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REVIEW

Sources, persistence, and current inactivation methods of foodborne pathogens in legumes

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Abstract

Legumes and legume-derived products are consumed in large quantities for their nutritional value as well as for their taste. Their sustainability and affordability are commendable, and legumes are a low-glycemic-index food that is high in protein and fibre while still being satiating. Legumes are commonly consumed as choice meals, particularly by vegetarians and vegans as a meat substitute, and they account for a significant portion of the dietary intake in many countries. The huge trend of vegan diets has prompted the industrial growth of legumes and pulses. The increased production within the shortest period to fulfil the expanding demand has put them at a higher risk of contamination with pathogenic microbes, rendering consumer safety uncertain. The objective of this review was to draw attention to the sources of microbiological contamination in legumes, the persistence of foodborne pathogens in legumes, and the existing techniques of foodborne pathogen inactivation. Water, surfaces, and human contact are all factors to consider, as is the risk linked with inadequate sanitation, poor equipment design, and improper handling. The foodborne pathogen, though seemingly unavoidable, can be minimized or inactivated using a variety of thermal and nonthermal procedures used prior to and after harvesting of legumes, as well as throughout the manufacturing and processing process.

1. Introduction

A legume is any plant from the Leguminosae (Fabaceae) family, which makes up the third-largest plant family in the world (Tekdal, 2021). Legumes are consumed as vegetables, dry grains, or extracted for oil. Legumes that are primarily consumed as dry grains are called pulses (Snapp *et al.*, 2018) and those primarily used for oil are called oilseeds (Rawal and Navarro, 2019). Legume crops offer a combination of feasible agricultural practices, food security, and nutritious diets (Rubiales *et al.*, 2018). The whole legume plant is often used in agriculture for cover crops, livestock feed, or fertilizer. Legume works as a fertilizer by fixing the nitrogen in the atmosphere and releases high-quality soil organic matter (SOM) to facilitate soil nutrients that are essential to the long-term survival of soil systems (Lebrazi and Fikri-Benbrahim, 2018). On top of that, legumes are capable of growing even in extreme conditions and under low nutrient sources, which are favourable attributes against climate change. The demand for plant-based proteins is also on the rise to limit greenhouse emissions by livestock cultivation. Legumes are a good staple that can provide food security and nutritional security to humans (Rubiales *et al.*, 2018) common beans, chickpeas, dry peas, lentils, cowpeas, and mung beans are predominantly cultivated and consumed worldwide (Rawal and Navarro, 2019). Sustainable agricultural practices and a growing demand for plant protein increase legume consumption.

Legumes are a traditional ancient food source packed with complex carbohydrates, protein, dietary fibre, and a wide range of vitamins and minerals (Monnet *et al.*, 2019). Legumes are also a good source of protein and micronutrients, particularly for consumers who cannot afford to have meat in their diets. For instance, the

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consumption of 20 g of grain legume daily has contributed a total of 10% protein intake and micronutrient sufficiency to Ghanaian infants, in the age of 6 to 8 months old (De Jager *et al.*, 2019). Starch with a low glycemic index, dietary fibre, phenolic compounds, and oligosaccharides are attributed to the beneficial effects of legumes. Legume phenolic compounds have potent antioxidant and antimicrobial properties. In the assessment of gluten-free foods high in legumes, special focus is placed on the functional properties of bean components such as starch and protein, among other things (Foschia *et al.*, 2017).

In general, dried legumes are typically dehulled, soaked, boiled, fermented, or sprouted before being consumed to increase palatability and digestibility. More importantly, these processes are capable of reducing the anti-nutrient compounds including tannin, cyanide, saponin, and lectins that are commonly present in most legumes (Sharma, 2021). Fermented legumes involve the use of endogenous microorganisms (natural fermentation), microbial starter, or the addition of fermented material (black slopping). The fermentation of leguminous seeds not only can reduce anti-nutritional compounds and allergenicity, but also improve protein, starch digestibility, and antioxidant capacity (Amarowicz, 2020). Sprouting legumes involves water absorption, followed by seed de-coating, and enzymatic metabolism which results in an overall increase in nutrient bioavailability (Devi *et al.*, 2015).

Legumes are a source of nutrients for human health but, it is also important to ensure safety. Unsanitary techniques in legume cultivation, harvesting, processing, and preparation are the causes of danger for human consumption (Adley and Ryan, 2016). Dried legumes are typically stored in less stringent storage conditions which could enhance pathogen growth. Subsequent processing of contaminated dried legumes poses a high risk of adverse health effects. The adverse health effects range from acute poisoning to long-term effects such as immune deficiency and cancer, because exposure to water for long hours during soaking can further enhance the growth of the pathogens, in which fermentation or cooking may not be able to reduce the pathogen to a safe level. Moreover, the formation of mycotoxin or spores, which are known to be heat resistant, could amplify the risk if the fermentation or cooking period is not adequate. Pathogenic fungi, *Clostridium botulinum*, *Salmonella* sp., *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes* are among the five major foodborne pathogens causing illness in legumes and derived products have been included in this literature synthesis. Legumes are prone to microbial contamination; therefore, prevention strategies for outbreaks are required. The objective of this review was to identify the sources of microbiological contamination, the persistence of foodborne pathogens, and the latest inactivation methods of foodborne pathogens in legumes and derived products.

2. Sources of microbial contamination in legumes

Consumption of previously contaminated legumes is frequent among consumers, and it has severe consequences on human health, economy, and society. Table 1 presents a number of cases of selected legumes that have been associated with previous outbreaks and product recalls. Most of the cases were reported for peanut butter previously contaminated with *Salmonella enterica* (Harris *et al.,* 2018). Legume contamination affects the access to important markets such as Europe and the United States, and the availability of safe foods for the population.

According to Izumi (2011), environmental variables (e.g., temperature, relative humidity, dew point), product characteristics (e.g., pH, water activity, humidity content), and product morphology and physiological state, all have a role in determining the degree of microbial contamination. Coliform germs (e.g., *Enterococcus* spp., *Salmonella* sp.) were found on legumes. Other Gram-negative rods such as *Stenotrophomonas* and *Xanthomonas*, Gram-positive bacteria such as *Bacillus* and *Staphylococcus*, and lactic acid bacteria such as *Enterococcus* and *Leuconostoc* are found in a variety of legume crops. It is common to find these bacteria isolated from vegetables in soil, agricultural water, and pesticide solutions among others.

In addition, contaminated water, surfaces, and hand workers are all known sources of bacterial contamination in legumes. Facility conditions such as the application of good agricultural practices, the equipment design, and the raw materials are also associated with possible causes of bacterial contamination in legumes. From this analysis of literature, there have been identified two main factors associated with contamination in legumes: environmental and facility factors.

2.1 Environmental factors

2.1.1 Water

During commercial operations, leguminous seeds are soaked in water, drained, and spread out in drums or on trays. The seeds are continuously irrigated and collected three to 10 days after sowing, depending on the accurate degree of maturity. After washing, the seed cover is removed, and the sprouts are placed into bags or plastic trays. As a result, different parts of legumes are in direct contact with water, and as previously stated, sprouts or

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Table 1. Outbreaks associated with legume consumption.

Legume type	Product (sources)	Foodborne pathogens	Year	Number of cases	Outbreak location	References
Peanut	Peanut Butter	*S. enterica* serovar Mbandaka	1996	15	Australia	Scheil *et al.* (1998)
	Peanut butter	*S. enterica* serovar Tennesee	2006-2007	715	USA (48 states)	CDC (2007)
	Peanut Butter	*C. botulinum* (serotypes A and B)2	2006-2008	5	Canada	Sheppard *et al.* (2012)
	Peanut Butter	*S. enterica* serovar Typhimurium	2008-2009	714	USA (46 states), one case in Canada	CDC (2010)
	Peanut Butter	*S. enterica* serovar Bredeney	2012	42	USA (20 states)	CDC (2012)
	Peanut Butter / Almond butter	*S. enterica* serovar Braenderup	2014	6	USA (CT, IA, NM, TN, TX)	CDC (2014)
Soynut (roasted soybean)	Soynut butter	*Escherichia coli* O157:H7	2017	32	USA (12 states)	CDC (2017)

seeds are easily contaminated if there is a degree of water contamination (Laborde, 2005).

The stem of the legume plant collects water, which is subsequently distributed throughout the plant by its vascular system. The process is described as a tube of water that runs through the plant, passing through the stems, and eventually arriving at the top of the tallest bud (Jongman and Korsten, 2018). There are a variety of factors that contribute to polluted water; one of them is the fertilizer runoff from agriculture. Irrigation water has the same effect on the plant. Surface water is used for irrigation as well; however, multiple studies revealed the presence of several enteric bacterial pathogens such as enterohemorrhagic *E. coli*, *Salmonella* sp., and *Listeria monocytogenes* which infect fruits and vegetables (Markland *et al.*, 2017).

In addition, microorganism transportation from contaminated soil to produce is facilitated by the splash of water droplets on the surface of the legume plant. Researchers have discovered that both rain and irrigation water droplets transport contaminated soil particles to the surface of the plant which has as a result the contamination of the harvested legume. The use of different sources of irrigation water is becoming increasingly popular around the world. Agricultural water must be used wisely as it is a vital and limited resource. As the population of dry areas continues to grow, there is an increased need for water, which has led to the development of wastewater applications and value-added in agriculture to minimize the strain on freshwater supplies (Balkhair, 2016).

2.1.2 Soil and animal manure

When soil is intended for agriculture, it is frequently fertilized with treated or untreated animal manure or human bio-solids. These fertilizers provide a cost-effective nitrogen source while being capable of harbouring pathogenic microbes. A direct contact of the plant with the soil surface contributes to the transfer of microorganisms from contaminated soil to produce. It is also possible that legume plants are splashed by rainwater. Researchers have shown that soil particles are transported to the surface of plants by both rain and irrigation water droplets; as a result, there is contamination of harvested produce. There is also a risk of contamination when plant surfaces come into direct contact with manure (Machado-Moreira *et al.*, 2019).

Besides, Laborde (2005) also discussed that, in the field or during postharvest storage, legumes may become contaminated by bacteria present in animal dung. The use of animal manure in fields of legumes meant for non-human consumption is harmful if seeds from those legumes are intended for fermentation and germination. During the germination process, nutrients, high amounts of moisture, and the heat generated by the process contribute to survival and bacterial growth. Rahman *et al.* (2021) claimed that the most common pathogens present in the agricultural crops associated with soil and animal manure are *Escherichia coli* and *Salmonella* spp. from a search that has been conducted.

2.1.3 Human contact

A way to introduce pathogens into food is via workers' hands who follow basic hygiene procedures. In this way, microorganisms are transferred to legumes through trace amounts of faecal matter present on workers' hands or any other parts of the body that have encountered it. Researchers have found more than 250 foodborne microorganisms that are possible to transfer from workers' hands to legumes (Lee *et al.*, 2020), among them bacteria, viruses and parasites. In addition to chemical contamination, food contamination and foodborne illness occur also because of cross-contamination (Centers for Disease Control and Prevention (CDC), 2022).

Specifically, the general manipulation of foodstuffs in the field is a major concern, with a special emphasis on the postharvest phase of the farm-to-fork supply chain. As a result of previous contact with contaminated soil or product, it is possible to transfer pathogens from the soil or product to the workers' hands (Machado-Moreira *et al.*, 2019). The application of prevention measures such as immunization and biosecurity of handlers helps to decrease the risk of disease transmission. This also contributes to good farm management because healthy workers increase the efficiency and sustainability of food production, as well as the overall level of food security.

2.4 Facilities

A large proportion of foodborne diseases occur during food preparation at home or food production sites, primarily because of the use of poorly cleaned equipment during processing and unhygienic practices. The environment where food is produced is also important. In fact, foodborne diseases are avoided if the standards that control Food Safety and Hygiene Regulation 2009 are adhered to in all steps of the food manufacturing process (Medeiros *et al.*, 2001). When food manufacturers and merchants comply with Codex standards, consumers can have trust, satisfaction and quality of the items they purchase, and importers can be assured that the food they ordered will fulfil the specifications. The World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures recognizes Codex standards as the international benchmark for food safety. The Hazard Analysis Critical Control Point (HACCP) system is a food safety assurance method that has played a significant role over the past three decades. In 1993, Codex adopted application guidelines for the HACCP system to harmonize the approach internationally (Lee *et al.,* 2021). The availability of clean water, a clean environment and adequate sanitation facilities are the principal measures for preventing foodborne diseases. It was affirmed that poor cleaning and sanitation are factors that contribute to outbreaks of foodborne diseases. It has been suggested that dry surfaces reduce the risk of cross-contamination, in part because bacterial growth and survival are also reduced. *Salmonella*, on the other hand, may be able to survive on dry surfaces for an extended period (Beumer and Kusumaningrum, 2004). Legumes contaminate during the growing and harvesting of raw materials, storage, and transportation to the factory, as well as throughout the process. It is possible that the final product will get contaminated again during subsequent storage and transportation to retail outlets, as well as during storage and preparation by the consumer.

2.4.1 Equipment design

Legume processing technologies are divided into two categories which are the traditional and the modern. This classification is based on the complexity of the procedures and the type of equipment. Traditional procedures use simple technologies and equipment at the household level, on the other hand, modern processing uses sophisticated industrial processes and equipment (Clark *et al.*, 2014). Practices such as cutting and coring legumes after harvest have as a result a high risk of plant surface contamination. Field core harvesting knives are vectors of infection that allow pathogenic bacteria present in the soil to be transferred to the edible parts of the plant. Finally, reusable crates are also potential sources of cross-contamination among different batches of products (Machado-Moreira *et al.*, 2019).

2.4.2Raw materials handling and control

Dried legumes have a long shelf life in comparison to other foods. Dried legumes are stored in a cool dry place and cold rooms with optimal storage conditions. However, fermented legumes are stored in cold places that have the conditions for further fermentation, and they have an average storage time of three months. Whereas, sprouted legumes are stored for ~3 days, and it is advised to consume them freshly. Nevertheless, pathogens already present in the sprouts continue to grow in the absence of suitable food-handling practices throughout harvest, processing, preparation, and storage (Laborde, 2005).

In the case of a lethal intervention step or a thermal and nonthermal inactivation method in the process, contamination of low-moisture meals occurs because of exposure to the processing environment or the addition of previously contaminated raw materials. The presence of a small number of bacteria in low-moisture food increases the risk of infections such as salmonellosis, even if the development is inhibited. The absence of microorganisms with a low infectious dose is necessary in food immediately prior to consumption at any point in time (Finn *et al.*, 2013).

3. Persistence of foodborne pathogens in legumes

3.1 Dried legumes

Dried legumes have low water activity (a_w), and they are known as low-moisture food. Low-moisture food has an a_w of 0.85 or below (United States Food and Drug Administration (FDA), 2014; Codex Alimentarius, 2015). Generally, products in this category have a long shelf life and remain stable over many years. Dried legumes are susceptible to microbial contamination if they have improper handling and storage conditions. Under these conditions, they are a risk to consumers. Dried legumes are commonly misconstrued to be inherently safe from foodborne pathogens because of having low water activity (a_w) and this perception leads manufacturers to improper preparation practices that render the product unsafe for consumption due to microbial proliferation (Finn *et al.*, 2013). Strains of foodborne pathogens resistant to a variety of antibiotics could become a major health concern (Kiessling *et al.*, 2002). For instance, 48 strains of *Bacillus cereus* in legume-based Indian fermented food were found to be resistant to a minimum of nine different antibiotics (Roy *et al.*, 2007). Nevertheless, fermented legumes play a prominent role in vegan diets and contribute to the health benefits associated with similar diets.

Undesired changes on legumes, such as off-flavours, discolouration, and rotting, are predominantly caused by mould-producing mycotoxins, particularly under dry conditions. Mycotoxins can persist not only in the field and the raw components but also in the processing and storage environment of the finished product (Houbraken and Samson, 2017). Mycotoxins enter the food chain because of mould infection in the legume plants prior to harvesting. Most mycotoxins are chemically stable and resistant to food processing. Aflatoxin is the most prevalent mycotoxin in legume plants, which typically infects peanuts. Food deterioration caused by fungi has a significant impact on the food industry, with annual losses of ~50% (Adeyeye, 2016). As a result, fungal propagules (spores, conidia and mycelium fragments) penetrate and infect the legume at various phases of the manufacturing process. Nevertheless, fungi such as *Aspergillus oryzae* and *A. sojae* among others can produce enzymes and break down soybeans, as well as add flavour. Specifically, they have been used in the manufacturing of koji and soy sauce for at least three thousand years, which is beneficial to humans (Bamforth and Cook, 2019).

3.2 Fermented legumes

Fermented foods are subjected to the action of microorganisms or enzymes that cause desirable biochemical changes and a significant modification to the original raw material. Some examples are soybeans fermented to “tempe” in Indonesia, and peanut press cake fermented to “oncom”. Molds formed on these foods, and their mycelium connected the individual beans into a mat of product. Fermented foods may have begun as 'natural' processes in which available nutrients and ambient factors selected certain microorganisms that changed and preserved the food. Raw products for fermentation are a variety of beans, including soybeans, green beans, and black beans, among others. Every type of fermented bean has its own distinct flavour and texture.

Microbial hazards and disability-adjusted life years because of regular consumption of fermented foods are largely unknown, despite the fact that they promote health (Cocolin *et al.*, 2016). Different ethnic communities in India consume a wide range of fermented foods as a delicacy. Pathogens like *C. botulinum* is widely distributed in the natural environment and is easily transmitted to many types of fermented foods. It causes food spoilage as well as two types of food poisoning such as diarrhoea and emetic.

3.2.1 Risk of Clostridium botulinum outbreak by consumption of fermented legumes

Clostridium botulinum is an anaerobic, rod-shaped spore-forming bacterium that produces a neurotoxin protein. When toxin-containing foods are consumed, botulism, a severe form of food poisoning, occurs. *Clostridium botulinum* is a very common type of bacteria, but when left in an anaerobic environment free of oxygen, the toxins grow and present a risk. *Clostridium botulinum* remains in fermented legumes because of the lack of oxygen during the fermentation process (Alizadeh *et al.*, 2020). *Clostridium botulinum* is also present in improperly preserved and canned foods. Traditional foods are produced through unsanitary, uncontrolled natural fermentations, and sold in local markets. Although the consumption of these fermented foods caused outbreaks, the cases have been reported on a regular basis in India's Northeast region (Keisam *et al.*, 2019). An outbreak reported 6 cases associated with the consumption of fermented legumes, paste of soybeans, and wax gourds (Motarjemi *et al.*, 2001).

3.3 Sprouted legumes

3.3.1 Risk of salmonellosis by consumption of sprouted legumes.

Salmonella sp. is a facultative anaerobic gram-negative rod-shaped bacterium of the Enterobacteriaceae family. The process of identifying food-borne and clinical *Salmonella* isolates involves the use of biochemical and serological techniques. Both techniques are complicated and labour-intensive procedures in which the surface antigens of *Salmonella* are agglutinated with antibodies unique to *Salmonella* (D'Aoust, 2009).

Salmonella enterica has been found on sprouting legume seeds such as alfalfa, and it has been a source of food safety concern for decades. Between 2000 and 2002, seven outbreaks of *Salmonella* Enteritidis

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infections connected to mung bean sprouts were recorded in the United States, Canada, and the Netherlands, with the majority occurring in the United States (Mohle-Boetani *et al.*, 2009). Furthermore, in the previous 20 years, at least 40 serious outbreaks of foodborne disease have been connected to contaminated bean sprouts, alfalfa sprouts, and other sprouts in the United States, Canada, and Europe. On sprouting legume seeds as well as in the surrounding sprouted seed exudation, which offers a rich variety of resources such as carbohydrates and amino acids, *Salmonella* serovars demonstrate different development logs (Wahlig *et al.*, 2019).

3.3.2 Risk of Escherichia coli outbreak by consumption of sprouted legumes

Escherichia coli is a species of pathogen that ordinarily dwells in the intestines of humans and other animals. *Escherichia coli* is a genus of gram-negative, non-spore producing, facultatively anaerobic, rod-shaped bacteria of the Enterobacteriaceae family. Most *E. coli* strains are non-pathogenic and beneficial to the health of the digestive tract. On the other hand, it is also a cause of diarrhoea if contaminated food, drink, or water is ingested (Felson, 2020).

Outbreaks associated with *E. coli* contamination were reported in the United States, Canada, and Europe in the last 20 years and at least 40 severe outbreaks of foodborne illness have been linked to tainted bean sprouts, alfalfa sprouts, or other kinds. They included what was once thought to be the worst *E. coli* outbreak in the world, in 1996 an epidemic was reported in Sakai, Japan. Nearly 10,000 persons were infected with *E. coli* O157:H7 most of them were students and teachers who were served school meals prepared in a central kitchen. At least 12 people died because of renal failure and its side effects.

Health officials eventually identified radish sprouts as the source of the outbreak, which were cultivated on a Japanese farm from Oregon. The seeds sprout never have any signs of the harmful bacterium. However, epidemiologists used a process of elimination to discover the reason. This process was applied in previous cases when the contaminated food was long gone. Since then, Japan has tried to retrain officials to respond rapidly by interviewing outbreak victims. Table 2 shows that most outbreaks in sprouts were caused by *Salmonella* sp. and pathogenic *Escherichia coli* (Laborde, 2005).

3.3.3 Risk of Bacillus cereus outbreak by consumption of sprouted legumes.

Bacillus cereus produces toxins that cause two different types of gastrointestinal illness, one is emetic (vomiting) syndrome and the other is a diarrheal syndrome. Vomiting happens when the emetic toxin (cereulide) is generated in meals. The diarrheal syndrome arises when enterotoxins are created in the intestine because of consuming *B. cereus*-contaminated food. Pao *et al.* (2006) found that there is a significant incidence of enterotoxigenic *Bacillus* spp. in sprouting seeds intended for at-home production of sprouts. Pao *et al.* (2006) further elaborates that a food poisoning epidemic connected to tainted vegetable sprouts was linked to *B. cereus* as the primary agent. Food poisoning outbreaks caused by *B. cereus* have been linked to the ingestion of contaminated rice on repeated events. It was also reported that some seed legumes, such as red clover, have antimicrobial activity that could prevent *B. cereus* growth in food production or storage systems. More research is recommended in the isolation and identification of inhibitory compounds in legume seeds, as well as their safe use in food protection applications (such as essential oils) (Pao *et al.*, 2006).

3.3.4 Risk of listeriosis by consumption of sprouted legumes

Listeria monocytogenes is present in damp habitats, soil, water, rotting vegetation, and animals. *Listeria monocytogenes* can survive and proliferate in a cold room and in other difficult environments. The human disease transmitted by *L. monocytogenes* is listeriosis (Oxaran *et al.*, 2017). *Listeria monocytogenes* recalls cause concerns among consumers since these bacteria

Table 2. *Salmonella* sp. and pathogenic *Escherichia coli* outbreaks associated with the consumption of legume sprouts from 2015 to 2020 in the United States.

Sprouts type	Foodborne pathogens	Year	Number of cases	Outbreak location	References
Clover sprouts	*E. coli* O103 (STEC)	2020	51	USA	CDC (2020)
Raw sprout	*S. enterica* serovar Montevideo	2017-2018	10		CDC (2018)
Alfalfa sprout	*S. enterica* serovar Reading and *S. enterica* serovar Abony	2016	36		CDC (2016a)
Alfalfa sprout	*E. coli* O157 (STEC)	2016	11		CDC (2016b)
Alfalfa sprout	*S. enterica* serovar Muenchen and *S. enterica* serovar Kentucky	2015-2016	26		CDC (2016c)
Raw and sprout butter	*S. enterica* serovar Paratyphi B variant L(+) tartrate(+)	2015	13		CDC (2016c)

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lead to deadly infections in susceptible individuals such as pregnant women, elderly people, and immunosuppression subjects (Oxaran *et al.*, 2017). Since the early 1980s, large-scale human listeriosis outbreaks have been connected to contaminated foods in multiple countries. Several studies on the detection of *L. monocytogenes* in various food types, including legumes, have been developed. Between 2015 and 2018, an outbreak of *L. monocytogenes* serogroup 4 generated 53 cases in five European countries. Whole genome sequencing (WGS) revealed that the sickness was caused by frozen sweet corn from a supplier in Hungary (Willis *et al.*, 2020).

4. Current foodborne pathogens inactivation methods in legumes

4.1 Thermal technologies

Changes in the properties of key microorganisms, changes in production procedures, climate change, ecological changes, and an increase in global food traffic have all contributed to the emergence of new threats. Food processing technology has progressed as a response to shifting customer demands and food safety concerns (Misra *et al.*, 2011). In an illustration by Van Impe *et al.* (2018), there are numerous methods for preventing the emergence of food-borne pathogens, including pasteurization. However, the production of highly resistant spores by stressed vegetative cells raises serious food safety and spoiling concerns. It is possible to inactivate foodborne pathogens in legumes by non-commercial boiling, autoclave, roasting and microwave methods.

Additionally, the process selected contributes to the quality, digestibility, nutrition, and health benefits. However, processing has a few advantages, including the inactivation of heat-labile antinutritional factors such as pulse antitrypsin factors, which reduce protein bioavailability (Avilés-Gaxiola *et al.*, 2018), the reduction of undesirable factors such as phytates in pulse, and the modulation of pulse amino acid composition and protein digestibility. Smelt and Brul (2014) analysed that minimum thermal treatment parameters for food products should be established within the context of a microbiological risk assessment. Exposure assessment is one component of risk assessment. In general, exposure evaluation considers contamination of raw materials, contamination during processing, growth or inactivation of the microbe, storage and distribution circumstances, and pathogen infectivity.

4.1.1 Boiling

Boiling is one of the most ancient techniques of moist-heat regulation of germs, and it is successful at killing vegetative cells as well as some viruses, among other things. Culinary methods such as boiling are less successful at killing endospores, which can live more than 20 hours under boiling conditions in some cases. Additionally, boiling is less efficient at higher altitudes since the boiling point of water is lower at higher altitudes and the boiling time required to kill germs is longer at higher altitudes. These are the primary reasons why boiling is not recommended for sterilizing purposes in the laboratory or clinical context. Peng *et al.* (2019) affirm that boiling water kills or inactivates pathogens causing damage to structural parts, and it stops essential life processes, (e.g., protein denaturation). According to Imaizumi *et al*. (2019), it is recommended a boiling time of 30 minutes at 100°C. Boiling is not sterilization, and it is better stated as pasteurization.

Cooking food is also a way to make it safe. It is considered cooked food when it is heated up to at least the pasteurization temperature for a target microorganism and held for a period to make pasteurization work. Temperature and time are directly related to the effectiveness of pasteurization. Boiling temperatures range from 190°F (88°C) to 212°F (100°C). Blanching at a temperature is from 50°C to 70°C, from one to 10 minutes. Water-borne pathogens are inactivated or killed at temperatures below the boiling point (212°F or 100°C); these are not typically associated with water-borne diseases such as *Clostridium botulinum* spores (Peng *et al.*, 2017).

4.1.2 Autoclaving

In an autoclave, which is a sealed container and a large pressure cooker, steam under pressure is used as the sterilizing agent. Steam reaches higher temperatures and has greater heat content and sterilizing power because high pressure makes it possible for the steam to reach those temperatures (Oyawale and Olaoye, 2007). Autoclave is being done for 60–90 mins at 121°C, depending on the loading circumstances, to achieve a wet temperature of at least 115°C for 20 mins. The length of time, temperature, and volume of steam that is permitted to enter the autoclave determine the efficacy of the device as a sterilizing method (Scott and Walls, 2003). Because the autoclaving process raises the temperature of the food, microbial proteins, nucleic acids, and lipids are harmed by these high temperatures. It causes protein and nucleic acid denaturation, which disrupts cell metabolism (Wason *et al*., 2021).

4.1.3 Roasting

During rousting food is kept at a high enough temperature for an extended period to destroy

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microorganisms. Roasting is also a process of cooking. Roasting is also comparable to baking; however, the two procedures are not the same. Roasting is often used for meat and vegetables, while baking is typically used for bread in a closed oven. Roasting requires a higher temperature than baking and the use of fat either butter or oil.

Roasting is a scheduled process in which a sequence of continuous temperature combinations has the potential to be a successful method. The process of dry roasting needs 60 mins at 160°C (Prestes *et al.*, 2019). Roasting is an effective method for microbial death because of the combination of high temperature and time. In addition, the use of oil or butter to conduct heat is recognized as an efficient means of heat transfer. As a result, temperature, time, and heat conduction mean inactivating pathogens in legumes. Roasting involves heating food until it turns a non-enzymatic browning or caramelizes to a deep amber colour. These changes produce flavour. The tasty crust of the food is obtained by the application of fat to keep moisture (Lee, 2021). The growth of pathogens is governed by a connection between time and temperature.

4.1.4 Microwave heating

Microwaves are utilized to irradiate living organisms. They have both thermal and non-thermal effects. Thermal effects are the result of the absorption of microwave radiation by cell molecules, which causes them to vibrate faster and causes the cell to heat up in general. A cell dielectric constant and electrical conductivity are both important factors in determining how much microwave radiation absorbs. A theory about the non-thermal effects of microwaves was developed after studies revealed that microwaves cause microbial death. These studies showed that bacterial cultures were significantly more destroyed by microwave-induced heating than by other heating methods that produced the same working temperature (Park *et al.*, 2017). Despite significant progress, the mechanism of microwaves' non-thermal impact remains a mystery. Although it appears that changes in the secondary and/or tertiary structure of functional proteins occur because of rotation and lining-up of the molecules with a fast-alternating electric field (> billion times/s), further research is required to confirm this.

4.2 Nonthermal technologies

In tandem with changes in customer demands and food safety concerns, advancements in food processing technologies have been made to maintain continued food safety. Further, when it comes to the quality of thermally processed meals, there are a few factors to consider, including the loss of nutritional content, and the negative impact on sensory quality. As a result, so-called nonthermal technologies have emerged as a viable alternative. An effective technology at ambient or sublethal temperatures is known as nonthermal technology. This type of preservation treatment reduces the detrimental effects of heat on the nutritional and quality factors of food (Chacha *et al.*, 2021).

4.2.1 Cold plasma

The sterilizing qualities of plasma were initially proposed towards the end of the 1960s, and it was patented in 1968. The first work with plasma created from oxygen was proposed in 1989. A great deal of investigation has been done into the mechanism of microbial inactivation by plasma agents. The interaction of the plasma agents with the biological material contributes to microbial death and inactivation (Scholtz *et al.,* 2015). According to Hati *et al*. (2018), plasma produces a large number of reactive species and free radicals which interact with the plasma membrane. Reactive oxygen species produced by plasma have oxidative reactions on the membrane. These reactions have as a consequence damage to cell organelles and mitochondria, which leads to severe cell damage.

A wide spectrum of microorganisms such as spores and viruses are efficiently inactivated and eliminated by cold plasma. Balthazar *et al.* (2019) revealed that *Bacillus subtilis, Erwinia,* and *E. coli* are examples of pathogens successfully shown to have been inactivated by the cold plasma method. In addition, this method has been also evaluated on *S. enterica* ser. Typhimurium and *L. monocytogenes*. The effect of plasma is selective; it either causes damage to pathogenic organisms while causing no damage to the host or activates different pathways in different organisms, depending on the situation. It is possible to use the pathogen-inactivating properties of cold plasma as a treatment step for fresh produce to lower microbial burden while maintaining nutritional and other essential features (Balthazar *et al.*, 2019).

4.2.2 Irradiation

Foodborne infections in vegetables are effectively controlled with the use of irradiation; it is restricted to killing pathogens because of the potential impact on food quality, especially at high doses. Microbiological decontamination occurs during irradiation through radiolysis, which breaks DNA and produces reactive chemicals (hydroxyl radicals, hydrogen atoms, and hydrogen peroxide), which harm cellular metabolic pathways cause intracellular oxidation and ultimately lead to cell death. The use of ionizing irradiation to kill bacteria considers the amount of treatment, the dose, and the rate of absorption. Other factors also considered include the physiological state of the bacteria and the environment. It is mostly used for microbial decontamination of perishable food products such as fish and meat as it is a non-thermal treatment (Dikici *et al.*, 2021). A decrease in treatment dose leads to a better quality of life for the treated items because the sensory quality degradation caused by ionizing radiation is dose-dependent. Because of an increase in the radiation sensitivity of the target microorganisms, smaller doses of radiation are required for lethality, and vice versa. Foodborne pathogens such as *E. coli* O157:H7 and *S. enterica* ser. Typhimurium are radio-sensitized more effectively if they are exposed to irradiation in the presence of active chemicals, such as bio-active edible coatings, and in the presence of modified atmosphere packaging (Correa *et al.*, 2019). As an example, gamma rays cause damage to the microbial DNA and render the cell inactive UV-C radiation method of microbial inactivation generated by a mercury lamp influences microorganisms. The inactivation and reduction in the pace of microbial growth are mostly caused by the synthesis of pyrimidine dimers (Thymine-Cytosine).

Batra *et al.* (2019) found that legumes are widely cultivated and provide an affordable and significant source of nutrients. The absence of consumer-friendly adjustable technologies for lowering non-nutritional components in the pulse has been a deterrent to the consumption of a variety of sections of legume plants (discomfort and other difficulties). Legumes are very susceptible to microbial pathogen contamination and play an important role in pathogen transmission. The global consumption of these products is increasing, as the number of produce-related disease outbreaks, has a significant socioeconomic impact (Batra *et al.,* 2019).

5. Conclusion

In summary, dried, fermented, and sprouted legumes can pose microbiological hazards from different foodborne pathogens. Routes of microbial transmission via contaminated water, processing facilities, processing equipment, and human contact should be controlled stringently to avoid pathogens in the legumes. By recognizing the most common pathogen associated with these products and the source of microbial contamination, appropriate inactivation methods for legumes can be effectively utilized to guard the safety of legumes.

One possible intermediate intervention to ensure the safety of legumes is a reliable food safety management system such as Good Agricultural Practice (GAP). GAP is important because it reduces the risk of outbreaks. GAP is applied to activities such as cultivation, harvesting, sorting, packaging, and storage operations for fresh fruits and vegetables. They are developed in a stepwise manner, and they take into consideration the risk associated with the product type and cultivar as well as the scientific data available. They contribute to identifying potential sources of bacterial contamination on the fruit or vegetable surface. In other cases, the risk is also related to the produce or preharvest practices associated with the absence of GAP, and poor production and handling practices, such as the use of untreated manure, contaminated irrigation water, or unsanitary handling. During a hepatitis A outbreak in 2003, farms that had adopted GAP maintained a rather stable volume of sales and demand for their other products in comparison to farms that had not implemented GAP (Wheeler *et al.*, 2005). The same author affirmed the contrary on farms that had not implemented GAP. As a result, the volume of green onion sales declined by 50%, and the demand for other products decreased by 30%. Sedaghati and Hokmabadi (2014) reported that the implementation of Good Agricultural Practices (GAP) on a consistent basis limits the risk of microbiological contamination of seeds used in legume production and processing. In addition, Machado-Moreira *et al.* (2019) affirmed that GAP during on-farm production and post-production procedures that have as a result safe agricultural products are critical to ensure a safe food supply.

Conflict of interest

The authors declare no conflict of interest.

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