1. INTRODUCTION

1.1 Background

Pulses belong to *Leguminosae* family and rank sixth as major grain legume crop in the semi-arid tropics of Asia, Caribbean and Africa, serving major amount of nutrition in form of proteins and carbohydrates in diet. Pulses are well consumed in form of snacks, sausages and fermented product worldwide [1]. Pulses are affluent with carbohydrates up to 65 % and comprises of basis nutritive components such as protein, vitamins, chains of fatty acids, dietary fibers and phytochemicals. Pulses contributes to several additional health benefits such as treating diabetes, protect reproductive system from infection, skin irritation, stabilize menstrual issues, and other microbial properties like antibacterial, antioxidant, anti-inflammatory, antitumor, *etc* [2].

The major drawback limiting the utilization of this proteinous rich food is presence of α-galactosides in high amount. This includes raffinose, also stachyose, verbascose and ajugose are considered as α-galactosides. Localization of these galactosides is in seed and in different parts of tissues in plant. These galactosides are not digested by humans, due to lack of α-galactosidase (α-gal) enzyme [3]. The microbes present in intestine metabolize this oligosaccharide producing considerable amount of gases such as CH_4, H and CO_2 in large intestine that leads to pain in abdomen creates flatulence causing diarrhea, *etc*. Thus, to get it better, use of microorganism mainly lactobacilli related species are known to produce α-gal enzyme in degrading this oligosaccharides during fermentation of pulses [3]. Presently, *Lactobacillus* (*L.*) *helveticus, Limosilactobacillus* (*L.*) *reuteri*, *Lpb. plantarum*, *Limosilactobacillus* (*L.*) *fermentum*, *Lactobacillus* (*L.*) *acidophilus* and *Lev. brevis* can hydrolyze galactosides into simple palatable form. In organism, this enzyme may be cell associated i.e. intracellular or extracellular, producing enzyme using α-D- galactopyranosyl groups as carbon source. Extracellular enzymes are advantageous as they show better yield and good stability than intracellular enzymes. Hence, these strains can be implemented during fermentation to make attractive product available for consumer [4].

From nutritional perspective, lactobacilli, falls under group of lactic acid bacteria (LAB) that has immense contribution as starter in food fermentation. They also promote food with longer shelf life, better odor and taste due to production of organic acids in fermented products. Lactic fermentation mostly gives lactic acid (LA), acetic acid (AA), CO_2 and ethanol as end products. Lactic acid, well known as 2-hydroxypropanoic acid, falls under Generally Regarded as Safe category (GRAS) [5]. It has broad application in food and non-food industries from medical to cosmetic. LA is usually synthesized chemically and by fermentation using lactobacilli, and prefers mono-disaccharide sugar for product formation. The major reason for production of acid in fermentation is a drop in pH. This acid benefits food by getting contaminated and hence improves quality of final product. Therefore, it is mandatory to detect organic acid level, growth of microorganism and enzyme activity for nutrition point of view in fermented foods [5].

Another factor that hinders pulses for consumption is anti-nutrients. Anti-nutritional factors (ANFs) can be reduced using food treatments like soaking, dehulling, roasting as well as fermentation. Fermentation has lead to decrease in many anti-nutrients such as tannins, saponins. This is due to absorption mechanism that occurs between fermenting flora and some phytochemicals might be responsible for the depletion [6].

Fermented foods usually are naturally fortified functional foods. They are considered superior for their high nutritional content, maintains boy homeostasis and helps to fight with against diseases [7]. Various fermented products from food using cereals are commonly prepared in India as well as throughout world. By using lactobacilli in food fermentation as carrier with pulses, would provide food with high calorie and better sensory characteristics.

REVIEW OF LITERATURE

Lactobacilli belong to group lactic acid bacteria. The genus is Gram-positive organism, non-sporeforming rods shaped, negative for catalase, non-motile, fastidious organisms and some nitrate reducers. The genus belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*. Lactobacilli fall in the category of GRAS organisms [8].

1.2 Morphology of lactobacilli

Lactobacilli are usually in rods and diverge in length among species. Few of them are coccobacilli and/or seem as in curve shape. Hetero-fermentative lactobacilli sometimes appear as coccoid, which makes it difficult to distinguish between *Leuconostocs*. Homo-fermentative anaerobs isolated from intestine often resembles with certain *Bifidobacteria* morphologically [9]. Lactobacilli are thus characterized by product outcome obtained via fermentation from glucose, whereas homo-fermentators mainly produce LA; morphological variations often appear within species. For instance, isolated cells of *Latilactobacillus* (*Lat.*) *sakei* from meat occur in very short rods observed to have a bacillary growth after they are subcultures. Similarly, *L. delbrueckii* subsp. *bulgaricus* and *Latilactobacillus* (*Lat.*) *curvatus* were found to form long spiral and curved cell, respectively [9].

1.3 Phylogeny of lactobacilli

A reclassification was done of the genus *Lactobacillus* (March, 2020) into 25 genera that includes *Lactobacillus delbrueckii* group, *Paralactobacillus* and 23 novel genera such as *Holzapfelia,Amylolactobacillus,Bombilactobacillus,Companilactobacillus,Lapidilactobacillus, Agrilactobacillus,Schleiferilactobacillus,Loigolactobacilus,Lacticaseibacillus,Latilactobacillus, Dellaglioa,Liquorilactobacillus,Ligilactobacillus,Lactiplantibacillus,Furfurilactobacillus, Paucilactobacillus,Limosilactobacillus,Fructilactobacillus,Acetilactobacillus,Apilactobacillus, Levilactobacillus,Secundilactobacillus and Lentilactobacillus* are proposed [10]. A general overview on genomic feature of lactobacilli is given in Table1 [11].

1.4 Habitat of lactobacilli

Based on numbers, lactobacilli are important group of living organisms. They grow in various habitats where high levels of soluble carbohydrates are present, vitamins, minerals and protein breakdown products as amino acid occurs. Ideally, acidophilic in nature, however, some species managed to adapt themselves in different environmental conditions [11]. They produce LA in high amount that suppresses growth of rest organisms and lowers pH in medium. These factor accounts to their wide distribution and establish markedly in different habitats. Lactobacilli have been recognized in human intestine, oral cavity and stomach that usually have pH between 2.2 to 4.2. *Lacticaseibacillus* (*L.*) *casei*, *Lacticaseibacillus* (*L.*) *rhamnosus*, *L. acidophilus* and *L. fermentum* exist in mouth in tooth surfaces,

Table 1: Genome features of lactobacilli

Species	Length (MB)	G+C content (%)
L. acidophilus NCFM	1.9	34.7
L. helveticus DPC 4571	2.0	37.0
L. gasseri ATCC 33323	1.8	35.2
L. crispatus ST1	2.0	36
L. johnsonii FI 9785	1.7	34.4
L. johnsonii NCC 533	1.9	34.6
L. delbrueckii ssp bulgaricus ATCCBAA 365	1.8	49.6
L. delbrueckii ssp bulgaricus ATCC 11842	1.8	49.7
L. casei ATCC 334	2.9	46.5
L. casei BL 23	3.0	46.3
L. rhamnosus GG	3.0	46.6
L. rhamnosus Lc 705	3.0	46.6
Lat. sakei 23k	1.8	41.2
Lev. brevis ATCC 367	2.3	46.0
Lpb. plantarum JDM 1	3.1	44.6
Lpb. plantarum WCFS 1	3.3	44.4
L. fermentum IFO 3956	2.0	51.4
L. reuteri DSM 20016	1.9	38.8
L. reuteri JCM 1112	2.0	38.8
L. salivarius	2.1	33.0

L. salivarius : *Ligilactobacillus* (*L.*) *salivarius* (Stefanovic et al., 2017)

cheeks, tongue of infants and young children, in small numbers. These organisms tolerate strong acidic conditions and some are ingested at the time of eating and swallowing saliva. These organisms are killed by HCL, minimizing population to 10^3 cfu/ml and include lactobacilli. The role of lactobacilli within intestinal ecosystem plays beneficial role on human health, i.e. as probiotics. Components of bacterial cell surface like polysaccharides, adhesions and proteins helps lactobacilli to adhere to intestinal epithelium, leading to elimination of pathogen and immune-modulation of host cells (Table 2) [12].

Lactobacilli were also found from other source including fermented vegetable food, sausages and beverages like kimchi, sauerkraut, silage, mixed pickles, olives, wine, juices, beer, *etc*. Mostly isolated species are *Lpb. plantarum, Lat. curvatus, Lat. sakei, Lev. brevis, Lacticaseibacillus paracasei* and *L. fermentum*. They occur often with *Pediococci*, *Leuconostocs*, *Weissella* and yeast [12]. Several species like *L. fermentum, L. salivarius, L. delbrueckii subsp. Lactis, Lpb. plantarum, L. helveticus, Lev. brevis, L. casei* contribute to dairy products like milk, cheese, whey, *etc*. Water sewage and manure or cow dung are considered secondary habitat for lactobacilli. Lactobacilli found in primary habitat or intestine is more likely the same (Table 3) [12].

Table 2: Source of lactobacilli

Species	**Source**
L. acidophilus NCFM	Infant faeces
L. helveticus DPC 4571	Cheese
L. gasseri ATCC 33323	Human Gut
L. crispatus ST1	Chicken faeces
L. johnsonii FI 9785	Human faeces
L. johnsonii NCC 533	Human faeces
L. delbrueckii ssp bulgaricus ATCCBAA 365	Yoghurt
L. delbrueckii ssp bulgaricus ATCC 11842	Yoghurt
L. casei ATCC 334	Cheese
L. casei BL 23	Cheese
L. rhamnosus GG	Human Gut
L. rhamnosus Lc 705	Cheese
Lat. sakei 23k	Meat
Lev. brevis ATCC 367	Human
Lpb. plantarum JDM 1	Human saliva
Lpb. plantarum WCFS 1	Adult Intestine
L. fermentum IFO 3956	Adult Intestine
L. reuteri DSM 20016	Silage
L. reuteri JCM 1112	Fermented plant material
L. salivarius	Terminal ileum of human

(Stefanovic et al., 2017)

Table 3: Ecology of lactobacilli

Species	Human body	Fermented food	Probiotic food
L. acidophilus	+	+	+
L. delbrueckii	+	+	+
L. casei	-	+	-
L. gasseri	+	-	-
L. fermentum	+	+	-
L. jensenii	+	-	-
L. paracasei	+	+	+
L. johnsonii	+	+	+
L. rhamnosus	+	+	+
Lpb. plantarum	+	+	+
Lev. brevis	+	+	-
L. salivarius	+	-	-

L. jensenii : *Lactobacillus* (*L.*) *jensenii* (Vos et al., 2011)

1.5 Metabolism of lactobacilli

Lactobacilli are strictly fermentative organism with complex nutritional requirement for fastidious growth. They require mostly carbohydrate, proteins, vitamins, minerals, fatty acids esters and nucleic acid derivatives for survival. Carbohydrate metabolism using lactobacilli leads to LA, AA, carbon dioxide and ethanol as end-product [13,14]. Metabolism of carbohydrate using different pathways for lactic acid production is as follows.

1.5.1 Glucose metabolism: Homo-lactic fermentation utilizes glycolytic pathway, while hetero-lactic species follows 6-phosphogluconate pathway. Obligate homo-fermentors does not requires phosphoketolase enzyme but facultative hetero-fermentative species does. During glycolysis formation of fructose1,6-diphosphate (FDP) occurs by FDP aldolase into dihydroxyacetone-phosphate (DHAP) and glycerinaldehyde-3-phosphate (GAP); GAP is converted to pyruvate (Figure 1) [13]. Fermentable sugars and its product depend on surrounding environment. Thus, ketolases is repressed by glucose in facultative hetero-fermentative organisms and pH is also affected on the type of sugar used. The nature of end-products is strongly affected in carbohydrate metabolism by oxidants. Presence of oxygen leads to H_2O_2. The enzymatic activities like pyruvate oxidase, NADH peroxidase, NADH: H_2O oxidase, α-glycerophosphate oxidase, *etc* [13].

Catalase activity has been detected in some known species like *Lpb. plantarum*, *Lev. brevis, Lentilactobacillus* (*L.*) *buchneri*, *L. delbrueckii*, *Lat. sakei*, and *L. fermentum*. The metabolism of glucose is a main source for energy and includes substrate-level phosphorylation. ATP thus synthesized during glycolysis can be derived from acetylphosphate. Homo-fermentative lactobacilli include *L. delbruckii*, *L. acidophilus*, *L. salivarius* and *L. helveticus*; hetero-fermentative lactobacilli are *Lpb. plantarum*, *Lat. curvatus*, *L. buchneri*, *Lat. sake*, *L. casei*, *L. fermentum*, *L. reuteri* and *Lev. brevis* [15].

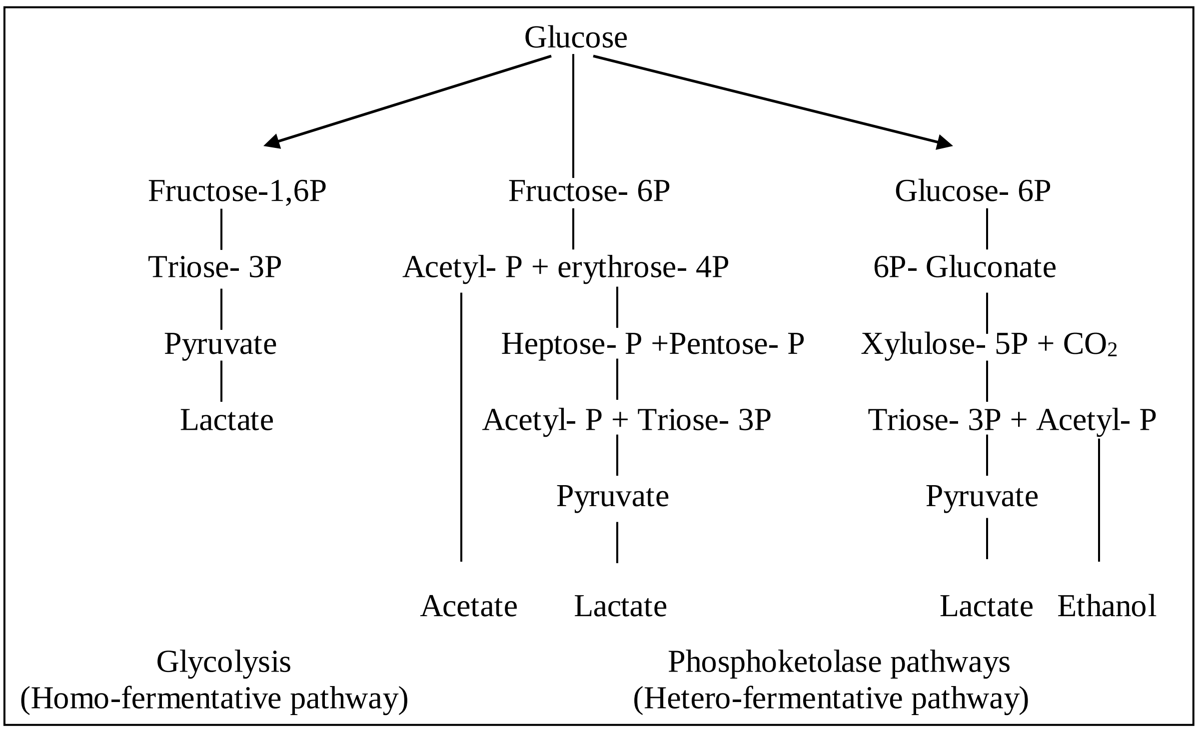


Figure 1: Major fermentation pathways for glucose by lactobacilli

1.5.2 Starch metabolism: Starch occupies a major position as storage polysaccharide in pulses. Starch is comprises of amylase and amylopectin. Amylolytic degradation occurs by α- β-amylase and amyloglucosidase linked to glucose, maltose, and maltodextrin during fermentation. Extracellular amylase activity was expressed in various lactobacilli species like *Lpb. plantarum*, *L. fermentum*, *Lactobacillus* (*L.*) *amylovorus, and L. gasseri*. During fermentation, maltose accumulates in the starting stage, once pH is reduced maltogenic amylase is inhibited but glucose from starch and maltodextrins continuous to releases from glucoamylases (Figure 2) [16].

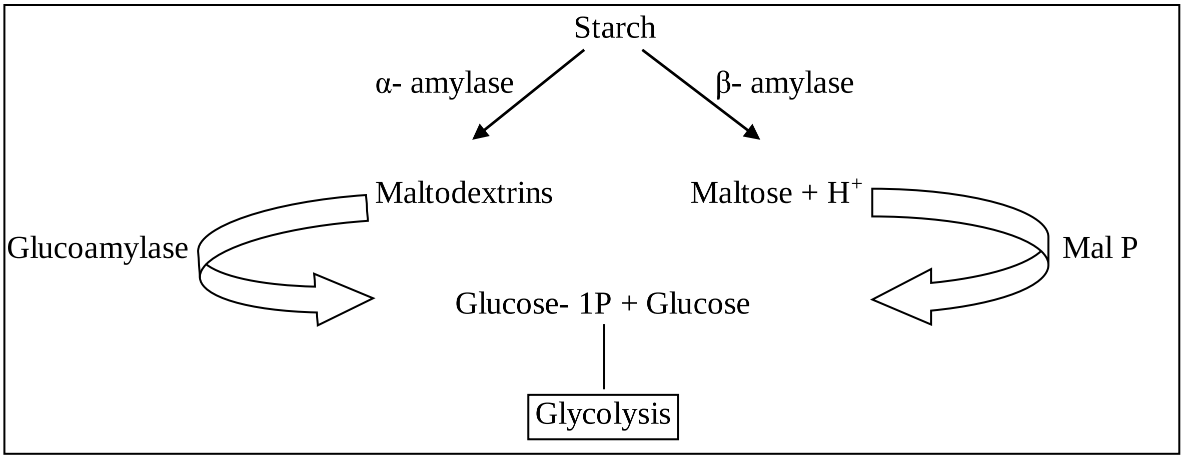


Figure 2: Starch conversion by lactobacilli

1.5.3 Sucrose metabolism: Sucrose is widespread in plants and abundantly found in pulse grain. Sucrose metabolism occurs by three steps; (i) hydrolysis by fructansucrases or glucansucrases (ScrR, transcription regulator) (ii) hydrolysis and phosphorylation of sucrose by ScrA, sucrose PTS transporter and (iii) hydrolysis by phosphor- fructo- furanosidases ScrB, sucrose-6 phosphate hydrolase. All genome of these species harbored function in sucrose metabolic pathway as sucrose is highly preferred substrate. Sucrose is carried away by PTS transporter in cell to sucrose- 6P, hydrolyzing into glucose- 6P, subsequently to fructose by ScrB [16].

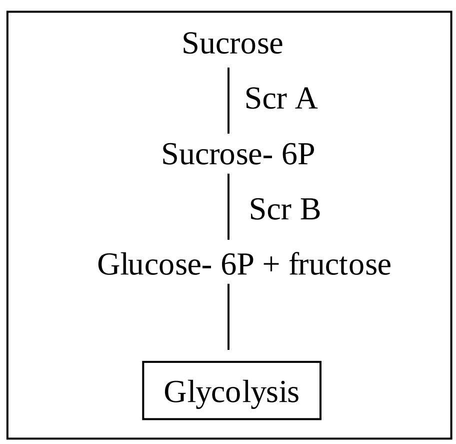


Figure 3: Sucrose utilization by lactobacilli

1.5.4 Raffinose metabolism: Raffinose is widely distributed in plants. Raffinose consists of α-(1 → 6) D- galactose units liked to sucrose. According to studies, metabolism of raffinose by lactobacilli aims at allowing fermentative removal. Conversion of raffinose to α-galactooligosaccharides (αGOS) by lactobacilli is encoded by mel A genes. Mel A is widely present in lactobacilli, reflecting importance of α-gal in intestinal ecosystem and plants. The enzyme acts as homo-tetramer, and validates unbranched oligosaccharides as substrate [16]. Breakdown of raffinose by enzyme α-gal releases sucrose while degrades raffinose using sucrose metabolic enzymes. Some lactobacilli strains convert raffinose to α-GOS by levansucrase without further galactosides metabolism. There are two alternative pathways for raffinose degradation that strains expressing mel A, levansucrase and sucrose phosphorylase or fructo-furanosidase (i) conversion of raffinose to α-GOS and fructose by levansucrase (ii) hydrolysis to sucose and galactose by mel A activity and conversion of sucrose by sucrose phosphorylase or fructo-furanosidase (Figure 4) [16].

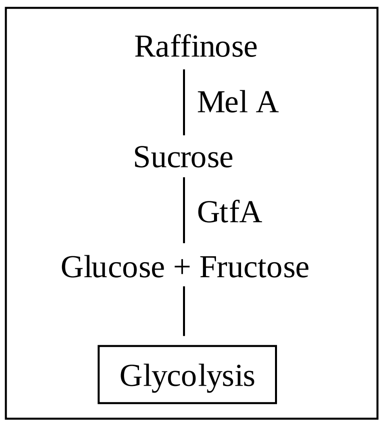


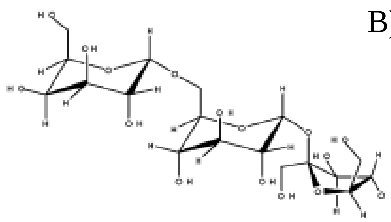
Figure 4: Raffinose utilization by lactobacilli

1.5.4.1 α- Galactosides

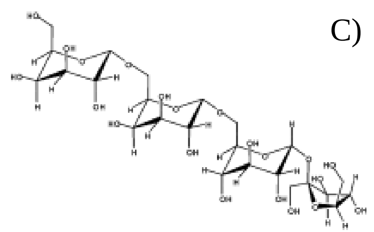
Galactosides are also familiar by raffinose family of oligosacchraides (RFO). They have less molecular mass, water soluble and water with alcohol solutions. α- galactosides are glycosides, linked with D- galactosyl residues attached with glycone molecule by acetyl linkage. The adjoining monosaccharide and oligosaccharide substrate is termed glycone, and involved in catalytic metabolism [17]. The α- D- galactose group or non-reducing terminal monosaccharide are ubiquitous in higher plants, occurs in combined form and found in various oligosaccharide, polysaccharide and some non-sugars like glycerol, and certain lipids. The known α- galactosides are raffinose, stachyose and verbascose ranked next to sucrose in plant kingdom. The function of these oligosaccharides is to store or transport carbohydrates. Accumulation of these galactosides in plants contributes to cold acclimation, membrane protein protection and denaturation because of desiccation process during dormancy [17].

The International Union Pure and Applied Chemisty (IUPAC) name of raffinose is α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2) -β-D- fructofuranoside; stachyose is α-D-galactopyranosyl-(1→6)-α-Dgalactopyranosyl- (1→6) -α-D-glucopyranosyl- (1 → 2)-β-D-fructofuranoside and verbascose is α-D-galactopyranosyl-(1→6)-[α-D-galactopyranosyl-(1→6)]2-α-D-glucopyranosyl-(1→ 2)-β-D-fructofuranoside. Hence, α- galactosides are considered important substrates for α- galactosidase enzyme [17].

A)



B)



C)

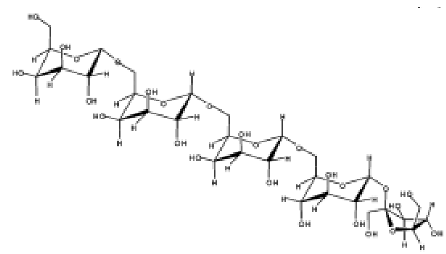


Figure 5: Chemical structure of α-galactosides. (A) raffinose (B) stachyose (C) verbascose

1.5.4.1.1 α- Galactosidase enzyme: α- D- galactose- galactohydrolase referred to as α- gal with E.C.3.2.1.22, hydrolyze galactosidic linkages in oligosaccharides like raffinose, stachyose, melibiose and verbascose and polysaccharides i.e. galactomannans and glycoconjugates. There higher derivatives like ceramide trihexosides broke by enzyme α- gal. α- gal is widely distributed and genes encoding its genes encoding enzyme were isolated from plants, animals and microorganisms [17].

1.5.4.1.2 Microbial source and substrate specificity of enzyme: In recent year, enzymes from microbes have emerged as an important property for industrial point of view. The advantages of this enzymes from microbes is its high production yield and cheap production cost because of extracellular synthesis. The production of α- gal enzyme is also done by plants and animals but in lower amounts; while higher production is seen in microbes [18]. The enzyme has been isolated from various microbial sources such as in fungi from *Aspergillus* sp., *Humicola* sp., actinomycetes, *Saccharomyces* sp., *Candida* sp., *Pencillium* sp., *Trichoderma* sp., *Mortierella* sp., mesophilic and thermophilic bacteria i.e. *Sulfolobus solfataricus* [18]. Enzymes have also been obtained from bacteria that have GRAS category like *Lpb. plantarum*, *Lev. brevis*, *etc*. α-gal enzyme is located both intracelluarly and extracellularly. Table 4 shows list of bacterial sources of alpha-galactosidase enzyme. The role of bacterial α- gal is to degrade complex oligosaccharides and polysacchraides into palatable carbon source [18].

Table 4: Bacterial sources of α-galactosidase enzyme

Microbial source	Location
Lpb. Plantarum	Cytoplasm
Lev. Brevis	Intracellular
Streptomyces olivaceus	Extracellular
Sulfolobus solfataricus (Thermophilic)	Intracellular
Saccharopolyspora erythraea	Culture supernatant
Aspergillus terreus	Extracellular
Saccharomyces cerevisiae	Extracellular

(Kartolia et al., 2014)

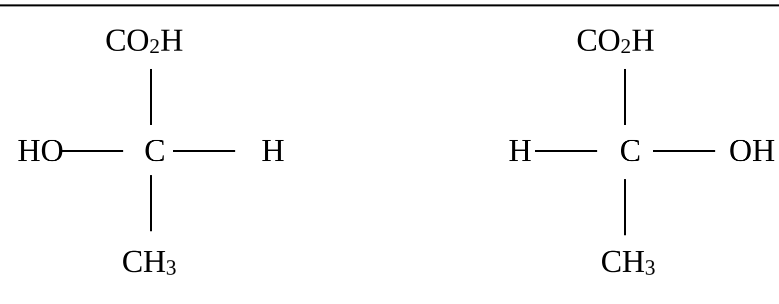
Bacterial α- gal displays specificities to D- galactosides of RFO. This also includes galacto-manno oligosaccharides such as Gal3Man3, Gal3Man4; branched polysaccharides like galactomannan present in locust bean gum, guar gum. These enzymes are shows active response towards artificial substrate like p- nitrophenyl - α- galactopyranoside (pNPG) e.g in lactobacilli. Another group of α- gal consist of polymeric substrates that attacks short oligosaccharides fragments of degraded polymers and artificial α- galactosides [18].

1.5.4.2 Physiological effect of α- galactosides: High level of α- galactosides in diet reported to have several negative effect on nutrition of human body like flatulence. α- Galactosides or RFO from pulses have been known as crucial contributor to flatus. Members of RFO cannot be digested by human digestive enzymes due to lack of hydrolytic enzymes like α- gal by intestinal mucosa. Therefore, RFO like sugars are unable to get through intestinal wall by them. High consumption of galactosides produces flatulence; but its low consumption does not create discomforts, in fact provides health benefits to the consumer [19]. Another problem related to this is osmotic effect of RFO in colon and its involvement with other nutrients in body. High raffinose consumption (6.7 %) develops in osmotic pressure. This creates imbalance and loss in raffinose before getting it fermented by microbes. The incline in fermentable carbohydrates at lower gut in body can lead to microbial imbalance and causes diarrhea. There are reports of these enzyme appeared in soybean created negative effect on consumption of protein from body. Studies on removal of these RFO from pulses like lupin showed significant improvement on digestion of amino acids [19].

1.6 Biotechnological application of lactobacilli

There are various applications of lactobacilli including medical as probiotics, feed for animals, food industry and other well known industries. The following segment will focus on application of lactobacilli in different areas related to foods.

1.6.1 Lactic acid production: Lactic acid (LA) or 2- hydroxypropanoic acid have widespread appearance in surrounding environment. The molecular formula of LA is $CH_3CHCOOH$, a colorless liquid. There are two types of isomers produced, in humans and mammals only L (+) isomer is produced, where as bacteria can synthesize both enantiomers D (-) and L (+) (Figure 6) [20].



(i) L (+) - Lactic acid (ii) D (+) - Lactic acid

Figure 6: Isomers of lactic acid

As a chemical, LA has received a significant attention in applications related to food, pharmaceuticals, cosmetics and chemical industries. About 70 % of LA is produced worldwide but its major use is in food industry for production of cheese and yogurt. LA is classified as GRAS as food additive by United States (US) Food and Drug Administration (FDA) and most important compound produced from lactobacilli. LA is considered in giving better shelf life to food, enhances flavor as well as controls food-borne pathogens especially in meat and poultry industries [21]. LA is mild acidic in taste that is the reason it is used as acidulant in pickled vegetables, salads and beverages. LA is not only used for flavors in food items but also in setting pH of cooked food to correct point. In food industry, LA is used for mineral fortification of food products. LA acts as natural ingredients in cosmetic industry as pH regulators, moisturizers; multiple properties such as antimicrobial activity, skin hydration, skin lightening, as well as oral products, *etc*. LA is used in anti-acne preparations as ethyl lactate, as it contains solvency power against oil, with no toxicological impact on environment. The use of LA in pharmaceutical industry is popular in making tablets with desired targets, surgical sutures, drug delivery systems, *etc* [22].

1.6.1.1 Methods for lactic acid production: LA can be produced chemically or microbial fermentation showed in Figure 7. Pure LA is commercially generated by microbial fermentation using glucose, starch/maltose, lactose, sucrose derived from feed-stocks like molasses, beet sugar, whey, barley malt, *etc*. Usually homo-lactic, modified or optimized strains lactobacilli are generally preferred for high LA production [22].

Other routes for chemical synthesis of LA includes base- catalyzed breakdown of sugars, CO and H_2O combined at highest temperature, oxidation of propylene glycol, oxidation of propylene, hydrolysis of $CH_3CHClCOOH$, HNO_3, *etc* [23].

Lactic acid fermentation using microorganisms are faster; with high yields that ends up to two stereo isomers of LA or racemic mixtures. After nutrient supplementations, carbon source is inoculated with selected organism, and fermentation occurs. Hence, low-cost raw materials for promoting development of competitive processes are selected for LA production [23].

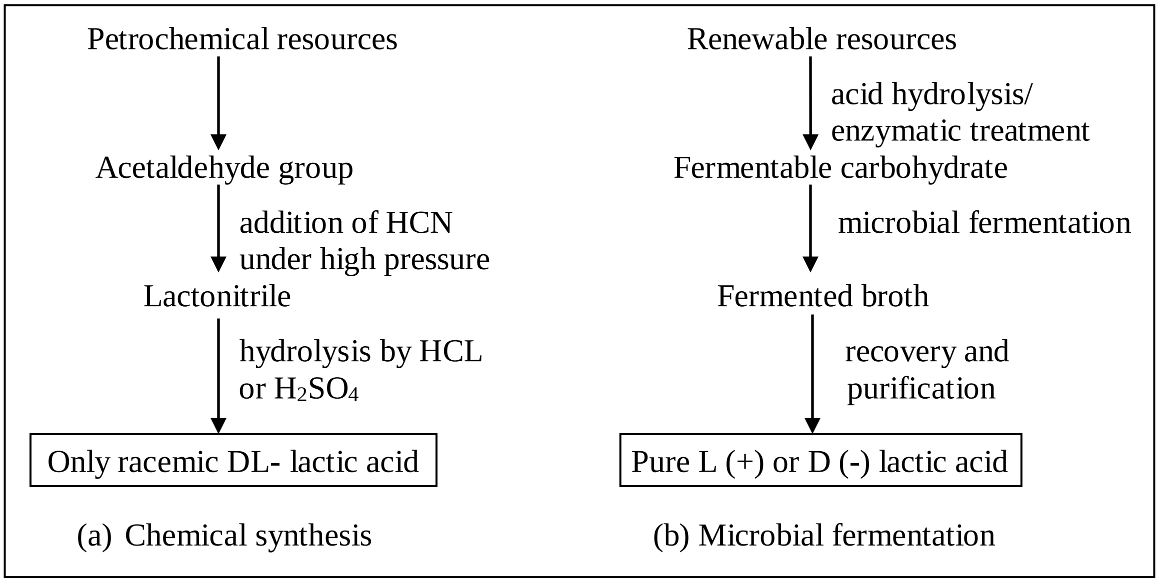


Figure 7: Methods for production of lactic acid

1.6.1.2 Fermentation using lactobacilli and raw materials for lactic acid production: Now a day, carbohydrates from raw materials are gaining more attention for production LA at low cost. Any carbon source containing hexose or pentose can be utilized in food industry; whey, molasses as by products are highly preferred (Table 5). Molasses contains high sucrose content, while whey has high lactose. Another by product used as substrate for LA production was date juice. Pulses are now gaining attention due to high starch content and presence of other components like sucrose in it [23]. In addition to this, there are other carbon sources like galactose, mannose or fructose which are consumed by lactobacilli present in synthetic medium for lactic acid production Table 6.

1.6.2 Lactobacilli in fermented foods

Fermented foods are unique group of microorganisms that enhances nutritional quality of food such as vitamins, proteins, fatty acids and essential amino acids. They impart function properties to consumers because of presence of function microorganism that possess probiotics features, antimicrobial, peptide production and antioxidant activity. Fermented foods have nutritive and non nutritive compounds that have capacity to modulate specific functions for well being and betterment of consumers [24]. Initially, fermentation was aimed to preserve food, production of inhibitory metabolites like organic acid, bacteriocins, ethanol with low water activity. However, fermentation helps in improving food safety by inhibiting pathogens, which degrades formation

Table 5: Lactobacilli and raw materials used in the production of lactic acid

Source	**Microorganism**	**Carbon**
Molasses	*L. casei*	Saccharose
	L. lactis	Saccharose
Pineapple syrup	*L. lactis*	Saccharose
Cow milk	*L. delbrueckii*	Lactose
Whey	*L. acidophilus*	Lactose
	L. bulgaricus	Lactose
	L. delbrueckii	Lactose
	L. casei	Lactose
	L. helveticus	Lactose
Date juice	*L. rhamnosus*	Saccharose
Corn	*L. amylophilus*	Starch
Potato	*L. amylophilus*	Starch
	L. delbrueckii	Glucose
Wheat bran flour	*L. amylophilus*	Starch
	L. bulgaricus	Glucose
Tapioca	*Lpb. plantarum*	Glucose
Bamboo	*Lpb. plantarum*	Glucose
Soy fiber	*L. delbrueckii*	Glucose
	Lpb. plantarum	Glucose

(Martinez et al., 2013)

of toxic compounds. Hence, increases the nutritional value and organoleptic quality of such foods. In addition to this, fermentation provides organic way to lessen amount of material to be transfered by destroying unwanted compounds and appearance of food. This also reduces the energy required to cook food to make a safer product. Lactic fermentation is mainly carried out by lactobacilli and other lactic acid bacteria (LAB), e.g. fermented cereals, sauerkraut, *kimchi*, *gundruk*, *etc* [24].

Globally fermented foods have been categorized into major nine groups such as fermented milk, fermented meat, fermented fish, fermented vegetables, fermented beverages, fermented cereals and fermented pulses products. List of commercially available fermented food using lactobacilli is shown in table 7 [25].

1.6.2.1 Fermented milk products: These are distributed into two parts; lactic fermentation involves LAB species including thermophilic (e.g. yogurt), mesophilic (e.g. cultured milk) and probiotics (e.g. acidophilus milk) type and fungal-lactic fermentation, comprises of LAB (e.g.

Table 6: Lactobacilli used for lactic acid production from synthetic medium

Microorganism	**Carbon**
L. amylophilus	Glucose , Starch
L. bulgaricus	Fructose, Galactose, Glucose, Lactose
L. casei	Glucose, Lactose
L. coryniformis	Glucose, Fructose, Glucose, Sucrose
L. delbrueckii	Glucose, Fructose, Galactose, Lactose, Maltose, Saccharose
L. helveticus	Lactose
L. lactis	Glucose, Xylose, Lactose, Saccharose
L. manihotivorans	Starch
L. paracasei	Glucose
L. pentosus	Glucose, Xylose
Lpb. Plantarum	Starch
L. rhamnosus	Galactose, Glucose, Maltose, Xylose
Lactococcus lactis	Glucose, Xylose, Lactose, Maltose, Saccharose

(Martinez et al., 2013)

kefir) and moldy milk products (e.g. viili). Starter cultures in preparing products includes *L. delbrueckii subsp. bulgaricus*, *Lactococcus lactis* subsp. *cremoris*, *Lc. Lactis* subsp. *lactis*, *L. helveticus*, *Leuconostoc* spp., and *Streptococcus thermophiles* [25-27].

1.6.2.2 Fermented meat products: There products are highly prone to bacterial spoilages. So to extend the life of meat, process like smoking, curing and packaging like applying low gas volume by vacuum or controlling temperature and refrigeration will depress growth of all this putrefactive type microorganisms. Sometimes condition may create growth of lactobacilli and results in souring of production, formation of slime layer, off odor as well as greening may occur. They are classified into categories like dried meat and jerky prepared from whole meat pieces and sausages made by chopping the meat. *Lat. curvatus*, *Lpb. paraplantarum*, *Lpb. plantarum*, *Lev. brevis*, *Lat. sakei*, *Lat. curvatus*, *L. casei*, *Lactobacillus* (*L.*) *sanfransiscensis* and *Lactobacillus* (*L.*) *divergens* are dominant lactobacilli found in fermented meats [25-27].

1.6.2.3 Fermented fish products: Traditional preparations of fish products were carried out through similar fermentation process of sun drying, prepare by smoking and salt addition than preserved and consumed during seasoning, condiments and curry by people living near coastal regions. Fermentation is brought about by auto-catalytic enzymes present in fish and microbes during high-salt concentration. Fermented fish products *ngari*, *tungtap* and *hentak* include lactobacilli namely *L. amylophilus*, *L. coryniformis* and *Lpb. plantarum* [25-27].

1.6.2.4 Fermented vegetable products: Fermented vegetable product like *sinki*, *goyang, kimchi, khalpi, sauerkraut* and *inziangsang* and various other naturally fermented bamboo productions are dominated by presence of lactobacilli and *Pediococcus*, followed by *Weissella*, *Tetragenococcus, Leuconostoc*, and *Lactococcus*. These products are mostly consumed in India, Nepal and Korea. At the time of fermentation, salt, anaerobiosis and cell death increases bioavailability of nutrients that helps drop in pH, where LAB gains dominance. Products like fermented olives, cucumber, silage, whole fruits or vegetables undergoes similar procedure and *Lev. brevis* and *Lpb. plantarum* starts to grow [25-27].

1.6.2.5 Fermented beverage products: Fermented beverages consisting of ethanol have been consumed from thousands of years by humans. This includes beer, wine, tea and other products. Fermented tea such as *miang*, in Thailand is dominated by *Lactobacillus* (*L.*) *thailandensis*, *Lpb. pentosus*, *Lactobacillus* (*L.*) *vaccinostercus*, *Lactobacillus* (*L.*) *camelliae*, *Lactobacillus* (*L.*) *pantheris*, *L. fermentum*, *Lpb. plantarum*, *Lactobacillus* (*L.*) *suebicus*, *etc.* In spite of high ethanol content, low pH (3.2-3.8) and SO_2, lactobacilli mange to survive the medium, exerts profound effects on quality of wine. Cocoa bean fermentation showed predominance of *L. fermentum* at initial stages [25-27].

1.6.2.6 Fermented cereal products: Fermentation of cereals occurs through complex ecosystem i.e. by LAB. Products like bread require leavening of dough. Breads prepared from wheat many be leavened by using yeast, but sourdough contained lactobacilli as predominant one followed by *Weissella*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Lactococcus*, and *Streptococcus*. *Ambali*, *chilra*, *bahtura* are well-documented food worldwide. While *idli*, dosa and jalebi are most common indian food made using cereal pulses mixture [25-27].

Table 7: List of commercially available fermented food using lactobacilli

Microorganism	**Products**
L. acetotolerans	Ricotta cheese, vegetables
L. acidophilus	Fermented milks, probiotics, vegetables
L. alimentarius	Fermented sausages; ricotta; meat, fish
Lev. brevis	Bread fermentation; wine; dairy
L. buchneri	Malolactic fermentation in wine; sourdough
L. casei subsp. casei	Dairy starter; cheese ripening; green table olives
L. delbruecki subsp. bulgaricus	Yogurt and other fermented milks, mozzarella
L. fermentum	Fermented milks, sourdough, urease (food additive)
L. ghanensis	Cocoa
L. helveticus	Starter for cheese; cheese ripening, vegetables
L. hilgardii	Malolactic fermentation of wine
L. kefiri	Fermented milk(kefir), reduction of bitter taste in citrus juice
L. kimchii	Kimchi
O. oeni	Wine
L. paracasei subsp. paracasei	Cheese fermentation, probiotic cheese, wine, meat
Lpb. pentosus	Bio-preservation of meat; green table olives; dairy, wine
Lpb. plantarum subsp. plantarum	Fermentation of vegetables, malolactic fermentation, green
Lat. sakei subsp. sakei	Fermentation of cheese and meat products; beverages
L. salivarious subsp. salivarius	Cheese fermentation
L. sanfranciscensis	Sourdough
L. versmoldensis	Dry sausages

O. oeni : *Oenococcus oeni* (Tamang et al., 2016)

1.6.2.7 Fermented pulses products: Pulses belong to family *Fabaceae* or *Leguminosae*. The family *Leguminosae* involves variety of species. The word pulse is applied to seed of these plants. All pulses can be recognized as legumes but not all legumes can be considered as pulses. So these terms called pulses and legumes are used interchangeably. The term pulses described by FAO are absolutely for the crops yield solely for dry seeds from leguminous plants. Legumes harvest for food such as peas, sprouts, green beans and for extraction of oil such as soybean, peanut is not included in pulses category [28]. Pulses are grown throughout the world and Asia is considered the largest producer. One of the most important features that distinguishes legumes is the proportion of carbohydrates, although present in remarkable amount, are slowly digested,

Table 8: Common pulses consumed globally

Indian name	English name/ Common name	Scientific name
Arhar / Rahar / Tur / Tuar	Pigeon pea / Red gram	*Cajanus cajan*
Soybean	Soyabean	*Gylcine max*
Chana/ Cholia / Hara chana / Kabuli Chana / Chhole	Chickpeas (Brown, Green, White)/ Garbanzo beans	*Cicer arietinum*
Chawli / Lobhia	Black-eyed beans / Cowpea	*Vigna unguiculata*
Rajma	Pulses / Split beans / Beans / Kidney beans	*Phaseolus vulgaris*
Urad / Kaali	Black gram / Black lentil (whole) / White lentil (dehusked)	*Vigna mungo*
Masoor	Red lentils	*Lens culinaris*
Moong	Green gram / Mung bean	*Vigna radiata*
Vaal	Field beans	*Vicia faba*

(Kamboj and Nanda, 2017)

hence are low-glycemic index foods. The most common pulses consumed worldwide are beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), lentils (*Lentis esculenta*), chickpeas (*Cicer arietinum*), broad beans (*Vicia faba*), peas (*Pisum sativum*) and peanuts (*Arachys hipogea*) (table 8) [28]. Table 9 refers to some common fermented legume products across the world.

1.6.2.7.1 Nutrient composition in pulses: The mature seeds of pulses are a seed coat, cotyledon and an embryonic axis, within these protein bodies and starch granules are embedded that serves as energy reserve. The composition of these pulses varies between species to species. This make them vary in concentration of carbohydrate, proteins, fibers as well as lipid, for each one (Table 10) [28]. Pulses are barely cultivated plants. They have capacity to fix nitrogen amount from surroundings by the action of bacteria present in nodules on their roots. This nitrogen is utilized by plants to form protein to be made available to human. This is the reason for accumulation of large amount of proteins in plants during their development. Pulses are superior when it comes to proteins quantity in comparison to other plants. They comprise twice the dietary content of protein than cereals grains [28]. During germination, protein is accumulated mainly in seed cotyledon, providing free amino acids NH_3 and C during growth. The major storage proteins in pulses seeds are classified as globulins representing 70 % of total proteins; albumins, 10-20 % as water- soluble proteins; glutelins, 10-20 % soluble in diluted acids and alkalis and prolamins up to 50-80 % soluble in ethanol. These fractions of storage proteins are usually low in pulses.

Pulses have low sulphur and amino acid like Trp while high Lys content. Therefore, combination of cereals and pulses are provided in diet which sums up for adequate nutrition to body [29].

The fiber content in pulses depends on variety of species and seed processing. In most pulses, concentration of fibers varies from 8-27.5 % and 3.3-13.8 % corresponds to soluble fibers. Variety of polysaccharides, peptic substances, cellulose, non cellulosic glucans are present in the cell wall of cotyledon. Comparatively, seed coat contains higher cellulose content up to 35-75 % [30]. On the other hand, lipids content in pulses accounts 1-6 % as low fat in seeds like lentil, chickpea, broad beans, while high fat content is found in peanuts and soybeans. Some pulses seed contains high oil content, as triglycerides; presence of mono-unsaturated and poly-unsaturated fatty acids. The ash content in pulses accounts up to 2-5 % and differs variety to variety [31]. Vitamins are present in appreciable amount in pulses. Vitamins like thiamin, carotene, pyridoxine, niacin, choline, riboflavin, folic acid, vitamin E, C and K are mostly found in pulses. The role of vitamins is to maintain stability on cell membrane; vitamin K acts as blood clothing factor. Red gram is rich in carotene with 469 µg. Level of thiamine and riboflavin is high in all pulses and range between 0.20-0.73 mg and 0.01-0.39 mg, respectively. Folic acid is present in ambient amount in black gram, cowpea, Bengal gram, green gram, lentils, dry peas and red gram in range between 12.10-220.00 µg. Bengal gram, moth bean and horse gram contains vitamin C up to 1-3 mg. Choline range up to 20-352 mg in black gram, bengal gram, cowpea, field beans, lentil, dry peas and green peas [32].

Carbohydrate accounts to about 6-62 % in pulses seeds; to which starch, source of storage carbohydrates in routine diet. During processing, the structure of starch might change and transform itself to resistance starch, which can acts as fiber. Other carbohydrates like monosaccharide's- ribose, glucose, fructose and galactose; disaccharides- maltose and sucrose; α-galactosides are present in seeds [31].

Table 9: Lactobacilli isolated from fermented legume products across world

Products	**Raw Material**	**Sensory features**	**Microorganisms**	**Country**
Bhallae	Black gram	Mild acidic, side Dish	*B. subtilis, L. fermentum, Leuc. mesenteroides, Rhiz. marina, S. cerevisiae, Ent. faecalis, Trichosporon beigelii, T. pullulans, W. robertsii*	India
Dhokla	Bengal gram	Mild acidic, spongy, steamed, snack	*Leuc. mesenteroides, L. fermenti, Ent. faecalis, Tor. candida, Tor. Pullulans*	India
Doenjang	Soybean	Alkaline, paste, Soup	*B. subtilis, B. licheniformis, B. pumilis, Mu. plumbeus, A. oryzae, Deb. hansenii, Leuc. mesenteroides, Tor. halophilus, Ent. faecium, Lactobacillus* spp.	Korea
Kawal	Leaves of legume (Cassia sp.)	Alkaline, strong flavored, dried balls	*B. subilis, propionibacterium sp., Lpb. plantarum, Staph. sciuri, yeasts*	Sudan
Maseura	Black gram	Dry, ball-like, brittle, condiment	*B. subtilis, B. mycoides, B. pumilus, B. laterosporus, Ped. acidilactici, Ped. pentosaceous, Ent. durans, L. fermentum, L. salivarius, S. cerevisiae, Pic. burtonii,*	Nepal, India
Ogiri/ Ogili	Melon seeds, castor oil seeds		*B. subtilis, B. pumilus, B. licheniformis, B. megaterium, B. rimus, Pediococcus sp., S. saprophyticus, Lpb. plantarum*	Africa
Tempe	Soybean	Alkaline, solid,fried cake, Breakfast	*Rhiz. oligisporus, Rhiz. arrhizus, Rhiz. oryzae, Rhiz. stolonifer, Asp. niger, Citrobacter freundii, L. fermentum, L. lactis, Lpb. plantarum, L. reuteri*	Indonesia (Origin), Japan, USA

(Tamang et al., 2016)

Table 10: Proximate composition of pulses (g/100g)

Composition/ Pulses	**Chickpea (chana)**	**Lentils (udad, Masoor)**	**Broad beans (vaal)**	**Green gram (mung)**	**Red gram (tuvar)**
Total Carbohydrate	62.3	56.4	35.4	53.3-61.2	57.3-58.7
Starch	37-50	32.2-52.8	40-50	37.0-53.6	40.4-48.2
Sucrose	4.3	1.5	2.1-2.3	0.65	1.1-2.1
Raffinose	0.4-1.2	0.3-1.0	0.1-0.3	1.65	0.24-1.05
Stachyose	2.0-3.6	1.7-3.1	0.7-3.1	2.75	0.35-0.86
Verbascose	0.6-4.2	0.6-3.1	1.7-3.1	-	1.60-2.30
Protein	23.6	20.6	26.6	23.86	17.9-31.0
Fat	5.3	1.4	0.8	1.3	1.0
Fibers	3.8	6.83	31.3	15.2	15.0
Minerals	3.0	3.2	3.2	3.5	1.0
Ash	3.7	2.8	4.1	3.32	3.8

(Hall et al., 2017)

Researcher from Japan suggested use of oligosaccharides from pulses as substitute for table sugar. The concentration of galactosides in pulses varies between 2 to 10 g/ 100 g in dry pulses seeds. Galactosides when they enter intestine are fermented by unique bacteria and produce methane, H and CO_2 gas, which eventually causes flatulence. Therefore, pulses seeds are traditionally prepared after various processing which includes, soaking than cooking this helps in palatability of pulses by inactivating protease inhibitors and haemagglutinins and improves its bioavailability of nutrients [31].

1.6.2.7.2 Anti-nutritional factors in pulses: The term anti-nutrient is a defense metabolite, from maximum molecular weight compounds to minimum molecular weight amino acids as well as galactosides. Pulses are higher in proteins than cereals and provides cheap protein source. Pulses proteins, have inferior quality, because of deficiency of amino acids lack in sulphur, also they lack in digestibility, ANFs, *etc* [19]. ANF causes detrimental effect in humans and animals and utilization or uptake of other feeds and food components or by creating discomfort and stress. ANFs, divided into different groups based on the physical and chemical properties like quinolizidine alkaloids, non-protein amino acids, cyanogenic glycosides, isoflavones, tannins, oligosaccharides, pyrimidine glycosides, saponins, lectins, phytates and protease inhibitors [19].

1.6.2.7.2.1 Positive impact of anti-nutrients in human nutrition: ANFs effects gastrointestinal tract and affect microbial count in intestine by promoting growth of beneficial bacteria. Lupins (*Lupinus campestris)* seeds contain anti-mutagenic activity and prevent development of cancer. ANF reduces heat shock protein level in gut epithelial cells once they expose to plant lectins. It is suggested that consumption of certain anti-nutrients in low level produces health benefits, avoiding adverse effect related to large intake [19].

1.6.2.7.2.2 Negative impact of anti-nutrients in human nutrition: There are toxic nutrient in pulses produces serious pathological effect on humans. These may cause serious paralytic diseases affecting lower limbs, known as lathyrism and hemolytic factor associated to fauvism occurs due to hemolytic anemia which is caused after cooking broad beans [19].

1.6.2.7.3 Kinds of anti-nutritional factors

ANFs have been characterized in to two heat labile and heat stable or toxic anti-nutritional and potentially toxic anti-nutritional factors. These include saponins, tannins, lectins, protease inhibitor and phytic acid. Here, focus will be given on heat stable anti-nutritional factors.

1.6.2.7.3.1 Tannins: Tannins are phenolic non-nitrogenous organic components, classified into two, hydrolysable and condensed tannins. Proteins are bind to tannins by hydrogen binding and hydrophobic interactions, hence reducing nutritional quality. They decrease palatability and decreases growth rate. Condensed tannins have profound digestibility, whereas hydrolysable tannins cause toxic manifestations, because of hydrolysis in rumen. Tannin acts in defense mechanism to surrounding environmental attack. Tannins act as an antioxidant in faba beans mostly by chain-breaking ability with transition metal elements. When concentration of tannin in diet increases, cellulose and intestinal digestion i.e. microbial enzyme activities may get depressed. Tannins are known to form certain insoluble complexes with proteins and this protein complex is responsible for ANF effect in foods [33].

1.6.2.7.3.2 Saponins: This word is derived from Latin word “sapo”, meaning soap. Saponins are widely distributed in nature, non-volatile secondary compounds, occurring widely in plants. They are diverse with non polar aglycones molecules, coupled with moieties of monosaccharide. The mixture of polar and non-polar elements resembles to soap like behavior in solutions. It has number of properties as foaming and emulsifying agent, sweetness and bitterness, haemolytic properties, pharmacological and medicinal, as well as insecticidal and antimicrobial activities [33]. Saponins are naturally occurring foam producers of steroidal glycosides or triterpene that occurs widely in plants, including oilseeds and pulses such as lentil, pea, kidney bean, chickpea, soybean, groundnut, lupine, alfalfa and sunflower. Saponins are reported for their hypocholesterolemic effects, by reducing uptake of certain nutrients like cholesterol and glucose at the gut, via intralumenal physicochemical interaction. Saponin containing foods control plasma cholesterol, osteoporosis, preventing peptic ulcer and reduces risk of heart disease. Toxic saponins may cause nausea, vomiting and head acne in human. Within grain legumes, dry weight of saponin content ranges between 0.5-5 % [33].

1.6.2.7.4 Processing of Anti nutritional factors

Processing of ANFs improves nutrient profile of pulses seed, increasing *in vitro* digestibility of carbohydrates and proteins up to 40-98 % same time decrease in anti nutritional compounds occurs. The following are some traditional processing techniques; non-heat processing method includes soaking, dehulling, germination, imbibition, or fermentation and heat processing method includes cooking, autoclaving or roasting. Heat-stable ANFs can be decrease by soaking, dehulling, germination or fermentation [33].

1.6.2.7.4.1 Soaking: To facilitate processing method, Soaking of pulses is done, which lasts 15–20 min or for longer time. Generally, pulses are soaked in water overnight. This process enhances naturally occurring nutrients in pulses. It was reported that during soaking tannins hydrolysis are greatly influenced by pH and temperature. The optimal pH for soaking was found between 5.0-6.0 and temperature between 45-65℃ for intrinsic ANFs [34-36].

1.6.2.7.4.2 Dehulling: Dehulling, a common method in desi type, (split beans or dal) then cooked and/or milled to flour. This process removes seed coat which contains many ANFs like tannins by 90 %. Therefore, dal has higher carbohydrate, vitamin and protein concentration [34-36].

1.6.2.7.4.3 Imbibition: Imbibition means increase in volume of pulses due to absorption of water. It is a common way of cooking grains, to reduce time of cooking. However, soaking reduces ANFs, leaching galactosides, tannins and protease inhibitors in to soaking medium. Amount of leaching mainly depends on soaking medium such as salt solution, water or bicarbonate solution and also the soaking time. Reports found to reduce tannin content in black gram by almost 50 % after overnight soaking in water [34-36].

1.6.2.7.4.4 Fermentation: Food fermentation is bacterial and enzymatic-based method for processing of food to increase shelf life. Product of plants such as cereals, vegetables and legumes are usually used in preparing fermented foods. Lactic fermentation is most known method in which pH drops as consequence growth of bacteria occurs leading to formation of lactic and acetic acids which favors tannins and saponin activities, results in lowering of this

ANFs [37]. The use of fermentation as food detoxification process highly practiced. Fermentation is one such method for food preservation and can be done at both household and industrial scale. Food fermentation is carried out by a very simple technique and includes grass roots technology that facilitates their refinement and adoption in under-developed countries, usually for detoxification of food sources. The incorporation of such fermentation processes into simple food technologies, offers better prospects for detoxification of food source, simultaneously giving flexibility in adapting good flavor, texture and color of prepared food. Fermentation using microorganism decreases protein size attributed to enzyme involved with organism and fermentation process, this also increases *in vitro* trypsin digestibility and antioxidant activity, thereby improving functional and nutritional properties of food product [38].