2. REVIEW OF LITERATURE

The literature pertaining to different aspect of the present study is reviewed under the following headings:

2.1. Pumpkin – General aspects
2.2. Constituents of the pumpkin seeds
2.3. Effect of processing on nutrients
2.4. Bread formulation
2.5. An overview about optimized products using Response Surface Methodology
2.6. Health benefits of pumpkin seeds

2.1. Pumpkin- General Aspects

Classification of pumpkin includes;

- **Family:** Cucurbitaceae
- **Botanical Name:** Cucurbita Maxima
- **Kingdom:** Plantae
- **Order:** Cucurbitales
- **Family:** Cucurbitaceae
- **Genus:** Cucurbita
- **Colour:** Light yellow-orange to bright orange
- **Best Season:** Throughout the year

Pumpkin (English), Kumbra (Bengali), Kohlu (Gujarati), Kaddu (Hindi), Kumbala (Kannada), Paarimal (Kashmiri), Mathan or Chakkara kumbalanga (Malayalam), Lal bhopla (Marathi), Kakharu (Oriya), Sitaphal (Punjabi), Purangikkai or Pooshanikai (Tamil), Gummadi kayi (Telugu) Dangaree (Sanskrit) (Gopalan et al., 2011).

Pumpkin is one of the most important crops of family Cucurbitaceae. *Cucurbitaceae* used as vegetable and medicine throughout the world. *Cucurbita maxima* (C. maxima) are an extremely diverse species. It has been suggested that it has more cultivated forms than any other crop. This species originated in South America from wild, free-living *Cucurbita maxima* species andreana, over 4000 years ago, and apparently did not migrate from its continental origin during pre-Columbian era (Sanjur
et al., 2002). India, Bangladesh and Myanmar are considered to be secondary centers of diversity for Cucurbita maxima (Ferriol et al., 2004).

Fokou, et al., (2004) assessed that pumpkin fruit has many nutritional components including pumpkin polysaccharides, active proteins, essential amino acids, carotenoids, and minerals. It has received considerable attention in the recent years because of the nutritional and health protective value of these components. Pumpkin origin in Beijing, containing 100g of edible part with protein 0.6g, fat 0.1g, carbohydrates 5.7g, crude fibre 1.1g, calcium 10mg, phosphorus 32mg, iron 0.5mg, carotene, 0.53mg, vitamin B 10.04mg, B 2 0.05mg, niacin 0.7mg, vitamin C 5mg.

Attarde et al., (2011) reported that pumpkin is very rich in carotenoid, which is known for keeping the immune system of an individual strong and healthy. Beta-carotene, found in pumpkin, is a powerful antioxidant as well as an anti-inflammatory agent. It prevents build up of cholesterol on the arterial walls, thus reducing chances of strokes. Being rich in alpha-carotene, pumpkin is believed to slow down the process of aging and also prevent cataract formation. Pumpkins have been known to reduce the risk of macular degeneration, a serious eye problem than usually results in blindness. The high amount of fibre, present in a pumpkin, is good for the bowel health of an individual. Being loaded with potassium, pumpkin is associated with lowering the risk of hypertension. The presence of zinc in pumpkins boosts up the immune system and also improves the bone density. Pulp of pumpkin applied to burns, scalds, inflammations, abscesses, boils and is remedy for migraine, neuralgia, haemoptysis & hemorrhages.

2.2. Constituents of the pumpkin seeds

Nuts (and seeds) are good foods. Most are rich in unsaturated fats, magnesium, and copper, with smaller amounts of protein, fiber, and iron. But they’re also high in calories (http://www.cspinet.org/nah, accessed on /10/09/2011). Food trends come and go. But today, we are still eating some things that our caveman ancestors were eating centuries ago. Seeds have been an important, quick-and-easy source of nutrition for at least 12,000 years. Seeds are rich in nutrients and they are powerful weapons for fighting diseases and lowering cholesterol. A little seed can go a long way (www.perfectfit.ws, accessed on /20/7/2011).

A study conducted by Balkaya et al., (2009), showed the genotypic variation in Cucurbita maxima seed in their shape, colour, brightness, dimensions, and weight. Seeds displaying a wide elliptic shape (62.5%) were the most common in the collection. Most
populations displayed a cream seed colour (46.9%), followed by white (25.6%), brown (20.0%), and tawny (7.5%). This study demonstrated that substantial differences in seed dimensions exist in Turkish winter squash populations. Seed length ranged from 15.0 to 25.7 mm, 7.6-15.5 mm for seed width, and 1.4-6.1 mm for seed thickness. For seed length to thickness ratio and seed width to thickness ratio, populations showed a range of 3.2-14.2 and 2.2-8.5, respectively.

Pumpkin seeds are an excellent natural source of essential vitamins and minerals, healthy oils and fiber. Boiled, baked, or even raw, pumpkin seeds are packed full of vitamins, minerals and amino acids. Just one serving (about 1/4 cup) pumpkin seed gives you almost half the recommended daily amounts of manganese, magnesium, phosphorus, iron, copper, vitamin K and zinc. Pumpkin seeds are a good source of protein and monounsaturated fats (http://www.squidoo.com/pepitas, accessed on 20/9/2012).

2.2.1. Chemical composition of Pumpkin seed

Mohammed (2004) investigated the nutritional and oil characteristics of pumpkin seed kernels. The pumpkin seeds contained 39.25% crude protein, 27.83% crude oil, 4.59% ash, and 16.84% crude fiber; the corresponding values for the kernels were 39.22%, 43.69%, 5.14%, and 2.13% respectively. Glew et al., (2006) found that pumpkin seed is a rich natural source of proteins. Evaluation of the nutritional value of pumpkin seeds revealed that it contained 45.4% crude oil, 32.3% crude protein, 12.1% crude fiber and 4.65% ash while the defatted flour of the pumpkin seeds contained 55.4% crude protein.

The pumpkin seeds contained relatively large amounts of potassium (5790 μg/g dry weight) and chromium (approximate 3 μg/g dry weight). However, the sodium content of pumpkin seeds was low (6.9 μg/g dry weight). Pumpkin seeds contained relatively large amounts of magnesium (5690), zinc (113), copper (15.4), molybdenum (0.805) and another minerals: phosphorus (1570), calcium (346), iron (106), manganese (49.3), aluminum (9.21), barium (1.16), cobalt (0.29), strontium (1.83), nickel (0.53), arsenic (0.45) (in μg/g dry weight). Noteworthy are the low amounts of calcium in the seeds (Glew et al., 2006).

Amoo et al., (2004) stated that proximate composition of the pumpkin powdered seed was oil 52.13±0.13%, protein 14.31±0.10%, moisture 3.08±0.09%, ash 3.60±0.15%, crude fiber 2.55±0.08% and carbohydrate 24.45±0.12%. The mineral composition of the seed was sodium 296.9ppm, potassium 358.7ppm, magnesium
348.7ppm, calcium 294.7ppm, phosphorus 2241.5ppm, manganese 17.9ppm, zinc 39.9ppm, iron 42.7ppm.

Pumpkin seed kernel contain oil content up to 50%, high-quality cooking oil can be used as raw materials. White melon seeds (fried) (Beijing) per 100 g of protein 35.1 g, fat 31.8 g, carbohydrates 23.3 g, 520 kcal energy, crude fiber 2.3 g, calcium (C) 235 mg, phosphorus 630 mg, iron 67 mg, carotene 0.47 mg, Vitamin B10.15 mg, B2 0.15 mg, niacin 30 mg (http. home health care us.blogspot.com/accessed on 26/10/2010).

2.2.2. Fatty acid composition

The composition of fatty acids varies depending on several factors: variety of areas in which the plants are grown, climate and state of ripeness. The variability in the oil content is very high resulting from a broad genetic diversity.

The pumpkin seed contains over 80% of unsaturated fatty acids, mainly linoleic (C18:2; 42-64%) and oleic (C18:1; 20-38%) acids, and 19% saturated fatty acids, mainly palmitic (C16:0; ca. 13%) and stearic acids (C18:0; ca. 6%) (Andrikopoulos et al., 2004).

The fatty acid composition of Cucurbita maxima with four fatty acids (FA) accounting for more than 97% of the fatty acid in total. It includes palmitic acid (C 16:0; 13.0%), stearic acid (C 18:0; 7.9%), oleic acid (C 18:1 n-9; 45.4%) and the essential fatty acid linoleic acid (C 18:2n-6; 31%). On a percentage basis, oleic acid was the predominant fatty acid whereas linoleic acid accounts for nearly one-third of the total fatty acid in pumpkin seeds, only a trace amounts of α- and γ- linolenic acids were found (Glew et al., 2006) and several studies have reported similar proportions of total fatty acids or free fatty acids (Bravi et al., 2006).

Younis et al., (2000) stated that Pumpkin (Cucurbita pepo) seeds found to be rich in oil (42.3%). It contained the following fatty acid composition: C16:0 -14%, C18:0-6.93%, C18:1-35.8%, 18:2-40.70%. The four dominant fatty acids found in pumpkin seeds are: palmitic C16:0 (13.3%), stearic C18:0 (8.0%), oleic C18:1 (29.0%) and linoleic C18:2 (47.0%). The oil contains an appreciable amount of unsaturated fatty acids (78.0%) and found to be a rich source of linoleic acid (47.0%). The total oil content of the pumpkin seed analyzed was 42.3% (Ryan et al., 2007).

The major fatty acids (FA) in the cold pressed roasted pumpkin seed oil were linoleic, oleic, palmitic, and stearic acids at levels of 47.2%, 36.35%, 8.9%, and 6.4%, respectively. These four fatty acids were the primary fatty acids reported in pumpkin seed oils with concentrations of 54.6%, 27.6%, 5.43%, and 12.4% for linoleic, oleic,
palmitic and stearic acids, respectively (Murkovic 2004). Makni (2008) stated that pumpkin seed contains 40.4-55.6% of linolenic acid 18:2 n-6, ω-6 fatty acid.

Applequist *et al.*, (2006) determined the presence of stearic, palmitic, oleic, and linoleic acids in *C. pepo* seed. *C. maxima* seeds contained 3-fold more PUFA than MUFA, which was significantly higher than *C. pepo* and *C. moschata* (*P* < 0.05). The linoleic acid concentration in *C.moschata* seeds was higher than *C. pepo* in other studies (Applequist *et al.*, 2006).

### 2.2.3. Amino Acid Composition

*Cucurbita maxima* contain 1.63% of tryptophan (Vickery, 1940). The following amino acids were released from proteins by HCl hydrolysis in pumpkin seeds were: alanine (23.4), arginine (93.2), aspartic acid (52.8), cysteine (6.73), glutamic acid (104), glycine (28.3), histidine (13.8), isoleucine (23.0), leucine (40.9), lysine (22.0), methionine (12.4), phenylalanine (31.4), proline (20.2), serine (31.7), threonine (18.4), tryptophan (15.3), tyrosine (22.1) and valine (28.2) (in mg/g dry weight) (Glew *et al.*, 2006).

According to Mansour *et al.*, (1994) the dehulled and defatted pumpkin seed (*Cucurbita pepo* Kakai 35), has an excellent pattern of amino acids, contain high levels of most essential amino acids including: isoleucine 2.66, leucine 6.13, lysine 5.20, cystine 1.52, methionine 1.25, tyrosine 2.94, phenylalanine 4.00, threonine 2.75, tryptophan 1.56, valine 3.40, histidine 3.62 and non-essential amino acids: arginine 16.70, aspartic acid 10.19, glutamic acid 18.13, serine 5.46, proline 4.34, glycine 5.86 and alanine 4.29 (in g per 16 g N).

Pumpkin seeds have been reported to be deficient in sulfur containing amino acids (cysteine and methionine), but relatively higher in rare amino acids such as citrullin, carboxyphenylalanine, β-pyrazolalanine, γ-aminobutyric acid and ethylasparagine (Fruhwirth and Hermetter 2007). Trace amounts of vitamins A and E, and the minerals potassium, magnesium, calcium, magnesium, zinc, iron, selenium, copper and molybdenum have also been detected in pumpkin seeds. High enough levels of iodine and selenium were measured in pumpkin seeds to cover the recommended daily value for adults (Kreft *et al.*, 2002).

### 2.3. Effect of Processing on Nutrients

The simultaneous reduction in carbohydrate content indicates that many carbohydrate molecules are broken down during sprouting to allow absorption of atmospheric nitrogen and reforming into amino-acids. The resultant protein is the most
easily digestible of all proteins available in foods. The remarkable increase in sodium content supports the view that sprouted foods offer nutritional qualities. Sodium is essential to the digestive process within the gastro-intestinal tract and also to the elimination of carbon dioxide. Together with the remarkable increase in vitamins, sodium materially contributes to the easy digestibility of sprout (www.healthlibrary.com. accessed on, 12/10/11).

They supply food in predigested form, that is, the food which has already been acted upon by the enzymes and made to digest easily. During sprouting, much of the starch is broken down into simple sugars such as glucose and sucrose by the action of the enzyme ‘amylase’. Proteins are converted into amino acids and amides. Fats and oils are converted into more simple fatty acids by the action of the enzyme lipase (www.healthlibrary.com. accessed on, 12/10/11).

Autoclave helps to sterilize the food by killing harmful bacteria and other microorganisms, and it increases the availability of nutrients. Proteins are denatured by heat. In this form they are more easily digested by proteolytic enzymes; cellulosic cell walls that cannot be broken down by monogastric animals like man are broken down, and some anti-nutritional factors such as enzyme inhibitors are inactivated. However, processing may reduce the nutritional value of some root crops as a result of losses and changes in major nutrients, including proteins, carbohydrates, minerals and vitamins (www.healthlibrary.com. accessed on, 12/10/11).

**Effect of Processing on Nutrients**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Effect of Processing</th>
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<tbody>
<tr>
<td>Fat</td>
<td>Oxidation accelerated by light</td>
</tr>
<tr>
<td>Protein</td>
<td>Denatured by heat (improves digestion)</td>
</tr>
<tr>
<td>Vitamin C (ascorbic acid)</td>
<td>Decreases during storage, drying, heating, oxidation, cell damage (chopping and slicing). Losses due to oxidation catalyzed by copper and iron.</td>
</tr>
<tr>
<td>Vitamin B₁ (Thiamine)</td>
<td>Destroyed by high temperatures, neutral and alkaline conditions, Lost in cooking water</td>
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</table>
An increase in the bioavailability of minerals and vitamins has been observed due to germination (Suleiman). Germination is a simple biochemical enrichment tool to enhance the palatability result in increasing the digestibility and nutritive value (Ramakrishna et al., 2006).

### 2.3.1. Chemical compositions

Germination increases the crude fiber in seeds due to the disappearance of starch. The crude fiber, a major constituent of cell walls increases both in percentage and real terms, with the synthesis of structural carbohydrates, such as cellulose and hemicelluloses. The increases in crude protein could be due to enzyme hydrolysis of the insoluble protein available (Echendu et al., 2009).

Mubarak (2005) reported that increase in protein during germination was due to the release of free amino acids after enzymatic hydrolysis for the synthesis of new protein. The increase in the protein content of the sprouted flour samples were mainly due to the use of seed components during the germination process, breakdown of complex proteins into simpler form and breakdown of nutritionally undesirable constituents (Chavan and Kadam, 1989). Germination significantly (p<0.05) decreased

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Sensitivity to Temperature and pH Conditions</th>
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<tbody>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt; (Riboflavin)</td>
<td>Sensitive to light at neutral and alkaline conditions. Moderately heat stable under neutral conditions. Sensitive to heat under alkaline conditions.</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;3&lt;/sub&gt; (Niacin)</td>
<td>The most stable vitamin -- stable to heat and light. Lost in cooking water.</td>
</tr>
<tr>
<td>Folate</td>
<td>Decreases with storage or prolonged heating. Lost in cooking water. Destroyed by use of copper utensils.</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt; (Pyridoxine)</td>
<td>Heat stable in alkaline and acidic conditions. Pyridoxal is heat labile.</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Destroyed by light and high pH.</td>
</tr>
<tr>
<td>Carotenes</td>
<td>Easily destroyed by heat. Oxidizes and isomerizes when exposed to heat and light.</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Very heat labile – easily destroyed by heat; easily oxidized.</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Oxidizes when exposed to heat and light.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Oxidizes readily.</td>
</tr>
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(Morris et al., 2004)
the total carbohydrate contents. Mubarak (2005) showed that sprouting resulted to a reduction in the carbohydrate content in mungbean.

Soaking did not interfere with the Net Protein Retention (NPR) of the experimental diets containing the common bean as protein source, nor did it reduce the tannin content. However, soaking reduced the phytate levels in the common bean (Helbig et al., 2003). Boiling and blanching, like other methods for processing of food reduce to minimal levels these antinutrients (Onwuliri et al., 2002). Urbano et al., (2006) said that germination results an appreciable reduction in the factors responsible for flatulence, thus increasing the intake and improving the utilization of available proteins and carbohydrates.

Mubarak (2005), revealed that boiling increased the moisture content due to the absorption of water by the seeds during cooking. There was a slight reduction in the moisture contents of the toasted flour but no significant difference (p>0.05) existed among them. The sample toasted for 90 minutes had the least moisture content of 5.36% which could be as a result of the high temperature and time it was subjected to (120°C and 90 minutes respectively) and it significantly differed from the moisture content of the raw flour.

2.3.2. Minerals

Urbano et al., (2006) revealed that soaking prior to the germination is responsible for the loss of magnesium and zinc that continuously emptying from the seed during the sprouting. Magdi (2007) claims that when seeds are sprouted, minerals chelate or merge with protein, in a way that increases their function. Processing like germination and roasting significantly (p < 0:05) increased the zinc and phosphorus and germination shows the increased iron into the pumpkin seeds. El-Adawy and Taha (2001), reported that iron content significantly (p<0.05) decreased when seeds were roasted and it was found to be 17.36mg/100g, however, after processing iron extractability increased to 13.48. Urbano et al., (2006) stated that soaking prior to the germination is responsible for the loss of magnesium and zinc that continuously emptying from the seed during the sprouting.

Roasted pumpkin seed increases the extractable sodium and phosphorus. Iron content significantly deceased when seeds were roasted and it was found to be 17.36 mg/100g (Giami, 2005). El-Adawy and Taha (2001) reported in their study that a successive increase in minerals extractability of pumpkin seed occurred after roasting the seed.
According to Hamed (2008) potassium content of untreated pumpkin seed was found to be 1078.55 mg/100 g and out of this amount about 70.11% was found to be extractable. Giami et al., (2005) reported that pumpkin seed contain 1379 mg potassium in 100 g which was higher than that (982 mg/100 g) stated by El-Adawy and Taha (2001). He reported that total and extractable potassium significantly (p<0.05) increased when the seeds were roasted and it was found to be 1331.7 mg/100 g and 85.23% respectively. Both roasted and unroasted pumpkin seeds contained significantly (p < 0.05) high amount of calcium (134.0 and 152.5 mg/100 g) and out of this amount about 77% and 84% were found to be extractable for unroasted and roasted seeds, respectively. Iron content significantly (p<0.05) decreased when seeds were roasted and it was found to be 17.36 mg/100 g, however, after processing iron extractability increased to 13.48 mg.

2.3.3. Amino Acids

Dehulling and germination processes caused a slight increase in total essential amino acids, but there were only slight decrease by other processes. All processes decreased the concentrations of isoleucine, tryptophan (except germination) and total sulfur amino acids (except dehulling and germination (Mubarak, 2005). Taraseviciene et al., (2009) has reported the increase in amino acid content in germinated broccoli seeds corresponding with crude protein content increase. The result showed by Khare et al., (2007) the dehulled and defatted pumpkin seed has an excellent pattern of amino acids, contain high levels of most essential amino acids. Young and Varner (1959) and Asiedu et al., (1993) stated that germination and fermentation improve the amino acids of the seed flour while boiling and roasting reduce the essential amino acids. The result agreed with the observation on lettuce seeds, blends of soybean, corn meal, maize and sorghum respectively.

Fagbemi, (2007) study found that fluted pumpkin seeds were processed into the raw, boiled, fermented, germinated and roasted seeds, dried at 50°C, milled and sieved. Predominant amino acids in the seed are glutamic acid, 132.04-152.30 mg/g cup; and aspartic acid, 124.61±130.78 mg/g cup. The limiting amino acids are methionine, 6.02–8.11 mg/g cup and tryptophan, 0.08–13.78 mg/g cup. Fermentation and germination improved the protein quality while boiling and roasting reduced them. Processing reduced deleterious metals and improved some of its nutrients

2.3.4. Fatty Acids

Out of the unsaturated fatty acids oleic acid decreased to a greater extent, whereas linoleic acid and linolenic acid increased during the germination. This is very
important because linoleic acid, linolenic acid and arachidonic acid are essential to the human organism. Linoleic acid is capable of transporting bioactive compounds and it can be converted into arachidonic acid from which hormone-like compounds are forming. Summarized, it was established that the majority of the fatty acids of buckwheat are the unsaturated fatty acids, out of which linoleic acid occurred in the highest amount.

Tokiko and Koji (2006) examining fat content and fatty acid composition of various sprouts established that the fat content ranged from 0.4% to 1.6%. During the examination of the fatty acid content it was found that linolenic acid was present in the highest concentration, in 23% case of buckwheat, in 48% the soyabean 47.7% the clover and in 40.6% the pea.

Fagbemi, (2007) study found that fluted pumpkin seeds were processed into the raw, boiled, fermented, germinated and roasted seeds, dried at 500ºC milled and sieved. Processing affected the levels of nutrients in the seed. The energy values ranged between 26.55 ± 0.7–30.06 ± 0.8 KJ/g and the seed is a good source of some essential minerals. Deleterious elements are very low and significantly reduced by processing. The fatty acids consist of oleic acid, 34.52– 46.39%, linoleic acid, 11.0–30.94%, palmitic acid, 13.61– 19.56% and stearic acid 11.84–18.9%.

2.3.5. Antinutrients

Plant phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals and man (Amadi et al., 2006). Most of the toxic and anti-nutrient effects of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008).

The reduction of tannin and phytic acid content of roasted samples may be due to the effect of the heat treatment. Reduction of polyphenols during roasting could be attributed to thermal degradation and denaturation, changes in chemical reactivity or to formation of insoluble complexes during heating (Sidduraju and Becker, 2001). Tannins cause a decrease in the digestibility of proteins and carbohydrates due to the formation of insoluble enzyme-resistant complexes. Sangronis and Machado, (2007) stated that an enzymatic hydrolysis by polyphenolase causes loss of tannins in grains during germination. Reduction in tannin content may be due to effects of soaking and germination of seeds. These results are in accordance with those reported by Majed et
Magdi (2007) reported that reduction in germination may be attributed to enzymatic hydrolysis of polyphenols by polyphenol oxidase.

Fagbemi et al., (2005) reported that processing significantly reduced antinutritional factors of fluted pumpkin seed. Total phenols of *curcas* seeds significantly reduced (p<0.05) due to roasting at 160°C for 30 minutes. These results are in agreement with those reported by Ibrahim et al., (2002). Reduction of polyphenols during roasting could be attributed to thermal degradation and denaturation, changes in chemical reactivity or to formation of insoluble complexes during heating (Siddhuraju and Becker, 2001).

Tannins cause a decrease in the digestibility of proteins and carbohydrates due to the formation of insoluble enzyme-resistant complexes. An enzymatic hydrolysis by polyphenolase causes loss of tannin in grains during germination. Reduction in tannin content may be due to effects of soaking and germination of seeds (Sangronis and Machado, 2007). The results obtained for pumpkin seeds was higher than that of pumpkin seed flour reported by El-Adawy and Taha (2001). Roasting of seeds significantly (p<0.05) reduced tannin and phytic acid content to be 125mg and 56.1mg respectively. The reduction of tannin and phytic acid content of roasted samples may be due to the effect of the heat treatment. Hassan et al. (2010) reported that the application of processing such as cooking have been effective in reducing antinutritional factors of lupine seeds.

Hassan and El-Sayed (2010) stated that all heat treatments significantly reduced the levels of all the investigated antinutrients of peanut seeds. Of the attempted treatments, autoclaving, boiling, roasting-salting and oil-roasting were the most effective in reducing the levels of antinutrients of peanut. Roasting in both brown and white sesame seeds partially eliminated the antinutrients (the reduction ranged from 15.6% to 61.2% in all antinutrients).

### 2.4. Bread formulation

Bread has always been one of the most popular and appealing food product due to its superior nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness (Giannou and Tzia, 2003). Bread is consumed in large quantity in the world in different types and forms depending on cultural habits (Cayot, 2007).

Bread products and their production techniques differ widely around the world (Dewettinck et al., 2008). The objective of bread making is to convert cereal flours into
attractive, palatable and digestible food. The earliest breads were unleavened or flat, but
the first major technical innovation was the introduction of leavening processes, which
yielded breads of superior palatability. The foremost quality characteristics of leavened
wheat breads are high volume, soft and elastic crumb structure, good shelf life and
microbiological safety of the product (Cauvain, 2003).

Palatability of bread is mainly due to its flavour. Bread flavour is the result of
many factors, such as the type of wheat flour, ingredients and the process used in bread
formulation (Hansen and Schieberle, 2005). There are many sensory parameters
affecting the quality of bread and its acceptance by the consumers like colour, flavour,
texture and appearance. The characteristic aroma of bread is one of the most important
factors (Quilez et al., 2006). The simplest bread can be created by basic formula dough
which includes flour, water, leavening agent (yeast or chemicals) and sodium chloride.
Other ingredients such as fat, emulsifiers, sugars and dough conditioner may be added to
improve the dough and bread quality. Each ingredient has its own function in bread and
if slightly changed will alter final bread quality. Therefore, a proper balance of
ingredients needs to be obtained to produce high quality bread (Giannou et al., 2003).

2.5. An overview about optimized products using Response Surface Methodology

Response Surface Methodology (RSM) is a collection of statistical and
mathematical techniques useful for developing, improving, and optimizing processes. It
also has important applications in the design, development, and formulation of new
products, as well as in the improvement of existing product design (Myers and
Montgomery, 2002). The most extensive applications of RSM are in the industrial
world, particularly in situations where several input variables potentially influence some
performance measure or quality characteristics is called the response. It is typically
measured on a continuous scale, although attribute responses, ranks, and sensory
responses are not unusual. Most real world applications of RSM will involve more than
one response. The input variables are sometimes called independent variables, and they
are subject to the control of the engineer or scientist, at least for purposes of a test or an
experiment (Myers and Montgomery, 2002).

Response Surface Methods are designs and models for working with continuous
treatments when finding the optima or describing the response is the goal (Oehlert,
2000). The first goal for Response Surface Method is to find the optimum response.
When there is more than one response then it is important to find the compromise
optimum that does not optimize only one response (Oehlert, 2000). When there are
constraints on the design data, then the experimental design has to meet requirements of the constraints. The second goal is to understand how the response changes in a given direction by adjusting the design variables. In general, the response surface can be visualized graphically. The graph is helpful to see the shape of a response surface, hills, valleys, and ridge lines.

RSM has also been shown to be a useful tool in the food field, for the development of new products and processes, the enhancement of existing products and processes, the optimization of quality and performance of a product, the optimization of an existing manufacturing procedure and the minimization of production cost, as highlighted by different authors. This technique was used to evaluate the effects of different factors on quality attributes, to optimize different process conditions or in the optimization of particular formulations (Oehlert, 2000).

Demirekler (2004) stated that basically RSM is a four-step process. First, the critical factors that are important to the product or process under study are identified. Second, the ranges of factor levels which will encompass the physical specifications of the samples are defined. Third, the specific test samples are determined by the experimental design and then tested. Fourth, the data from these experiments are analyzed by RSM and then interpreted.

RSM is a statistical technique that uses quantitative data to determine and simultaneously solve multivariate equations, which specify the optimum product for a specified set of factors through mathematical models. These models consider interactions among the test factors and can be used to determine how the product changes with changes in the factor levels. RSM is more efficient than traditional experimental procedures because it decreases the time and cost required to determine the optimum product (Demirekler, 2004).

2.6. Health Benefits of Pumpkin Seed

2.6.1. Antioxidant activity of pumpkin seed

Xanthopoulou et al., (2009) studied on four commercially available pumpkin seeds (Cucurbita pepo, Cucurbita moschata, Cucurbita maxima and Cucurbita mixta) extracts were screened for their antioxidant activity and their inhibitory activity against lipid peroxidation. The results of his study showed that radical scavenging activity of the water extracts related to their total phenolic content (up to 85-92% of total extractable phenolic). The highest antioxidant activity was found in water and methanol fractions, the lowest for ethyl-acetate fractions. Pumpkin seed water extracts inhibited lipid
peroxidation at 1.5 mg/ml, while the acetone extracts inhibit 50% of lipoxygenase activity at the range from 0.16 to 0.80 mg/ml.

2.6.2. Intestinal antiparasitic effect of pumpkin seed

Marie et al., (2009) study was carried out to evaluate the in vitro effect of Cucurbita moschata seed against the parasitic nematode of small ruminants Haemonchus contortus. Three extracts (aqueous, methanolic and dichloromethane) of C. moschata seed were tested in vitro on four developmental stages of H. contortus using Egg Hatch Assay (EHA), Larval Development Assay (LDA), L3 Migration Inhibition (LMI) Assay and Adult Worm Motility (AWM) assay. The highly significant (P<0.001) ability to stop larval development (inhibition >90% for each extract) and the negative effect of the dichloromethane and methanolic extracts on adult worm motility (inhibition of motility >59.2% after 24 hour of incubation) compared to the negative controls, suggest anthelmintic properties of C. moschata seed against H. contortus.

2.6.3. Pumpkin seed for Prostate and bladder treatment

Gossell et al., (2006) revealed that the oil from the pumpkin seed is claimed to be useful in the management of benign prostatic hyperplasia. Hyperplasia was induced by subcutaneous administration of testosterone (0.3 mg/100 g of body weight) for 20 days. Simultaneous oral administration of either pumpkin seed oil (2.0 and 4.0 mg/100 g of body weight) or corn oil (vehicle) was also given for 20 days. Testosterone significantly increased prostate size ratio, and this induced increase was inhibited in rats fed with pumpkin seed oil at 2.0 mg/100 g of body weight. The protective effect of pumpkin seed oil was significant at the higher pumpkin seed oil dose.

2.6.4. Pumpkin seed for Protection for Men's Bones

One of the important reasons for older men to make zinc-rich foods, such as pumpkin seeds, a regular part of their healthy way of eating is bone mineral density. Hyun, (2004) reported the correlation between low dietary intake of zinc in the form of pumpkin seeds, low blood levels of the trace mineral, and osteoporosis at the hip and spine.

2.6.5. Anti-inflammatory activity of pumpkin seed

Fahim et al., (1995), described an anti-inflammatory activity of pumpkin seed oil administered in intramuscular injection in experimental arthritis in male Sprague-Dawley rats. Pumpkin seed oil (100 mg/kg body weight). Pumpkin oil administration inhibited paw oedema during the chronic phase in about 44% as compared to the control.
untreated group. No potentiating of the anti inflammatory effects of indomethacin combined with pumpkin seeds oil was observed.

2.6.6. Cardiovascular activity of pumpkin seed

Hypertensive rats treated with pumpkin seed oil for 4 weeks (40 mg/kg), significant retardation of progress of hypertension with concomitant administration of felodipine or captopril was observed (Al Zuhair et al., 2000).

2.6.7. Pumpkin seeds for depression

The World Health Organization (WHO) defines depression as a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration (WHO, 2006). The majority of the people who suffer from depression are young people, merely 6 million of the 121 million people suffering from depression worldwide are elderly (WHO, 2001).

Depression has for some time now been known to be associated with deficiencies in neurotransmitters such as serotonin, dopamine, noradrenaline, and GABA (Fric et al., 2007). As reported in several studies, the amino acids tryptophan, tyrosine, phenylalanine, and methionine are often helpful in treating many mood disorders, including depression (McLean, 2004). Tryptophan is a precursor to serotonin and is usually converted to serotonin when taken alone on an empty stomach. Therefore, tryptophan can induce sleep and tranquility and in cases of serotonin deficiencies, restore serotonin levels leading to diminished depression (Hibbeln et al., 2006).

2.6.7.1. Prevalence

The depression is an all too common disorder that can affect anyone. Indeed chances of developing a depressive illness are estimated to be 1 among 5 for women and 1 among 10 for men for making it a particularly common affliction of mankind. The World Health Organization estimated that within 20 years, recurrent depressive disorder will be the second most serious cause of morbidity and burden of disease in the world (Malcolm et al., 2001).

According to world health report, about 450 million people suffer from a mental or behavioral disorder, which amounts to 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020. Depression is a major cause of morbidity worldwide. Lifetime prevalence varies widely, from 3% in Japan to 17% in the US. In most countries the number of people who would suffer from depression during their lives falls within an 8-12% range (Kessler, 2005). In North America the probability of
having a major depressive episode within a year-long period is 3-5% for male and 8-10% for female (Murphy et al., 2000). The occurrence of depression 100 years ago was rare, occurring primarily in the elderly. Only 1% of Americans born before 1905 developed depression before they were 75 years old, while 6% of Americans born in 1955 developed depression by the time they were 24 years old (Meyer et al., 2005).

In India the prevalence of serious mental disorders is about 10 to 20 per 1000 of the population (Murthy, 2004). There have been a number on studies of mental disorder the prevalence in community and primary or general health care settings in South Asia and the reported rates vary considerably from 5% to over 50% (Patel, 1999; Mirza and Jenkins, 2004). As might be expected, rates are generally higher in primary care settings and when these were reported for common mental disorders (CMD), rather than the narrower diagnostic construct of depression. A review of 8 epidemiological studies of CMD in South Asia had shown that the prevalence in primary care was 26.3% (95% confidence interval [CI] 25.3%-27.4%) (Patel, 1999).

Mental health disorders have emerged as a major public health problem in India. Conditions such as schizophrenia, mood disorders (depression and bipolar mood disorders) and mental retardation account for 8.5% of the total burden of diseases. The National Commission on Macro Economics and Health (NCMH) estimated that nearly 7% of the adult population suffers from a serious mental disorder, with no considerable rural urban difference. The age group of 25-44 years was more vulnerable (Prabhakaran, 2011; Patel, 2011).

Studies from India have reported prevalence rate that vary from 21% to 83% in primary care. A recent study covering an urban general population, with around 25,000 participants constituting the largest survey, reported an overall prevalence of 15% (Poongothi et al., 2009). Depression may begin at any age, but average age at onset is late 20’s. The symptoms of major depressive disorder typically develop over days to weeks. The prodromal symptoms including generalized anxiety, panic attacks, phobias, or other depressive symptoms that do not meeting the diagnostic threshold may occur over the preceding several months. In some cases sudden onset may occur. The duration of major depressive episode was also variable, if untreated; the episode typically lasts 6 months or longer.

2.6.7.2. Symptoms of depression

The key symptoms of depression are feeling sad most of the time, losing interest in things you used to enjoy, and feeling very tired. If you are depressed, you will have
some of these symptoms most of the time, for at least two weeks. That was,

- Problem sleeping, or sleeping too much
- Finding it hard to concentrate or make decisions
- Little confidence in oneself
- Either little appetite or more appetite than usual
- Feeling guilty for no reason
- Feeling either agitated or sluggish
- Thinking about suicide (http://besttreatments.bmj.com/btuk/about/acssessed on 12/12/2011).

2.6.7.3. DSM-IV-TR and ICD-10 Criteria for depression

The most widely used criteria for diagnosing depressive conditions are found in the American Psychiatric Association's revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-), and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems) (ICD-10), which uses the name recurrent depressive disorder (www.who.int. ICD-10:cited, Accessed on 2008/11/08) The latter system is typically used in European countries, while the former is used in the US and many other non-European nations, and the authors of both have worked towards conforming one to the other (American Psychiatric Association 2000a).

Major depressive disorder is classified as a mood disorder in DSM-IV-TR (American Psychiatric Association, 2000). The ICD-10 system does not use the term major depressive disorder, but lists very similar criteria for the diagnosis of a depressive episode (mild, moderate or severe); the term recurrent may be added if there have been multiple episodes without mania (WHO, 2004).

2.6.7.4. Classification of Depression

According to the classification system of the American Psychiatric Association’s (APA) Diagnostic and Statistical Manual IV-Text Revised Edition (DSM-IV-TR), unipolar depressive disorders can be divided into three groups, major depressive disorder, dysthymia and depressive disorder (Fernandez, et al., 2005).

2.6.7.4. 1. Major depressive episode

A major depressive episode is characterized by the presence of a severely depressed mood that persists for at least two weeks (American Psychiatric Association 2000a). Episodes may be isolated or recurrent and are categorized as mild (few symptoms in excess of minimum criteria), moderate, or severe (marked impact on social
or occupational functioning). An episode with psychotic features - commonly referred to as *psychotic depression* - is automatically rated as severe. If the patient has had an episode of mania or markedly elevated mood, a diagnosis of bipolar disorder is made instead. Depression without mania is sometimes referred to as unipolar because the mood remains at one emotional state or "pole" (WHO, 2004).

### 2.6.7.4.2. Subtypes

The DSM-IV-TR recognizes five further subtypes of MDD, called specifies, in addition to noting the length, severity and presence of psychotic features:

**Melancholic depression** is characterized by a loss of pleasure in most or all activities, a failure of reactivity to pleasurable stimuli, a quality of depressed mood more pronounced than that of grief or loss, a worsening of symptoms in the morning hours, early-morning waking, psychomotor retardation, excessive weight loss (not to be confused with anorexia nervosa), or excessive guilt (American Psychiatric Association 2000).

**Atypical depression** is characterized by mood reactivity (paradoxical anhedonia) and positivity, significant weight gain or increased appetite (comfort eating), excessive sleep or sleepiness (hypersomnia), a sensation of heaviness in limbs known as leden paralysis, and significant social impairment as a consequence of hypersensitivity to perceived interpersonal rejection (American Psychiatric Association 2000a).

**Catatonic depression** is a rare and severe form of major depression involving disturbances of motor behavior and other symptoms. Here the person is mute and almost stuporous, and either remains immobile or exhibits purposeless or even bizarre movements. Catatonic symptoms also occur in schizophrenia or in manic episodes, or may be caused by neuroleptic malignant syndrome (American Psychiatric Association 2000c).

**Postpartum depression**, or mental and behavioral disorders associated with the puerperium, not elsewhere classified (American Psychiatric Association 2000e) refers to the intense, sustained and sometimes disabling depression experienced by women after giving birth. Postpartum depression has an incidence rate of 10–15% among new mothers. The DSM-IV mandates that, in order to qualify as postpartum depression, onset must have occurred within one month of delivery. It has been said that postpartum depression can last as long as three months.

**Seasonal affective disorder** (SAD) is a form of depression in which depressive episodes come on in the autumn or winter, and resolve in spring. The diagnosis is made
if at least two episodes have occurred in colder months with none at other times, over a two-year period or longer (American Psychiatric Association 2000b).

2.6.7.5. Depression symptom rating scales and diagnostic interview tools

The General Health Questionnaire can be used as a screening instrument to determine the mental health symptoms affecting the individual’s physical health and their perception of their health (Baldwin, 2002).

Many scales are available for the assessment of the presence of depressive symptoms. These include the Beck’s, Hamilton’s, Montgomery-Asberg, Zung and the Patient Health Questionnaire (PHQ). A few have been validated in the Caribbean context such as the Zung scale (Maharaj et al., 2005; Hutchinson, 2003). Assessment tools are therefore available for use by depressants in the Caribbean. However, it must be emphasized that these tools only indicate the presence of depressive symptoms and do not diagnose the disorder. Two simple questions do you feel sad or down and do you still enjoy the things you usually do can also be used in contexts where quick screening is required. This instrument the PHQ-2 has also been validated for use in adolescents (Richardson et al., 2010).

Beck Depression Inventory is a depression test to measure the severity and depth of depression symptoms as listed in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV; 1994) in patients with clinical depression. Beck Depression Inventory II is a revised version, containing some items, which bring the BDI–II into alignment with DSM–IV criteria (Beck, 1996).
Depression symptom rating scales and diagnostic interview tools

<table>
<thead>
<tr>
<th>Depression symptom rating scales and diagnostic interview tools</th>
<th>Abbreviation</th>
<th>Number of items</th>
<th>Scoring range</th>
<th>Typical Cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptom Rating Scales</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beck Depression Inventory</td>
<td>BDI</td>
<td>21</td>
<td>0-63</td>
<td>11 mild 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>borderline clinical 21 moderate 31 severe</td>
</tr>
<tr>
<td>Center for Epidemiological Studies Depression Screen</td>
<td>CESD</td>
<td>20</td>
<td>0-16</td>
<td>16</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>GDS</td>
<td>30</td>
<td>0-30</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>GDS-15</td>
<td>15</td>
<td>0-15</td>
<td>5</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale (Clinician rating tool)</td>
<td>HAMD</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montgomery-Asberg Depression Rating Scale (Clinician rating tool)</td>
<td>MADRS</td>
<td>10</td>
<td>0-60</td>
<td>None found</td>
</tr>
<tr>
<td>PRIME-MD Patient Health Questionnaire Brief</td>
<td>PHQ-9</td>
<td>9</td>
<td>0-27</td>
<td>5 mild 10 moderate 15 moderately severe 20 severe</td>
</tr>
<tr>
<td>Primary Care Evaluation of Mental Disorders, Mood Module screening items</td>
<td>PRIME-MD</td>
<td>2</td>
<td>0-2</td>
<td>2</td>
</tr>
<tr>
<td>PRIME-MD brief screen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite International Diagnostic Interview, two “stem” items for depression section.</td>
<td>CIDI</td>
<td>2</td>
<td>0-2</td>
<td>2</td>
</tr>
<tr>
<td>CIDI 2-item</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diagnostic Interview Tools</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite International Diagnostic Interview</td>
<td>CIDI</td>
<td>(varies, depending on responses)</td>
<td>Diagnostic code</td>
<td>NA</td>
</tr>
<tr>
<td>Primary Care Evaluation of Mental Disorders, Mood Module</td>
<td>PRIME-MD</td>
<td>(varies, depending on responses)</td>
<td>Diagnostic code</td>
<td>NA</td>
</tr>
<tr>
<td>PRIME-MD (varies, depending on responses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BDI = Beck Depression Inventory; CES-D = Center for Epidemiological Studies Depression Scale; GDS = Geriatric Depression Scale; ZSDS = Zung Self-rating Depression Scale; ICC = intraclass correlation coefficient; HADS = Hospital Anxiety and Depression Scale; VAS = Visual Analog Scale (Spalletta et al., 2006; Salter et al., 2007).
2.6.7.6. Nutritional aspects to depression

Diet has an effect on mood and cognitive function (Rogers, 2001). There is some evidence that deficiency or supplementation of nutrients can affect not only mood, but also behavioral patterns.

A double-blind placebo-controlled trial with 30 patients showed that omega-3 essential fatty acid supplements alleviated symptoms in patients with bipolar disorder. In a recent double-blind, placebo-controlled trial on 231 young adult prisoners, by comparing the number of their disciplinary offences before and during the supplementation, antisocial behavior was reduced by the supplementation of vitamins, minerals and essential fatty acids (Gesch, 2002). Vitamin D supplementation during winter was reported to improve mood in a double-blind, placebo-controlled trial on 44 healthy volunteers (Lansdowne and Provost, 1998).

Tolmunen and Voutilainen, (2003) reported in number of studies that acute tryptophan depletion produces depressive symptoms and results in worsening of mood. Folic acid deficiency may also correlate with depression, and it has particular effects on mood, cognitive as well as social functioning (Reynolds, 2003). Recently, it has been reported that low levels of dietary folic acid was associated with elevated depressive symptoms in middle-aged men.

Depressed subjects tend to consume more carbohydrates in their diets than non-depressed individuals, and they show heightened preference for sweet carbohydrate or fat rich foods during depressive episodes (Christensen, 2001). High carbohydrate intakes increase brain uptake of tryptophan, which in turn stimulates the synthesis of serotonin (Rogers, 2001). At present, there are some studies focusing on the use of dietary supplements in individuals with mental disorders, but there is a lack of consistent data concerning the impact of nutrition, diet and eating habits on mental health.

A food high in amino acids, tryptophan is a precursor to serotonin and melatonin. Tyrosine is a precursor to norepinephrine and may stimulate thyroid hormone synthesis. Numerous studies have found that low levels of essential fatty acids can lead to depression. Good sources of EFA’s include flax seed oil, borage seed oil, pumpkin seeds and cold-water fish such as salmon, tuna, cod and sardines. Supplementation can also be helpful (www.depressionwellness.com).
Complementary and Alternative Medical Treatment for Depression
Brief review of natural supplements for depression

<table>
<thead>
<tr>
<th>Botanical</th>
<th>Dosage Possible</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypericum perforatum (St. John’s wort)</td>
<td>300–600 mg of the extract</td>
<td>Reduces the degradation rate of acetylcholine, sedative actions, weak serotonergic activity, sigma 1 receptor blockade</td>
</tr>
<tr>
<td>Gingko biloba</td>
<td>80 mg</td>
<td>Antioxidant, cerebrovascular protection, etc.</td>
</tr>
<tr>
<td>Chromium</td>
<td>200 μg qd</td>
<td>Glucose balance, serotonin modulation</td>
</tr>
<tr>
<td>Fish oil (omega-3 fatty acids)</td>
<td>1 tablespoon of fish oil qd, or 2 g</td>
<td>Platelet clotting ability, inhibits sympathoadrenal activation and normalizes membranes</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>0.5–1.0 mg qd</td>
<td>Lowers homocysteine</td>
</tr>
<tr>
<td>Inositol</td>
<td>4–12 g qd</td>
<td>Serotonin and acetyl cholinergic modulation</td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.5–5 mg hs</td>
<td>Circadian rhythm restoration and antioxidant</td>
</tr>
<tr>
<td>Selenium</td>
<td>200 μg qd</td>
<td>Antioxidant, helps convert T4 to T3, enhances immunity</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>0.5–1.0 g qd</td>
<td>Serotonin precursor</td>
</tr>
<tr>
<td>5-HTP</td>
<td>100–200 mg</td>
<td>Serotonin precursor</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>400 IU qd</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Zinc</td>
<td>25 mg qd</td>
<td>Neurologic and immune modulator</td>
</tr>
</tbody>
</table>


Hallahan et al., (2005) stated the effects of n-3 Essential fatty acids supplementation on depression sample. The baseline BDI scores were used as covariates in the subsequent statistical analysis (for all psychometric variables, baseline scores were entered as covariates). The significant improvements in BDI scores at 12 weeks in the n-3 EFA group. Moreover, more patients in the n-3 EFA group attained more than 50% and 70% reduction (response and remission respectively) in symptoms.
2.6.7.6.1. Tryptophan and Depression

Tryptophan is the amino acid precursor to 5- HTP and serotonin Shaw et al., (2002). Tryptophan depletion is a widely used paradigm to study the role of the serotonergic system in the pathophysiology and treatment of depression (Neumeister, 2003). 5- HTP is commonly given to rats or mice to test the SSRI potency of putative antidepressants (Neil and Moore, 2003). This simple in vivo test measures the potency of a compound in potentiating the serotonin syndrome induced by the administration of 5- HTP. The behavioral and physiological features of this syndrome include hypolocomotion, head twitch, forepaw treading, tremors, hind limb abduction, flat body posture or hunched back, cyanosis, and hyperthermia. In rodents, 5- HTP induces a serotonin syndrome at dosages of 100–200 mg/ kg (Casal et al., 2000).

5- HTP has not been directly investigated as an “antidepressant” compound in rodents, 5- HTP has been used to manipulate serotonin levels in rats.

Behavioral studies show that 5- HTP administration reverses the increases in avoidance responding induced by sleep deprivation and serotonin depletion in rats. These findings indicate that 5- HTP attenuates the hyperalgesia induced by sleep deprivation, thus suggesting that the administration of 5- HTP restores serotonin levels in rats (Smith & Kennedy, 2003).

Tolmunen and Voutilainen, (2003) reported that acute tryptophan depletion produces depressive symptoms and results in worsening of mood. Folic acid deficiency may also correlate with depression, and it has particular effects on mood, cognitive as well as social functioning (Reynolds, 2003). Recently, it have been reported that low levels of dietary folic acid are associated with elevated depressive symptoms in middle-aged men.
<table>
<thead>
<tr>
<th>Author/ year</th>
<th>N</th>
<th>Patients</th>
<th>Design</th>
<th>Dose (mg/day)</th>
<th>Duration (days)</th>
<th>Results by drug group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alino et al., (1976)</td>
<td>30</td>
<td>Unipolar</td>
<td>5-HTP vs. placebo Augmentation of nialamide</td>
<td>50-300 BDI</td>
<td>15-20</td>
<td>12/14Nmarkedly improved 58% reduction in HAM-D after 15 days</td>
</tr>
<tr>
<td>Barlet and Pailard, 1973 Mendlewicz &amp; Youdim, 1980</td>
<td>25 in 5-HTP group</td>
<td>5-HTP vs. placebo 5-HTP vs placebo</td>
<td>200-300</td>
<td>10-240 32</td>
<td>19 of 25 improved 5-htp and maoi better than placebo, 5 HTP alone not better</td>
<td>placebo rate not known same as 5-htp</td>
</tr>
<tr>
<td>Nardini et al., 1983</td>
<td>26</td>
<td>Unipolar</td>
<td>5-HTP vs. placebo Augmentation of clomipramine</td>
<td>300</td>
<td>28</td>
<td>56% reduction in HAM-D 7/13 marked improved (GGI) 0/13 worsened</td>
</tr>
<tr>
<td>Rousseau, 1987</td>
<td>50</td>
<td>Elderly depressed</td>
<td>Dihydroergocristine (DA) and 5-HTP vs placebo 5-HTP vs placebo</td>
<td>200-300 BID-</td>
<td>50</td>
<td>12% reduction in HAM-D</td>
</tr>
<tr>
<td>Van Praag et al., 1972</td>
<td>10</td>
<td>Severely depressed</td>
<td>5-HTP vs placebo</td>
<td>200-3000</td>
<td>21</td>
<td>34% reduction in HAM-D 3/5 improved on 5-HTP</td>
</tr>
<tr>
<td>Van Praag, 1984</td>
<td>15 in HTP group</td>
<td>Unipolar and Bipolar</td>
<td>5-HTP vs. tryptophan vs. placebo</td>
<td>200</td>
<td>28</td>
<td>67% reduction in HAM-D</td>
</tr>
</tbody>
</table>
## Open-label trials of 5-HTP for depression

<table>
<thead>
<tr>
<th>Authors/year</th>
<th>N</th>
<th>Patients</th>
<th>Dose (mg/day)</th>
<th>Length (days)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujiwara &amp; Otsuki, 1973</td>
<td>20</td>
<td>Endogenous depression</td>
<td>50-200</td>
<td>7-28</td>
<td>10/20 Markedly improved</td>
</tr>
<tr>
<td>Kaneko et al., 1979</td>
<td>18</td>
<td>12: Unipolar; 3: bipolar; 3: first episode</td>
<td>150-300</td>
<td>1</td>
<td>10/20 improved</td>
</tr>
<tr>
<td>Kline &amp; Sacks, 1963</td>
<td>50</td>
<td>20: Classical depression; 30: schizoaffective</td>
<td>10–50 mg/ Dose i.v. single dose</td>
<td>1</td>
<td>18/20 Typical depression marked improvement, 1 partial 7/30 Schizoaffective, marked 15/30 Partial</td>
</tr>
<tr>
<td>Kline et al., 1964</td>
<td>59</td>
<td>Depressive element</td>
<td>DB, 5-HTP vs. 5-HTP 100–200 i.v</td>
<td>1</td>
<td>11/31 marked; 6/31 partial;14/31 no change</td>
</tr>
<tr>
<td>Matussek et al., 1974</td>
<td>23</td>
<td>21: Unipolar; 1: bipolar; 1: schizoaffective</td>
<td>300 TID</td>
<td>14</td>
<td>7/23 Good improvement or symptom-free</td>
</tr>
<tr>
<td>Sano, 1972</td>
<td>107</td>
<td>Endogenous depression</td>
<td>50-300</td>
<td>3-35</td>
<td>74/107 Markedly improved</td>
</tr>
<tr>
<td>Zmilacher et al., 1988</td>
<td>25</td>
<td>Depressed</td>
<td>300</td>
<td>14</td>
<td>13/25 Very good or good improvement No difference between groups</td>
</tr>
</tbody>
</table>
2.6.7.7. Animal models of Depression

Animal models of depression are research tools built to relate depression and action of antidepressants that may be as a simulation to investigate the symptomatology and pathophysiology of depressive illness or used to screen novel antidepressants (http://en.wikipedia.org/wiki/file:animal model of depression.jpg). The forced swim test and tail suspension test are perhaps the most validated (Cryan et al., 2005) and is sometimes also used as a putative rodent model of depression. Either FST or TST is an aversive stressful situation that generates behavioral despair, i.e., immobility.

2.6.7.7.1. Behavioral despair

Behavioral despair might be assessed with tests such as the forced-swimming test or the tail suspension test (Hasler, et al., 2004).

2.6.7.7.1.1. Forced-swimming test (FST)

Forced Swimming Test is based on the observation that animals develop an immobile posture in an inescapable cylinder filled with water. In this paradigm, immobility is interpreted as a passive stress-coping strategy or depression-like behavior (behavioral despair). After antidepressant administration, the animals will actively perform escape-directed behaviors with longer duration than animals with control saline treatment. FST is the most widely used tool in depression research, more specifically as a screen for acute antidepressants (Benoit et al., 2005).

2.6.7.7.1.2. Tail suspension test (TST)

The TST, also known as tail suspension test, shares a common theoretical basis and behavioral measure with the FST. In the TST, mice are suspended by their tails using adhesive tape to a horizontal bar for a certain couple of minutes, and the time of immobility is recorded. Typically, the suspended rodents perform immediately escape-like behaviors, followed by developing an immobile posture. If antidepressants are given prior to the test, the subjects will be engaged in escape-directed behaviors for longer periods of time than after saline treatment, exhibiting a decrease in duration of immobility. A major advantage of the TST is that it is simple and inexpensive. A major disadvantage of the TST is that it is restricted to mice and limited to strains that do not tend to climb their tail. Besides, like FST, TST is sensitive to acute treatment only, and its validity for non-monoamine antidepressants is uncertain (Cryan et al., 2005).
Umadevi, et al., (2010) revealed that, aqueous and alcoholic extract of *Cucurbita pepo* produced significant antidepressant like effect in mice in Forced Swim Test (FST) and its efficacy was found to be similar to imipramine. This test is quite sensitive and relatively specific to all major classes of antidepressant drugs. In FST, rats are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. The results show that *C. pepo* seed extracts can decrease immobility time in forced swim test. It is found that *C. pepo* can produce antidepressant like activity at a dose of 100mg/kg body weight after 30 days of treatment. The decrease in the immobility time is accompanied with the increase in swimming time. The precise mechanisms by which *C. pepo* extracts may produce antidepressant like effect are not completely understood.

2.6.8. Pumpkin seeds for Hypercholesterolemia

Hypercholesterolemia is a major cause of cardiovascular disease (CVD), such as atherosclerosis and coronary heart disease. CVD is the most common cause of mortality and morbidity worldwide. Hyperlipidemia refers to either increased serum total cholesterol level or serum triglyceride level or both. Hyperlipidemia or hypercholesterolemia leads to the development of atherosclerosis and later leads to the progression of Coronary Heart Disease (CHD), which will cause cardiovascular morbidity and mortality (Yokozawa, et al., 2003). Although several factors, such as cigarette smoking, high-fat diet, high blood pressure, physical inactivity, age, and heredity have significant roles in causing CVD, high blood cholesterol is mainly responsible for the onset of CVD (Yokozawa et al., 2003). Lowering serum cholesterol levels by drug or dietary interventions could reduce the risk of CVD. Therefore, it is worthwhile to develop new safe and effective cholesterol-lowering agents from natural products.

2.6.8.1. Prevalence of hypercholesterolemia

The Indian Council of Medical Research (ICMR, 2004) surveillance project reported that a prevalence of hypercholesterolemia (defined as a ratio of total to HDL cholesterol >45) of 37.5per cent among adults aged 15-64 years, with an even higher prevalence of hypercholesterolemia (62%) among young male industrial workers.
According to Kasliwal et al., (2003), prevalence of hypercholesterolemia in India showed that hypercholesterolemia presentation 85.6%-23.3% had elevated LDL (>100g/dl), 72.5% had low HDL and 37.01% had elevated triglycerides. Even when 130mg/dl was used as cut-off to define high LDL, 81.4% were found to be having hypercholesterolemia.

The ICMR/WHO study on non communicative disease risk factors estimated that there are 8.72 million hypertensive subjects in Kerala. About 52.1% of male and 61.4% of female populations have a total cholesterol of >200mg/dl. The average cholesterol level in any given segments of the Kerala state’s population was found to be 225mg (desirable level is 180mg) (The Hindu, 2005).

Epidemiologists in India and international agencies such as the World Health Organization (WHO) have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India, with 2.6 million Indians predicted to die due to CVD (Goenka, 2009: Reddy, 2006).

Basu and Datta (2001) assessed the lipid profile and atherogenic factors in 75 adult male (31 to 60 years) in Calcutta and reported that out of 75 subjects, 41 had elevated total cholesterol and triglyceride levels. Also, the elevated blood lipid profile vs. body weight values, age-group wise indicated that number of subjects with normal body weight and elevated blood lipid gradually decreased as the age decreased. almost 55% of the adult subjects in the study were at risk of CVD with hyperlipidemia.

Mishra et al., (2002) studied the association of anthropometric profile with hyperlipidemia in 50 non-obese Asian Indian male (Body Mass Index- BMI <25 kg/m2) and compared with that of normolipidemic male (n=50; BMI < 25 kg/m2). Results revealed that BMI, waist circumference, Waist Hip Ratio (WHR), skin folds and percentage of body fat were significantly higher in hyperlipidemic subjects as compared to normolipidemic controls. Hence Asian Indian male, defined as “non-obese” based on BMI, had adverse profile of anthropometric parameters compared to normolipidemic male.

2.6.8.2. **Studies on role of Pumpkin seed in Hypercholesterolemia**

Pumpkin seed contain phytosterol, which help for reduce hypercholesterolemia.
Styrian oil pumpkin seeds contain about 1.5–1.9 mg/g dry seeds of phytosterols which become enriched in the corresponding styrian pumpkin seed oils (3.5–4.0 mg/g oil) (Murkovic et al., 2004). Grundy, (2005) stated that reduction of LDLC obtained by phytosterols and stanols in doses of 0.7 g/day to 2.5 g/day ranges from 6.7% to 11.3%.

Many plant-derived homologs of cholesterol (phytosterols and phytostanols) have been reported to have a cholesterol-lowering effect. Several studies have investigated the cholesterol-lowering mechanisms of phytosterols, which were reviewed only recently (Marinangeli et al., 2006; Moruisi, et al., 2006: Ostlund, 2006). Dietary uptake of phytosterols appears to be as important in cholesterol lowering as reduction in consumption of saturated fats. Since phytosterols are in the seeds of the Styrian oil pumpkin as well as in the corresponding pumpkin seed oils, it can be speculated that these components together with the high content of linoleic acid can exert beneficial health effects in lipid-associated disorders like atherosclerosis.

In a study involving 30 male rats, consumption of a diet enriched with flaxseeds and pumpkin seeds was found to exert anti-atherogenic and hepatoprotective properties. Increases in PUFAs (ALA and LA) and MUFAs (oleic and eicosaneoic acid) and decreases in SFA (palmitic and stearic acids), decreases in levels of malondialdehyde in plasma and liver and improvements in antioxidant defense system were found. Rats fed the 1% cholesterol diet without flax and pumpkin seeds were found to have lipid storage in hepatocytes - this was found to be improved in rats fed flax and pumpkin seeds (Makni et al., 2008).

Al Zuhair et al., (2000) reported that dietary induced hypercholesterolemia in rabbits pumpkin seed oil (40 mg/kg body weight) given together with simvastatin for three weeks significantly increased aortic contractile response to norepinephrine and prevented elevated activity of serum Alanine Amino Transferase (ALAT), Aspartate Amino Transferase (ASAT) and Creatine Phosphokinase (CPK). The administration of simvastatin as solitary treatment induced a significant increase of activity of ALAT, ASAT and CPK. This experiment exhibited the potentiation of the antihypercholesterolemic effect of statins with a possible future application to decrease their therapeutic dose together with a reduction of the number of side effects.
Cerqueira et al., (2008) reported in their study, four groups of twenty recently weaned male Wistar rats received for 10 days commercial whole, sifted (sieve 0.5 mm) and residual pumpkin seed flour to maintain the rate of 30% of the total starch and dextrin in the control group. Blood samples have been evaluated in 48 hours intervals to measure cholesterol, triglycerides and glucose levels. The ingestion and animals’ growth were similar in all groups along experiment. Glucose levels were significantly decreased in group treated of whole, triglycerides in group receiving sifted pumpkin flour.

2.6.8.3. Phytosterols as cholesterol-lowering agents

The phytosterols (including sitosterol, campesterol, and bressicasterol) and their saturated derivatives, the stenols, are naturally occurring plant derivatives. Stanols are less abundant in nature. The primary sources of phytosterols are vegetables, nuts, fruits and seeds. The amounts of the plant sterols present in diets are recommended on the treatment of hypercholesterolemia. In order to use the claim, the food product must contain at least 0.65 grams of vegetable oil sterol esters per serving for a total of 1.3 g/day (at two servings), or 1.7 grams of plant stanol esters per serving for a daily total of 3.4 grams (Watkin, 2002).

Yankah and Jone, (2001) stated that 0.74g/day soybean phytosterols mixed in butter (36% fat, 0.4g/day) lowered total cholesterol by 10 and 15%, respectively in male after 4 weeks. In hypercholesterolemic male 0.6-0.8g/day of phytosterols has been shown to reduce the same components by 4 and 7%, respectively, over 9 weeks.

Phytosterols are a natural mixture of sterols containing a minimum 70% β-sitosterol. They differ from cholesterol by the presence of an extra methyl or ethyl group on the cholesterol side chain at the C-24 position. The planar sterol structure with a hydroxyl group and a hydrophobic tail is characteristic for the molecular structure. Over 200 different types of phytosterols have been identified, of which β-sitosterol; campesterol and stigmasterol are the major dietary sterols (Moreau, 2002). Phytostanols are saturated phytosterols, that is, they have no double bonds in the sterol ring.

Phytosterol or phytostanol intake from functional foods (e.g. bread spreads) is usually 1.5-3g/day. Phytosterol and phytostanol products reduce the serum concentration of total cholesterol by up to 15% and that of LDL cholesterol by up to 22% (Ostlund, 2002; Christiansen et al., 2001). Cholesterol derives the intestinal tract from two major
sources. A normal western diet provides 300-600 mg cholesterol per day to the intestine and an additional 1000-1500 mg/day is derived from endogenous sources, mostly from the bile (Trautwein et al., 2003).

**Molecular structure of some phytosterol, phytostanols and a fatty acid ester** (Gilbert et al., 2005).

Phytosterols and especially sterols from the kernels oils: such as olive and nuts (walnut), wheat and pumpkin are investigated in reduction of risks in the cardiovascular disease and might lower the risk of coronary artery disease when integrated into diet that is low in saturated fat and cholesterol. Mechanisms of action of plant sterols in cholesterol absorption are observed. Low plant sterols diet also is implicated in a host of emotional and behavioral problems, including depression. The consumption of the low energy lipid products such as different margarine is useful in the hypercholesterolemic therapy (Maki, et al., 2001).
Devaraj et al., (2006) added plant sterols (1 g/240 ml) to a reduced calorie orange juice beverage to study the effect of plant sterols on cholesterol, C-reactive protein and lipid levels. The reduced calorie beverage (with and without sterols) was fed to 72 subjects two times per day with meals for eight weeks. Supplementation with sterols decreased total and LDL cholesterol (5% and 9%) compared to baseline and also lowered C-reactive protein levels (12%). There were no significant changes in triglycerides, plasma vitamin E and carotenoids.

**Clinical studies with Phytosterols**

<table>
<thead>
<tr>
<th>Phytosterol (carrier)</th>
<th>Dose (g/day)</th>
<th>Time (week)</th>
<th>Changes in LDL-C (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterol ester</td>
<td>1.8</td>
<td>3</td>
<td>-6.5</td>
<td>Mussner et al., 2002</td>
</tr>
<tr>
<td>Sterol (butter)</td>
<td>1.8</td>
<td>3</td>
<td>-11.3</td>
<td>Vanstone et al., 2002</td>
</tr>
<tr>
<td>Sterol (margarine)</td>
<td>1.5</td>
<td>26</td>
<td>-11.6</td>
<td>Christiansen et al., 2001</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterol (butter)</td>
<td>1.0</td>
<td>4</td>
<td>-6.2</td>
<td>Volpe et al., 2001</td>
</tr>
<tr>
<td>Sterol ester (fat spread)</td>
<td>2.5</td>
<td>8</td>
<td>-10.0</td>
<td>Neil et al., 2001</td>
</tr>
<tr>
<td>Sterol ester (butter)</td>
<td>2.4</td>
<td>4</td>
<td>-12.2</td>
<td>Nestel et al., 2001</td>
</tr>
<tr>
<td>Sterol ester (butter)</td>
<td>2.4</td>
<td>4</td>
<td>-12.2</td>
<td>Nestel et al., 2001</td>
</tr>
</tbody>
</table>

A randomized, double-blind, placebo controlled, 3 group parallel design study of 224 subjects with an initial LDL cholesterol level between 3.4 and 5.2 mmol/l over a 5 week study period. Subjects followed a low fat Step I diet from the National Cholesterol Education Programme (NCEP). The groups received 14g of reduced-fat spread either enriched with a low dose plant sterol esters (1.1 g plant sterols/day) or a higher dose (2.2 g plant sterols/day), or the control spread. Subjects in the low- and high-sterol groups who consumed 80% of the scheduled servings had total cholesterol reductions of 5.2% and 6.6%, and LDL-cholesterol reductions of 7.6% and 8.1% respectively. This study shows that a reduced-fat spread containing plant sterol esters incorporated into a low fat
NCEP diet is a beneficial adjunct in the dietary management of hypercholesterolemia (Maki et al., 2001).

Scholle et al., (2009) in this meta-analysis, the effect of phytosterols on lipid parameters was investigated in hypercholesterolemic patients on statin therapy. Eight studies were eligible for inclusion: 5 parallel trials and 3 crossover trials with plant sterols/stanol doses ranging between 1.7 and 6.0 g/d. In the presence of statins, the use of plant sterols/stanols significantly lowered total cholesterol by -0.36mmol/L and LDL-C by -0.34mmol/L (-8.4%). HDL-C and TG were not significantly affected. Similar effects on blood lipids were found for plant sterols vs. plant stanols and for presence of diet medication vs. no diet medication. It was concluded that phytosterols have an additive beneficial effect on TC and LDL-C on top of statins.