UNIVERSITY OF NAIROBI

FOURTH YEAR PROJECT

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EFFECT OF SPROUTING AND FERMENTATION ON PHYTIC ACID, IN COMMON BEAN (Phaseolus vulgaris)

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A fourth year project proposal submitted in partial fulfilment requirements for the award of a Degree in B.Sc Food Science and Technology.

SUPERVISOR:

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DECLARATION

I declare that the fourth year project submitted is purely my work with help of literature and research, and therefore as submitted in partial fulfillment requirements for the award of a degree in B.sc Food Science and Technology. In department of FOOD SCIENCE, NUTRITION AND TECHNOLOGY

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One hundred grams (100 g) of bean seeds were soaked in a glass beaker for twelve hours in distilled water (1:5 w/v) at 24 °C. The soak water was discarded.

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CHAPTER 1: INTRODUCTION

1.1 Background information
BEANS

Beans belong to the family fabaceae (alternately leguminoseae) used for human food or animal feed. The term "bean" originally referred to the seed of the broad bean, but was later expanded to include members of the genus phaseolus, such as the common bean and the runner bean and the related genus Vigna. The term is now applied in a general way to many other related plants such as soybeans, peas, lentils, chickpeas (garbanzos), vetches, and lupins.

Common bean (Phaseolus vulgaris) are affordable good source of protein and calories and they are widely consumed all over the world, together with other legumes, they have long been one of the most important sources of proteins in rural population and satisfy a considerable proportion of population's protein requirement. However like other pulses beans contain several anti-nutritional factors which limit their consumption and affect the digestibility and bioavailability of nutrient.

Beans production in Kenya was 38403 tonnes in 2008, (FAOSTAT 2008) an indication of its high consumption.

Different procedures have been proposed to eliminate or reduce anti nutritional factors in legumes. Homepractices such as soaking, dehulling and cooking effectively improve the nutritional value of legumes. Moist heating generally destroys the trypsin inhibitor (Oke, 1987; Steinkraus, 1979). Germination and fermentation have been reported to improve legume nutritional and sensory values. Germination of peas for 10 days decreased phytates by 75% and increased phytase activity 12-fold (Beal & Mehta, 1985). Sprouting high tannin bean varieties for 72 h significantly reduced the tannin content and increased the in vitro protein digestibility (Maeda, Sule, & Samwel, 1991)

1.2 PROBLEM STATEMENT

This study was designed to reduce the level of phytic acid in common beans, the locally affordable source of plant protein. The presence of phytic acid limits the consumption, digestibility and bioavailability of nutrients (particularly the essential minerals like iron and calcium) in common beans. Due to the problem of binding of nutrients by phytic acid, in this affordable source of protein in rural areas, many investigations have been carried out to develop techniques in order to reduce or even remove the content of phytic acid and enhance the nutritional quality.

1.3 JUSTIFICATION

The study is aimed at reducing phytic acid in common beans, a popular legume in Kenya. This project is aimed at evaluating the effects of sprouting and fermentation in the reduction of phytic acid in common bean. Establishing these effects on phytic acid levels in common bean and its utilization will lead to:
• Higher preference in choosing common bean as the source of protein.
• Due to affordability of beans, there will be better digestibility and availability of essential minerals.

The common bean is one of the most affordable sources of protein in rural areas and has been very vital in the nutrition and diets of many poor communities. Legumes such as common beans have been used in the alleviation of hunger, micronutrient deficiencies and protein energy malnutrition in Kenya. Research efforts have been directed towards the identification of suitable processing technologies for the reduction of phytic acid levels in beans. Therefore, the results of this study will contribute towards the growing body of knowledge on the reduction of phytates in foods.

1.4: MAIN OBJECTIVE
The main purpose of the study is to evaluate the effects of sprouting and fermentation on phytic acid content in common bean.

1.4.1 Sub objectives
• Determine moisture content and phytic acid content of common beans before processing.
• Determine levels of phytic acid after sprouting.
• Determine the content of phytic acid after fermentation.

1.5 Hypothesis
Sprouting and fermentation can reduce the levels of phytic acid in common bean.

CHAPTER 2: LITERATURE REVIEW
2.1 Phytic acid
Phytic acid is found within the hulls of nuts, seeds, and grains. In-home food preparation techniques can reduce the phytic acid in all of these foods. Simply cooking the food will reduce the phytic acid to some degree. More effective methods are soaking in an acid medium, fermentation, and sprouting. Phytic acid has a strong binding affinity to important minerals, such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be non absorbable in the intestines. This process can therefore
contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries. Contrary to that, one study correlated decreased osteoporosis risk with phytic acid consumption. It also acts as an acid, chelating the vitamin niacin, the deficiency of which is known as pellagra. In this regard, it is an anti nutrient, despite its possible therapeutic effects. For people with a particularly low intake of essential minerals, especially those in developing countries, this effect can be undesirable. Phytates constitute 1 to 2%, by weight, of many cereals and oilseeds. Typically, 60 to 90% of the total phosphorus in seeds occur as phytic phosphorus.

Phytic acid (known as inositol hexakisphosphate (IP6) or phytate when in salt form) is the principal storage form of phosphorous in many plant tissues, especially bran and seeds. Phytate is not digestable to humans or non ruminant animals, however, so it is not a source of either inositol or phosphate if eaten directly. Moreover, it chelates and thus makes un absorbable certain important minor minerals such as zinc and iron, and to a lesser extent, also macro minerals such as calcium and magnesium. Phytic acid (inositol hexaphosphate, IP_6) is considered to be the major cause of impaired absorption of several essential minerals turning them into insoluble forms which are unavailable to the human body.

**Phytic acid**

![Phytic acid structure](image)

**MW: 660.03**

**Formula:** C_{6}H_{18}O_{24}P_{6}
The bioavailability of phytate phosphorus can be increased by supplementation of the diet with the enzyme phytase. Also, viable low-phytic acid mutant lines have been developed in several crop species in which the seeds have drastically reduced levels of phytic acid and concomitant increases in inorganic phosphorus. However, reported germination problems have hindered the use of these cultivars thus far.

The use of sprouted grains will reduce the quantity of phytic acids in feed, with no significant reduction of nutritional value.

Some kind of raw beans and especially red and kidney beans contain harmful toxin (lectin phyto haemaglutinin) that must be destroyed by cooking. Many edible beans contain oligosaccharides (particularly raffinose and stachyose), a normal human digestive tract does not contain any anti-oligosaccharide enzymes, consumed oligosaccharides are typically digested by bacteria in the large intestine, and this digestion produces flatulence-causing gases as by products. Some species of molds produce α-galactosidase, an anti oligosaccharide enzyme. Tannins have been reported to cross link with proteins, carbohydrates lowering their biological effectiveness and forming complexes with enzymes such as trypsin, lipase, α-amylase and polysaccharides and their content can be reduced by soaking to sprout.

2.2 Processing methods for phytic acid reduction
There has been traditional and other treatments that were earlier used to curb or reduce anti nutrients in beans like cooking which is still used up to date reducing most toxins like hema glutinins, soaking that reduces flatulence factors (particularly those of raffinose, stachyose). Germination increases activity of hydrolytic enzymes, which decreases phytates and protease inhibitors on other hand some species of molds produce alpha-galactosidase enzyme which is an anti oligosaccharide enzyme and consequent removal of toxins like gluttinin. Phytic acid present in common bean impairs the absorption of essential minerals turning them into insoluble forms making them unavailable to body.

"Probiotic lactobacilli, and other species of the endogenous digestive microflora, as well, are an important source of the enzyme phytase which catalyses the release of phosphate from phytate and hydrolyses the complexes formed by phytate and metal ions or other cations, rendering them more soluble, ultimately improving and facilitating their intestinal absorption"
CHAPTER 3: RESEARCH DESIGN & METHODOLOGY

3.1 Materials and methods

Sample collection

Dry seeds of red kidney beans were obtained from Tusky supermarket in Nairobi town and stored in a polythene bag till they were required for treatment.

- Raw dry kidney beans.
- Soaked kidney beans.
- Fermented kidney beans.
- Sprouted kidney beans.

The samples were then air dried at 65°C in an oven for at least 16 hrs.
- The samples were then ground individually using a laboratory blender into fine powder.
- They were stored separately in tight capped bottles until required for analysis.

Preparation of processed samples

3.1.1 Cleaning and removal of surface contamination.

When required for treatment seeds were first hand sorted to remove all stones, trash and deformed seeds. Then immersed in 65% ethanol for 1 minute and will be cleaned 5 times using tap water so as to remove any residual ethanol from the seeds.

3.1.2 Soaking

One hundred grams (100 g) of bean seeds were soaked in a glass beaker for twelve hours in distilled water (1:5 w/v) at 24°C. The soak water was discarded.

3.1.3 Sprouting

One hundred grams (100 g) of bean seeds were rinsed five times with distilled water (1:3 w/v) and soaked.

For 9 h in a glass beaker in tap water (1:3 w/v). The presoaked seeds were allowed to sprout on sterile germinating perforated trays. Germination was carried out at 25°C with 8 h of daylight per day. Samples were collected at 0 h (control), 12 h, 24 h, 48 h, 72 h and 96 h.
Soaking (15hrs) Sprouting was carried out on a perforated tray as follows:
- A damp cheese clothe was spread on tray and soaked seeds in turn spread on clothes.
- The seeds were then be covered with another cheese clothe to exclude day light
- The tray were kept at room temperature in open to allow free flow of air though seeds
- During sprouting period seeds were washed once every 12 hrs with water containing 2.5% acetic acid to keep off mold growth.
- Sprouting was carried out and stopped when the seed attained an average radical length of 5mm.

3.1.3 Fermentation

After cleaning with distilled water Seeds were then suspended in distilled water in a proportion of 1:4(w/v). Natural fermentation was carried out at 42°C and Samples collected at 0 h (control), 12 h, 24 h, 48 h, 72 h and 96 h.

3.1.4 Preparation of samples for laboratory analysis

Samples of raw dry; soaked and sprouted; and fermented seeds were drawn from respective treatments as indicated in design.

The samples were then air dried at 65°C in an oven for at least 16 hrs.
- The samples were then ground individually using a laboratory blender in to fine powder.
- They were then stored separately in tight capped bottles until required for analysis.

3.2 Research design

Procuring the raw dry kidney beans

Cleaning and sorting

Soaking (15hrs)

Fermentation
Sprouting under natural conditions

Air drying at 65°C

4.0 METHODS OF DETERMINING PHYTIC ACID DETERMINATION BASED ON AOAC 1990

Determination of phytic acid contents

Standard AOAC method was used to quantify phytic acid in unprocessed and processed red kidney bean samples.

STATISTICAL ANALYSIS OF RESULT

In order to quantify the influence of different processing techniques, data were subjected to analysis of variance using GRAPH PAD PRISM computer package (1992-2010) a two way ANOVA was applied.

- As elaborated below

4.1 Phytic acid determination

Chemicals and equipment

- Prepared and treated samples of kidney bean.
- Petroleum ether,
- Conc. 37% HCl
- The Wade reagent 0.03% FeCl₃·6H₂O, 0.3% Sulfosalicylic acid
- Spectrophotometer
- Centrifuge machine
- Eppendorf tube

Method of analysis.

- 100mg of seed flour was taken in an eppendorf tube
- 10ml of petroleum ether was added and kept in ultra sonic bath for 30 minutes for defatting.
- After centrifugation at 13000 rpm for 5 minutes, the supernatant was discarded and the residue was air dried.
- Residue was extracted with 1ml of 2.4% HCl for 10 minutes in ultra sonic bath-1ml (this procedure repeated for 3 times.)
• All the supernatants were pooled together and made up to a known volume (10ml) with distilled water.

Estimation

• The extract (500μl) was taken in a test tube and diluted to 2.5 ml with distilled water.
• 1 ml Wade reagent (1ml of 0.03% FeCl$_3$.6H$_2$O and 1ml of 0.3% salfosalicylic acid) was added.
• The contents were vortexed and centrifuged at 3500 rpm at 5 minutes.
• Then the absorbance of the supernatant was measured at 500nm
• Blank was prepared using distilled water
• The phytic acid content was calculated by using the standard curve prepared with phytic acid and expressed in mg/g extract on dry matter basis.

5.0 RESULTS

<table>
<thead>
<tr>
<th>Hours</th>
<th>sprouting</th>
<th>fermentation</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.000 ±0.000$^A$</td>
<td>82.000 ±0.000$^A$</td>
<td>100.000 ±0.000$^B$</td>
</tr>
<tr>
<td>12</td>
<td>81.000 ±0.000$^A$</td>
<td>79.000 ±0.000$^A$</td>
<td>100.000 ±0.000$^B$</td>
</tr>
<tr>
<td>24</td>
<td>79.000 ±0.000$^A$</td>
<td>77.000 ±0.000$^A$</td>
<td>100.000 ±0.000$^B$</td>
</tr>
<tr>
<td>48</td>
<td>78.000 ±0.000$^A$</td>
<td>64.000 ±0.000$^B$</td>
<td>100.000 ±0.000$^C$</td>
</tr>
<tr>
<td>72</td>
<td>66.000 ±0.000$^A$</td>
<td>62.000 ±0.000$^B$</td>
<td>100.000 ±0.000$^C$</td>
</tr>
<tr>
<td>96</td>
<td>65.000 ±1.000$^A$</td>
<td>60.000 ±0.000$^B$</td>
<td>100.000 ±0.000$^C$</td>
</tr>
</tbody>
</table>

Data presented as the mean and ± standard error of mean of the two separate determinations (n=2). Values in the same row with different upper case superscript letters are significantly different (p<0.05)

Soaking had 86μg/g phytic acid concentration.
6.0 DISCUSSION

- The level of phytic acid in unprocessed samples was 100.0 μg/g. The only significant (P<0.0001) reduction in phytic acid content of red kidney beans was noted with Germination and fermentation.

- However, the reduction with sprouting was only 36.0%, whereas fermentation resulted in 43% reduction. The other processing methods (soaking) reducing 12% did not alter the concentration of phytic acid contents significantly.

- These reductions due to sprouting are mainly due to an increase in the phytase activity, leading to a solubilization of phytates.
7.0 CONCLUSION:
• the tested anti-nutritional factors (phytates) were significantly reduced with different processing techniques,
• phytic acid was least reduced by soaking (12%).
• Phytic acid were reduced significantly (P<0.0001) with sprouting and fermentation.

8.0 RECOMMENDATION
• The simple and inexpensive technique of sprouting and fermentation has been therefore recommended for both in the home and by industries that produce food products for nutritionally vulnerable persons with high Ca and P requirements.
• for some practically meaningful reduction in phytic acid contents, in this bean needs sprouting prior to cooking
• With continued research there is need for a suitable culture to be used for fermentation.

8.1 other benefits of phytic acid
• Excess iron is known to have disease causing effects, so the fact that phytate prevents some of the iron absorption may actually be a good thing. Phytic acid is also an antioxidant (like vitamin C).
• Some research suggests that phytate has the potential ability to lower blood glucose, reduce cholesterol and triacylglycerols, and reduce the risks of cancer and heart disease, and it may play a part in reducing colon cancer through its absorption of iron and other minerals that cancer cells need for growth.
• However, it also deprives non-cancerous cells of the minerals needed for health.
• With studies phytic acid is also an anti oxidant
9.0 REFERENCES

1. Google Scholar and Wikipedia.eplantScience.com


