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Introduction

Food is any substance of either plant or animal origin consumed to provide nutritional support to the body for the sustenance of life. Indian traditional foods deserve special mention as they are of immense variety. They are a combination of vegetables, cereals, millets, pulses, milk, yogurt, eggs, animal protein and spices to satisfy our energy and dietary requirements.

Grain legumes have been grown since millennia and have been an important component of the human diet especially in India. They are an inexpensive source of protein which can be substituted for dietary animal protein (Anderson *et al.*, 1999). A cup of cooked beans provides 25% of the daily requirement for the amino acids at a cost much less than the cost of meat protein. Given to the important role of legumes in populations that consume plant-based diets, it is not surprising that legume intake is higher in vegetarians than in non-vegetarians (Ridout *et al.*, 1988; Donovan and Gibson, 1996).

1.1. Overview of legumes

Legumes belong to a large family Leguminosae and have a long history of their use in agriculture. The legume fruit is commonly called a pod that splits into two halves. The term "legume" is derived from the Latin word "*legumen*" which means "to gather". The dicotyledonous seeds of legumes without seed coat are called pulses. Legumes are among the first crops cultivated and mentioned even in the Old Testament. Archaeologists have discovered traces of pulse production around the river Ravi in Punjab, the seat of the Indus Valley civilization, dating back to around 3300 BC.

1.1.1. Food legumes in India

Pulses have been grown through millennia and they form an important component of the human diet in India. These provide a nutritionally balanced food with essential proteins and calories to the people in India where the consumers avoid animal protein either due to its high cost or for religious reasons. Besides their high nutritional value, pulse crops have unique capacity of enhancing soil fertility through biological nitrogen fixation (Kannaiyan, 1999). Virtually all Indians belonging to different economic strata consume pulses and these are served at both homes and eateries.

India has been growing 12 different pulse crops (Nene, 2006) and is now ranked as the world's single largest producer, importer and consumer, accounting for about 25% of global production, 15% of international trade and 27% of consumption (Price *et al*., 2003). The maximum production of pulse in the country occurs in the state of Madhya Pradesh, with about 26% of total production from 1996-1997 to 1999-2000 followed by the states of Uttar Pradesh (18%), Maharashtra (14%), Rajasthan (14%), Karnataka (5%), Andhra Pradesh (5%) and Bihar (5%) (Meenakshi *et al*., 1986). It is an important Indian crop, with an annual production of about 13 million tons from an average productivity of 565 kg/ha from 23 million hectares (Srivastava *et al*., 2010).

1.1.2. Nutritional and economic importance of legumes

Legumes are a rich source of protein with varying proximate compositions depending on the variety, species and region of cultivation (Liu, 1997). For this reason, it is known as poor people's food and is nutritionally important for people who cannot or choose not to eat meat. Although the protein of legumes is adequate in essential amino acid lysine, it is deficient in the sulphur-containing amino acids such as methionine and cystine (Friedman, 1996). This means that a smaller proportion of the plant proteins, compared to animal proteins, may be used for the synthesis of protein in humans. According to the protein combining theory, legumes should be combined with another protein source such as a cereal to balance out the amino acid levels as cereals are low in the essential amino acid lysine. Cereal and food grain legumes are the staple food of the people of India and this combination provides a good balance of carbohydrates and proteins (Nout and Sarkar, 1999). Thus a vegetarian meal often comprises of legumes along with cereals, creating a more complete protein diet than either the beans or the grains on their own. Common examples of such combinations are dal (dehulled split beans) with rice, beans with corn tortillas,tofu with rice, and peanut butter with wheat bread.

Legumes are a good source of different minerals such as calcium, potassium and phosphorus (Liu, 1997). The bioavailability of these minerals can be improved through processing. Not only are legumes an excellent source of essential minerals, but they are also a good source of dietary fibers, niacin, thiamine, riboflavin, pyridoxine and other phytochemicals which are important in human health. All of these nutrients are necessary for normal growth and for the building of body tissues. Dietary fiber is made up of polymers like cellulose, hemicellulose, pectin and lignin that help in water absorption from the digestive tract (Fennema, 1996a). It also has health benefits such as lowering of blood pressure and serum cholesterol, protection against cardiovascular diseases, diabetes, obesity and colon cancer (Ubom, 2007). Consumption of lentils, peas and beans by diabetic persons helps with blood glucose management. Compared with some other carbohydrate sources, pulses have a lower glycemic index. Studies have also shown that consuming pulses can result in a more stable blood glucose level after meals. Soybeans have attracted the most scientific interest, mainly because they are unique source of isoflavones which work synergistically with other phytochemicals to reduce the risk of chronic diseases (Messina, 1999).

Recent research suggests the presence of 'anti-aging' agents or antioxidants in the seed coats of legumes. While animal proteins are often rich in saturated fatty acids, most legumes are low source of fat with the exception of soybean and groundnut. The small quantities of fats in legumes are mostly unsaturated. So, pulses are a perfect diet component for people interested in weight management as they are high in fiber and protein, low in fat and moderate in calories. Legumes play an important role in human nutrition especially in tropical countries like India where they are the next important food crop after cereals (Uzoechina, 2009). They are processed into various semi-finished and finished products (Ojimelukwe, 2009). Indians are credited for using legumes and different types of legume-based foods from time immemorial. There are several legume-based traditional foods from several parts of India that have become popular with time throughout the country. Some of these foods like dhokla, dosa, idli, kinema, papad and wadi are fermented, while others like besan and sattoo are non-fermented. There are several legume-based foods which have now been commercialized as a ready-to-eat or packaged food product and retailed under different brand names, while some are still manufactured at the cottage-scale level by employing traditional, technologically less advanced methods. The principal legumes used in the preparation of a variety of foods in India include Bengalgram, blackgram and soybeans. A scale-up in the processing technologies of some of the foods have been attempted successfully with the aim of commercializing them, and the foods like dhokla, dosa and idli that were earlier indigenous to the southern parts of India are now equally relished in other parts of India. In the modern era, these foods are making a niche for themselves in the rapidly evolving fast-food sector in India. With more and more people turning health conscious, there is a growing demand for these food products which are nutritious, delicious and an economic source of protein.

In spite of being a rich source of protein, legumes have lower utilization mainly due to the presence of several antinutritional and toxic factors like allergens, antivitamins, cyanogenic glycosides, enzyme inhibitors, goitrogen, haemagglutin, lectins, oxalates, phytates, polyphenolic compounds and trypsin inhibitors (Apata and Ologhobo, 1997). These compounds reduce protein digestibility and availability. The oligosaccharides present in legumes, are the RFOs which include raffinose, stachyose, verbascose and ajugose. In mature legume seeds, RFOs are present at a level of 31%-76% of the total soluble sugar content (Reddy *et al.*, 1984). These RFOs consist of one or more galactose units attached to a sucrose unit via α-D-1,6 linkages. Ajugose, being a higher homologue, is present in traces in the seeds (Girigowda *et al.*, 2005). The RFOs are the principal cause of flatulence in humans and monogastric animals (Jood *et al.*, 1985). The gastrointestinal tract in them lacks the enzyme α-galactosidase which is required to hydrolyze the α-1,6-galactosidic linkage of these RFOs (Gitzelmann and Auricchio, 1965). Consumption of inadequately processed legumes containing higher oligosaccharide contents in them leads to gastronomical discomfort. These saccharides escape digestion and absorption in the stomach and small intestine and reach the colon where they undergo anaerobic fermentation by bacteria to produce several gases such as carbon dioxide, hydrogen and a small amount of methane (Rackis, 1975). Besides social implications, flatulence is characterized by abdominal cramps, diarrhoea and nausea (Albersheim and Darvill, 1985). Hence, it is necessary to determine the conditions for reducing the effect of these RFOs and improve the nutritional quality and digestibility of legumes. Reduction or elimination of these factors would make them more acceptable for human consumption. Some antinutritional factors in legumes such as tannin, a polyphenolic compound, is reported to possess antioxidative activity (Amarowicz and Pegg, 2008). Raw legumes have higher content of antinutritional factors but can be eliminated or reduced by processing.

1.1.3. Processing of legumes

Legumes are used for the preparation of several semi-finished and finished food products, but at the beginning all legumes need to be processed adequately to convert the raw material into the final edible product. Some of the techniques employed are traditional and still carried out without much alteration. Legumes can be processed at both cottage-scale and industrial level to enhance productivity in terms of yield and quality to ensure food security, income generation and food safety. Some of the different methods of processing employed are dehulling, sorting and cleaning, soaking, blanching, cooking or boiling, roasting or frying, and fermentation. There are several practices like malting, dehulling, cooking, and enzyme treatment which help to reduce the RFO content in legumes (Price *et al.*, 1988). There are some reports of the effects of soaking, germination and cooking on the RFO content of blackgram (*Vigna mungo* (L.) Hepper; synonym *Phaseolus mungo* L.) (Iyengar and Kulkarni, 1977; Rao and Belavady, 1978). Of the several techniques practised, dehulling, the primary processing, is an important post-harvest operation in which dal, consumed in various forms, is obtained from the whole seed of pulses (Singh, 1995). The seed coat of pulses is often indigestible and sometimes bitter in taste (Singh and Singh, 1992). Even though dehulling of the pulses is carried out to improve the taste and acceptability of the foods prepared from it, the most beneficial effect is the considerable reduction of cooking time (Williams *et al.*, 1993). Some potent antinutritional factors like polyphenols present in the seed coats are also considerably reduced due to dehulling (Rao and Deosthale, 1982; Singh, 1993). Besides, dehulling facilitates improvement in appearance, texture and digestibility of the grain legumes (Kon *et al.*, 1973; Deshpande *et al.*, 1982). Legumes are sorted by hand or through machines and cleaned to remove broken and spoilt seeds and foreign materials.

The most common household practice of processing legume beans prior to preparation of different foods is soaking them in water, preferably overnight. The soak water is decanted off and the soaked beans are used for cooking or for the preparation of various dishes. Besides being used as a popular soup, dal-based traditional fermented foods such as dosa, idli, vada, wadi and papad are relished all over the country. During the preparation of soup, soaked beans are cooked, however the cook water, which contains further leached out RFOs, is not discarded before consumption. Hence, reduction of RFO content at the soaking stage is imperative. Different seeds are soaked in water for different periods of time. Even though dehulling and splitting of pulses have reduced the cooking time considerably, the cooking process is time-consuming. Also a hardening defect, called "hard-to-cook", arises with the storage of the grain legumes at high temperature and humidity (Parades-López *et al.*, 1991). In order to overcome these defects, a soaking process in water is recommended which reduces the cooking time. Soaking in water allows the seeds to absorb water and decrease antinutritional factors. Soaking also helps in the leaching out of the complex sugars that cause flatulence thereby improving digestibility.

Blanching is a mild heat treatment of seeds in which legumes are soaked in hot water or boiled in water for a few minutes – the treatment which destroys food enzymes and some antinutritional factors in the legumes.

After these treatments, legumes are cooked by boiling for some minutes which improves their sensory properties. Boiling tenderizes the seeds through water absorption and eliminates the heat-labile antinutritional factors such as trypsin inhibitors (Bishnoi and Khetarpaul, 1993). Legumes are also roasted and fried which improves digestibility and reduces antinutritional factors (Abd El-Moniem *et al.*, 2000). Roasted and fried legumes have unique flavours due to which their sensory appeal is enhanced.

Fermentation employed in the preparation of some legume-based foods improves the nutritional quality, flavour, colour and texture of legumes. It destroys enzymes causing food spoilage thus extending shelf-life of the food and enhances the food safety by deactivating spoilage and pathogenic microorganisms in the food products. This process increases the digestibility of plant proteins by proteolytic conversion of proteins to the more digestible peptides and amino acids (Nout, 1994). It also reduces the antinutritional factors such as phytate.

1.1.4. Improving processing steps for better product quality, safety and shelf-life

In a developing country like India, the processing of legumes into different products is the source of income for small-scale producers to food outlet entrepreneurs. However, it is necessary to upgrade the processing techniques to bring about increased productivity, reduced drudgery and efficient time management. In most household and cottage processing activities, a lack in the standardization of processing methods leads to variation in the quality of the product produced from different batches. Most operations are done using subjective judgements and ingredients are added according to the taste of the processor and not according to a standardized formula. Besides, sometimes these products are prepared under poor hygienic condition which leads to contraction of various foodborne diseases.

Optimization of processing conditions is a crucial stage if one wants to develop an efficient and economic process (Sanjay *et al.*, 2007). Traditionally, an optimization process was achieved by an empirical method in which one factor was varied at a time while others were kept constant (Juntachote *et al.*, 2006). This one-factor-at-a-time approach was cumbersome, expensive and less effective as interactions between the variables could not be studied. According to Montgomery (2005), RSM is a powerful tool which is useful for applications in which a response is influenced by several factors. Unlike the traditional empirical method, RSM is the preferred and effective optimizing tool that helps to understand and quantify the interactions between variables with fewer experimental runs. It uses data from the experimental designs to find the combination of factors that give the optimum response. Thus, RSM is less time-consuming than the other optimization processes and, therefore, finds a wide application in various sectors including food industry (Luna-Solano *et al.*, 2005).

Modern food industries have also been using sensory analysis both in the development of new products and in the optimization of the existing processes (Chang *et al.*, 1998). The responses of the consumers can be analyzed using RSM which generates predictive models that help to correlate the consumer responses to the test variables. These models help in optimizing a process and predicting responses for a combination of variables not tested (Moskowitz, 1994).

Food processing methods can be employed successfully to enhance the safety of foods. For instance, heating a food at a temperature high enough for microbial viability for a sufficient length of time ensures microbial safety. At the industrial level, heat treatment at a particular temperature-time combination destroys the vegetative forms of the pathogens without affecting the quality and nutritional aspects of the food. Modulation of pH, a_w and the use of bacteriocins help to prevent the growth of organisms or the production of microbial toxins in the food. pH can be reduced below 4.0 by fermentation or acidification with acid, while a_w can be reduced by the addition of sugar or salt to the food. With the growing pressure to replace the use of chemical preservatives in foods, bacteriocins are an ideal alternative. These are usually low molecular weight proteins produced by some LAB to inhibit the growth of other bacteria. Packaging is also useful to protect the foods against moisture and subsequent attack by the pathogens. So, it is very essential to educate and train the processors about improving processing methods and food safety practices to ensure consistent quality products for increased income generation.

Water is one of the most important components determining the physical, chemical and sensory properties of foods (Fennema 1996b). a_w, which is defined as the ratio of the vapour pressure of water in equilibrium with a material to the vapour pressure of pure water at the same temperature, is used to express the amount of water available in a food system for microbial growth and biochemical reactions (Fennema 1996b). An important aspect of a_w concept is the moisture sorption isotherm which is a graphical representation of the relationship between moisture content of a product and a_w at a particular temperature. Moisture sorption isotherms give vital information about sorption behaviour and interaction of various components of a food with water (Kaymak-Ertekin and Gedik, 2004). It is also an important property of dried food products as it helps to determine the degree of drying required to obtain a stable product (Lerici *et al.*, 1985). Various conventional methods of drying are used to increase the shelf-life of foods. Air-drying has been reported to be frequently used in the food industry for drying various fruits and vegetables (Konopacka, 2006) as it is a feasible method to preserve them. However, drying is an energy-intensive process and a proper knowledge is required to optimize the drying efficiency (Pérez-Francisco *et al.*, 2008). In any drying process, the main aim is always to obtain a product of desired quality at a minimum cost and to optimize these factors (Fernandes and Rodrigues, 2006).

Dried low-moisture legume products like besan, sattoo, wadi and papad have excellent keeping qualities at ambient temperature when stored away from moisture. However, the freshly prepared products such as dosa, dhokla, kinema, idli, etc. have shorter shelf-life under ambient conditions. Hence, it is important to understand the sorption characteristics and thermodynamic functions of different foods for the prediction of their quality, and shelf-life during their packaging and storage to prevent both microbial and chemical degradation of the food (Viadles *et al*., 1995). Research results can then be utilized for developing low-cost techniques for extending the shelf-life and safety of these products, as costly and sophisticated preservative techniques are usually not adopted by local processors.

1.2. Foodborne illness

In the past, humans used to obtain food by hunting and agriculture but today most of the food consumed by the world population is supplied by the food industry that developed with the emergence of new processing technologies such as preservation, packaging and transportation during the industrial revolution in the 19th century. Until then, the traditional food processing techniques such as salting, curing, curdling, drying, pickling, fermenting and smoking were used to prevent bacterial growth. With the emerging trends in food manufacturing process, growing international trade, globalization of the food supply over large geographic distances, industrialization, and constant travels across the world, foodborne diseases have increased (Nyachuba, 2010). WHO defines foodborne illness as "diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food."

Food spoilage and foodborne illness caused by microorganisms were problems that must have continually pre-occupied early humans. In the early 1900s, some foodborne infections recognized were typhoid fever, tuberculosis, brucellosis and septic throat (Tauxe, 2002). The causal organisms behind food poisoning and human gas gangrene infections were identified as *Bacillus* spp. and *Clostridium perfringens*, respectively; *Yersinia enterocolitica* was identified as the gastroenteritis-causing bacterium. (Hartman, 1997). Foodborne pathogens not only affect public health but also burden the food industry with steep economic losses incurred due to order recalls and compensation payments. Most of the foodborne diseases have been controlled by sanitation, pasteurization and other measures (Tauxe, 2002). However, the burden of emerging pathogens like *Listeria monocytogenes* and *Escherichia coli* O157:H7 with increased resistance to control measures and some *Salmonella* serotypes resistant to common antibiotics like tetracycline and streptomycin still exist (Antunes *et al*., 2003; Wilks *et al*., 2005; Knøchel, 2010; Uhlich *et al*., 2010; Riazi and Matthews, 2011; Van Elsas *et al*., 2011). A better understanding of hardiness and survivability of pathogens causing foodborne diseases is therefore essential. During storage and retail, nearly every food product has contact with one or more packaging materials, which may be a source and reservoir of foodborne pathogens. With a better understanding of long-term survival of pathogens, risk assessment recommendations will have more valid scientific backing, the food industry will be better prepared to combat recalls and economic losses, and consumers will better understand the risk and danger of improper food handling and storage.

1.2.1. Classification of foodborne illnesses

Foodborne illnesses can be broadly classified into two categories, foodborne infections and foodborne intoxications. Infections occur when a food contaminated with bacteria, viruses or parasites are consumed by a person. The ingested pathogen causes infection in the person by invading and colonizing various tissues of the body. The bacterial pathogens on successful multiplication within the body of the host releases toxins which cause infection. On the other hand, foodborne intoxications are caused by consuming foods or beverages already contaminated with a toxin such as poisonous chemicals or toxins produced either by bacteria or naturally occurring in some plants and fungi. One type of foodborne intoxication is characterized by nausea, vomiting and abdominal cramp and has an incubation period of 1-6 h. This is the emetic form of the illness and resembles the *Staphylococcus aureus* food poisoning. The other form of the illness is known as the diarrhoeal form having an incubation period of 8-16 h and resembles the food poisoning caused by *C. perfringens*. While the foodborne infections can be transmitted from person to person and are chiefly caused due to poor personal hygiene and cross-contamination, the foodborne intoxications are not communicable and caused due to inadequate cooking and improper holding temperatures of the foods before consumption.

1.2.2. Transmission of foodborne pathogens, and toxins

Foodborne diseases can originate by the consumption of foods contaminated with different pathogenic organisms like bacteria or viruses. Illnesses can arise from these foods irrespective of whether they are home-cooked or eaten out at eateries. The common factor leading to bacterial foodborne illness is cross-contamination of food from other uncooked foods. Use of the same utensils or cutlery while cooking several dishes without washing them in between can also be a probable cause of cross-contamination in the kitchen. The other factor is temperature abuse during the storage of foods, in which contaminated food is left out at room temperature for many hours, allowing the bacteria to multiply to high numbers, and then inadequately heated before eating (CDC, 2010). Heating of foods to an internal temperature above 78°C for even a few seconds can kill various pathogens except the sporeformers like *Bacillus* and *Clostridium* which are heat-resistant . Toxins produced by *S. aureus* remain unaffected by boiling, whereas boiling completely inactivates the toxin produced by *Clostridium* (CDC, 2010). Refrigeration or freezing arrests the vegetative growth of all bacteria except *L. monocytogenes* and *Y. enterocolitica.* Contamination with *Shigella*, hepatitis A virus and Norwalk virus is also possible if infected humans handle the food with unwashed hands. Washing fruits and vegetables with water containing animal manure or human sewage can also be a source of contamination. Fresh manure used in the agricultural fields can also contaminate several crops like legumes, cereals and vegetables. Some legume-based foods in India were reported to be contaminated by bacterial pathogens like *Bacillus cereus* group (s.l.), *S. aureus*, *C. perfringens*, *E. coli*, *Salmonella* and *Shigella* due to unhygienic handling, contaminated water and inferior quality of raw materials (Roy *et al.*, 2007). This report indicated the effect of manufacturing processes and conditions on the growth and survival of the foodborne bacterial pathogens. The processing methods did not support the growth and survival of *S. aureus,* while the detection of a high count of TAMB, *B. cereus* group s.l. and Enterobacteriaceae in all the foods suggested the need for improving the manufacturing processes both at the urban and rural levels by bringing about some changes in its processing, storage and distribution to enhance their microbial safety.

1.2.3. Prevention of foodborne disease outbreak

Even though the prime responsibility to produce safe foods lies with the producers, the authorities also need to regularly test them both at the site of production and at the site of sale. Foodborne illness is largely preventable if suitable public health initiatives such as food inspection are designed to educate the producers and consumers about the necessity of food safety measures. Apart from food contamination, transmission of foodborne diseases can also occur by improper storage and handling of raw and cooked foods. Lack of adequate food safety education among the masses is yet another reason for such disease outbreaks. Hence, the most important measure to be adopted in the prevention of foodborne illness is to educate the consumers and train the food handlers about the necessity of food safety measures. Besides this, environmental measures should also be adopted to avoid the use of fresh manures in the agricultural fields for the cultivation of various crops (WHO, 2007). In order to check the spread of foodborne diseases it is essential to rapidly detect and identify the causal pathogens, and the research results will be beneficial in formulating measures to combat foodborne pathogens that cause problems for public health and in the food industry (Scallan *et al*., 2011). WHO has framed several policies to promote the safety of food from production to consumption.

1.3. Objectives of the present investigation

The present study was aimed at evaluating (1) the occurrence and survival of foodborne bacterial pathogens during the production and storage of some legume-based traditional foods of India, (2) the conditions required for optimizing the processing steps of some legume-based foods using RSM to obtain microbiologically safe foods with adequate sensory acceptability, (3) moisture sorption behaviour of some legume-based foods during storage at different temperatures and a_w in order to determine the appropriate storage conditions of those foods, and (4) whether modulation of the traditional technologies has any positive role on the safety of these foods.

The strategies adopted for accomplishing the above objectives were as follows:

1) Obtaining information about the processing methods of various legume-based traditional Indian foods;
2) Collecting raw materials, intermediate products and final products from sites of production for evaluating the microbiological and physical qualities of the foods;
3) Isolation of foodborne bacterial pathogens and testing of the isolates to confirm their taxonomic status;
4) Determining survivability and growth following intentional inoculation of raw material, processing intermediates and foods with the selected bacterial pathogens;
5) Response surface optimization of the process parameters of some selected legume-based foods using central composite designs;
6) Determining moisture sorption characteristics of some foods by the static gravimetric method; and
7) Studying the influence of added preservative and a_w modulation on the safety and quality of one traditional food as a model.