the flavour of lupin.

It was decided that further research into the use of lupin sprouts as a food ingredient in soup was justified.
APPENDIX E: PRELIMINARY SOUP TRIALS

TRIAL 1: LUPIN SOUP

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
750ml chicken stock

VARIATIONS:
1. 120g gungurru seeds  
Soak for 24 hours
2. 120g chick peas  
then drain and rinse
3. 120g lentils
4. 120g frozen day two lupin sprouts

METHOD:
1. Place all ingredients into a pressure cooker and cook for 25 minutes.
2. When cooked, puree until smooth.

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>N=4</th>
<th>SEEDS</th>
<th>CHICK</th>
<th>LENTIL</th>
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<tr>
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<td>24</td>
<td>28</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>20</td>
<td>7</td>
<td>31</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: The lentil soup was found to be much more acceptable than the other soups regardless of its unpleasant appearance. The chick pea soup was found to be the most unacceptable. Both of the lupin soups were rated more acceptable than the chickpea but not as acceptable as the lentil soup.

RECOMMENDATION: Use a better control to test lupins against, chickpea and lentil were very different from lupin. Suggest using split pea soup as a control.
TRIAL 2:

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
750ml chicken stock

VARIATIONS:
1. 120g gungurru seeds  ]Soak for 24 hours
2. 120g green split peas ]then drain and rinse
3. 120g frozen day two lupin sprouts

METHOD:
1. Place all ingredients into a pressure cooker and cook for 25 minutes.
2. When cooked, puree until smooth.

<table>
<thead>
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<th>SOUPS</th>
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<tr>
<td>Flavour Pleasantness</td>
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<tr>
<td>Sweetness</td>
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<td>19</td>
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<tr>
<td>Saltiness</td>
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<td>Beaniness</td>
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<td>22</td>
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<td>Mouth texture</td>
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<tr>
<td>Overall acceptability</td>
<td></td>
<td>25</td>
<td>35</td>
<td>18</td>
</tr>
</tbody>
</table>

CONCLUSION: Regardless of the green colour of the pea soup it is rated as having a more pleasant appearance. The soup was also rated higher for flavour pleasantness and overall acceptability. The lupin soup made from the seeds was rated higher overall than the sprouts. The texture of both lupin soup was rated poorly and may have effected overall acceptability.

RECOMMENDATION: Improve the texture of the lupin soups - cook soups for longer or dehull the lupins
TRAIL 2A: lengthen cooking time

Variations: use 60g of lupins and cook for 40 minutes.
Result: lupins were softer, but not enough liquid.
Recommendation: increase liquid content to 1L and cook for 40 mins

TRAIL 2B: increase liquid and cooking time

Variation: use 60g of sprouts and 1 litre of water and cook for 40 minutes.
Result: lupins were softer and enough liquid to make a soup.
Recommendation: use the method for cooking sprouts with the hull.
TRAIL 3: 22/3/91

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L chicken stock

VARIATIONS:
1. 60g Day 2 sprouts with hull cooked for 40 mins
2. 60g Day 3 sprouts with hull
3. 60g Day 6 sprouts with hull
4. 60g Day 6 sprouts without hull - cooked for 30 minutes

METHOD:
1. Place all ingredients into a pressure cooker and cook for at least 30 minutes.
2. When cooked, puree until smooth.

<table>
<thead>
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<th>SOUPS</th>
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<th>Day 2 hull</th>
<th>Day 3 hull</th>
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<td>Appearance</td>
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<tr>
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<td>Mouth texture</td>
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<td></td>
<td>25</td>
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</tbody>
</table>

CONCLUSION: The flavour pleasantness and overall acceptability was very similar for the Day 2 no hull soup, the day 3 with hull soup and the day 6 with hull soup. The day 2 soup with the hull was not rated as high, this may be related to its grainy texture, which was rated low. The day 6 with hull sprout also had a low rated texture because of its graininess, however it was still rated quite high overall. This suggests the older day 6 sprout is preferred to the day 2 sprout.

RECOMMENDATION:
Try to improve the texture, strain the soup through a metal strainer.
TRAIL 3a:

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L chicken stock

VARIATIONS:
1. 60g Day 2 sprouts with hull [cooked for 40 mins]
2. 60g Day 2 sprouts with hull - strained cooked for 40 mins
3. 60g Day 2 sprouts without hull - cooked for 30 minutes

METHOD:
1. Place all ingredients into a pressure cooker and cook for at least 30 minutes.
2. When cooked, puree until smooth.

<table>
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<tr>
<td>Overall acceptability</td>
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<td>22</td>
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</tbody>
</table>

CONCLUSION: The texture of the day 2 soup that was strained was much higher rated than the other soups. Though it rated overall similar to day 2 with hull, even though it had a grainy texture. Day 2 with hull less overall acceptable, suggesting hull adds flavour.

RECOMMENDATION: Leave hull in soup when cooking and strain after cooked.
TRAIL 3b:

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L chicken stock

VARIATIONS:
1. 60g Day 3 sprouts with hull
2. 60g Day 6 sprouts with hull
3. 60g Day 6 sprouts with hull - strained

METHOD:
1. Place all ingredients into a pressure cooker and cook for 40 minutes.
2. When cooked, puree until smooth.

<table>
<thead>
<tr>
<th>SOUPS</th>
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<tr>
<td>Overall acceptability</td>
<td>25</td>
<td>24</td>
<td>24</td>
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</tr>
</tbody>
</table>

CONCLUSION: All rated very similar. Day 6 strained had the smoothest texture, day 3 was next then day 6 not strained.

RECOMMENDATION:
Try different ages with the hull to see if the older sprout has a less grainy texture.
TRAIL 4:

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L chicken stock

VARIATIONS:
1. 60g Day 1 sprouts with hull
2. 60g Day 4 sprouts with hull
3. 60g Day 6 sprouts with hull

METHOD:
1. Place all ingredients into a pressure cooker and cook for 40 minutes.
2. When cooked, puree until smooth.

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<tr>
<td>Overall acceptability</td>
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<td>21</td>
<td>24</td>
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</tr>
</tbody>
</table>

CONCLUSION:
The day 1 sprout seems to be preferred over the day 4 and 6 sprout for flavour pleasantness, texture and overall acceptability.

RECOMMENDATION:
Continue to compare different ages of sprouts. Day 4 and 6 need to be compared again to seen if there is a difference and whether texture also makes a difference to which age sprout is preferred.
TRAIL 5:

BASIC INGREDIENTS:
100g chopped carrot
80g diced onion
60g chopped celery
1L chicken stock

VARIATIONS:
1. 60g Day 4 sprouts with hull
2. 60g Day 6 sprouts with hull
3. 60g Day 6 sprouts with hull - strained

METHOD:
1. Place all ingredients into a pressure cooker and cook for 40 minutes.
2. When cooked, puree until smooth.

<table>
<thead>
<tr>
<th>SOUPS</th>
<th>N=9</th>
<th>Day 4 hull</th>
<th>Day 6 hull</th>
<th>Day 6 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>28</td>
<td>29</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Flavour Pleasantness</td>
<td>23</td>
<td>29</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Sweetness</td>
<td>22</td>
<td>20</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Saltiness</td>
<td>22</td>
<td>24</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Beaniness</td>
<td>28</td>
<td>21</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Unusual Flavours</td>
<td>29</td>
<td>26</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Mouth texture</td>
<td>26</td>
<td>26</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>21</td>
<td>26</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>21</td>
<td>26</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION:
Day 6 sprout was preferred over day 4 sprout soup. The day 6 strained soup was slightly higher rated than the unstrained day 6 sprout.

RECOMMENDATION:
Continue to try and find which age sprout is most preferred.
APPENDIX F: FOOD PRODUCT DEVELOPMENT CALCULATIONS

TRIAL A

TEXTURE
\[
\sqrt{2 \times 18.2 \times 2.62 \times 2/16} = 3.45
\]

TRAIL B

APPEARANCE
\[
\sqrt{2 \times 50.4 \times 2.62 \times 2/21} = 5.01
\]

BEANINESSNESS
\[
\sqrt{2 \times 80.2 \times 2.62 \times 2/21} = 6.3
\]

TEXTURE
\[
\sqrt{2 \times 24.6 \times 2.62 \times 2/21} = 3.5
\]

OVERALL ACCEPTABILITY
\[
\sqrt{2 \times 72.1 \times 2.62 \times 2/21} = 5.998
\]

TRIAL C

BEANINESSNESS
\[
\sqrt{2 \times 58.1 \times 2.62 \times 2/19} = 5.66
\]

TEXTURE
\[
\sqrt{2 \times 36.3 \times 2.62 \times 2/19}
\]
TRIAL D

APPEARANCE
\[
\sqrt{2 \times 50.8 \times 2.62 \times 2/20}
\]
= 5.15

BEANINESS
\[
\sqrt{2 \times 63.1 \times 2.62 \times 2/20}
\]
= 5.75

MOUTH TEXTURE
\[
\sqrt{2 \times 55 \times 2.62 \times 2/20}
\]
= 5.3

TEXTURE
\[
\sqrt{2 \times 21.3 \times 2.62 \times 2/20}
\]
= 3.06

TRIAL E

APPEARANCE
\[
\sqrt{2 \times 56.7 \times 2.62 \times 2/18}
\]
= 5.7

FLAVOUR PLEASANTNESS
\[
\sqrt{2 \times 57 \times 2.62 \times 2/18}
\]
= 5.9

SALTINESS
\[
\sqrt{2 \times 88 \times 2.62 \times 2/18}
\]
= 7.1
MOUTH TEXTURE
\[ \sqrt{2 \times 52.7 \times 2.62 \times 2/18} \]
= 5.34

OVERALL ACCEPTABILITY
\[ \sqrt{2 \times 72 \times 2.62 \times 2/18} \]
= 6.47

TRIAL F
APPEARANCE
\[ \sqrt{2 \times 72.9 \times 2.62 \times 2/16} \]
= 6.9

FLAVOUR PLEASANTNESS
\[ \sqrt{2 \times 81.2 \times 2.62 \times 2/16} \]
= 7.29

BEANINESS
\[ \sqrt{2 \times 93.4 \times 2.62 \times 2/16} \]
= 3.8

TEXTURE
\[ \sqrt{2 \times 35.3 \times 2.62 \times 2/16} \]
= 4.8

OVERALL ACCEPTABILITY
\[ \sqrt{2 \times 53.7 \times 2.62 \times 2/16} \]
= 5.93
TRIAL G

FLAVOUR PLEASANTNESS
\[ \sqrt{2 \times 70.5 \times 2.62 \times 2/16} = 6.7 \]

BEANINESS
\[ \sqrt{2 \times 78.9 \times 2.62 \times 2/16} = 5.0 \]

UNUSUAL FLAVOURS
\[ \sqrt{2 \times 67.4 \times 2.62 \times 2/16} = 6.6 \]

OVERALL ACCEPTABILITY
\[ \sqrt{2 \times 81.2 \times 2.62 \times 2/16} = 7.3 \]

TRIAL H

APPEARANCE
\[ \sqrt{2 \times 50.3 \times 2.62 \times 2/24} = 4.68 \]

UNUSUAL FLAVOURS
\[ \sqrt{2 \times 108 \times 2.62 \times 2/25} = 6.73 \]

MOUTH TEXTURE
\[ \sqrt{2 \times 47 \times 2.62 \times 2/25} = 4.4 \]

TEXTURE
\[ \sqrt{2 \times 29.8 \times 2.62 \times 2/25} = 3.5 \]
OVERALL ACCEPTABILITY

\[
\sqrt{2 \times 56.6 \times 2.62 \times 2/25} = 4.48
\]

TRIAL I

APPEARANCE

\[
\sqrt{2 \times 58.7 \times 2.62 \times 2/20} = 5.5
\]

BEANINESS

\[
\sqrt{2 \times 57.9 \times 2.62 \times 2/20} = 5.5
\]

MOUTH TEXTURE

\[
/ 2 \times 40.856.6 \times 2.62 \times 2/20 = 4.8
\]

OVERALL ACCEPTABILITY

\[
\sqrt{2 \times 62.8 \times 2.62 \times 2/20} = 5.7
\]

TRIAL J

TEXTURE

\[
\sqrt{1 \times 49.2 \times 2.62 \times 1/15} = 2.93
\]
APPENDIX G: MIDLAND GATE GRAIN EXPO DISPLAY

Plate 3. Grain Expo display

Plate 4. Lupin soup tasting and surveying