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Assessment of quality variation on germination of chick pea in probiotic solution: A review

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Abstract
The thesis is based on the investigations made on the qualitative analysis of chickpea seeds. The chickpea seeds are part of the Leguminosae family that is basically adapted to the category of pulses. The introductory discussion is also made on the probiotics and their useful effects since chickpea seeds also contribute to their proportion to it. Moreover, legumes and their germination are also considered an important part of this project work. The germination is basically linked with the amount of protein extraction from chickpea seeds and other legumes. Also, other cooking treatment methods such as pressurized cooking, boiling, microwave cooking, etc. affect the qualitative geometry of protein concentration from chickpea seeds. Various methods of cooking treatments affect chickpea in a different manner and the conditions that support the better extraction and consumption of protein from chickpea have been discussed. The different forms of chickpea that are used as the raw material in different categories of foods have been discussed in the thesis. This helps us to fulfill the requirement of protein without being dependent on other sources such as meat for its fulfillment. A major part of chickpea is generally not suitable for human consumption and therefore, it can be used as a source of food for animals. Ch chickpea's composition helps us know where it can be used in the best way, so the requirement of amino acids essential for a living being can be fulfilled.

Keywords: Chickpea, probiotics, health benefits, nutritional composition

1. Introduction

From earlier times, we have been using biological means in order to enhance the quality of food as well as to prepare new food products. The Romans and Greeks were well recognized for the use of fermented products and cheese, who also recommended for their consumption (Gismondo *et al*., 1999) [27]. The preparation of curd for our consumption has been a part of our daily routine for centuries. Bacteria, not only help with the formation of curd but also, are very helpful for our gut health. Many scientific conclusions had been made to establish the relationship between these types of biotic reagents and human health, more clearly in the branch of science dealing with 'Probiotics' (B Mombelli *et al*., 2000) [39]. From earlier times, we have been using probiotics in one or the other forms for our benefit. Based on the history of our early life, we are quite aware of the intake of yogurt or buttermilk that we used to take when we had any stomach-related problems like irregular bowel movements, constipation, indigestion, dysentery problems, etc. This ritual has been thriving for many years, more clearly, our ancestors started it as a form of cure for the problems discussed, maybe very ago (R Fuller, 1992) [23].

In 1917, during an outbreak of the disease *shigellosis*, Alfred Nissle, a German professor, obtained a bacterial strain of *E.coli* from the faces of a soldier who did not show any symptoms of the disease in spite of being targeted by the *shigella* bacteria. Probiotics have also emerged as a potent source for medical therapies in gastrointestinal and non-gastrointestinal diseases such as diarrhea, constipation, irritable bowel syndrome, inflammatory bowel syndrome, asthma, atopic dermatitis, peptic ulcer, colon cancer, coronary heart disease, and urinary tract infections (Syngai *et al*., 2016) [63].

Economically, if we observe, then, the consumption of functional food products has risen from $33 billion in 2000 to $176.7 billion in 2013. If we analyze the data, the growth in the economy of functional food has achieved a market of $143.7 in just an interval of 13 years which creates a tremendous impact on the global economy. The major products that contributed to the boost in the economy of functional foods are dairy products containing probiotic bacteria such as cheese, buttermilk, ice cream, flavored milk, fermented milk, infant food, and whey-based beverages (CE Hoesl *et al*., 2005) [28].

When there comes something new and beneficial in the scientific community, researchers of different nations start binding up all the findings and research in order to develop its role. It is done in order to take benefits from all possible ways they can extract so that the growth of the nation takes place as well as the growth of the people globally (C Hill *et al*., 2014) [30]. Similar is the case regarding the probiotics, where, related field investigations are being carried out in Asian, North American, Australian and European countries in order to provide a sound body with cheap and best healthful diet of the people of their respective nations. These steps from the scientists of different parts of the world are trying to provide satisfaction for the required diet (Salminen S *et al*., 1998) [61].

E.coli or Escherichia coli is one of the most abundant species of bacteria that is found in the large intestine of humans which are also responsible for the vitamin production also (Jean Guy LeBlanc *et al*., 2012). These vitamins are generally vitamin K and vitamin B. After production, these vitamins are directly absorbed into the blood. The amount of vitamins produced by these bacteria is quite low, but these vitamins produce is really helpful in the case when these vitamins are not being taken in a proper amount through diet (AE Axelrod, 1971) [3]. If an individual just depends on these intestinal bacteria for the vitamin demand, individual becomes vitamin deficient and many health problems start arising. One such example can be seen in the case related to disorders of blood clotting. Since, vitamin K is very essential for blood clotting, therefore, lack of vitamin K may lead to blood clotting disorders. The condition may be fatal in few cases when the wound or cut is very deep and blood clotting does not take place. This will lead to excessive loss of blood from body. If somehow, other symptoms occurs in response to deficiency of vitamins that are synthesised by the large intestine and antibiotics are taken to reduce the symptoms and these antibiotics can even kill the good bacteria also which can lead to more severe condition when an individual is wholly dependent on the intestinal bacteria for vitamin production (L Maier *et al*., 2018) [40].

Antibodies are synthesised and produced by special types of cells, that are, T-cells and B-cells (DN Posnett *et al*., 1988) [46]. Sometimes, these cells are also called T-lymphocytes and B-lymphocytes as these cells are a kind of lymphocytes. Lymphocytes are generally responsible for fighting mechanism of body with several diseases and infections. Since, we know that our large intestine is a home to different types of species of bacteria; therefore, these are also treated as foreign bodies for our body. In response to this, our body's immune system releases antibodies (B Dugas *et al*., 1999) [21]. These antibodies are beneficial in the case if we get infected from other bacterial infections. Antibodies released are helpful to kill other pathogens which are harmful for our body. Therefore, we can conclude that intestinal flora is also responsible for our immunity against several other pathogenic infections. Taking probiotics intake with our regular diet is able to increase the capability of our body against pathogenic infections naturally without any use of antibiotics which may show some side effects also in most of cases.

Lactic Acid Bacteria (LAB) and bifidobacteria are manufactured on a commercial level to compensate for the demand of customers for probiotic dietary supplements. The probiotic supplement that is being produced commercially must have the highest possible yield, stability and consistent performance for the intended application. It should be stable with the environmental conditions such as humidity, temperature, and pressure with rapid action without any significant delay (F Abe *et al*., 1995) [1]. The basic machinery for production of LAB and bifidobacteria is quite similar involving the following processes in the sequential order. Frozen seed stock, which can be regarded as the producers of progenies consist of a single pure strain that is to be cultured (L De Vyust *et al*., 2007). The pure strain is checked by Quality and Control (QC) department to counter any contamination if present in the colony, so that the cells are not at a risk for genetic drift.

As discussed earlier, the process involving the production of probiotic cells are significantly accompanied with the use of bioreactors for the large scale commercial production (SY Wu *et al*., 2006). The bioreactors or fermentation vessels are specially designed for the growth of microbes in them at commercial level. Even after fulfilling the primary requirement, i.e. fermentation vessels, it is very important to maintain particular conditions in them so that there is no lag in the biomass production. These conditions include optimum temperatures, pressures and pH levels essentially (G Liden, 2002) [37]. These conditions are different for different types of probiotic cells growth. The process of freezing is done in order to check the metabolic activities of the probiotic culture, so that the cells can be used for other purposes later on. The process of freezing however, can lead to the damage of the cells. In order to ensure their longer lifespan, these cells after preparations are added with some stabiliser solutions as discussed earlier (MW Townsend *et al*., 1988) [64].

The legumes are the plants that produce a pod with seeds inside them. The term 'legume' is used to describe the seeds of these plants. The common varieties of legumes those are edible are lentils, peas, chickpeas, beans, soybeans, and peanuts. The different types of legume differ greatly in their nutrition values, structure and shape appearance, taste as well as their significant use for dietary purposes. The presence of both protein and starch in correct proportions, along with fibre, vitamins and micronutrients, has made the research over legumes to look after them (Duranti, 2006) [20]. The nutritive values of pulses can also further be improved by physically fractionating the grain into basic constituents such as protein, starch and fibre. These improved food substances may further be used for improving the nutritive value of other food products by adding into them (Rochfort *et al*., 2007) [47].

The researched have also been made to identify several properties of pulses that work efficiently in health problems. Recent studies have demonstrated that many anti-nutrients present in pulses may be beneficial, when taken on a regular basis in the diet, to prevent diseases like cancer and coronary diseases (Champ, 2002) [11]. Legumes are known to possess a large number of compounds that are in biologically active state. They can be proteins, glycosides, tannins, saponins, or alkaloids (Muzquiz, 2000) [41]. The methods of extracting these bioactive compounds vary greatly based on their source and nature (Pedrosa *et al*., 2012) [45]. Current studies shows that the balance between harmful and beneficial effects of different compounds obtained from legumes or pulses significantly depends on their chemical structure, concentration, time of exposure and their interaction with other dietary substances (Balkazar *et al*., 2007) [6]. The role of nutrients is very important in a regular diet, as they must be balanced in order to fulfil our body needs completely. This concept is already highlighted by the experts of FAO and

WHO (Choudhary *et al*., 2004). The concept basically includes the following major requirement as; protein must not exceed 15% of the total calories, carbohydrates 60%, fat 25%, comprising 75% of plant foods and 25% animal foods. Dietary fibre in legumes have been successfully proved its health related functions in the prevention of risks of chronic diseases such as cancer, CVD and diabetes mellitus (disorder with excess amount of urination and very less absorption of water by the kidneys). It comes from the family of carbohydrates, not digested in the small intestine but may be fermented in the colon into acetate, propionate and butyrate (Roberfroid, 1997) [48]. They enhance water absorption in the colon, and thus prevent constipation. Propionate has been shown to inhibit the activity of the enzyme hydroxyl-3-methylgutaryl-CoA reductase, the limiting enzyme for cholesterol synthesis (Chen *et al*., 1984) [14]**.** Dietary fibre's viscous and fibrous structure can control the release of glucose with time in the blood, thus helping in the proper control and management of diabetes mellitus as well as obesity in different individuals (Jenkins *et al*., 1982) [32].

Chickpea was found to be originated in the location of present-day southeastern Turkey and its neighbor Syria. The cultivated chickpea has its scientific name as *Cicer arietinum*. The three varieties have been found in the originating place as *C. echinospermum*, *C. Bijugum*and *C. reticulatum*. Among these, the species described at last is known to be the ancestor of other two. Further there have been indications that the large seeded, cream coloured chickpea reached India only two centuries ago, apparently through Afghanistan, as its Hindi name is *Kabuli chana*, because the capital of Afghanistan is Kabul. The small seeded, dark coloured chickpea is called *Desi*, and these two names are popularly used in India to distinguish these two varieties of chickpeas (LJG Van der Maesen, 1987) [71]. The production of plant protein has a very high demand among the industries because of the vast application of plant isolated proteins in food as well as non-food markets. The European Union is also trying to develop its own plant based protein market in order to avoid any dependency of proteins form any other source or other countries based on commercial treaty (Chominot, 1992) [15].

The chickpea seeds are generally used for human consumption for its quality of providing large amount of protein. But, during the cultivation of chickpea, about 20% of its harvest is damaged and cannot be used for human consumption. Therefore, this important percentage of damaged production harvest is usually used for feeding the livestock because it is considered as a by-product and therefore, sold at very cheap prices (Ulloa *et al*. 1988) [68]. The protein quality of chickpea has been found very close and equivalent to that of soybean meal (Friedman, 1996) [24]. Also, the chickpea seeds have been found to contain several anti-nutritional factors that decrease the potential for protein utilisation (Singh, 1988) [59]. Most of these compounds or factors are responsible for inhibiting the digestive enzymes of our gut or they sometimes react with essential amino acids thereby limiting the application of the whole content of the chickpea seeds in many food products directly. This problem can be checked if the chickpea proteins are isolated from inhibitory anti-nutritional factors or compounds which are responsible for its side-effects. The studies have been established among two types of its isolated forms as Isolate-A and Isolate-B.

Isolate-A is easier and cheaper to prepare than Isolate-B because Isolate-A is obtained by the extraction of the proteins without using sodium sulphite. Also, it does not make the use of either ethanol or acetone for washing. However, Isolate-B has various benefits that are, milder extraction conditions, and sodium sulphite inhibits polyphenol oxidation thereby avoiding the further reaction between proteins and oxidised polyphenols (Cheftel *et al*., 1989) [16]. Solubility of chickpea proteins that were extracted in the isolated form were, 80.9% for Isolate-A and 87.1% for Isolate-B. Moreover, the precipitation at the pI of the extracted proteins led to the losses of those that are soluble at pH 4.3. These soluble proteins were especially known to be as albumins (Berot *et al*., 1996) [17].

Seed germination can be defined as a process by which an organism (plant) grows from a seed or a similar type of structure. The basic example can be visualised as the sprouting of seedling from a seed of an angiosperm or gymnosperm. Germination also includes the growth of a sporeling from a structure that is similar to a seed known as the spore. The spore formation can be seen in the Fungus, ferns, bacteria, etc. The basic needs of seed germination are fulfilled by the endosperm present inside the seed which is also responsible for its nutrition. When the seed absorbs water, hydrolytic enzymes are activated which breakdown these stored food resources into metabolically useful chemicals (Raven *et al*., 2005) [49]. Even the presence of endosperm is not sufficient for the seed germination, along with it many basic nutrients are also needed for the seed germination process. Oxygen is one of the most important nutrients which is required for metabolic processes in the seed germination (Siegel *et al*., 1962) [60].

The seed germination finally leads to the formation of shoot from a region called plumule and root from the region called the radical. The angiosperms are the plant types which on their seed germination give rise to a plant that will bear flowers on them. The other type called the gymnosperms bear no flowers. Favourable temperature conditions are also necessary for the germination of seed. Some seeds are germinated in the range of 16-24 C, while some at very cold temperature in between the range of -2 to 4 C. Cold stratification is the process that is responsible for inducing dormancy breaking before light emission thereby promoting germination (Baskin *et al*., 2014) [72]. The presence of light or its absence can also play a significant role in the seed germination process. Most seeds are not affected by light or darkness, but many seeds need direct light exposure in order to facilitate the germination process (Raven *et al*., 2005) [49].

The seed germination process of chickpea, also possess its germination conditions that are very important for its optimum growth after germination. Chickpea is a cool season annual crop which is optimally grown in between the 70 to 80 F temperature range. They have tap root system therefore they are good in yielding with drier conditions, optimally less than 30mm of rainfall. Chickpea are best suited with fertile sandy, loam soils with internal drainage. Drainage is very important because even short periods of flooded or waterlogged fields reduce its growth (E.S. Oplinger *et al*., 1990). The germination of chickpea is very helpful in maintaining the protein digestibility, although at a lower level than the cooking. Germination helps to degrade proteins to simple peptides, thereby improving its raw protein content. Also, germination is also responsible for decreasing the percentage of lysine, tryptophan, sulphur and aromatic amino acids, but most contents are still higher than proposed by FAO/WHO reference patterns (El-Adawy *et al*., 2002). In some parts of the world, young chickpea leaves are also consumed in the form of cooked green vegetables. Chickpea leaves are very nutritive in nature since these leaves have a significantly higher mineral content than spinach leaves (Ibrikci *et al*., 2003) [31].

2. Biochemical Composition of Chickpea

2.1.1 Effect of cooking and germination on Nutritional Composition of Chickpea

As already discussed, chickpeas have been found to contain low nutritive value. This is due to the fact that the amount of sulphur-containing amino acids is quite less. Also, nutritive value decreases due to the presence of anti-nutritional factors because it is responsible for decreasing the digestibility of the protein content. Therefore, methods such as, cooking is performed in order to remove or decrease these damaging factors which are responsible for reduced protein consumption. Generally, heating is done to inactivate the anti-nutritional factors present along with the protein content in the chickpeas. The process of cooking is performed on the chickpeas before consumption for humans (Vijayakumari P *et al*., 1998) [69]. However, cooking also causes a deficient in the considerable loss in the concentration of soluble solids, generally which contains vitamins and minerals. Similarly, the process of germination can also be carried in order to reduce concentration of anti-nutritional factors as well as other undigestable factors that inhibit the performance of other beneficial components such as the protein content (Barampama Z *et al*., 1995) [7].

In Egypt, the consumption of chickpea seeds is usually considered to be in its raw form or the green tender form which is the unripe stage of chickpea. The chickpea at this stage is termed as Malana. Also, they consume it in its mature seeds form after drying them. In addition to these forms, chickpea seeds are converted to make them into the flour which is used as a basic ingredient in various dishes. It may be used in biscuits bread or food mixes (Van der Maesn *et al*., 1972) [70]. The quality and quantity of heating affects much when we talk for the nutritive value of the chickpeas. Therefore, considerable and optimum heating is required to isolate the beneficial components of the chickpea for consumption. More or less heating is undesirable as it either promotes an increase or decrease in the undigestable factors. It has already been noticed in an experiment that increasing the duration and temperature of cooking can reduce the nutritive value and the availability of amino acids such as lysine in the chickpeas (Kon S *et al*., 1981) [33].

When we take the process of cooking using microwave radiations, the case has not been studied so extensively yet. But, many indirectly related studies have showed that, the anti-nutritional factors as well as the undigestable factors have been found to be reduced after conducting the process of microwave heating on the chickpeas. This has also been in agreement with other legumes also. Doing this therefore can increase the intake for the protein content in the chickpea. A study on chickpeas which are cooked by microwave has the uncertainty that they would be helpful in increasing the nutritive value of the chickpeas or not and also would they be able to reduce the content of anti-nutritional factors or not. Also, the process of microwave heating is somewhat difficult and consumes a greater amount of energy. Due to this reason, the practice of microwave heating was discarded when other methods such as cooking (heat treatment) and germination are already available. Moreover, these are easier methods (Chau *et al*., 1997) [19].

Different experimental methods were applied on the chickpea seeds for reducing the concentration of anti-nutritional factors so that the nutritive value can be enhanced. The methods that were performed on different samples gave a rough estimation for the efficiency of different methods. The methods included; germination, cooking treatments, boiling, autoclaving, microwave cooking. After treating chickpeas with the above methods, chemical composition of the chickpeas was determined. This was done so as to compare the chemical composition of the samples after treatment with different methods so that they can be compared with their initial form or the raw form. In this way, observation can be made for the nutritive values and conclusion can be made for different methods to know their efficiency with regard to the process applied. The tests that were conducted to know the chemical composition mainly included those components which were truly essential for making a comparison with the raw form of chickpeas. The tests were performed for the B-vitamins, minerals, anti-nutritional factors, amino acids, energy production values. Moreover, the value for *in vitro* protein digestibility was also recorded (Tarek *et al*., 2000) [65].

The main points that were made out of testing and observation to give a conclusion out were tabulated. The cooking treatments significantly decreased the non-protein nitrogen, ash and fat contents. This reduction in the contents could be due to the fact that contents might have diffused into the cooking water because of the solute concentration gradient setup between the internal and external environment of the chickpea. Crude fibre was significantly increased by cooking treatments. This increment could be due to the formation of several protein-fibre complexes during the process of cooking (Bressani, 1993) [10]. Moreover, germination process led to the increase in the crude protein content, non-protein nitrogen and crude fibre when it was compared to the raw seeds. This increment could be mainly due to the use of seed components and rupture of complex proteins into simpler form which may be in the form of peptides. However, germination process was also responsible for the decrease in the total fat content as well as total carbohydrate content in the chickpea. This decrement of fats and carbohydrates could be attributed to their use as an energy source for stating the process of germination (El-Beltagy *et al*., 1996) [22].

In reference to the carbohydrate contents, raffinose, stachyose, and verbascose were completed diminished in the case when chickpea seeds were allowed for germination process. The process of germination significantly decreased the concentration of reducing sugars such as starch and sucrose by 28.57% and 25.77%, respectively. These losses in the levels of reducing sugars could be assumed for the fact, due to hydrolysis of these components by hydrolytic enzymes to their respective monosaccharides, which may be the essential source of energy for the germination process. Similar results were also observed in the case of soybean sprouts (Bau *et al*., 1997) [8]. In the context of cooking treatments, it was observed that reducing sugars, sucrose, raffinose and stachyose were significantly reduced but, concentrations of verbascose were completely negligible. These reductions could be attributed to the fact that these components might have diffused into the cooking water.

Moreover, the process of germination was more effective in the reduction of oligosaccharides, basically raffinose and stachyose, than the process of cooking treatments when compared with the concentration in the raw chickpea seeds.

When talking about anti-nutritional factors, the activity of trypsin, an inhibitor enzyme, was significantly reduced by both the processes, that is, by germination as well as by the cooking. The highest decrement was observed after the autoclaving process which was 83.67%. The boiling process followed the process of autoclaving in which the reduction was nearly about 82.27% which was followed by the microwave cooking and germination processes in which, the reductions were 80.50% and 33.9% respectively. For soybeans, the trypsin inhibitor activity was decreased about 12% when the germination process was carried out for the 12 days (Chandrasiri *et al*., 1987) [12]. Haemagglutinin activity was completely inhibited by cooking and was significantly reduced in the case of germination. The reduction in the case of germination was 77%. In case of faba beans seeds, the method of boiling and autoclaving completely eliminated the activity of haemagglutinin (Khalil *et al*., 1995) [34]. Tannins, phytic acid and saponins in chickpeas were reduced to a larger extent when method of cooking was applied. Moreover, the process of germination was less effective than cooking treatments in the reduction of tannins and saponins, but it was more effective in reducing the concentration of phytic acid (Vijayakumari, *et al*., 1998) [69].

In the context of vitamins, if we observe, then concentration of vitamins such as riboflavin, thiamine, niacin and pyridoxine in chickpea seeds were significantly decreased by the process of cooking. These losses in the concentration can be characterised due to the combination of leaching and chemical destruction. The losses in the concentration of such vitamins when observed for the sample treated with microwave cooking were smaller when compared with the process of boiling and autoclaving. This improvement in vitamin sustainability in case of microwave cooking could be attributed to the reason that the microwave cooking is performed for a shorter period of time when compared to the other heating methods. The nature of vitamins to show their removal from the cooking process can be visualised in a descending order as: pyridoxine, riboflavin, thiamine and niacin (Salama *et al*., 1997) [55]. The process of boiling resulted in a larger loss for each of the vitamins when compared to the other cooking methods. Also, the conventional methods of cooking leads to a greater loss of vitamins such as thiamine, riboflavin and ascorbic acid in case of vegetables, but microwave cooking and autoclaving methods improved the retention of vitamins when compared to boiling (Uherova, *et al*., 1993) [67]. Although, the method of germination significantly reduced both thiamine and niacin, where as riboflavin and pyridoxine is associated with the increment in their concentration (Khalil, *et al*., 1995) [34].

When talking about mineral concentration in different of heat treatment processes, the minerals are moved from the chickpea seeds into the water at different rates to cooking water. However, microwave cooking gave the results for the retention of all the minerals in the greatest amounts. This result is successively followed by the case of autoclaving and boiling. Also, the process of boiling the chickpea seeds in water resulted in great losses of minerals as K (24%), Cu (15%) and Fe (8%), (Salama *et al*., 1997) [55]. The reported losses for copper and magnesium were 31% and 22% respectively from mature chickpea seeds when the method of autoclaving was applied. The chickpea seeds which were obtained as a result of germination process, showed a significant decrement in the concentration minerals such as potassium, calcium, magnesium, manganese and copper. However, germination method was successful in increasing the concentrations of iron, phosphorus and zinc by 2.46%, 5.75% and 14.12%, respectively (Longe, 1983) [36].

In the context of amino acids, the changes that came out as a result of different cooking processes were also recorded. Chickpea protein was rich in essential amino acids concentration such as isoleucine, lysine and tryptophan. Therefore, chickpea seeds are very helpful in the case for consumption when deficiency of amino acids such as lysine and tryptophan has to be compensated. However, leucine, total sulphur amino acids, threonine, and valine were found to be low in concentration in the chickpea. Above mentioned concentrations were found to have in the crude chickpea seeds which were not affected with any type of cooking treatments (FAO/WHO, 1973) [25]. The method of boiling and microwave cooking caused a very small amount of increment in the concentration of total essential amino acids. The amino acids concentrations were not affected by the process of autoclaving and germination. However, cooking treatments and germination with an exception of microwave cooking reduced the levels of lysine. Also, cooking treatments with an exception of germination reduced the levels of tryptophan. The chickpea seeds which were subjected to the process of cooking and germination were found to have higher concentrations lysine and isoleucine except the process of autoclaving (Ziena, 1989). All the treatments were found to involve in the increment of concentrations of leucine, but the concentration of valine was not affected. Also, it has been showed that excess concentrations of leucine in foods are attributed to interfere with the utilisation of isoleucine and lysine (Deosthale *et al*., 1970).

From the above observations made as per the experimental results, we can draw a rough conclusion about the impact of various methods of cooking as well as germination on the nutritive values of the chickpea seeds. As per the study, boiling, autoclaving, microwave cooking and germination significantly affected the chemical composition, concentrations of anti- nutritional factors and nutritional quality of chickpea seeds. However, microwave cooking and germination caused very little losses in the concentrations of vitamins and minerals, while boiling and autoclaving caused greater losses. All cooking processes as well as germination method has been successful in improving the *in vitro* protein digestibility and protein efficiency ratios of chickpeas. Therefore, from the observations gathered, we can conclude that microwave cooking should be recommended for chickpea preparation in households and restaurants for increasing the nutritive values of the chickpea seeds. The microwave cooking method not only increases the nutritive values, but also, decreases the cooking time. Therefore, energy consumption for chickpea seeds preparation becomes less (Tareka *et al*., 2002).

2.2 Chickpea

2.2.1 Basic types

The chickpea is annual leguminous plant. It is present in the family of Fabaceae and its sub-family is Faboideae. Its botanical name is *Cicer arientinum* where Cicer defines about is specific epithet and arientinum signifies the genus it belongs to. Chickpea seeds are high protein containing seeds and are one of the earliest cultivated legumes because archaeological studies clearly revealed that its remains were found to be more than seven thousand years old. These remains were extracted from the sites in the Middle-East (Berger *et al*., 2007) [9]. There are different varieties of chickpea that are popularly known by their different vernacular (common) names. These include,

- Gram
- Bengal Gram
- Garbanzo bean
- Egyptian pea

2.2.2 Major uses

Chickpea are one of the main ingredients many cuisines prevailing in India, Mediterranean and the Middle-East. However, in the year 2018, India shared its 66% for the total production of chickpea globally (UNFAO, 2019) [58]. Chickpea is a very important ingredient of two very import cuisine dishes such as hummus and falafel. The name chickpea has been derived from the French word *pois chiche* in which *chiche* comes from the Latin cicer. Another variety that is garbanzo is derived from the Spanish language. The protein quality of chickpea has been found very close and equivalent to that of soybean meal.

2.2.3 Geographical distribution of different varieties of Chickpea

The physical features different of the plant does not vary to a very large extent. The plant height is about 20-50 cm in length. It has small feathery leaves on either sides of the stem. *Desi chana*variety closely corresponds to the other two types of chickpea found in the world. Chickpea are actually a type of pulses. One of its seed pod contains generally two or three peas. The plant bears flowers containing pink, violet or blue veins (S Cho *et al*., 2002) [18]. *Desi chana* has small, darker seeds and a rough cover. They are mostly grown in India and various parts of Indian subcontinent. Also, they are found in Ethiopia, Mexico and Iran. Desi chana can be black, green or speckled. On the other hand, Garbanzo beans or "kabuli" chana are light in colouration, larger and with a gentle cover. These are mainly cultivated in the areas of Mediterranean, Southern Europe, Northern Africa, South America, and the Indian subcontinent also.

Chickpea demand has raised several folds in a way that it was the part of American household that counted for about 17%.Usually, American and Iranian chickpeas have more sweet flavour than the Indian chickpeas. A very rare variety of chickpea, *ceci neri* is grown in some parts of Italy. More specifically, these are grown in the regions of Apulia and Basilicata. Ceci neri variety of chickpea has same size as that of those of the Garbanzo beans. But, this variety is actually darker in appearance than the desi variety of chickpea that is mainly found in India. Chickpeas have also been a source of energy and protein for the animals also (Bampidis *et al*., 2011) [5].

2.2.4 Raw material in various edible recipes

As we have already discussed about some of its culinary features, that these are basically used as a protein source in various dishes. These dishes have been originated in various part of the world in their own combinations. Generally, chickpeas are kept in water for a period of 12-24 hours and then boiled or pressure cooked before its use. Doing this, chickpeas are easy to cook. Some of the dishes containing chickpea as their key ingredient are falafel, cecina, panelled, rancho, tapas, hummus. Among these, hummus is one of the most popular dishes basically from the Arabic tradition. According to a survey, hummus has been consumed on a regular basis by 5% Americans on a regular basis (Grosglik., 2011).

Chickpeas and Bengal grams have been also the part of many Indian curries and are one of the most popular foods in India. Also gram flour, called as 'besan' in India are used to make sweets. Moreover, chickpea flour is also used in the preparation of 'Burmese tofu' which originated in Burma among the Shan people. A liquid made from chickpea called as 'aquafaba' can be used as an alternative for the egg white to make meringue. In the Philippines also, chickpeas preserved in syrups are eaten as sweets as well as in desserts such as 'halo-halo'. Guasanas or garbanzo is popular in Mexico as a Mexican street snack. Aquafaba can also be used with residual pomace to make a smooth texture flour (Shim *et al*., 2018) [57].

2.2.5 Use of chickpea as a nutrition source for animals

As protein rich diet is essential for humans, they are needed for the animal growth also. Protein rich grains such as peas and faba beans are very popular in use among the industries which produce something related with animal nutrition. Since they have much less anti-nutritional factor than those of soybeans, therefore, they can be used for the nutrition directly for animals. However, if the process of isolation is carried out to extract the protein supplement form the peas or faba beans, the nutritive value of the food increases many times (Paredes, 1991) [44]. The chickpeas due to their high protein content are grown in arid regions so that they may an advantage over other protein rich grains. Chickpea has been also considered as a better source of fat when compared to other leguminous grains and also it contains calcium and phosphorus in an assimilatory form so that they can be easily absorbed in the gut of animals. Further, the nutritive value of chickpea can be further increased by cooking it (Singh, 1991) [53].

When horses have a diet that are added with chickpeas, they develop a soft coat and a soft skin, which clearly depicts that they possess the good health. At the earlier times of British Empire in India, more specifically, the British era, chickpea was the main ingredient in the food of horses. These horses were needed good health so that they can perform their best as a part of cavalry. In Spain also, chickpea was used as food for the pigs and for lambs in France. In many countries, due to low production of chickpea because of restricted area of favourable conditions, chickpea production is meant only for the human consumption. This helps to fulfil their protein requirements. However, in Mexico, the case is just opposite. In Mexico, due to very large with favourable conditions of cultivation, 70% of chickpea production is used for the animal consumption (Cordesse *et al*., 1990).

In a study, the effects of protein rich diet among the rats were conducted. The study was conducted on four groups of rats. Each group was distributed with two rats among it. The rats were enclosed in four different cages, each containing groups of two rats. These rats were initially getting 10 to 15% protein rich diet. All the groups of rats were fed to another protein diet in different manners. One group of rats was fed on the diet containing untreated chickpea. The second category of rats was fed on the chickpea which was treated with sterilisation process at 110 C for 15 minutes. The third category of rats was fed on the chickpea which was treated with sterilisation or autoclaving process at 140 C for 30 min. The fourth category of rats was fed to a diet rich in casein based protein. The results were very clear after observation that, the chickpeas which were sterilised before the consumption by the rats gave the best performance among the first three. This result was very much similar to that of the casein based protein diet. But, one more observation was also recorded that the heat treatment if follows a wrong procedure, it would lead to poor performance. The chickpeas which were untreated or more specifically, the raw chickpeas showed slightly lower performance among the others. These results have been also proved by others also (Wang N, 2010) [73].

The level at which untreated chickpea shows its performance is not known yet with proper information. Many studies have been conducted by using untreated spring dry pea to feed the pigs and poultry. The results were somewhat similar to that, which were obtained with the use of sterilised chickpeas based protein diet. This study was made by using 16% chickpea content in the diet for the normal ones and 24% for the lactating ones (Gatel *et al*., 1987). Chickpeas have been used for the experiments involving in the diet of other animals also, such as, sheep, goats and cattle (LA Rubio *et al*., 2005) [52]. The results were quite specific as they were in the favour to support the diet with chickpea based protein when compared with any other source of protein for these animals. The experiments were conducted on the rabbits. Two varieties of chickpea were selected for this test. One variety of chickpea contained 22.5% concentration of protein and the second variety of chickpea contained 26.2% protein concentration. These two varieties of protein diet in the chickpea were used in a growth test on a 30 days old rabbits. The results were not so different for these two varieties of chickpeas when they were compared to the soybean meal. The performance on the basis of their digestion, consumption and efficiency were very similar among these when compared with the soybean meal. The energy obtained from the chickpea was in the range of 3100 to 3200 kilocalories per kilogram. This energy content was similar to the energy content obtained with the intake of soybean meal and wheat meal. Moreover, the digestion of nitrogen content from the diet in case of chickpea with higher protein intake concentration was 70% and the digestion of nitrogen content from the diet in case of chickpea with lower protein intake concentration was about 82% (Lebas, 1988) [35].

Another experiment was also conducted on lambs. The chickpeas used in this case were of the two types. The first type of chickpea was crushed and the second type of chickpea was remained uncrushed for the experimental use. The raw protein content in the experiment with the crushed chickpeas was found to be 16% and the raw protein content in the experiment with the uncrushed chickpeas was found to be 17%. Therefore, it was concluded that the process of crushing did not make a very larger difference in the protein content when it was compared with the protein content of the uncrushed chickpeas in the case of lambs. The lambs used in this experiment were 60 to 100 days old males for the first experiment and 75 to 120 days old males were used for the second experiment (Cordesse *et al*., 1986).

From the above experiments on various ruminants and non-ruminants animals, we come to a rough conclusion that the presence of anti-nutritional factor does not appear to be a limiting factor when it comes for the use of ruminant animals for their consumption. But, when we look at the case of non-ruminant animals, it is very desirable precursor for them that the level of anti-nutritional factors as well as the fiber content in the diet must be reduced when incorporated with the chickpeas based protein. A relatively higher fat content is desirable in the case when higher energy demand is needed (Cordesse *et al*., 1990).

Table 1: Chemical composition of chickpea seeds (g/100g dry weight basis)

S. No.	Nutritional component	Quantity
1.	Total Protein	$23.64^{a} \pm 0.50$
2.	Non-Protein Nitrogen	$1.82^{b} \pm 0.10$
3.	Ash	$3.72^{b} \pm 0.04$
4.	Fat	$6.48^{b} \pm 0.08$
5.	Crude Fibre	$3.82^{a} \pm 0.13$
6.	Moisture	$10.35^{a} \pm 0.31$
7.	Reducing sugars	$0.97^{b} \pm 0.04$
8.	Sucrose	$1.89^{b} \pm 0.08$
9.	Raffinose	$1.45^{b} \pm 0.07$
10.	Stachyose	$2.56^{b} \pm 0.08$
11.	Verbascose	$0.19^{b} \pm 0.06$
12.	Starch	$36.91^{a} \pm 0.60$

a, b Means in the same column with different letters are significantly (P< 0.05) different

± Means standard deviation of three determinants

Table 2: Anti-nutritional factors in Chickpea seeds

S. No.	Factor	Quantity
1.	Trypsin Inhibitor	$11.90^{d} \pm 0.10$ (TIU/mg protein)
2.	Haemagglutinin	$6.22^{b} \pm 0.22$ (HU/mg sample)
3.	Tannins	$4.85^{b} \pm 0.05$ (mg/g sample)
4.	Phytic Acid	$1.21^{b} \pm 0.09$ (mg/g sample)
5.	Saponins	$0.91^{b} \pm 0.10$ (mg/g sample)

b, d Means in the same column with different letters are significantly (P< 0.05) different

± Means standard deviation of three determinants

TIU means Trypsin Inhibitory Unit

HU means Haemagglutinin Unit

Table 3: B-Vitamins content in chickpea seeds (μg/100g dry weight)

S. No.	Vitamin Component	Quantity
1.	Riboflavin	$173.33^{b} \pm 6.66$
2.	Thiamine	$453.33^{c} \pm 6.51$
3.	Niacin	$1602.67^{d} \pm 12.22$
4.	Pyridoxine	$466.33^{d} \pm 7.52$

b, c,d Means in the same column with different letters are significantly (P< 0.05) different

± Means standard deviation of three determinants

Table 4: Minerals content in chickpea seeds (mg/100g dry weight basis)

S. No.	Mineral	Quantity
1.	Na	121
2.	K	870
3.	Ca	176
4.	Mg	176
5.	P	226
6.	Mn	2.11
7.	Zn	4.32
8.	Cu	1.10
9.	Fe	7.72

Average of two determinant

Table 5: Amino acid composition in chickpea seeds (g/16g N)

S. No.	Amino Acid	Quantity
1.	Isoleucine	4.1
2.	Leucine	7.0
3.	Lysine	7.7
4.	Cystine	1.3
5.	Methionine	1.6
6.	Total sulphur A.A.	2.9
7.	Tyrosine	3.7
8.	Phenylalanine	5.9
9.	Total aromatic A.A.	9.6
10.	Threonine	3.6
11.	Tryptophan	1.1
12.	Valine	3.6
14.	Histidine	3.4
15.	Arginine	10.3
16.	Aspartic Acid	11.4
17.	Glutamic Acid	17.3
18.	Serine	4.9
19.	Proline	4.6
20.	Glycine	4.1
21.	Alanine	4.4

Source: Journal of food composition and analysis (2006).

3. Conclusion

From the research work of thesis, conclusion can be made on various factors that are responsible for affecting the nutritional composition of chickpea seeds. In the above studies and observation we saw that nutritional composition is basically based on the treatment of chickpea with different types of cooking methods. They may include germination, microwave heating, pressurised cooking, boiling, etc. All the factors that are involved in the treatment processes contribute to the variation in the probiotic content of chickpea. On analysis of various components of chickpea that may be nutritional or anti-nutritional factors, it can be concluded that different methods of cooking treatment can be applied as per the requirement of the components present in it. A rough measure of efficiency can also be made to a conclusion for its use in the particular requirement. Moreover, protein concentration and its extraction are very important are the basic parameters on which this study is based for.

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