Antimicrobial potential of LEGUMES extracts against foodborne pathogens: A review

M.C. Pina-Pérez* and M.A. Ferrús Pérez

Dpto. Microbiología, Universitat Politècnica de València, Camino de vera s/n, 460022 Valencia, SPAIN.

*corresponding author: conpipe@upvnet.upv.es

ABSTRACT

Background: Alternative protein sources are being investigated under the increasing consumer demand of innovative and healthy food products from vegetable origin to replace the non-sustainable animal exploitation. In that sense, *Leguminosae* family includes a wide variety of plants and nutritious seeds, very rich in protein with high biological value, carbohydrates, vitamins and minerals. Not only the seeds, but also the aereous parts, pods, hulls and roots have demonstrated to be natural sources of antioxidants, anti-inflamatory and antimicrobial compounds.

Scope and Approach: The present article overviews the antimicrobial potential of the most popular legumes wordlwide against foodborne pathogens.

Key Findings and Conclusions: According to the reviewed literature, soybean and chickpeas are the two consumed legumes with highest antimcirobial activity. Long-chain soy peptides (IKAFKEATKVDKVVVLWTA) resulted with high antimicrobial potential against both Gram positive and Gram negative bacteria at concentration level of 37.2 mM. Also, a wide spectrum of proteins and peptides in raw chickpeas and processed extracts have exerted antimicrobial activity against foodborne pathogens applied in the range 8-64 µg/ml. These results open a new research line regarding the development of a new generation of natural preservative ingredients and extracts to be included in novel formulated products based on the extraction and functionality assessment of phytochemicals from legumes, mainly proteins and peptides.

Keywords: antimicrobial potential, antioxidants, proteins, peptides, legumes, pulses, novel food.

1.INTRODUCTION

Generally speaking, **legumes** are the fruits and grain seeds belonging to the *Fabaceae* (or *Leguminosae*) family. Forage, grain, blooms, pharmaceutical/industrial, fallow/green manure, and timber species are all of them farmed legumes, that depending on their mature stage are used with several applications. The word "legume" is derived from the Latin verb *legere* which means to gather. According to FAO the term "pulse" is reserved for crops harvested solely for the dry seed. This word derives from a porridge, cooked bean dish which the ancient Romans use to eat in early 301 B.C. (Allaire and Brady, 2008). With about 13.000 species, leguminosae family is the second largest in vegetables and ranks among the most economically important ones.

Legumes are an essential food in the traditional and modern diet of people worldwide. It seems that the earliest human-domesticated plants were legumes (APO, 2003; Polak et al., 2015). Asian, Indian, or American cuisine was based on soybeans, lentils, black beans, and peas, as main ingredients in traditional dishes (Shuster-Gajzágó, 2002). It is in recent years, as the result of consumer concern for a healthy diet, rich in nutraceutical compounds when the development of new ingredients and products derived from legumes is emphasized (Ghadge et al., 2008; Gowen, 2008). The balanced ratio between proteins (13-15 %) and carbohydrates (4-23 %) present in legumes put these ingredients in the base of the food pyramid for specific populations, i.e vegetarians and vegans. Due to the high biological value of their proteins, legumes are named as the "*poor people's meat*".

Last year 2016 was declared by the 68th UN General Assembly as the International Year of Pulses (IYP 2016) with the aim to increase the general knowledge about the benefits derived from legumes consumption by the general public, and to emphatize the industry research and integration of these ingredients into their products.

The most relevant pulses around the world include the followings: road bean or fava bena (*Vicia faba*), lima bean (*Phaseolus lunatus*), moth bean (*Vigna aconitifolia*), adzuki bean (*Vigna angularis*), urad bean (*Vigna mungo*), mung bean (*Vigna radiate*), black eye peas (*Vigna *unguiculata*), chickpeas (*Cicer arietinum*), pea (*Pisum sativum*), lentil (*Lens culinaris*), soybean (*Glycine max*), pigeon pea (*Cajanus cajan*), lupine bean (*Lupinus albus*) and the peanut (*Arachis hypogaea*). In spite of the many species and sub-species of legumes that are known, only about a dozen of them are important as commercial food crops. Amongst them, beans and peas account for about 25 per cent of the total legume crops production.

Nowadays, the preference for proteins of vegetable origin, and the need to improve the formulation of protein enriched and energetic products aimed to infant, senior or sportsman sectors, constitute a new line of research and guides the trending products development. In the same line, the R&D of food industry, academia and pharmaceutics is focused on the investigation and analysis of extracted bioactive phytocompounds from legumes, with innumerable and valuable technological and functional properties, taking advantage of the nutritional benefits associated to them (Malaguti et al., 2014; Ponnusha et al., 2011).

Among the technological properties of legumes, one line of research still remains unexplored. Answering to the requirements of the food industry to formulate new clean-labeled products with alternative antimicrobial compounds from natural sources, legumes and their by-products appear to be a potential group rich in a wide variety of phytochemicals with demonstrated antibacterial activity (Nair et al., 2013; Nguyen et al., 2014). The present work overviews the most relevant studies published in lasts years [2002-2017] including raw legumes, and its derivatives as bioactive compounds with antimicrobial potential, to be introduced in food and pharmaceutic formulated products; and remarks the uncovered points to deal with in the near future. The specific capability of the generally consumed legumes worldwide against several of the most concerning foodborne pathogens is detailed in Table 1.

2.MAIN BIOACTIVE COMPOUNDS IN LEGUMES WITH ANTIMICROBIAL POTENTIAL

2.1. Polyphenols in legumes

Polyphenols are a structural class of natural chemicals present in vegetables (leaves, fruits, sedes) characterized by phenol structural units. As secondary metabolits in plants, phenols play significant physiological and defensive roles in superior plants and could positively impact human health by means vegetables consumption. These phytochemicals show highly diverse structures and over 500 polyphenol different molecules are known in foods (Neveu et al., 2010). Polyphenols are present in plant tissues mainly as glycosides. Also these molecules could appear associated with various organic acids and/or as complex polymerized molecules with high molecular weights, such as tannins (Daglia, 2012). Among the most relevant polyphenols are flavonoids, phenolic acids, qoumarine, quinone, stilbenes, lignans, tannins, and others, remarked by their biological functions, including anti-allergic, anti-inflammatory, anticancer, antihypertensive, also with antimicrobial and antioxidant activities (Fidrianny et al., 2014). It is highly valuable the potential of polyphenols to protect against oxidative cell damage. In this sense, and taking into account the richness in polyphenols of legumes, some studies have been carried out in recent years dealing with the antioxidant potential of legume extracts (Zhao et al., 2014; Gujral et al., 2012, 2013). The antioxidant properties of this phytochemicals group are justifying the boosted interest of the scientific community to use specific vegetables and extracted polyphenols in the prevention of several major chronic diseases associated with oxidative stress, such as cardiovascular diseases, cancers, type II diabetes, neurodegenerative diseases or osteoporosis (Pérez-Jiménez et al., 2010).

Some polyphenols have been extensively studied and related with an antimicrobial potential against a wide spectrum of microorganisms (Pina Pérez et al., 2011; Pereira et al., 2007). Flavan-3-ols, flavonols, and tannins received most attention due to their effectiveness to suppress a number of microbial virulence factors (such as inhibition of biofilm formation, reduction of host ligands adhesion, and neutralization of bacterial toxins) and their synergism with antibiotics (Daglia, 2012). Among the most accepted mechanisms responsable for the polyphenols antimicrobial capability are the followings: desestabilization of the cytoplasmic membrane, the permeabilization of the cell membrane, the deprivation of essential mineral micronutrients such as iron and zinc by means quelation and the inhibition of extracellular microbial enzymes, and direct interferences on microbial metabolism (Daglia, 2012; Heinonen, 2007)

The antioxidant and antimicrobial potential of aqueous hull extracts from mung bean (*Vigna radiate*), chickpea (*Cicer arietinum*), and pigeon pea (*Cajanus cajan*) was evaluated by Kanatt et al. (2011). The pigeon pea hull extract was the richiest one in total phenolic and flavonoid content as compared to mung and Bengal gram hull extracts, being this fact associated with the highest antimicrobial potential of this extract against *B. cereus*.

Food processing affects the content in polyphenols of grains and legumes mainly by the dehulling of legume seeds and decortication. The main reasons to commercialize dehulled legumes are both the reduction of the cooking time and the removement of the bitterness. In that sense, and taking into account that decortication of seeds could represent up to a final level of 20% bio-wastes, food processors are also interested in the re-valorization of these by-products (e.g. legumes hulls) by means their antioxidant and antimicrobial potentials (Kanatt et al., 2011).

3.2. Proteins in legumes

Proteinase inhibitors (work storage proteins) present in plants and particularly in legumes are also recognized to exert antimicrobial capability. Natural defense compounds from plants against pests and pathogens are nowadays excellent candidates for use as alternative sources of antimcirobial compounds in food. In this sense, the mechanism of action for these compounds is regarding the suppression of the enzyme activities in response to attack by proteinases produced by phytopathogenic microorganisms (Kim et al., 2009).

The specific antimicrobial potential of proteins from soybean and chickpea has been studied against *Listeria monocytogenes* and *Salmonella enteritidis*. Methylated proteins from both legumes revealed a concentration-dependent antimicrobial activity. It was observed that under the optimal growth temperature of the bacterium, the studied proteins inhibited close to 97 and 91 % of the *L. monocytogenes* and *S. enteritidis* growth, respectively, after an exposure period of 6–12 h. The antimicrobial potential was attributed to the methylated subunits that act with cell wall and cell membrane, producing channels and pores and affecting the integrity of the cell, and finally achieving the lysis and the death of the studied microorganisms (Sitohy et al., 2013). Also the reduction in bacterial growth by means the effect of broad bean, soybean and chickpea proteins isolated and esterified with methanol was observed by Sitohy et al. (2013). Applying methylated proteins at concentration levels in the range [0.1-10] mg/ml it was observed a concentration dependent inhibition zone both against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeroginosa* and *Escherichia coli*) bacteria by means the agar diffusion method.

This research group also investigate the antimicrobial potential of esterified legume proteins in a real food matrix, raw buffalo milk kept at 4 ºC, to control the growth of psychrotrophic bacteria (Sitohy, Mahgoub, Osman, 2011). The addition of esterified legume proteins to raw buffalo milk samples enhanced the shelf-life of the product from 2 days (in control samples) up to 6 days in supplemented samples. Significant reduction in psychrotrophic bacteria count (PBC) and *Pseudomonas* spp. was detected in supplemented samples, being the total bacterial counts in the range of [5.1-5.3] $\log_{10}$ cfu/ml. The casein coagulation in supplemented milk was delayed up to 10 days at storage temperature of 4ºC.

3.2.1. Peptides from legumes. Peptides defined as released protein molecules (smaller than 10 kDa) through digestive enzymes have been associated with several positive effects on human health, being among the most relevant ones their capability to low blood pressure levels (ACE inhibitory), cholesterol-lowering effects, antithrombotic and antioxidant properties, and also an antimicrobial potential has been associated to peptides from legumes (Malaguti et al., 2014). Peptides with antimicrobial nature are known as antimicrobial peptides (AMPs). Roots, stems, leaves, flowers and seeds from a wide range of vegetable species are sources of plant antimicrobial peptides (AMPs) (Ye et al., 2002). AMPs are generally active against a broad spectrum of micro-organisms including Gram-positive and Gram-negative bacteria, fungi, viruses and some cancer cells (Barari et al., 2015). AMPs interact with the bacterial cell membranes leading to changes in permeability and finally the cell death (Li et al., 2012). Also, the partial cell membrane disruption, moreover to disturbed osmotic regulation are recognized as mechanisms of AMP action against bacterial cells (Li et al., 2012). The estructure and sequence of amino acids in AMP peptides are the main factors responsable for their effectiveness as antimicrobials. According to the studies of Dhayakaran (2014) long chain soy peptides (IKAFKEATKVDKVVVLWTA) (37.2 µM concentration) were more effective against *P. aeruginosa* and *L. monocytogenes* than short chain (PGTAVFK) peptides

3.2.2. Lectins in legumes. Lectins are proteins involved in plant defense (direct interaction with infectious agents, protecting plant from animal predators and pathogens). Lectins act by means of the breakdown of the invaders membranes (fungi, bacteria and viruses). These bioactive compounds have been associated with anti-cancerigen effects, being the richness in lectins one of the main reasons by which other vegetables additionally to legumes (tomatoes, corn, whole grain rice, wheat, oats, nuts, sunflower seeds, peaches, mangos, grapes, cinnamon, citrus, berries, tea) are healthy to human (Van Buul & Brouns, 2014). According to the estudies of Nair et al. (2013) legume lectins were able to inhibit the growth of bacteria but not to kill them. Lens culinaris (*lentil)* and Pisum sativum *(pea)* purified lectins were effective against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Although lentils showed the lowest antibacterial activity, both lentil and pea purified lectins were effective as bacteriostatic agents. The antimicrobial mechanisms of lectins against bacteria are based in the interaction of lectin with teicoic and teicuronic acids, peptidoglycans and lipopolysaccharides (Paiva et al., 2010).

4.LEGUMES WITH ANTIMICROBIAL POTENTIAL

4.1. Chicpeas (Cicer arietinum)

The antimicrobial effectiveness of cicer extracts depends on their composition, nature and origin (Kan et al., 2010). According to the studies of Kan et al. (2010) fruit skin extracts of cicer exerted the same antimicrobial capability than aerial part extracts against *E. coli, K. pneumoniae, and E. faecalis* at the concentration level of 32 µg/ml. Chickpeas seed extracts showed the highest antifungal activity against *C. albicans* at a concentration level as of 8 µg/ml (Kant et al., 2010).

Seed extracts and also hull extracts from chickpeas have demonstrated to be effective both against Gram positive (*S. aureus*, *B. subtilis*, *E. faecalis*) and Gram negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) (Kanatt et al., 2011) being more effective against Gram negative bacteria with MIC values in the range [16–64] µg/ ml, and requiering higher MIC values against Gram positive bacteria (close to 64 µg/ ml) (Al-Snafi, 2016). Chickpea seed methanol extracts showed a higher antimicrobial potential against Gram positive bacteria (*B. cereus* and *S. aureus*) than Gram negative, being the required MIC against *E. coli* equal to 582.2 µg / mL.

Even the crude water extracts of *Cicer arietinum* have shown significant antifungal activity at concentrations below 5 % (w/v). Barari et al. (2015) studied the antimicrobial potential of aqueous extracts from the chickpea seeds against several microorganisms, among them the foodborne pathogens *Escherichia coli* (*E. Coli*) (ATCC 25922), *S. aureus* (*S. aureus*) (ATCC 25923), *B. cereus* and *B. subtilis* (PTCC 1715).

The baseline of the antibacterial and antifungal properties of chickpeas was studied regarding its protein and peptide profile. Several proteins in *Cicer arietinum* have demonstrated antimicrobial potential including a glucanase, a chitinase and a cyclophyllin-like protein. Bioactive protein molecules from chickpeas have demonstrated effectiveness to inactivate bacteria, fungi, viruses and also carcinogenic cells (Montesinos, 2007; Tam et al. 2015). A wide spectrum of molecules, from less than 14.5 kDa to more than 100kDa, was detected by SDS-PAGE analysis of crude chickpeas protein. The antibacterial activity of 5 % chickpeas ammonium sulphate precipitated proteins was demonstrated against *B. cereus* and *S. aureus*, with inhibition zones of 8 and 10 mm, respectively, observed by means disk diffusion in Müeller Hinton Agar. The antimicrobial activity of these extracts was higher against Gram positive bacteria than against Gram negative.

Serine proteinase inhibitors (PIs) from *Cicer arietinum* (L.) seed extracts have shown antimicrobial activity, exerting antimetabolitic activity against *Helicoverpa armígera* (Nair & Sandhu, 2013). Also AMPs in chickpeas have been tested against spoilage microorganisms in food. The antifungal activity of cicerin and arietin (5-8 kDa) isolated from the seeds of the chickpea (Ye et al., 2002; Al-Snafi et al., 2016) have demonstrated their antifungal activity against *M. arachidicola*, *Botrytis cinerea*, and *Fusarium oxysporum,* being arietin the most effective.

Another compound with recognized antimicrobial potential, extracted from Cicer roots of wild species, is the cicerfuran. Cicerfuran is a phytoalexin with effective antifungal activity. This compound has been described to be the major defensive agent against *F. oxysporum* f.sp. ciceri, with antimicrobial activity also against *Botrytis cinerea*. However, the low levels at which this compound is present in nature has led to scientists to an artificial syntetization, being the antibacterial and antifungal activity of these cicerfurans compared to the antimcirobial protential of natural ones. The antimicrobial activity of these phytoalexins is finally dependant on the presence of one free hydroxyl group in the molecule, being moreover, the position of the hydroxyl very important to maintain the antimicrobial potential (Aslam et al., 2009).

According to the studies of Lopez-Amoros et al. (2006), the most relevant polyphenols found in chickpeas at different concentrations are hydroxybenzoic phenolic compounds, protocatechuic, p-hydroxybenzoic, vanillic acid, trans-ferulic acid, cis and trans p-coumaric acid, which have been associated with antimicrobial properties (Daglia, 2012). Ghiassi et al. (2012) studied the antimicrobial and antioxidant effect of phenolic compounds present in acetone and methanolic extracts of chickpeas, germinated and not germinated. According to their studies, ethanolic extracts from chickpeas showed the highest antioxidant potential, being in spite of this, the acetone extract the richest in polyphenols. The germination process increased the antioxidant potential of the chickpeas extracts, due to the synthesis of structural proteins and other bioactive compounds that happens during this process (Kuo et al., 2004). According to the studies of Fernandez-Orozco *et al*. (2009) the germination of chickpeas contributes to improve the antioxidant potential of these legumes, manifested by means of an increase in the total phenolic content, and a slight inhibition of lipid peroxidation. These results have been supported by several subsequent studies confirming the possibility to include chickpeas flours in new functional products formulation (Abou Arab et al., 2010).

Peas (Pisum sativum)

The antibacterial activity of seed/pod of *Pisum sativum* L. (garden pea) was demonstrated against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus vulgaris,* and *Pseudomonas aeruginosa* among others (Rehman & Khanum, 2011). Antimicrobial potential of purified studied peptides (~19 kDa, ~22 kDa, for seed peptides; and ~10 kDa and ~11 kDa, for pod peptides) was exerted at pH values in the range of 5-7. The effectiveness inhibiting bacterial growth of purified peptide seed S_4 and S_5 (tested by disc diffussion method) (0-200 µg/ml) and pod (P7 and P8) fractions was especifically high against *S. aureus*, with a MIC required value between 75 µg / ml and 100 µg /ml, respectively. High MIC values were observed against other bacteria, mainly Gram negative *S. typhi* and *E. coli.* The antimicrobial potential of seed and pod studied peptide fractions was dependent on temperature, being S_4 fraction effectiveness enhanced from 4ºC to 25ºC against *E. coli* and *S. typhi*. The effectiveness of purified peptides from pea against *S. aureus* was doubled regarding the antimicrobial potential of the antibiotic used as a control. Also the studies of Golla et al. (2016) revealed the potential of germinated seeds to accumulate peptides with antimicrobial potential. P*eptides (<10kDa)* of pea were effective against, *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. Among the studied germinated seeds extracts, phosphate buffered (PBS) *Pisum sativum* exerted the highest inhibition potential of the obtained ones in the study of Golla et al. (2016), showing 22.16±0.04 mm diameter zone against *Staphylococcus aureus*, 18.58±0.03 mm against *Escherichia coli*, and 9.35±0.05 mm against *Pseudomonas aeruginosa*.

According to the studies of Habib et al. (2016) the pea sativum 90 % aqueous extract was highly effective inhibiting *E. coli* growth, being the 60% fraction with moderate antibacterial activity, meanwhile 30% aqueous extract not revealed significant antibacterial effect. The antimicrobial potential of these extracts was attributed to the protease inhibitors present in these plant materials, that inhibit the protease secreted by microorganisms, causing the lost of cell viability due to the reduction in the available amino acids necessary for growth and development.

Also the specific lectins present in *Pissum sativum* have been recognized as antimicrobials against *Aspergillus flavus* and *Fusarium oxysporum* (Sharma et al. 2009).

The promising scientific results regarding the antimicrobial potential of pea sativum revealed that even novel antibiotic substances could be obtained and purified from this plant, especially from non-edible part of peas, contributing to the legumes by-products revalorization (Ayala et al., 2014).

Pigeon pea (Cajanus cajan)

Cajanus cajan leaves are specifically rich in flavonoids and stilbenes, and also contain saponins, tannins, and moderate quantities of reducing sugars, resins and terpenoids (Pal et al., 2011). The *in vitro* antimicrobial potential of *C. cajan* has been assessed against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans*, among others. The supercritical fluidextracts showed a marked inhibitory effect against *S. aureus* and *B. subtilis* (Pal et al., 2011).

The antimicrobial potential of this legume has been also related to its content in a coumarin, named as cajanus lactone, which exerts high antibacterial activity against *S. aureus* (Kong et al., 2010). Also isoflavanoids present in ethanolic extract of pigeon pea leaves show significant antimicrobial potential (Zu et al., 2010)

According to the studies of Okigbo, & Omodamiro (2007) the antimicrobial potential of leaf extracts of *Cajanus cajan* was assessed against several bacteria and fungi (including *Escherichia* *coli, Staphylococcus aureus, Salmonella typhi* and *Candida albicans)*. Organic solvent extracts (petroleum ether, ethanol, and chloroform/methanol mixture) were effective inhibiting *E. coli, S. aureus*, and *S. typhi*, being the aqueous extract only effective against *E. coli* and *S. aureus*. The most sensitive microorganism to the pigeon pea extracts was *S. typhi*, with MIC in the range of 0.0325–0.0625 mg/ml, being *E. coli* the most resistant with MIC values between 0.125 and 0.25 mg/ml. No antimicrobial effect of the assayed extracts was detected against *C. albicans* (Okigbo, & Omodamiro, 2007). In the same way, and according to *Obiorah* et al. (2012) *Cajanus* leaf extracts showed significant inhibitory activity against *S. aureus* (9 - 14 mm) and *Bacillus subtilis* (7 - 12 mm), being these fractions rich in reducing sugars, glycosides, saponins, tannins, resins and acidic compounds.

Mung Bean (Vigna radiata)

Certainly, the functionality and quality value (nutritional, organoleptical, technological) of vegetable food products is highly influenced and modified by the stage of breeding, post-harvest technological processing, and/or by modification of storage conditions. In legumes, it is well established that the induction of germination results in an improvement of the nutraceutical value, e.g. in soybeans, mung beans or lentils. During this process a wide variety of secondary metabolites are generated such as lignins, salicylates, coumarins, hydroxycinnamic amides, flavanoid phytoalexins, and pigments, enhancing also the protein and essential nutrients that are readily to be used by the body (Swieca, 2015). Specifically, sprouts like mung bean and fava bean are markadely rich in phenolics, vitamins and minerals with demonstrable increased amounts in slowly digestable carbohydrate and potent antioxidant activity after only one day of germination (Randhir et al., 2004). Accoridng to the studies of Randhir et al. (2004) mung bean sprout extracts and mung bean treated extracts with lactoferrin (LF) and oregano elicitors were specifically effective against *Helicobacter* *pylori.* The high antimicrobial potential of both*,* LF and OE elicited extracts was also correlated with their high antioxidant activity achieved after one day of germination.

The antimicrobial potential of mung bean sprout extracts to inhibit the growth of *Helicobacter pylori* results really interesting for the scientific community, taking into account the incidence of this bacteria worldwide, affecting even to the 80 % of the global population, with fatal consequences, gastritis, peptic ulcers, and stocmach cáncer (Eusebi et al., 2014). This microorganism has been recently classified as carcinogenic level I by the International Agency for Research on Cancer (IARC) (1994)**.** Randhir et al. (2004) and Mitchell & Megraud (2002) confirmed the antimicrobial activity of mung bean extracts against this bacterium.

Considering the Solid State Bioconversion (SSB) process as an alternative technology to enhance the functionality of cereals and legumes, new studies have been carried out with the objective to test the increased antimicrobial potential linked to sprouts of mung bean by means the application of this treatment. SSB implies the microbial bioprocessing in a solid food substrate that acts as a physical support and source of nutrients in the presence of low free liquid. This technology has demonstrated to be effective mobilizing the conjugate forms of phenolic precursors naturally found in several vegetable and fruit matrices such as mung bean, fava bean, cranberry pomace, and pineapple (Correia, McCue, Magalhães, Macêdo, & Shetty, 2004; Randhir, Vattem, & Shetty, 2004). Randhir & Shetty (2007) SSB was carried a study on *Rhizopus oligosporus* food grade. Agar-diffusion test was used to determine the antimicrobial potential of mung bean extracts against *H. pylori* at different concentrations. The SSB mung bean extracts effectiveness against *H. pylori* was assessed at different points during the solid-state growth (0-20 days). In that sense, it was observed a marked antimicrobial capability of mung bean extracts (100-200 µl) corresponding with 4 and 8 days being these extracts also the most antioxidant, and confirming consequently that antioxidant activity compounds mobilized during SSB could contribute to *H. pylori* inhibition. In the same way, phenolic polymerization during SSB also is contributing to *H. pylori* growth inhibition, similary to the observed for SSB fava bean and cranberry processing.

Soya (Soy Bean)

Soy bean is highly valuated in many countries worldwide by its nutraceutical potential and its healthy benefits to human (Friedman & Brandon, 2001; Xiao, 2008), mainly, reducing the risks of various cancers (Ahmad et al., 2013). This functional food crop is very rich in phytochemicals and has good acceptation between the general public, due to the increased awareness of consumers regarding the positive health benefits of this legume, boosting its introduction in many new developed food products. The isoflavon content of soy bean (3mg ⁄g dry weight) has been associated to the health promoting activity of this vegetable product, due the biological activity of soy isoflavones compounds such as genistein, daidzein and biochanin A (Chang et al., 1995).

According to the studies of Shankar-Ponnusha et al. (2011) soya mehtanolic extracts prepared at concentration of 24.6mg/ml showed antimicrobial potential against *Pseudomonas aeruginosa* and less potential against *Bacillus subtilis.*

Also soy isoflavones have exerted antimicrobial potential to effectively fight microorganisms biofilm formation. The capability of generally microbes to reside estructured in dynamic, complex, multicelular communities referred to as biofilms, represents a serious problem for the food industry, due to the derived persistent contamination and fouling that this films consist of. Although desinfetion and chemical-based decontamination strategies (Srey et al., 2013) are up-to-date the most effective measure to control foodborne pathogens proliferation in solid surfaces, the development of new material, including antimcirobial natural substances, to use for food packaging and fim/coating protection of industrial surfaces is nowadays a reality. In that sense, new alternative antimcirobial sources have emerged with potential application in this field and good prospects in biofilm erradication (inhibition or disruption). The antimicrobial potential of soy isoflavones (10 – 100 mg/mL) against *L. monocytogenes* (LMC379), *P. aeruginosa* (PA76), *E. coli* (ATCC 25922) and Methicillin Resistant *S. aureus* (MRSA M0535) was assessed by Priyadarshini et al. (2015). The results obtained revealed an antimicrobial capability against *L. monocytogenes* and *E. coli*, in the range of concentration of [10 - 100 mg/mL], being not effective against the growth/formation of MRSA and *P. aeruginosa*.

The studies of Shetty (2012) also demostrated the antimicrobial efficacy of high phenolic soybean sprouts and fermented extracts against *Helicobacter pylori in vitro*. According to Shetty and co-workers, bioactive food phenols have the potential to inhibit specific steps in the microaerobic metabolic stages of *H. pylori*, such as proline oxidation linked respiration, also interrupting membrane related functions. This finding results very relevant becoming possible the development of novel therapies based in the formulation of legumes-based products against this pathogen in combination with the traditional use of antibiotics.

Other soy bean phytochemicals have also exerted antimicrobial potential, being the soy peptides among them. The antimicrobial activity of soy based peptides (PGTAVFK and IKAFKEATKVDKVVVLWTA) was assessed by Dhayakaran et al. (2016) against *Listeria monocytogenes* and *Pseudomonas aeruginosa.* It was observed that *P. aeruginosa* and *L. monocytogenes* growth was stopped/slowed by the exposure to soy short-chain peptides, being its antimicrobial activity exerted at concentrations above 625 mM. On the other hand, long-chain soy peptides (IKAFKEATKVDKVVVLWTA) were more effective against both microorganisms, with inhibitory effects exerted at concentration level of 37.2 mM.

Derivated peptides from soy have been also associated with hypocholesterolemic, antiobesity, antioxidant, anticancer and immunomodulatory, moreover to antimicrobial properties (Feng-Liu & Pan, 2011).

Lentils (Lens culinaris)

Lentils are a vegetable group highly valuated in the cuisine worldwide by their capability to readly absorb a variety of wonderful flavors and seasonings, being also relatively easy to prepare compared with other types of beans. Their nutritional value is very attractive, being remarkable their richness in fibre, functional minerals such as cooper, phosphorous, manganese, and magnesium, and also valuable by its content in vitamins, mainly folate (Butu et al., 2014).

The low glycemic index of these vegetables, and the pre-biotic propierties associated to them, morevoer to its richness in high biological value protein, make this vegetable group valuable for the scientific commnity to search for novel processing and presentation forms to be attractive to the general public. Physical, chemical and enzymatic processing of legumes are being applied to improve the nutritional and palatability spectra of these vegetables. Germination, UV radiation or High Hydrostaitc pressure treatment are influencing significantly the digestibility and solubility of proteins from legumes and also are favouring the accumulation of valuable secondary methabolites on their structures (Li et al., 2011). Regarding the secondary metabolites responsable for the antimcirobial potential in legumes, mainly polyphenols and peptides, scarce literature exists related to lentils and the study of their bioactive compounds (Raj et al., 2016; Swieca et al. 2014). However, in the reviewed literature a positive correlation is stablished between the antioxidant potential and polyphenol content of this *Leguminosae* and their antimcirobial capability (Raj et al., 2016). In that sense, and according to the studies of Swieca et al. (2014) the antioxidant and polyphenolic content of lentils sprouts could be enhanced by means an elicitation processing. However, the capability of sprouted lentils to inhibit food microorganisms has not been confirmed yet.

The studies of Nguyen et al. (2014) evaluating a great variety of vegetable material against Gram positive and Gram negative bacteria, revealed that lentils were not specifically effective inhibiting *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria innocua*, and *Escherichia coli*, among others.

In spite of this, and according to the studies of Shenkarev et al. (2014), specific peptides with antimicrobial potential (named defensins) have been encountered in *Lens culinaris*. According to these researchers, the termed Lc-def defensin (5440.4 Da; 47 amino acid residues) found in germinated seeds of lentils exhibited antifungal activity against *Botrytis cinerea* and *Neurospora crassa* but did not inhibit the growth of Gram-positive or Gram-negative bacteria. A concentration of 37 µM of Lc-def defensin was effective inhibiting the complete growth of *Neurospora crassa.*

Black bean and Black turtle bean (Phaseolus vulgaris)

Brazil is the most relevant black bean producer country. This food product has a priviligiate position in the Food pyramid of populations worldwide, being this fact boosted by the richness of this legume in uncountable bioactive compounds (Xu and Chang, 2009). Black beans are rich in molybdenum, ferrous (59µg Fe/g), phenolic acids (83.2 59 µg/g), folate (2.56 µg/g) and also in vitamin B_1 (4.2 µg/g). The non-digestible carbohydrates fraction (dietary fibers and oligosaccharides) in black beans is higher than the found in other legumes, like lentils or chickpeas. This indigestible fraction stimulates the growth of bifidogenic and lactic acid bacteria in the gastro-intestinal tract. The substances produced in the colon by microbiota (e.g. butyric acid) have been related to the manteinance of the gut tract health, being the consumption of black beans recently associated with a reduction of the colon cancer risk in rats (Hangen & Bennink, 2002).

Accoridng to the studies of Jati, Vadivel, and Biesalki (2013) it is remarkable the antioxidant capability of anthocyanins contained in colored legumes - black, purple, red and blue-colored seed coated. Individual anthocyanin compounds in legumes – delphinidin-3-O-glucoside, petunidin-3-O-glucoside, and malvidin-3-O-glucoside – have been reported to have anti-inflamatory, anti-proliferative and antioxidant potent activities, confirmed both *in vitro* and *in vivo* (Lin, Gong & Song, 2016). Despite the great number of papers dealing with the functional effects exerted by anthocyanins, only a limited number of studies is focused on the antimicrobial capability of these compounds from vegetable origin. To our knowledge, scarce information exist regarding the specific antimicrobial potential of legumes coats depending on the anthocyanin content. In spite of this, many authors have established a direct relationship between antioxidant potential of these bioactive compounds and their capability to inhibite bacterial growth. Cisowska, Wojnicz and Hendrich (2011) review the antimicrobial potential of pure anthocyanins from fruits and grains and confirmed the generally highest usceptibility of Gram positive bacteria than Gram negative.

According to the studies of Nguyen et al. (2014) among the *Fabaceae* family, ethanolic and methanolic extracts of black bean and black turtle bean were specifically effective inhibiting Gram + and Gram – bacteria. These results seem to be related with the bioactive compounds contained in the coatings of the coloured seeds. Coloured seed coats are rich in bioactive secondary metabolites such as anthocyanins, condensed tannins and flavonoids (Vázquez et al., 2007; Rocha-Guzman et al., 2007) and consequently exerted a stronger antibacterial activity regarding the not coloured ones (e.g. navy beans).

Ariza Ortega et al. (2013) assessed the antimicrobial potential of several varieties of *Phaseolus vulgaris* L. According to their results, black bean mehtanolic extracts (100 %, 80 %, and 50 %) showed the highest antixoidant potential, being also highly effective inhibiting *S. typhi* (50 % methanolic extracts), *S. aureus (*100 % metanolic extracts) and *B. cereus* (50 % methanolic extract).

Peanut (Arachis hypogaea)

The antimicrobial potential of peanuts shells was studied by Vaughn (1995). It was observed that the 5,7-dihydroxychromone flavonoid decomposition production from peanut shells was able to inhibit the 50 % of fungi growth at concentration levels in the range of 18-26 µM. Both *Rhizoctonia solani* and *Sclerotium rolfsii* were effectively inhibiting fungi growth and stablishing consequently an effective protection of the plant against phytopathogens. Although the research regarding the antimicrobial potential of peanut is scarce, there are some studies also associated to the antimicrobial capability of peanut by-products.

In the line of polymeric flavonols and tannins with antimicrobial potential, the capability of pro-anthocyanins present in the peanut skin were evaluated by Sarnoski et al. (2012) against *S. cerevisiae*, *Zygosaccharomyces bailii* (both obtained from an industry source, isolated from spoiled beverage), and *Zygosaccharomyces bisporus.* The *S. cerevisae* growth was significantly inhibited by means the exposure to whole peanut skins extracts at concentration level of 10 mg/ml by means an extensión of the lag phase. The fractioned extract of peanut skin was also studied in terms of its antimicrobial potential. According to the obtained results, one of the studied fractions, specifically rich in compounds of low molecular weight (570–600 Da) was the most inhibitory against *S. cerevisiae* even at lower concentrations [1-4] mg/ml (Sarnoski et al., 2012).

Also phytoalexins from peanuts have exerted antimicrobial potential (Holland & O'Keefe, 2010). Moreover to the phytoalexins antimicrobial properties, these compounds have been recently associated with anti-diabetic, anti-cancerigen and vasodilatory efffects (Lozano-Mena et al., 2014). These secondary metabolites are among the main defense groups in plants, which generation is modulated by the activation of specific biosynthesis pathways, answering to the expossure to phytopathogens. Phenols, terpenoids, furanoacetylenes, steroid glycoalkaloids, sulfur-containing compounds and indoles are some of the chemical families to which pytoalexins are belonging. Endogenous (such as the effects of sugars, sucrose, glucose and fructose) or exogenous (such as elicitors) signals are acting on the mechanisms regulating the biosynthesis and accumulation of phytoalexin in plants, as well as controlling the expression of biosynthetic genes.

Peterson et al. (2015) studied the antimicrobial potential of defensins from peanut. Minimal inhibitory concentration against bacteria (*E. coli* and *S. aureus* (clinical isolate)) and fungi (*Alternaria* and *Cladosporium*) was determined. Peanut defensins showed no inhibitory effect against bacteria at a máximum concentration level of 64 µg/mL peanut defensins. Similar results wer obtained after the exposure of several yeast such as *C. albicans*, *P. pastoris*, and *S cerevisiae* to the considered defensins. In spite of this, a potent anti-fungal activity was exerted by peanut defensins at low concentration levels (even atv 6.25 µg/mL) against *Alternaria* species, being the antimicrobial effect dose-time dependent. 70 % of *Alternaria* and *Cladosporium* growth was inhbiited by means of 25 to 100 µg/mL of the peanut defensin, showed prolongued along time of exposure (up to 150 h of study).

Conclusion

The *Leguminosae* family is a very promising group of plants and grains with demostrated antimicrobial potential against some of the most relevant foodborne pathogens. The future design of novel ingredients from legumes (e.g. proteinic flours, and polyphenols concentrates from legume hulls) leads to the development of a new generation of innovative products with nutritional and technological improved value. In that sense, the capability of bioactive compounds from legumes to inhibit bacterial growth in food matrices is one of the most promising applicabilities of this family of plants, especially in minimally processed products of added value, and to control the bacterial proliferation during the shelf-life of the food.

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