EFFECT OF PROCESSING ON ANTI-NUTRITIONAL FACTORS AND *IN VITRO* PROTEIN DIGESTIBILITY OF FABA BEAN (*Vicia faba*)

By

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To my parents, my husband and my kids, Sarah,
Mohammed, Mussaab and Israa, with love

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خلاصة الأطروحة

أجريت هذة الدراسة على أربعة عينات من الفول المصري استجلبت من محطـة ابحاث شمبات. أجرى التحليل التقريبي لهذة العينات لتحديد الرطوبة و الدهون و الألياف و الرماد و البروتين. كما أن العينات اخضعت لعدة معاملات هى النقع في الما لمدة 1، 4 و ايام 6؛ التزريع بعد النقع في الماء لمدة 24 ساعة لمدة يومين و4 ايام و 6 ايام؛ أزالـة القشرة و الطبخ للحبوب الجافة و الحبوب المنقوعة في ماء مقطر لمدة 12 ساعة و فـى الملح(يحتـوى (1.5 $NaHCO_3$, 0.5% Na_2CO_3, 0.75% citric acid) لمـدة 12، 18، 24 ساعة لمعرف تأثير هذة المعاملات علي نسبة حمض الفايتك و المواد الدابغـة و الهضم المعملي للبروتين.

فى العينات SML ، شمبات 616 و شـمبات 00104 اجـرى تخليـل للهـضم المعملي للبروتين لبذورها المطبوخة و غير المطبوخة بعد معاماتها بملح الصوديوم ثنائي الكبريتيت ($NaHSO_3$) بتركيز 0.1 M، 0.25 M و 0.5 M لمدة يـوم ، يـومين و ثلاث ايام.

أظهر التحليل التقريبي للأربعة عينات ان نسبة الرطوبة تتـراوح مـا بـين 7.6-7.8 و الدهون ما بين 1.2-1.7، نسبة الألياف الخام بين 5.2-6.1%، الرماد 3.1-3.6% امـا النشويات فتراوحت نسبتها ما بين 46.8-49.1% و البروتين ما بين 30.9-35.1%.

وجد أن محتوى المواد الدابغة في العينات الأربعـة يتـراوح مـا بـين -0.038 0.078% إذ قلت معنوياً بعد النقع لمدة 1، 2 و 3 ايام فى الماء المقطـر وايـضاً بعـد التزريع و التقشير و الطبخ للبذور الجافة و المنقوعة حيث لوحظ أن الطبخ بعد النقع فـي الملح لمدة 24 ساعة أكثر تأثيراً في نقصان نسبة المواد الدابغة في العينات الأربعة.دلـت النتائج علي أن حمض الفايتك يتراوح مابين 247.1-259.2% ملم/100جرام للأربعـة عينات و هذة النسبة انخفضت انخفاضاً معنوياً بعد الطبخ و بعد النقع في الماء المقطـر و التزريع حيث تقل نسبتة بزيادة مدة النقع و التزريع و كان التزريع أكثر فعالية في تقليـل نسبة حمض الفايتك. أما التقشير فأدى الي زياده معنوية في نسبة حمض الفايتك في كـل العينات.

نسبة الهضم المعملى للبروتين تتراوح مـا بـين 68.6-75.1% للبـذور غيـر المطبوخة حيث ذادت هذة النسبة بعد التقشير و مع الزيادة في زمن كل من النقع في الماء المقطر و التزريع، حيث اظهر التزريع أعلى نسبة للهضم المعملي للبـروتين. العينـات المطبوخة اظهرت نسب تتراوح ما بين 50.7%-54.8% للهضم المعملي للبروتين و هذة النسب تقل معنوياً عن تلك العينات غير المطبوخة. الطبخ للعينات بعـد النقـع لمـدة 12 ساعة في الماء المقطر و لمدة 12، 18، و 24 ساعة في الملح قد حسنت من نسبة الهضم المعملي للبروتين و كان النقع لمدة 24 ساعة في الملح اكثر فعالية في تحسين نسبة الهضم المعملي للبروتين.

البذور المطبوخة و غير المطبوخة للعينات SML و شـمبات 616 و شـمبات 00104 و التى سبق نقعها فى محلول $NaHSO_3$ بتركيز 0.1 M ، 0.5 M و 0.25 M لمدة يوم، يومين و ثلاث أيام أظهرت زيادة معنوية في نسبة الهضم المعملي للبروتين. و كانت أعلى زيادة في نسبة الهضم المعملي للبروتين في العينـات غيـر المطبوخـة و الطبوخة عند نقعها فى المحلول الذى تركيزة 0.1M.

ABSTRACT

Four faba bean cultivars; SML, SML 85/1/1, Shambat 616 and Shambat 00106, obtained from Shambat Agriculture Research Station, were used in this study. Proximate composition for the four cultivars was conducted to determine moisture, fat, ash, protein and carbohydrates contents. The four cultivars were subjected to soaking in distilled water for 1, 2 and 3 days, cooking of dry seeds, cooking after soaking in distilled water for 12 h, soaking in salt solution (1.5% $NaHCO_3$, 0.5% $NaCO_3$ and 0.75% citric acid) for 12, 18 and 24 h, dehulling and germination for 2, 4 and 6 days to study their effects on IVPD, tannin and phytic contents. The three cultivars, SML, Shambat 616 and Shambat 00104 were cooked and soaked in sodium bisulphite to study their effect on IVPD.

The proximate composition for these four cultivars showed that moisture content ranged from 7.6 to 7.8%,, fat content ranged from 1.2% to 1.7, crude fibre ranged from 5.2% to 8.1%, ash was in the range 3.1%-3.6%, protein fin the range 30.9% to 35.1% and carbohydrate in the range 46.8% to 48.1%.

Tannin content for the four cultivars ranged from 0.038% to 0.078 and it was significantly ($P \leq 0.05$) reduced after soaking in distilled water for 1, 2 and 3 days. Also tannin, was reduced by dehulling, germination (for 2,

4 and 6 days), cooking of dry seeds and soaked seeds in distilled water and salt solutions. Soaking for 24 h in salt solution effectively reduced tannins. Faba beab cultivars showed phytic acid content in the range of 247.1- 259.2 mg/100g. It was significantly ($P \leq 0.05$) decreased with time of soaking, by cooking and with time of germination. Dehulling significantly ($P \leq 0.o5$) increased phytic acid content in all cultivars. The initial *in vitro* protein digestibility of the four cultivars ranged from 68.6% to 75.1% for the uncooked samples, which increased by dehulling and time of soaking and germination. Germination gave higher values of IVPD. The cooked samples gave a range of 50.7% to 54.8 for IVPD, which is lower compared to the uncooked ones. Cooking after soaking in distilled water for 12 h and salt solution for 12, 18 and 24 h improved the IVPD. The highest IVPD was given by soaking in salt solution for 24 h. The cooked and uncooked seeds of the cultivars SML, Shambat 616 and Shambat 00104 when soaked in sodium bisulphite in different concentrations (0.1, 0.25 and 0.5 M) and for 1, 2, and 3 days, showed a significant ($P \leq 0.05$) improvement in IVPD. The *in vitro* protein digestibility for the uncooked faba bean seeds increased when soaked in 0.1 M sodium bisulphite for one day. Soaked seeds were higher in IVPD compared to unsoaked ones. Generally, the IVPD for the cooked faba bean was improved after soaking before cooking.

All salt concentrations improved IVPD, with 0.1 M concentration showing a remarkable improvement.

CHAPTER ONE

INTRODUCTION

Faba bean (*Vicia faba* L.) is a member of the family Leguminaceae. It also referred to as broad bean, horse bean, and field bean, windsore bean, tick beans (small types), Bakela (Ethiopia), Bobykurmouvje (Russia), Faveiva (Portugal), Ful masri (Sudan), Feve (France) and Yeshil Bakla (Turkey) (Muehlbauer and Tullu, 1997). It is considered as one of the most important pulse crops in the world after dry beans, dry peas and chick peas (Hawtin and Stewart, 1979).

The origin of faba bean is still debated (Duc, 1997). It is assigned to the Central Asia, Mediterranean and South America as centers of diversity. Cubero (1974) postulated a Near Eastern Centre of Origin with four radii; (1) to Europe (2) along the coast of North Africa to Spain (3) along the Nile to Ethiopia and (4) from Mesopotamia to India. Secondary Centres of diversity are postulated in Afganistan and Ethiopia. Ladizinsky (1975) reported an origin of central Asia. Geographically, the origin of the crop was the Near East. The wild form is presently found from Afghanistan to india. The large seeded types (*Vicia faba major*) have developed in south Mediterranean countries and China. These types expanded in the sixteen century toward Mexico and Southern America. Small seeded

(*V. faba minor*) are found in Ethiopian area and have been favoured by North European Agriculture (Duc, 1997). Medium seed types (*V. faba equina*) have developed through Middle East and North Africa with major concentration in Egypt. Faba bean is normally grown in spring in northern latitudes and in winter in warm temperate and subtropical areas. The crop requires a cool season for best development. It is grown on different types of soil, but grows best in loamy soil. Moderate moisture supply is necessary (Duke 1981). Also it is a popular crop at higher elevation.

The crop occupied nearly 3.2 x 10^6 ha world wide (FAO, 1992). The production in the world is concentrated in nine major agro-ecological regions, namely: Northern Europe, Mediterranean, the Nile valley, Ethiopia, Central Asia, Oceana, Latin America and North America (Bond *et al*., 1985). China is the largest producer with an area of 2 x 10^6 ha and estimated annual production ranging from 2.4-2.6 million MT (1161-1447 kg/ha) for 1979 and 1994 (FAO, 1994). Argentina reported the highest yield record of more than 9000 kg/ha for 1992 and 1994.

The area grown in Sudan was 7450 ha in 1965 and was increased to 30000 ha in 1994/1995 (Salih *et al*., 1995). Most of the production comes from the Northern States, where production is about

90% of the country. Small amounts are produced in Khartoum State, Central Sudan and Jebel Mara in Western Sudan.

Faba bean, like other food grain legumes, is characterized by relatively high contents of protein and carbohydrates. It also contains a significant amount of crude fibre, lipids, minerals and vitamins (Alonso *et al*., 2000; El Tinay, 1993).

The crop is an important source of protein in the diet of many people in developing countries (mainly Asia and Africa). In Europe and USA, it is used as animal feed mainly for horses, poultry and pigs. It can be used as vegetable, green or dried, fresh or canned. It is a common brakfast food in the Middle East, Medierranean region, China and Ethiopia (Bon *et al*., 1985). In Sudan, it is the most popular food legume and it is consumed in breakfast and supper in almost all parts of the country.

The most popular dishes of faba bean are Madamas (stewed beans), Flafel (deep fried cotyledon paste with some vegetable and spices), Bissara (Cotyledon paste poured onto plates) and Nebet soup (boiled germinated seeds) (Jambunathan *et al*., 1994). As a folk medicine, it can also be used as diuretic, expectorant, or tonic (Muehlbauer and Abebe Tullu, 1997).

Besides their high nutritional value, food grain legumes contain

naturally occurring compounds known as anti-nutritional factors, as phytic acid and tannins. These anti-nutrients negatively affect the nutritive value of the bean through direct and indirect reactions (Bressa, 19989, 1993).

Phytate myo-insitol hexakis phosphate is found in all plant seeds. In beans, phytic acid is mainly located in the cotyledons (Alonso *et al*., 2000). Phytic acid can complex protein as well as mono and divalent cations and lower their bio-availability and also inhibits proteases and amylases (Carnovale, 1987).

Tanins have been reported to occur in appreciable amount in faba bean. The anti-nutritive activity of tannin is due to its ability to make complexes with protein as well as with digestive enzymes (Liener, 1989).

Removal of these anti-nutritional factors is essential to improve the nutritive value of legumes. Different authors have reported that some processes such as soaking,, cooking, germination and dehulling improve the quality of legumes because of the removal of some of the anti-nutritional factors.

The objectives of this study were:

1. To study the differences in proximate composition of four faba bean cultivars.

2. To evaluate the effect of soaking in distilled water and salt on *in vitro* protein digestibility, tannins and phytic acid content of four faba bean cultivars.
3. To evaluate the effect of cooking on *in vitro* protein digestibility, tannins and phytic acid content of four faba bean cultivars.
4. To evaluate the effect of dehulling on *in vitro* protein digestibility, tannins and phytic acid contents of four faba bean cultivars.
5. To evaluate the effect of germination on *in vitro* protein digestibility, tannins and phytic acid contents of four faba bean cultivars.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutritive value of faba bean:

The nutritive value of food grain legumes is divided into two large groups: positive and negative factors. The positive factors include high protein and lysine contents, which allows legumes to serve as an excellent supplement to cereal grains (Bresani, 1989, 1993). The health related value of beans include their positive effect on blood cholestrol level and glucose levels (Walker, 1982; Leeds, 1982), possibly through the dietary fibre present in beans.

The negative factors fall into two groups; antinutritional factors which include enzyme inhibitors, flatulence factors, polyphenols, tannins and phytic acid. The negative nutritional factors include protein and carbohydrate digestibility, and sulfur amino acid deficiency (Bressani, 1989, 1993). Like other grain legumes faba bean is considered to be a good source of protein, energy and fibre. They are also rich in phosphorous, iron, potassium and vitamin B complex (Eden, 1968; Clarke, 1970).

The percentage of solids in the mature seeds of faba bean is over three times that of solids in the immature seeds. Although the mature seeds are much higher in calories, protein, minerals and starch,

the immature seeds are good sources of vitamin A and C (Askar, 1986). Faba bean is a good source of protein and lysine, except for the low content of sulfur containing amino acids in particular methionin (Ali *et al*, 1982). The amino acids content except for methionine is reasonably well balanced (Bond *et al*., 1985). Amino acid content as mg/g of nitrogen varies from 36-69 mg/g for methionine, 44-94 mg/g for cystine and 333-4000 mg/g for lysine (Chevan *et al*., 1989).

Faba bean can provide a very good supplement to weaning foods of high nutritive value (Ali *et al*., 1982). The high lysine content of grain legumes is very important nutritional attribute and probably more important than total protein content because it makes food grain legumes, a significant supplementary protein to cereal grain based diet, which are known to be deficient in lysine. (Berssani 1989).

The mineral content of food grain legumes is of interest from nutritional point of view, but iron content is of special interest because of its deficiency in the diet of many developing countries, particularly among rural dwellers (Berssani, 1989).

Food grain legumes when consumed reduced plasma cholesterol levels and slowly increase blood glucose levels (Leeds, 1982; Walker, 1982). These effects have been associated with the dietary fibre fraction of the beans.

2.2 Proximate composition:

Proximate composition of plant material consists of determining the major classes of chemical components which include moisture, crude fibre, fat content (ether extract), crude protein, ash and carbohydrates (by difference).

Proximate composition provides a good initial impression of relative nutritive value and utility of an agricultural product and allows basis of comparison between different species, plant parts and cultivation conditions.

2.2.1 Moisture content:

The determination of moisture content of foodstuff is very important for both commercial and scientific applications. The moisture content in food is so difficult to be determined accurately (Food Standard Committee, 1979).

Moisture content of faba bean ranges from 6-6.7% as reported by El Tinay *et al*. (1989). EL Skeikh *et al*. (1999) reported a range of 6.34-7.12%. El Sayed (1994) found that the moisture content of faba bean seeds ranging from 4.3-8.1%. Ali *et al*. (1982) reported a range of 8.3-8.7%. Fifteen genotypes of faba bean grown at Shambat gave a range of 4.6-6.7% moisture content (El Tinay, 1993)

2.2.2 Fibre content:

There are two terms for fibre known in the field of food composition; dietary fibre and crude fibre. Crude fibre is the insoluble organic residue that remains after boiling a defatted sample successfully with dilute sulphuric acid followed by dilute sodium hydroxide and ignited. It consists of cellulose and hemicellulose. The dietary fibre is defined as the non-starch polysaccharides and lignin that are not digested or absorbed in the human small intestine (ASP, 1987). In human diet, fibre has many health benefits such as lowering the blood cholesterol, aiding water retention during the food pass through the gut and benefits the colon function. On the other hand, crude fibre is found to influence the rate of digestion by decreasing the activity of proteolytic enzymes (Schneeman, 1990). The fibre content of faba bean ranges from 6.1-6.5% as reported by El Tinay *et al*. (1989) and from 8.7-9.9% as reported by Elsheikh *et al* (1999) and from 5.1-7.3% as reported by Ali *et al*. (1982). El Sayed (1994) reported a range of 6.8-12.4%.

2.2.3 Ash content:

The ash content of foodstuffs is the inorganic residue that remains after burning the organic matter. Faba bean contains about 2.2-3.4 ash as reported by El Tinay *et al*. (1989). Elsheikh reported a

range from 3.03-3.62% and Ali *et al*. (1982) reported a range of 2.7-3.0%.

2.2.4 Fat content:

Lipids (fats) are heterogenous compounds, which are classified according to their solubility in organic solvents as chloroform, ethyl ether, petroleum ether or benzine. This solubility differentiates them from other constituents such as proteins, carbohydrates and nucleic acids in seeds. Lipids include free fatty acids, mono-glycerides, di-glycerides, tri-glycerides, phospholipids, sterol ester, glycerols or glyecrides.

The fat content of faba bean is low and has no commercial value. The fatty acid composition of broad bean oil has been reported as 88.16% unsaturated (Duke, 1981). The range of fat content in faba bean is 1.1-2.2% (El Tinay *et al*., 1989), 0.7-1.36% (Ali *et al*., 1982) and 1.08-2.12% (Elsheikh *et al*., 1999).

2.2.5 Protein content:

Proteins are most important components in the organic matter. Since proteins are the principle constituents of the organic and soft tissues of the human body, its continuous supply is needed throughout the human life. Proteins are high molecular weight polymers of amino acids. The main functions of proteins are the provision of amino acids

for building and maintenance of body and synthesis of nitrogen containing substances which are important for body function, such as enzymes, antibodies, hormones, etc.. . Besides the building function, proteins are sometimes used for energy supply. Protein content of foodstuffs can easily be estimated by determining the nitrogen content, and multiplying that by 6.25 (since each 100 grams contain 16 mg nitrogen).

Protein content of faba bean ranges from 28.8% to 30.1% as reported by El Tinay *et al*. (1989). Esheikh *et al*. (1999) and Elsyed *et al.* (1994) obtained 31.8-39% and 28.0-37.8% ranges of crude protein in faba bean, respectively.

2.2.6 Carbohydrates:

Carbohydrates are a group of foodstuffs, which contains carbon, hydrogen and oxygen in their chemical composition. They contain three main groups: monosacharrhides, oligosacharrides and polysacharrides. Carbohydrates play an important role in human diet. They are source of energy and aid in utilization of body fats through metabolic process. They also exert sparing effect on proteins, help in the function of intestinal tract and add flavour to food (Fageer, 2003).

Carbohydrates in faba bean range from 42.7 to 48.3% as reported by Elsheikh *et al*. (1999) and 52.3 to 54.8 as reported by El

Tinay *et al*. (1989).

2.3 Anti-nutritional factors

Faba bean with other legumes has the capacity to synthesize various chemical compounds, which when consumed by animals or humans may result in a reduction in the nutritive value as compared to that predicted from amino acid data. A number of different classes of compounds such as phytic acid, lectines, protease inhibitors, tannins, polyphenols, α-amylase inhibitors, HCN, flatulence factors and allergen (Liener and Kakade, 1980; Liener, 1989), these factors negatively affect the nutritive value of bean through direct and indirect reactions: they inhibit protein and carbohydrate digestibility; induce pathological changes in intestine and liver tissue, thus affecting metabolism; inhibit a number of enzymes and bind nutrients making them unavailable (Bressani, 1989, 1993).

2.3.1 Tannins

The term tannin originally refers to substance with the ability to tan leather. It is now used to include any naturally occurring compounds of high molecular weight (500-3000 KD) and containing a large number of phenolic hydoxylic groups (100-200 molecular weight) to enable it to form effective cross-links with proteins and other molecules (Swain, 1979). The hydrolysable and condensed

tannins are the two groups of these compounds widely distributed in the plant kingdom, which may be differentiated by their structure and reactivity towards hydrolytic agents (Hulsam, 1966).

Condensed tannins are considered the major anti-nutritional factors in faba bean. The anti-nutritive activity may be due to complexation with digestible protein in general and with digestive enzymes, such as trypsin and chymotrypsin, in particular (Liener, 1989a). Tannins have bean implicated in adversely affecting the digestibility of dietary protein and to a lesser extent that of available carbohydrates and lipids (Mosely and Griffiths, 1979).

Tannin content of faba beans ranges from 0.26% - 0.46% as reported by Elsheikh *et al*.(2000). Helesper *et al*. (1992) reported a range of 0-0.74% tannin in faba bean. Babikir and El Tinay (1993) reported a range of 0.067-0.077%. Alonso *et al* (2000) reported 0.195% tannin in faba bean.

2.3.2 Phytic acid

Phytic acid is a polyphosphorylated polyol. It is commonly identified as myoinsitol hexaphosphoric acid and scientifically named myo-inositol 1, 2,3,4,5, 6 hexakis-dihydrogen phosphates) (IUDAC, IUB, 1968).

Phytic acid is widely distributed in seed legumes, cereals and

oil seeds. Due to its highly reactive structure at different pH levels, phytic acid can complex proteins as well as mono and divalent cations and lower their bio-availability and also inhibits proteases and amylases.

Faba bean contains about 710 to 1150 mg/100g phytic acid as reported by Carnovale *et al*. (1987). El Tinay *et al* (1989) reported a range of 1400 to 1500 mg/100g. Alnoso *et al*. (2000) gave a value of 2170 mg/100g DM of phytic acid in faba bean and Elsheikh *et al*. (2000) reported a range of 120 -270 mg/100g of phytic acid.

2.4 *In vitro* protein digestibility

Faba bean like other food grain legumes is characterized by relatively high protein content. The protein digestibility is determined to provide the most satisfactory indication of seed utilization (FAO/WHO/UNV, 1985).

The protein quality of feed or food is defined by its amino acid composition and its digestibility. The protein digestibility primarily determines the availability of its amino acids (Hahn *et al*, 1981).

Historically, protein digestibility has been determined by bioassays using rats or microorganisms. These procedures share the disadvantages of being time consuming and expensive (Hahn *et al*., 1981).

Alonso *et al*. (2000) reported 70.8% for protein digestibility of faba bean. Elsheikh *et al*. (2000) reported a range of 66.2%-80.1%. Babiker and El Tinay (1993) reported *in vitro* protein digestibility of two faba bean cultivars ranging from 80.7% to 81.7%.

2.5 Processing and cooking and their effect on the anti-nutritional factor and protein digestibility

Removal of undesirable components is essential to improve the nutritional quality of legumes, and effectively utilize their full potential as human food. Simple and inexpensive processing techniques are effective methods of achieving desirable changes in the composition of seeds (Vidal Valverde *et al*., 1994). Different authors have reported that soaking, cooking and germination improve the quality of legumes because of the removal of some anti-nutritional factors. In many instances, usage of only one methods may not affect the desired removal of anti-nutritional compounds, but a combination of two or more methods is required (Vidal Valverde *et al*., 1994).

2.5.1 Soaking

Soaking is one of the processes used to remove soluble anti-nutritional factors, which can be eliminated with the discarded soaking liquors, but some metabolic reactions can take place during soaking

affecting the content of some compounds (Vidal-Valverde *et al*., 1992).

Soaking, is an integral part of traditional methods of processing, saving energy cost by shortening cooking time, offers an additional advantage of rendering the grain nutritionally superior by removing certain anti-nutritional factors like phytic acid, saponin and polyphenols (Kataria *et al*.,1988). The decrease of these anti-nutrient contents during soaking may be attributed to leaching out into soaking water under the influence of the concentration gradient.

2.5.1.1 Effect of soaking on phytic acid content

Soaking in water or some other salt solutions reduces phytic acid as reported by different authors. Vidal-Valverde (1994) reported that soaking of lentils in distilled water, 0.07% sodium bicarbonate and 0.1% citric acid reduced phytic acid by 27%, 23% and 37%, respectively. Soaking of faba bean in water for 12 hours, significantly reduced phytic acid content by 32.7% (Alonso *et al*., 2000). Sharma and Sehgal (1992b) reported that soaking of two faba bean varieties for 12 hours reduced phytic acid by 4%. A reduction by 28% in phytic acid in blackgram (Kataria *et al*., 1988) and by 30% in mungbean (Karatia *et al.,* 1989) when soaked in water for 18 hours. Ibrahim *et al*. (2002) observed that soaking of cowpeas in water and bicarbonate

solution is efficient way of removing anti-nutrients as protease inhibitors, tannin, phytic acid, riffinose and stachyose. Long time soaking (16 h) in sodium bicarbonate solution caused remarkable reduction in the anti-nutritional factors.

2.5.1.2 Effect of soaking on tannin content

Soaking in distilled water or other solution generally affects polyphenol and tannins in grain legumes. Alonso *et al*. (2000) reported that tannin and polyphenols in faba bean seeds were reduced by 47.7 and 4.85%, respectively, after soaking in double-deionized water for 12 hours. Kataria *et al*.(1988) reported that soaking of black gram in plain water for 18 hours reduced polyphenols by 10%. Soaking mungbean for 18 hours in distilled water reduced phenols by 7% (Kataria, 1989). Babiker and El Tinay (1993) reported that soaking of faba bean seeds in distilled water and sodium carbonate for different times and at different temperatures reduced tannin content. They reported that soaking in sodium carbonate for 20 minutes at 100˚ C is the most effective in tannin reduction. Sharma and Sehgal (1992a) reported that soaking of two faba bean cultivars for 12 h reduced tannin content by 42% and 51% for both cultivars. However, Vidal-Valverde *et al*. (1994) reported that soaking of lentils in distilled water, 0.07% sodium bicarbonate and citric acid increased the

levels of tannin and catchin content.

2.5.1.3 Effect of soaking on the in vitro protein digestibility (IVPD):

Babiker and El Tinay (1993) reported that soaking of faba bean seeds in distilled water or sodium carbonate increased the IVPD. As the time of soaking and the concentration of sodium carbonate increased, IVPD increased. Arbab and El Tinay observed that soaking of sorghum grains in sodium bisulphite significantly increased the IVPD. Alonso *et al* (2000) reported that IVPD of faba bean increased by 0.71% after soaking. Kataria et al (1989) reported that soaking of mungbeans improved IVPD, which increased by period of soaking. It increased by 4, 15 and 21% for 6, 12 an 18 h of soaking, respectively.

2.5.2 Cooking:

Cooking generally inactivates heat sensitive factors such as trypsin and chymotrypsin inhibitors and volatile compounds. Cooking of beans may be done with or without soaking which typically reduce cooking time and energy cost.

2.5.2.1 Effect of cooking on phytic acid content:

Vidal-Valverde (1994) reported that cooking of lentils after soaking in distilled water reduced phytic acid by 39% while cooking after soaking in sodium bicarbonate resulted in 29% reduction. Kataria

et al. (1989) observed 20% reduction in phytic acid for pre-soaked cooked mungbean. The loss of phytic acid was 15% when unsoaked seeds were cooked compared to soaked seeds. Kataria *et al*. (1988) found that pressure cooking of soaked seeds of blackgram reduced phytic acid by 33%, whereas that of unsoaked seeds resulted in a reduction of 8%. Elsheikh *et al* (2000) reported a significant reduction in phytic acid content of faba bean after cooking. Saikia *et al.* (1999) observed a decrease in phytic acid of rice bean after cooking. Extrusion cooking of faba bean reduced phytic acid by 26.7% (Alonso *et al*., 2000).

2.5.2.2 Effect of cooking on tannin content:

Elsheikh *et al*. (2000) reported that cooking of faba bean seeds significantly reduced tannin content. Less than 10% of total tannin decomposed during cooking, while up to 50% were leached to the cooking liquor (Ziena *et al*., 1991). Sharma and Sehgal (1992a) reported that significant reduction in tannins (76-81%) after cooking of two faba bean cultivars. Tannin content in lentils increased after cooking (Vidal-Valverde *et al*., 1994).

2.5.2.3 Effect of cooking on IVPD:

Cooking beans in water with or without pressure increases the protein quality, the protein and carbohydrate digestibility and

inactivates protease and α-amylase inhibitors (Bressani, 1993). Cooking when done under controlled time and temperature improve protein quality of food grain legumes (Bressani, 1985). Bressani (1984) using *Phaseolus vulgaris* observed that increased cooking time reduced the IVPD of the whole and the hulled seeds. The cooking process affects *in vitro* protein digestibility of legume seed. Kataria *et al*. (1989) reported that IVPD was higher when soaked seeds of mungbeans were cooked than when unsoaked. Elsheikh *et al* (2000) reported that cooking of faba bean seeds significantly increased IVPD (66.2-80.1% for the unsooked to 74.6-84.66 for the cooked seeds).

Arbab and El Tinay (1997) reported that cooked sorghum has less IVPD than the uncooked control. They observed that soaking seeds in reducing agent (ascorbic acid or sodium bisulphite) improve the IVPD for the cooked and uncooked seeds. Rom *et al* (1992) reported an improvement in protein digestibility of sorghum after sodium bisulphite treatment before cooking. An increase of 23.5% in faba bean *in vitro* digestibility of proteins after extrusion cooking was reported by Alosno *et al*. (2000).

2.5.3 Dehulling:

In many countries of the world grain legumes are initially

processed by hull (seed coat) removal and splitting (Siegel and Fawcett, 1976). Removal of the hull (dehulling) facilitates a reduction of fibre and tannin contents and improvement in appearance, texture, cooking quality, palatability and digestibility of the grain (Kon *et al*. 1973; Deshpande *et al*., 1982).

2.5.3.1 Effect of dehulling on phytic acid:

Sherma and Sehgal (1992b) reported that dehulling of faba bean reduces phytic acid by 4%. Alonso *et al* (2000) found that dehulling of faba bean and kidney beans increases the level of phytic acid by 9.68 and 1.89%, respectively. Carnovale *et al*. (1987) reported that dehulling concentrated the protein and phytic acid contents. Dehulling of beans significantly elevated the percentage of phytic acid compared to the whole beans. (Deshpande *et al.,* 1982).

2.5.3.2 Effect of dehulling on tannin content

The tannins in the grains are mostly located in the hull. Removing the hull removes both crude fibre and tannins (Bressani, 1999). Since tannins are mainly located in the testa, the physical removal of the testa (dehulling) significantly decrease the tannin concentration (Alonso *et al*, 2000). Sharma and Sehgal (1992a) reported that dehulling of two faba bean varieties, VH-131 and WF, reduced tannin content by 70 to 73% in the two varieties, respectively.

Deshpande *et al*. (1982) reported that tannin contents of whole and dehulled bean (*Phaseoulus vulgaris* L.) ranged from 33.7-282.8 and 10-28.7 mg catchin equivalents/100 g beans, respectively. They found that removal of seed coats lowered the tannin content of bean by 68-95%.

2.5.3.3 Effect dehulling on IVPD

Dehulling improves protein quality and digestibility, most likely due to the removal of the seed coat tannins and dietary fibres (Bressani *et al.*, 1984) which may contribute to decreased protein digestibility.

Alonso *et al* (2000) reported that, dehulling of *Vicia faba* and *Phaseolus vulgaris* increased the protein content and the *in vitro* protein digestibility. The IVPD increased by 2.4 and 5.14%, respectively after dehulling of both beans.

Dehulled beans (*Phaseoulus vulgaris* L.) were significantly higher in *in vitro* protein digestibility, compared to whole bean. The *in vitro* protein digestibility ranged from 69.4-73.1% for the whole and dehulled beans, respectively (Deshpande *et al*., 1982).

2.5.4 Germination

Germination has bean documented to be an effective treatment to remove anti-nutritional factors in legumes and mobilizing

secondary metabolic compounds, which are thought to function as reserve nutrients (e.g. phytate and raffinose oligosacharrides) (Vidal-Valverde *et al.*, 1994). Germination and fermentation increase vitamins and reduce flatulence factors and phytic acid Berssani, 1993).

2.5.4.1 Effect of germination on phytic acid content:

Germination is the most effective process for the reduction of phytic acid in legumes. These losses may be attributed to the activity of the enzyme phytase. Phytic acid serves as important reserve of phosphorous generated by the action of phytase during seed germination in developing seedling (Vidal-valverdel *et al*., 1994). Reddy *et al* (1978) noticed that phytic acid was hydrolyzed during germination resulting in an increase in available inorganic phosphorus.

Vadal-Valverde *et al*. (1994) reported that germination reduced phytic acid by 44% and 66% for two lentil varieties. The reduction was more than that of cooking and soaking. The reduction of phytic acid is also affected by the time taken in germination. Kataria *et al*. (1988) reported that germination for 24 hours of soaked blackgram seeds reduced phytic acid by 32% and after 60 hours of germination the reduction was 54%. Germination of mungbean seeds for 24 hours

and 60 hours reduced phytic acid by 27% and 38%, respectively (Kataria *et al*., 1989). Alonso *et al*. (2000) reported that germination of faba bean for 24 and 72 hours resulted in a reduction in phtyic acid by 53% and 60.8%, respectively.

2.5.4.2 Effect of germination on tannin content:

Germination and time of germination affect tannin content in legumes. In this regard, Alonso *et al* (2000) reported that 24 hours germination reduced tannin content of faba bean and *Phaseolus vulgaris* by 55.9% and 43.5%, respectively. The reduction reached 60.0% for faba bean and 71.6% for and *Phaseolus vulgaris* after 72 hours germination. Sharma and Sehgal (1992a) reported that 48 hours germination of two faba bean varieties (VH-131 and WF) reduced tannin content by 90 and 91%, respectively. This reduction was more than for 24 and 36 hours germination. Ibrahim *et al*. (2000) observed that cooking of pre-germinated cowpeas was most effective in removing anti-nutritional factors (tannin, phytic acid, protease inhibitors, olygosacharrides).

2.5.4.3 Effect of germination on IVPD:

Germination of faba bean and *Phaseolus vulgaris* for 24 hours increased IVPD by 3.1% and 7.8%, which increased to 10.3% and 14.5% when germinated for 72 hours for the two beans, respectively

(Alonso *et al*., 2000). Kataria *et al*. (1989) reported that IVPD of germinated mungbean was increased by 25% after 24 hours of germination which increased to 44% after 60 hours germination.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Samples:-

Four faba bean (*Vicia faba* L.) cultivars, were obtained from Shambat Research Station, namely S.M.L, SML 85/1/1, Shambat 616 and Shambat 00104 were used in this study.

Chemicals: -

Chemicals used in this study were of analytical grade.

3.2 Processing and cooking methods:

3.2.1 Dehulling:

Faba bean hulls were removed mechanically by laboratory dehuller,

3.2.2 Soaking: -

Seeds were soaked in distilled water for 12 h., 1, 2 and 3 days, in sodium bisulphite for 1, 2 and 3 days and in salt soaking solution (1.5% $NaHCO_3$, 0.5% Na_2CO_3 and 0.75% citric acid, for 12, 18 and 24 h. The proportion of seed to soaking medium was 1:3 (w/v). The unimbibed soaking solution was discarded, the soaked seeds were washed using distilled water and then dried in an oven at 70° C for 24 h.

3.2.3 Cooking:-

Dry seeds and 12 h soaked seeds were rinsed in distilled water and placed in round mouthed conical flasks fitted with a condenser. Distilled water was added to the samples at a ratio of 1:3 (w/v) for the soaked seeds and 1:7 (w/v) for the dry seeds and then boiled until cooked soft as felt between fingers. Cooked seeds were dried at 70° C for 24 h. (Kataria *et al*., 1989).The salt soaked seeds were cooked in a 500 ml beaker containing 200 ml boiling distilled water. Boilling was continued until the seeds were soft as felt between fingers. All cooked seeds were dried at 70° C for 24 h.

3.2.4 Germination:

The germination of faba bean seeds was carried out according to the method of Bhise *et al*. (1988) with some modification. The seeds were soaked in distilled water for 24 h. The soaked seeds were drained and the wet seeds were soaked in 0.2% formaldehyde (1:2 w/v) for 40 min to prevent mould growth during germination. The soaking solution was drained and the seeds were washed several times and soaked for 20 min in distilled water to remove the remaining formaldehyde. The wet seed were then spread on a wet sterilized filter paper and germinated at room temperature (32 ±2° C), were sprayed with distilled water several times daily to avoid drying. Portion were

taken from the second day of germination every other day to obtain 2, 4 and 6 days old germinated seeds which were air-dried. The root portions were removed manually.

All processed and raw seeds were milled using laboratory mill, sieved to pass 0.4 mm screen and stored at 4˚C for further analysis.

3.3 Methods of analysis: -

3.3.1 Proximate analysis:

3.3.1.1 Moisture content

Moisture content was determines according to the AOAC (1984) as follows:

2 g of sample were weighed using a sensitive balance in clean dry and pre-weighed crucible and then placed in an oven at 105° C and left over night. The crucible was transferred to a desicater and allowed to cool and then weighed. Further placements in the oven were carried out until approximately constant weight was obtained. Moisture content was calculated using the following formula:

$$MC\% = \frac{(W_2-W_1) - (W_3-W_1)}{(W_2-W_1)} \text{ X } 100$$

where,

MC = Moistute content

W_1 = Weight of empty crucible

W_2 = Weight of crucible with the sample

W_3 = Weight after drying

3.3.1.2 Ash Content:

Ash content of the sample was determined according to the method of AOAC (1984) as follows:

2 g of sample were placed in a clean dry preweighed crucible. Then the crucible with its content ignited in a muffle furnace at about 550°C for 3 h or more until light gray ash was obtained. The crucible was removed from the furnace to a desicator to cool and then weighed. The crucible was reignited in the furnace and allowed to cool until constant weight was obtained. Ash content was calculated using the following equation:

$$AC\% \quad \frac{W_2 - W_1}{W_s} X\ 100$$

Where,

AC = Ash content

W_1 = Weight of empty crucible

W_2 = Weight of crucible with ash

W_s = Weight of sample

3.3.1.3 Fat content:

Fat was determined according to the method of AOAC (1984) using Soxhelt apparatus as follows:

An empty clean and dry exhaustion flask was weighed. About 2 g of sample was weighed and placed in a lean extraction thimble and covered with cotton wool. The thimble was placed in an extractor. Extraction was carried out for 6-8 h with petroleum ether. The heat was regulated to obtain at least 15 siphoning per hour. The residual ether was dried by evaporation. The flak was placed in an oven at 105 °C till it dried completely and then cooled in a desiccator and weighed. The fat content was calculated using the following equation:

$$AC\% \quad \frac{W_2 - W_1}{W_s} X\ 100$$

Where,

W_1 = Weight of extraction flask

W_2 = Weight of extraction flask with fat

W_0 = Weight of sample

3.3.1.4 Crude fibre:

Crude fibre was determined according to AOAC (1990). 2 g of deflatted sample were treated successively with boiling solution of H_2SO_4 and KOH (0.26 N and 0.28 N, respectively). The residue was

then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105° C. for 18-24 h. The crucible with the sample was weighed and ashed a muffle furnace at 500°C and weighed. The crude fibre was calculated using the following equation.

$$CF\% \quad \frac{W_1 - W_2}{W_s} X\ 100$$

Where,

CF = Crude fibre

W_1 = weight of crucible before ashing

W_2 = " " " after ashing

W_0 = " " " sample

3.3.1.5 Crude protein

The crude protein was determined by using the micro-Kjeldahal method according to AOAC (1984) as follows:

a. Digestion:

0.2 g of the sample was weighed and placed in small digestion flask (50 ml). About 0.4 g catalyst mixture (96% anhydrous sodium sulphate and 3.5% copper sulphate) was added. 3.5 ml of approximately 98% v/w of H_2SO_4 was added. The contents of the flask were then heated on an electrical heater for 2 h or till the colour

changed to blue-green. The tubes were then removed from the digester and allowed to cool.

b. Distillation:

The digested sample was transferred to the distillation unit and 20 ml of 40% sodium hydroxide were added. The ammonia was received in 100 ml conical flask containing 10 ml of 2% boric acid plus 3-4 drops of methyl-red indicator. The distillation was continued until the volume reached 50 ml.

c- Titration:

The content of the flask were titrated against 0.02 N HCl. The titration reading was recorded.

The crude protein was calculated using the following equation:

$$CP\% = \frac{(T-B) \times N \times 14 \times 100 \times 6.25}{W_s \times 1000}$$

Where,

CP = crude protein

T = titration reading

B = blank titration reading

N = HCl normality

W_s = sample weight

1000 : to convert to mg.

3.3.1.6 Carbohydrates:

Carbohydrates were determined by difference according to the following equation:

Carbohydrate = 100 - (MC+Ash content + Fat content + Fibre content + Protein content)

3.3.1.7 Phytic acid:

The phytic acid content was determined by the method described by Wheeler and Ferrel (1971). Two g of milled sample were weighed in 125 ml conical flask. The sample was extracted with 50 ml of 3% trichloroacetic acid (TCA) for 3 h with mechanical shaker. The suspension was centrifuged for 5 minutes and 10 ml aliquot of the supernatant was transferred to 40 ml tube. 4 ml of $FeCl_3$ solution (made to contain 2 mg ferric ion per ml in 3% TCA) were added to the aliquot. The tube was heated in boiling water bath for 45 min. One or two drops of 3% sodium sulphate in 3% TCA were added. The tube was cooled and centrifuged for 10-15 min. The clear supernatant was decanted, the precipitate was washed twice by dispersing well in 25 ml 3% TCA, heated for 10-15 min in a boiling water bath, then centrifuged again. Washing was repeated once more with water, the washed precipitate was dispersed in a few ml of water and 3 ml of 1.5 N NaOH were added and the volume completed to approximately 30

ml with water. Then the tube was heated in a boiling water both for 30 min. and hot filtered using Whatsman No. 2: the precipitate was washed with 60-70 ml hot water and the washings were decanted. The precipitate was disolved from the filter paper with 40 ml hot 3.2 N HNO_3 into 100 ml volumetric flask.

The paper was washed with hot water, the washing was collected in the same flask, then completed to volume. 0.5 ml aliquot was taken from the above solution and transferred into 10 ml volumetric flask, then 2 ml 1.5N KSCN was added and completed to volume by water, then immediately read at 480nm using a spectrophotometer within one min.

Calculation

A standard curve of different Fe $(NO_3)_3$ concentrations was plotted to calculate the ferric ion concentration. The phytate phosphorous was calculated from the ferric ion concentration assuming 4.6 iron : phosphorous molar ratio.

$$\text{The Phytate} = 6/4 \times \frac{A \times C \times 20 \times 10 \times 50 \times 100}{1000 \times 2} \text{ mg/100g}$$

where,

A = optical density, C = concentration corresponding to the optical

density.

3.3.1.8 Tannin content:

Quantitative estimation of tannin was carried out using the modified vanillin - HCl method of Price *et al*. (1978).

The vanallin - HCI reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% vanallin in methanol. The two solutions of the reagents were mixed just prior to use. It was discarded if a trace of colour appeared.

Catechin was was used to prepare the standard curve. This was done by adding 600 mg of catechin to 100 ml of 1% HC1 in methanol. From this stock solution various dilutions were prepared . Five ml of vanallin-HCl reagent were added to 1 ml of each dilution. The absorbance was read using a spectrophotometer at 500 nm after 20 min. from addition of the reagent at 30°C. The absorbance was plotted against catechin concentration.

0.2 g of ground sample were placed in a test tube. Then 10 ml of 1% concentrated HCl in methanol were added. The test tube was capped and continuously shaken for 20 min. One ml of supernatant after centrifugation was pipetted into each of the tubes and then proceeding as was described in the standard curve above.

For zero setting before absorbance was read, 1 ml of blank

solution (1% HCl in methanol) was mixed with 5 ml 4% concentrated HCl and 5 ml of vanillin-HCl reagent in a test tube and incubated for 20 min at 30°C. Absorbance at 500 nm was read on a spectrophotometer and the concentration of the condensed tannin was determined from the standard curve. Tannin concentration was expressed as catechin equivalent as follows:

$$\text{tannin\%} = \frac{\text{Cx 10 x 100}}{200}$$

Where:

C = Concentration corresponding to the optical density

10 = Volume of extract (ml).

200 = Sample weight (mg).

3.3.1.9 *In vitro* Protein Digestibility:

Determination of in vitro protein digestibility was carried out by the method of Maliwal (1983) with modification by Monjula *et al*. (1991).

A known weight of the sample containing 16 mg nitrogen was taken in triplicate and hydrolyzed with 1 mg pepsin in 15 ml of 0.1 M HCl at 37°C for 18 h. The reaction was terminated by addition of 15 ml of 10% w/v trichloroacetic acid (TCA).

The mixture was filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method. Digestibility was calculated using the following formula:

$$\text{Protein digestibility \%} = \frac{\text{N in supernatant - N in blank x 100}}{\text{N in sample}}$$

N in blank = N in pepsin enzyme and reagents

3.4 Statistical analysis

Analysis of variance was used to analyze the data (Snedecor and Cochran, 1987) and Duncan Multiple Range Test was used for mean separation (Duncan, 1955).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical composition of faba bean:

The chemical composition of faba bean seeds is shown in Table 1. Values were expressed on dry matter basis (DMB).

4.1.1 Moisture content:

The moisture content of the four faba bean cultivars were 7.65% for SML, 7.72% for SML 85/1/1, 7.60% for Shambat 616 and 7.68% for Shambat 00104. There was no significant difference between the four cultivars in moisture content.

Values obtained in this study were in agreement with values obtained by Elsayed (1994) and in close agreement with those reported by Elsheikh *et al*. (1999) who reported a range of 6.34-7.62%. These values disagree with those obtained by Ali *et al*. (1982) who reported a range of 8.3-8.7% for moisture cntent of faba bean and also disagree with the 6-6.7% range obtained by EL Tinay *et al.* (1989).

4.1.2 Fat content:

The fat content of faba bean ranged from 1.2 to 1.72%. There were no significant differences between the four cultivars in fat content.

Table 1

The results obtained in the study were in agreement with those obtained by El Tinay *et al* (1989) who reported a range of 1.1-2.7% and Elsheikh *et al.* (1999) who reported a range of 1.08-2.12% for fat content but disagree with theose obtaibed by Ali *et al*. (1982) who reported a range of 0.7-1.36%.

4.1.2 Fibre content:

The fibre content of SML 85/1/1 was 8.0% and for Shambat 00104 was 8.08%. There was no significant ($P \leq 0.05$) difference between those two cultivars but were significantly different ($P \leq 0.05$) from values obtained for SML and Shambat 616 which were 6.27% and 5.19, respectively.

Values obtained in this study were in agreement with that reported by Elsayed (1994) and in close agreement of those obtained by Ali *et al.* (1982) who reported a range of 5.1-7.3% of fiber content in faba bean.

4.1.4 Ash content:

The ash content of the four faba bean cultivars studied ranged from 3.07 to 3.6%. There were no significant differences between cultivars in ash content.

These results were in agreement with those obtained by Elsheikh *et al.* (1999) who reported a range of 3.03-3.64% for ash content; they were slightly higher than 2.6-3.03% reported by Ali *et al*. (1982).

4.1.5 Protein content:

The protein content ranged from 30.89% for cultivars SML 85/1/1 to 35.14% for cultivar Shambat 616. There were significant ($P \leq 0.05$) differences between the four cultivars. These results were in agreement with those obtained by Elsayed (1994) who reported a range of 28.0% to 37.8% and Elsheikh *et al*. (1999) who obtained a range of 31-39% for crude protein in faba bean. They also agree with the range obtained by Chaven *et al.* (1989) who reported 20-41% protein content for faba bean.

Protein content is influenced by both genetic and environmental factors (Bond *et al*., 1985).

4.1.6 Carbohydrate content:-

Carbohydrate content for SML and SML 85/1/1 was 47.1 and 48.12, respectively. There were no significant ($P \leq 0.05$) differences between these cultivars, but were significantly different from Shambat 616 and Shambat 00104 which were respectively 46.8% and 48.83%.

Values obtained in this study were in agreement with that obtained by Elsheikh *et al*. (1999) and were less than the values obtained by El Tinay *et al*. (1989) who reported a range of 52.3% to 54.8% for carbohydrate in faba bean.

4.2 Aninutritional factors:

4.2.1 Tannin contents:-

Tannin content of faba bean seeds are shown Table 2. The results were

expressed on dry matter basis (DMB).

Shambat 00104 had the highest value with 0.0777%m SML had 0.0553%, while SML 85/1/1 and Shambat 616 had 0.0380 and 0.0443%, respectively. There were no significant differences between these two cultivars, but they were significantly ($P \leq 0.05$) different from the other two cultivars.

Values obtained in this study were in agreement with the values obtained by Helesper *et al.* (1992) and were in close agreement with those obtained by Babiker and El Tinay (1993).

These results disagree with those obtained by Esheikh *et al* (2000) who reported a range of 0.26%-0.46% and also disagree with that obtained by Alnoso *et al*. (2000) who reported a value of 0.192% tannin in faba bean.

4.2.2 Phytic acid:

The phytic acid content of faba bean seeds is listed in Table 2. Values were expressed as on dry matter basis (DMB).

Table 2

Phytic acid was 247.07 mg/100g for SML 85/1/1, 255.20 mg/100g for Shambat 616, 257.75 mg/100g for Shambat 00104 and 259.21 mg/100g for SML. There were no significant differences between SML, Shambat 616 and Shambat 00104 cultivars. However, there was a significant ($P \leq 0.05$) difference between SML 85/1/1 and Shambat 00104 and between SML and SML 85/1/1.

These values were similar to that btained by Elsheikh *et al*. (2000) but were less than those reported by El Tinay *et al.* (1989) and Carnovale *et al.* (1987), who reported a range of 1400-1500 mg/100g and 710-1150 mg/100g, respectively. They were lower than the value given by Alsono *et al* (2000) who reported 2170 mg/100g DM for phytic acid in faba bean.

4.2.3 *In vitro* protein digestibility (IVPD):-

The *in vitro* protein digestibility of faba bean seed is listed in Table 2.

The IVPD for the four cultivars in the range of 68.62%-75.09% SML 85/1/1 showed the highest value, while Shambat 00104 showed the lowest value. There were no significant ($P \leq 0.05$) differences between SML, Shambat 616 and Shambat 00104 in IVPD but were significantly ($P \leq 0.05$) different from SML 85/1/1.

The IVPD results obtained in this study were in agreement with the range given by Elsheikh *et al.* (2000) and are in agreement with those obtained

by Alsonso et *al*. (2000) who gave a value of 70.8% for IVPD for faba bean but were less than those obtained by Babikir and El Tinay (1993) who reported a range of 80.7%-81.5% for IVPD in faba bean.

4.3 Effect of processing on the antinutrional factors and IVPD.

4.3.1 Soaking

4.3.1.1 Effect of soaking in distilled water on tannin content:

Tannins expressed as percentages of dry bean weight in raw and soaked faba bean cltivars are shown in Table 3. Significant ($P \leq 0.05$) reduction was noted with increasing soaking time. Reduction of SML cultivar was 49.40% after one day soaking and 57.1% after two and three days of soaking. For cultivar SML 85/1/1, 29.0% reduction was observed after one day soaking, 27.1% and 41.3% reduction was noted after 2 and 3 days, respectively. Tannins of Shambat 616 cultivars decreased by 46.5%, 56.4% and 66.1% after 1, 2 and 3 days soaking, respectively. Shambat 00104 cultivar showed the highest reduction in tannin conent due to soaking. Its tannin content reduced by 55.0%, 61.4% and 74.7% after 1, 2 and 3 days soaking, respectively.

Alonoso

Table 3

et al. (2000) reported that tannin contents in faba bean seeds was reduced by 47.7% after 12 hours soaking in doubl-deioized water. Sharma and Sehgal (1992a) observed that soaking of faba bean cultivars VH131 and WF for 12 h in distilled water reduced tannin content by 42% and 51%, for both cultivars, respectively. Vidal-Valverade *et al*. (1994) reported an increase in tannin content after soaking in distilled water, 0.1% citric acid and 0.07% sodium bicarbonate.

Soaking of blackgram and mungbean in plain water for 8 hours reduced polyphenols content by 10% and 7%, respectively (Kataria *et al.* (1988, 1989).

The loss of tannin content after soaking may be attributed to leaching out into soaking water under the concentration gradient (Kataria *et al*., 1988, 1989).

4.3.1.2 Effect of soaking in distilled water on phytic acid:-

The result of phytic acid content after soaking are shown in Table 4. Values were expressed as mg/100g dry matter.

Soaking for 1, 2 and 3 days significantly ($P \leq 0.05$) reduced phytic acid content in the four faba bean cultivars. The longer the time of soaking, the greater is the loss of phytic acid.

Table 4

Phytic acid of SML cultivar was reduced by 5.9%, 8.3% and 15.17% after 1, 2 and 3 days, respectively. For SML 85/1/1 phytic acid was reduced by 12% after one day and by 14.5% and 17.4%, respectively for 2 and 3 days. Shamabt 616 and Shambat 00104 showed a reduction of 6.2% and 10.48%, respectively after one day soaking; after 2 and 3 day, Shambat 616 lost about 15.6 and 20.8%, respectively from the initial amount in the raw seeds. Shambat 00104 showed a pronounced reduction in phytic acid after 2 to 3 day soaking. Its phytic acid content was reduced by 20.4% and 29.9% after 2 and 3 days soaking, respectively.

Reduction in phytic acid after soaking was observed by Vidal-Valverade *et al*. (1994) who reported that soaking of lentils in distilled water, 0.07% sodium bicarbonate and 0.1% citric acid reduced phytic acid by 27%, 23% and 37%, respectively. Sharma and Sehgal (1992b) reported a 4% reduction of phytic acid after soaking faba bean seeds for 12 hours. Ologhbo and Fetuga (1984) reported 14.4 to 28% reduction in phytic acid after soaking of 10 cowpea varieties for three days.

The decrease in phytate content of the legume during soaking may be attributed to leaching out in the soaking water (Kataria et al., 1989), 1988). Beleia et al. (1993) attributed the decrease in phytic acid content during soaking and germination to the leaching out effect during hydration.

4.3.1.3 Effect of soaking in distilled on IVPD:-

Effect of soaking treatment on IVPD was shown in Table 5. Values were expressed on dry matter basis.

The IVPD is significantly ($P \leq 0.05$) increased after soaking. For SML the IVPD increased by 4.9% after 1 day soaking and increased up to 20.9% after 2 days soaking and 9% after 3 days soaking. The IVPD of the variety SML 85/1/1 increased by 3.5%, 4.6% and 7.3% after 1,2 and 3 days soaking. Shambat 616 showed an increase of 6.2% in IVPD after one day soaking, 16.6% after 2 days soaking and 13.9% after 3 days soaking. For cultivar Shambat 00104 the IVPD increased by 6.9%, 17.6% and 8.3% after 1, 2 and 3 days soaking, respectively. It is clear that the two days soaking is more effective in increasing the IVPD than one and 3 days.

The increment in the IVPD after soaking was reported by Alonso *et al.* (2000) and by Kataria *et al*. (1989), who reported an increase in IVPD by 4,15, and 21% after 6.12 and 18 h of soaking of mung beans.

Table 5

4.3.2 Cooking:

4.3.2.1 Effect of soaking and/or cooking on tannin content:

Table 6 shows the effect of cooking treatment on tannin content of faba bean cultivars. There was a significant ($P \leq 0.05$) reduction in tannin content after cooking of soaked and raw seeds. For the raw seed the reduction was 36% for SML 85/1/1, 58% for SML, 69% for Shambat 616 and 78% for Shambat 00104. When seeds presoaked in distilled water for 12 h. cooking reduced their tannins by 37.6% for SML 85/1/1, 63.8% for SML, 88.9 % for Shambat 616 and by 78% for Shambat 00104. On the other hand, when seeds were soaked in salt solution (Containing 1.5% $NaHCO_3$, 0.5% Na_2CO_3 and 0.75% citric acid) for 12, 18 and 24 h. before cooking, SML tannins reduced by 52, 65, and 66%, respectively. The reduction for SML 85/1/1 was 49, 58 and 67% after 12, 18 and 24 h soaking. Shambat 616 showed a reduction of 64, 69 and 80% in tannins after 12, 18 and 24 h. soaking, respectively. Shambat 00104 showed a reduction in tannins by 85.5% after 12 h soaking, 94% after 18 h soaking and 95% after 24 h. soaking in salt solution before cooking.

The presoaking in salt solution before cooking was more effective in reducing tannins compared to distilled water.

Table 6

Loss of tannin after cooking was reported by Elsheikh *et al*. (2000), Sharma and Sehgal (1992a) and Zeina *et al.* (1991).

The loss of tannins after cooking was attributed to decomposition during cooking and leaching out to the cooking liquor (Zeina *et al*., 1991). Heat may destroy polyphenols (Kataria *et al*., 1989). Higher reduction in tannins in the presoaked cooked seeds may be due to the fact that soaking of seeds removes a significant amount of tannins leaving small amount when the soaked seeds were cooked (Kalataria *et al*., 1989).

Babikir and El Tinay (1993) reported a reduction in tannin content when faba bean seeds were soaked in either distilled water or Na_2CO_3.

4.3.2.2 Effect of soaking and/or cooking on phytic acid content:-

Results shown in Table 7 revealed that cooking without soaking significantly ($P \leq 0.05$) reduced phytic acid. The reduction was 16 and 16.8% for SML and Shambat 616 cultivars, respectively, while it was 18.8 for cultivar SML 85/1/1 and Shambat 00104. Cooking after soaking in distilled water caused a remarkable reduction in phytic acid. Cooking after soaking in distilled water reduced phytic acid by

Table 7

19.5, 22, 29 and 30% fo cultivar SML, Shambat 616, Shambat 00104 and SML 85/1/1, respectively. Cooking after soaking in salt solution reduced phytic acid by 7.4, 9.7 and 14.4% for cultivar SML when seeds were soaked before cooking for 12, 18 and 24 h, respectively. Cultivar SML 85/1/1 showed 8.4, 12.2 and 16% reduction in phytic acid when soaked for 12, 18 and 24 h, respectively before cooking. Shambat 616 and Shambat 00104 showed 17.5%, 21%, 17% and 21, 21.3, 25.8% reduction in phytic acid when soaked for 12, 18 and 24 h. before cooking.

These results revealed that soaking seeds in distilled water is more effective in reducing phytic acid than cooking without soaking or cooking after soaking in the salt solution. Similar results were reported by Vidal-Valerde *et al*. (1994) who observed that cooking after soaking lentils in distilled water reduced phytic acid by 39% while soaking in $NaHCO_3$ before cooking reduced phytic acid by 29% and soaking in citric acid before cooking reduced it by 32%.

Kataria *et al*. (1989) reported 20% reduction in phytic acid after soaking in distilled water and cooking of mungbean compared to 15% for the unsoaked cooked seeds.

Pressure cooking of soaked seeds of blakgram reduced phytic acid by 33% while that of unsoaked seeds was 8% (Kataria *et al*., 1988).

Decrease in phytic acid content of the lequme seeds during cooking may be attributed to the formation of insoluble complexes between phytate and other components (Kumar *et al*. 1978).

4.3.2.3 Effect of cooking after soaking in distilled water or salt solution on IVPD:

The results reveal that cooking significantly ($P \leq 0.05$) reduced the IVD (Table 8). When soaked seeds were compared with the unsoaked ones, the IVPD increased by 5.9% for SML 85/1/1, 6.5 for SML, 7.7% for cultivar Shambat 00104 and 7.9% for Shambat 616 when seeds were soaked in distilled water before cooking. For seeds soaked in salt solution, the IVPD improved by 8.2%, 14% and 16% for cultivars SML after, 12, 18 and 24 h soaking, respectively. For cultivar SML 85/1/1, it increased by 11.6%, 12% and 16.8% after 12, 18 an s24 h of soaking, respectively. For Shambat 616 and Shambat 00104 there was no improvement after 12 h. soaking. However, the IVPD for Shambat 616 increased by 5.9% and 10.3% after 18 and 24 h. of soaking, respectively. For Shambat 00104 it increased by 1.7%

Table 8

and 12.7% after 18 h. and 24 h. of soaking, respectively. Improvement of IVPD in seeds cooked after soaking in distilled water was reported by Kataria *et al*. (1989).

The negative effect of cooking on the IVPD could be due to the formation of disulphide bond in the protein (Oria *et al.* (1995) together with an increase in fibre contents. The IVPD presoaked cooked seeds could be due to the great reduction in tannin content and for further reduction in phytic acid.

4.3.3 Dehulling:

4.3.3.1 Effect of dehlling on tannin contents:

Table 9 shows tannin content of whole and dehuuled seed of faba bean cultivars.

Dehulling significantly ($P \leq 0.05$) reduced tannin content. For cultivar SML tannins were reduced by 54%. The reduction was 35%,
43% and 59% for cultivars Shambat 616, SML 85/1/1 and Shambat 00104, respectively.

The decrease in tannins after dehulling in this study is less than that observed by Alonso *et al* (2000) and Sharma and Sehgal (1992a).

Table 9

Since most tannins are located in the testa, physical removal of the testa reduced tannin content.

4.3.3.2 Effect of dehlling on phytic acid content:

Phytc acid content of whole and dehulled seeds is shown in Table 10.

Phytic acid increased by 4.7%, 5.8%, 6.6% and 7.7% for cultivar SML 85/1/1, Shambat 00104, SML and Shambat 616, respectively. Similar results were reported by Alonso *et al*. (2000) Cavnovule *et al*. (1988) and Deshpande *et al* (1982).

Increasing in phytic acid content after dehulling is due to the fact that phytates are mainly located in the coytledon (Alnoso *et al*., 200). Carnovale *et al*. (1988) reported that dehulling concentrated the protein and phytic acid content.

4.3.3.3 Effect of dehulling on IVPD:

Table 11 shows in vitro protein digestibility of whole and dehulled Faba bean seeds.

Dehulling significantly ($P \leq 0.05$) increased the IVPD in faba bean. IVPD was increased by 17.4% for cultivar Shambat 616, 18% for SML and SML 85/1/1 and 22% for cultivar Shambat 00104

Table 10

Table 11

. Similar results were reported by Alonso *et al.* (2000) for *Vicia faba* and *Phaseolus vulgaris*, in which IVPD increased by 2.4 and 5.14%, respectively after dehulling.

The improvement of IVPD after dehulling may be attributed to the removal of tannins and fibre content which are mainly located in the seed coot. (Alonso *et al*., 2000; Bressani,1984).

4.3.4 Germination:

4.3.4.1 Effect of germination on tannin content:

Table 12 shows the effect of germination on tannin contents of faba bean seeds for 2, 4 and 6 days significantly ($P \leq 0.05$) reduced the tannin content. The reduction was 47, 51 and 60.3% after 2, 4 and 6 days of germination for SML cultivar. For SML 85/1/1 the reduction was 28.7, 33 and 50% after 2, 4 and 6 days of germination, respectively. For Shambat 616 the reduction was 24, 50 and 59% after 2, 4 and 6 days of germination, respectively. Shambat 0010 shows the highest levels of reduction after germination; its tannin content was reduced by 66.9, 75 and 78% after 2, 4 and 6 days, repectively.

These results are in agreement with those obtained by Alonso *et al*. (2000) who reported reduction of tannin in faba bean and *Phaseolus vulgaris* after germination. Sharma and Sehgal (1992a)

Table 12

observed that, 24 h germination of two faba bean cultivars, VH131 and WF, reduced tannin contents by 90 and 91%, respectively.

Reduction in tannin content after germination may be attributed to the leaching out effect during hydration (Beleia *et al*, 1993).Rao and Deosthale (1982) reported that the activation of polyphenolase may reduce polyphenols during germination of food grain legumes.

4.3.4.2 Effect of germination on phytic acid content:

The effect of germination on phytic acid is shown in Table 13. Germination significantly reduced phytic acid content of faba bean. The two days germination reduced phytic acid content by 9% for Shambat 616, 10% for SML 85/1/1 and 11% for SML and Shambat 616 and Shambat 00104, respectively. After 4 days of germination, Shambat 616 lost about 17.7% of its phytic acid content. The loss was 21%, 22.8% and 27% for SML 85/1/1. SML and Shambat 00104, respectively. phytic acid content was greatly reduced after the 6^{th} day of germination:32% for SML, 34% for SML 85/1/1 and Shambat 616, and 34.5 for Shambat 00104.

A decrease in phytic acid content after germination for lentils was reported by Vidal-Vulerede *et al*. (1994) for faba bean by Alonso *et al*. (2000) for blackgram and mungbean by Kataria *et al*. (1988, 1989).

Table 13

Loss of phytic acid during germination could be attributed to leaching out effect during hydration (Beleia *et al*. 1993). And to phytase activity in the germinating legume seeds as reported for faba

bean by Michael Eskin and Wiebe (1983).

4.3.4.3 Effect of germination on IVPD:

Table 14 shows the effect of germination on the IVPD of faba bean. The IVPD was significantly ($P \leq 0.05$) increased by germination: 2, 4 and 6 days germination increased the IVPD of SML cultivar by 16, 26 and 29%, respetively. For cultivar SML 85/1/1 it respectively increased by 20.8, 26 and 29.7% after 2, 4 and 6 days of germination. The IVPD of Shambat 616 was increased by 12.7, 26.6 and 34.6%, while for Shambat 00104 the IVPD was increased by 14, 21 and 27.6%. Similar results were reported for faba bean and *Pgaseolus vulgaris* by Alonso *et al*. (2000) and for mungbean by Kataria *et al* (1989). Improvement in IVPD after processing could be attributed to the reduction of anti-nutrients. Germination is the most effective process in reducing phytic acid and improving the IVPD.

Table 14

4.4 Effect of treatment with sodium bisulphite on IVPD:

4.4.1 Effect of soaking in sodium bisulphate in varying concentration on IVPD:

Table 15 shows the effect of soaking in sodium bisulphate in varying concentration on IVPD of cooked and uncooked faba bean seeds. This experiment was conducted on three cultivars; SML, Shambat 616 and Shambat 00104. For SML cultivar, the IVPD for untreated seeds was 69.78, which increased to 84% after soaking in 0.1 M $NaHSO_3$ and to 81% after soaking in 0.25 M and 0.5 M $NaHSO_3$. There were no significant ($P \leq 0.05$) differences in IVPD after soaking in 0.25 M sodium bisulphate. Cooked SML sample treated with 0.1 M sodium bisulphate showed an improvement in IVPD by 31.6 compared to cooked untreated sample. Treatment with 0.25 M $NaHSO_3$ increased the IVPD by 31% while increment with 0.5 μ $NaHSO_3$ increased it by 24.8%.

For Shambat 616 the IVPD of uncooked untreated sample was 70.87% which increased to 80.3%, 77% and 75.4% when soaked in 0.1 M, 0.25 M and 0.5 M sodium bisulphite, respectively. Soaking in 0.1M $NaHSO_3$ showed pronounced increase in IVPD compared to

Table 15

0.25M and 0.5M $NaHSO_3$. For cooked samples treated (0.1M $NaHSO_3$), the IVPD increased by 20.6% over the cooked untreated seeds and by 16% after treatment with 0.25 M and 0.5 M NaHSO3. For Shambat 00104 the IVPD of the uncooked untreated sample was 69.4% which increased to 85% after treatment with 0.1M sodium bisulphite and to 82.4% and to 78.6% after soaking in 0.25 and 0.5 M sodium bisulphite, respectively., 0.1 M treatment gave the highest increment (22.5%) in IVPD over 0.25 and 0.5 M, respectively. For cooked treated samples, treatement with 0.1 sodium bisulphite increased the IVPD by 29% compared to untreated cooked samples. O.25 M and 0.5 M increased the IVPD by 27.8% and 23%, respectively.

Treatment with 0.1 M sodium bisulphite is more effective in improving the IVPD of both the cooked and uncooked samples compared to 0.25 and 0.5 M. This observation is in agreement with that obtained by Arbab abd El Tinay (1996) using sorghum grain. Improvement in IVPD in sorghum grain after treatment with sodium bisulphite was also reported by Humaker *et al*. (1987) and Rom *et al*. (1992).

4.4.2 Effect of soaking in sodium bisulphite in varying durations on IVPD:

Table 16 shows the effect of soaking in sodium bisulphite for 1, 2 and 3

days and cooking on the IVPD.

For the uncooked soaked seeds, the IVPD is significantly increased after one day soaking. For cultivar SML, the IVPD increased by 20.8% after one day soaking and it increased by 14% and 18% after 2, 3 soaking, respectively. For cultivar Shambat 616, 1 day soaking of uncooked sample improved the IVPD by 12%; there was no significant ($P \leq 0.05$) differences between 2 and 3 days soaking, which gave 8.5% and 7.7% increment, respectively. Cultivar Shambat 00104 showed an increase of 22% in the IVPD after one day soaking which dropped to 18% and 14% after 2 and 3 days soaking.

For cooked soaked seeds, the IVPD of all three cultivars is significantly ($P \leq 0.05$) higher after soaking seeds in sodium bisulphite before cooking compared to unsoaked cooked seeds. For cultivar SML, the IVPD increased by 26% over the control after one day soaking, which increased to 28% nd 33.7% after2, 3 days soaking, respectively. For cultivar Shambat 616 the IVPD increased by 10.6

Table 16

after one day soaking and improved by 14% after 2 and 3 day soaking showing 14% increasing in IVPD. For Shambat 00104, the one day soaked cooked seeds showed the highest value for IVPD compared to 2 and 3 days, its IVPD increased by 25% after 2 and 3 day soaking.

Results reveal that one day soaking in 0.1 M concentration is more effective in improving the IVPD of the uncooked seeds compared to 2 and 3 days soaking. For the cooked seeds the 3 day soaking is more effective in improving IVPD.

CONCLUSIONS AND RECOMMENDATIONS

Results of this study indicated that the four Faba bean cultivars did not show variations in moisture, fat or ash contents. Shambat 616 had the highest value for protein. Cultivar SML 85/1/1 had the highest value for carbohydrate and IVPD. The high IVPD of cultivar SML 85/11 could be attributed to its low tannin and phytic acid contents.

Phytic acid was reduced by soaking, cooking of dry and soaked seeds as well as germination. The longer the time of soaking and germination, the greater the reduction of phytic acid. Germination is most effective in reducing phytic acid. All treatments reduced tannin contents, however, soaking in salt solution was more effective in removing tannins. The IVPD increased after all treatments with germination being the most effective.

Cooking resulted in significant reduction in IVPD of faba bean. The deleterious effect of cooking was minimized by soaking seeds in salt solutions.

Recommendations:

1- Germination and dehulling treatments could be applied to make highly nutritious meals (high protein) out of faba bean.

2 Salt treatments before cooking of faba bean could be used at household and industrial levels to produce good quality meals and to minimize fuel

consumption.

3- Further work should be designed to optimize conditions for processing treatments so as to improve the nutritive value and maintain nutritional composition at its maximum level.

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Table 1. Proximate composition of faba bean cultivars

Cultivars	**Moisture content (%)**	**Fat (%)**	**Fibre (%)**	**Protein (%)**	**As**
SML	7.65[a](±0.05)	1.20[a](±0.09)	6.27[b](±0.08)	33.92[b](±0.05)	3.0
SML 85/1/1	7.75[a] (±0.10)	1.72[a] (±0.50)	8.00[a](±0.15)	30.89[d](±0.15)	3.5
Shambat 616	7.60[a](±0.10)	1.40[a] (±0.25)	5.17[c](±0.12)	35.14[a](±0.57)	3.3
Shambat 00104	7.68[a](±0.27)	1.52[a] (0.13)	8.08[a] (±0.08)	32.28[c] (±0.63)	3.6

Values are means (±SD)
Means with the same letter (s) within a column are not significantly different using Duncan Multiple Range Test at 0.05

Table 2. IVPD, tannin and phytic acid content for faba bean cultivars

Cultivars	**IVPD (%)**	**Tannin (%)**
SML	69.78^{b}(±0.92)	0.0553^{b} (±0.0085)
SML 85/1/1	75.09^{a} (±0.87)	0.0380^{c} (±0.0020)
Shambat 616	70.85^{b}(±1.53)	0.0443^{c} (±0.0012)
Shambat 00104	68.62^{b} (±1.67)	0.0777^{a} (0.0035)

Values are means (±SD)
Means with the same letter (s) within a column are not significantly different using Duncan Multiple Range
Test at 0.05 level.

Table 3. Effect of soaking in distilled water on tannin content (%) of faba bean cultivars

Soaking treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sha**
Unsoaked	0.0553[a] (±0.0085)	0.0380[a] (±0.0020)	0.0443[a] (±0.0012)	0.077
1-day soaking	0.0280[b] (±0.0046)	0.0270[b] (±0.0020)	0.0237[b] (±0.0029)	0.035
2-day soaking	0.0237[b] (±0.0075)	0.0277[b] (±0.0030)	0.0193[c] (±0.0012)	0.030
3-day soaking	0.0237[b] (±0.0015)	0.0223[c] (±0.0025)	0.0150[d] (±0.0017)	0.019
Overall cultivars means	0.0327[b] (±0.0015)	0.0288[c] (±0.0063)	0.0256[c] (±0.0119)	0.040

Values are means (±SD)

Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 4 Effect of soaking in distilled water on phytic acid content (mg/100 g) of faba bean cultivars

Soaking treatment	Cultivar			
	SML	**ML85/1/1**	**Shambat616**	**Sha**
Unsoaked	259.21[a] (±3.20)	247.07[a] (±6.32)	255.26[a] (±3.87)	257.
1-day soaking	243.95[b] (±2.25)	217.36[b] (±2.53)	239.53[b] (±3.86)	230.
2-day soaking	237.87[c] (±1.25)	211.35[bc] (±3.86)	215.45[c] (±8.01)	205.
3-day soaking	219.89[d] (±1.45)	204.05[c] (±10.37)	202.23[d] (±2.93)	180.
Overall cultivars means	240.24[a] (±14.83)	219.96[c] (±17.95)	228.12[b] (±30.34)	218.

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 5. Effect of soaking in distilled water on *in vitro* protein digestibility (IVPD) of faba bean cultivars

Soaking treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Shamb**
Unsoaked	69.78^{d} (±0.92)	75.09^{c} (±0.87)	70.85^{c} (±1.53)	68.62^{c} (±
1-day soaking	73.23^{c} (±0.47)	77.68^{b} (±0.83)	75.22^{b} (±1.70)	73.32^{b} (±
2-day soaking	84.38^{a} (±1.04)	78.53^{b} (±0.31)	82.62^{a} (±2.69)	80.72^{a} (±
3-day soaking	76.06^{b} (±1.62)	80.58^{a} (±1.58)	80.70^{a} (±0.96)	74.22^{b} (±
Cultivars means	75.86^{b} (±5.71)	77.97^{a} (±2.27)	77.35^{a} (±5.08)	75.22^{b} (±

Values are means (±SD)

Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 6. Effect of cooking after soaking in distilled water and in salt* solution on tannin content (%) of faba bean cultivars

Cooking treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**S**
Uncooked unsoaked	0.0553^{a} (±0.0085)	0.0380^{a} (±0.0050)	0.0443^{a} (±0.0012)	0
Cooking without soaking	0.0230^{b} (±0.0046)	0.0243^{b} (±0.0045)	0.0137^{b} (±0.0040)	0
Cooking after soaking in distilled water	0.0200^{b} (±0.0017)	0.0237^{b} (±0.0050)	0.0047^{c} (±0.0006)	0
Cooking after soaking for 12 hours in salt	0.0263^{b} (±0.0012)	0.0193^{bc} (±0.0012)	0.0160^{b} (±0.0036)	0
Cooking after soaking for 18 hours in salt	0.0197^{b} (±0.0021)	0.0160^{cd} (±0.0020)	0.0137^{b} (±0.0045)	0
Cooking after soaking for 24 hours in salt	0.0187^{b} (±0.0006)	0.0126^{d} (±0.0035)	0.0090^{bc} (±0.0056)	0
Overall cultivars means	0.0272^{a} (±0.0137)	0.0223^{b} (±0.0088)	0.00169^{c} (±0.0136)	0

Values are means (±SD)

* salt solution is 1.5% $NaHCO_3$ + 0.5% Na_2CO_3 + 0.75% citric acid

Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 7. Effect of cooking after soaking in distilled water and in salt* solution on phytic acid (mg/100g) of faba bean cultivars

Cooking treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	
Uncooked unsoaked	259.21^{a} (±3.20)	247.07^{a} (±6.32)	255.26^{a} (±3.87)	2
Cooking without soaking	217.83^{d} (±2.53)	200.68^{c} (±8.69)	212.31^{b} (±2.53)	2
Cooking after soaking in distilled water	208.77^{d} (±3.87)	172.90^{d} (±16.43)	199.26^{c} (±3.87)	1
Cooking after soaking for 12 hours in salt	240.05^{b} (±10.25)	226.24^{ab} (±14.41)	210.50^{b} (±3.19)	2
Cooking after soaking for 18 hours in salt	233.88^{bc} (±16.14)	216.95^{bc} (±08.79)	201.15^{c} (±1.27)	2
Cooking after soaking for 24 hours in salt	221.83^{cd} (±16.15)	207.54^{bc} (±17.51)	211.15^{b} (±2.54)	1
Overall cultivars means	230.26^{a} (±18.40)	211.90^{bc} (±25.84)	214.93^{b} (±19.43)	2

Values are means (±SD)
* salt solution is 1.5% $NaHCO_3$ + 0.5% Na_2CO_3 + 0.75% citric acid
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 8. Effect of cooking after soaking in distilled water and in salt* solution on *in vitro* protein digestibility (IVPD) (%) for faba bean cultivars

Cooking treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	
Uncooked	69.78^{a} (±0.92)	75.09^{a} (±0.87)	70.85^{a} (±1.53)	6
Cooking without soaking	50.68^{d} (±1.63)	54.47^{e} (±0.89)	54.79^{c} (±2.44)	5
Cooking after soaking in distilled water	53.93^{c} (±0.99)	57.68^{d} (±0.50)	59.16^{b} (±0.93)	5
Cooking after soaking for 12 hours in salt	54.81^{c} (±0.00)	60.76^{c} (±1.78)	52.77^{c} (±1.72)	4
Cooking after soaking for 18 hours in salt	57.80^{b} (±0.94)	61.03^{c} (±1.89)	58.03^{b} (±0.89)	5
Cooking after soaking for 24 hours in salt	58.80^{b} (±3.16)	63.64^{b} (±0.85)	60.44^{b} (±0.90)	5
Overall cultivars means	57.63^{c} (±6.37)	62.11^{a} (±6.74)	59.34^{b} (±6.06)	5

Values are means (±SD)

* salt solution is 1.5% $NaHCO_3$ + 0.5% Na_2CO_3 + 0.75% citric acid

Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 9. Effect of dehulling on tannin content (%) of faba bean cultivars

Treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sha**
Hulled	0.0553[a] (±0.0085)	0.0380[a] (±0.0020)	0.0443[a] (±0.0011)	0.077
Dehulled	0.0253[b] (±0.0006)	0.0217[b] (±0.0015)	0.0287[b] (±0.0045)	0.031
Overall cultivars means	0.0403[b] (±0.0125)	0.0298[c] (±0.0091)	0.0365[b] (±0.0091)	0.054

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 10. Effect of dehulling on phytic acid content (mg/100g) for four faba bean cultivars

Treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sham**
Whole seed	259.21[b] (±3.20)	247.07[b] (±6.32)	255.26[b] (±1.87)	257.75[b]
Dehulled	276.24[a] (±7.22)	258.63[a] (±1.48)	275.04[a] (±2.55)	272.80[a]
Overall cultivars means	267.724[a] (±6.58)	252.85[b] (±7.56)	265.15[a] (±11.22)	265.27[a]

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 11. Effect of dehulling on *in vitro* protein digestibility (%) of faba bean cultivars

Treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sh**
Whole seed	69.78[b] (±0.92)	75.09[b] (±0.87)	70.85[b] (±1.53)	69
Dehulled	82.31[a] (±1.58)	88.76[a] (±1.69)	83.17[a] (±0.93)	84
Overall cultivars means	76.04[b] (±6.96)	81.931[a] (±7.59)	77.01[b] (±6.84)	77

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 12. Effect of germination on tannin content (%) of faba bean cultivars

Germination treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Shamb**
Ungerminated	0.0553[a] (±0.0085)	0.0380[a] (±0.0020)	0.0443[a] (±0.0012)	0.07
Germinated for 2 days	0.0293[b] (±0.0025)	0.0267[b] (±0.0035)	0.0337[b] (±0.0029)	0.02
Germinated for 4 days	0.0270[b] (±0.0000)	0.0253[b] (±0.0035)	0.0220[c] (±0.0036)	0.01
Germinated for 6 days	0.0217[b] (±0.0025)	0.0190[c] (±0.0010)	0.0180[c] (±0.0020)	0.01
Overall cultivars means	0.0333[a] (±0.0141)	0.0273[b] (±0.0075)	0.0295[b] (±0.0110)	0.03

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 13. Effect of germination on phytic content (mg/100g) of faba bean cultivars

Germination treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sha**
Ungerminated	259.21[a] (±3.20)	247.07[a] (±6.32)	255.26[a] (±3.87)	257.
Germinated for 2 days	229.63[b] (±1.29)	221.63[b] (±1.27)	231.25[b] (±2.54)	229.
Germinated for 4 days	200.04[c] (±1.68)	195.46[c] (±5.83)	210.07[c] (±5.20)	187.
Germinated for 6 days	176.03[d] (±1.27)	163.31[d] (±18.71)	168.81[d] (±3.80)	168.
Overall cultivars means	216.23[a] (±32.68)	206.87[b] (±33.63)	216.35[a] (±1.27)	210.

Values are means (±SD)
Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 14. Effect of germination on in *vitro protein* digestibility (IVPD) (%) of faba bean cultivars

Germination treatment	Cultivar			
	SML	**SML85/1/1**	**Shambat616**	**Sham**
Ungerminated	69.78^{d} (±0.92)	75.09^{d} (±0.87)	70.85^{d} (±1.53)	69.45^{d}
Germinated for 2 days	81.20^{c} (±1.62)	90.69^{c} (±0.92)	79.88^{c} (±1.73)	79.19^{c} (
Germinated for 4 days	87.97^{b} (±0..01)	94.54^{b} (±1.09)	89.71^{b} (±0.95)	84.23^{b} (
Germinated for 6 days	89.98^{a} (±0.75)	97.42^{a} (±1.27)	95.40^{a} (±0.00)	88.62^{a} (
Overall cultivars means	82.23^{c} (±10.29)	89.44^{a} (±9.04)	83.96^{b} (±7.49)	80.37^{d} (

Values are means (±SD)

Means with the same letter (s) within a column for each cultivar and treatments mean and within row for cultivars means

are not significantly different using Duncan Multiple Range Test at 0.05 level.

Table 15. Effect of soaking in sodium bisulphate in varying concentrations on *in vitro* protein digestibility of cooked and uncooked faba bean cultivars

Salt* concentration M	Cultivar			
	SML		**Shambat616**	
	Cooked	Uncooked	Cooked	Uncooked
Control	50.68c (±1.63)	69.78c (±0.92)	54.79d (±2.44)	70.85d (±1.53)
0.1	66.71a (±2.32)	84.14a (±2.79)	63.51a (±2.21)	80.31a (±3.86)
0.25	66.46a (±1.91)	80.99b (±4.19)	61.37b (±1.00)	77.10b (±2.87)
0.50	63.27b (±2.88)	81.13b (±3.09)	60.47c (±0.79)	75.37c (±2.41)

Values are means (±SD)
Values for each concentration are means of the three soaking times.
* = Sodium bisulfite
Means with the same letter (s) within a column are not significantly different using Duncan Multiple Range
Test at 0.05 level.

Table 16. Effect of time of soaking (days) in sodium bisulfate solution on *in vitro* protein digestibility of cooked and uncooked faba bean cultivars

Soaking time (days)	Cultivar			
	SML		**Shambat616**	
	Cooked	Uncooked	Cooked	Uncooked
Control	50.68c (±1.63)	69.78c (±0.92)	54.79c (±2.44)	70.85c (±1.53)
1	63.92b (±1.68)	84.28a (±1.68)	60.60b (±0.79)	79.53a (±2.88)
2	64.80b (±3.21)	79.73b (±4.73)	62.36a (±1.62)	76.88b (±2.27)
3	67.76a (±1.86)	82.24a (±2.29)	62.39a (±2.53)	76.34b (±4.82)

Values are means (±SD)
Values for each soaking time are means of the three concentrations.
Means with the same letter (s) within a column are not significantly different using Duncan Multiple Range
Test at 0.05 level.