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Soymilk, probiotic tofu production and extension of shelf life

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ABSTRACT

Soybean products such as tofu, soymilk, soymilk powder, bean sprouts, dried tofu, soy sauce, soy flour, tempeh and soybean oil have been prepared and used through traditional ways and using modern processing techniques in the world. Soybeans are an important raw material for those seeking vegan, lactose-free products, such as soymilk and tofu. Soybean variety is one of the vital factors that affect the tofu quality and shelf life as well. The aim of this detailed review article is to provide an overview of production of tofu from soybean seeds and extension the shelf life. Tofu is an unfermented food product but at present it is available as fermented foam in market which is considers more beneficial than unfermented foam in many East Asian countries. It has been popular worldwide because of cholesterol-free, low in saturated fats, high in protein, carbohydrates, calcium, iron, and several potential health benefits to humans. It is prepared by soymilk after coagulation and pressing the resulting curds into white blocks of varying softness; it can be silken, soft, firm, or extra firm. Physical, chemical and sensory parameters are being used to ensure the quality and improve product yield. The shelf life of tofu is very important factor for the tofu in this review paper we are focus on extension of shelf life. Current preservation methods for extension of shelf life of the tofu: include low temperature storage, high pressure processing and addition of preservatives such as chitosan, plant extracts, and nisin etc.

KEYWORDS: Soymilk, tofu, shelf life, soybean and sensory analyses,

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1. INTRODUCTION

The high nutritional value and low cost of soybean products make them a suitable inexpensive way to overcome malnutrition problems among rural masses in India. Tofu, a fundamental part of Asian food culture, is a traditional soybean food composed of protein and lipid. Currently, its consumption and popularity is not much more among poor people. Soy foods were also given a significant boost when FDA in 1999 approved a health claim linking soy foods with heart disease risk reduction [1]. There are a wide range of soy products around the world and they can be broadly classified into two main categories: fermented (e.g. tempeh, natto, miso, soy sauce, etc.) and unfermented (e.g. soy milk and tofu) products. In fact, soy contains a number of biologically active compounds, including isoflavones, essential fatty acids, antihypertensive, antioxidative, opioid agonistic peptides and tocopherols [2]. Tofu is the nutritive food made up of soybean has the potential health benefits. It is produced from water-extracted and salt- or acid precipitated soybean in the form of a curd, resembling a soft white cheese or a very firm yogurt. Tofu can also be further processed into various secondary tofu products, including deep-fried tofu, grilled tofu, frozen tofu, dried tofu, fermented tofu, and more. In most cases, these processed tofu products have different characteristics, such as taste, texture, end uses, and commercial identities with respect to the original plain tofu [3]. Tofu is a perishable highly hydrated, gelatinous product having relatively high pH (5.8 to 6.2) and moisture content (80 to 88%) [4]. Its water content can be varied to produce an array of tofus with different characteristics.

Soybean, *Glycine max* (L.) Merrill belongs to family Fabaceae and subfamily Faboideae, globally known as Golden Grain. It is an important type of legume that has been used for a long period as a protein source in Asia countries [5]. It is one of the world's most important oil producing crops having significant amount of protein 35–40%, carbohydrates 30.16g, fat 19.94g etc. Variety is one of the major factors that influence food product making potential of soybeans. Soybean products have an excellent nutritional status based on their high protein content and all the essential amino acids to meet biological requirements. Soybean products such as tofu, soymilk, soymilk powder, bean sprouts, dried tofu, soy sauce, soy flour, tempeh and soybean oil have been prepared and used through traditional ways and using modern processing techniques: Thermal and non-thermal processing high temperature, low temperature pasteurization, sterilization blanching, caning, drying in the world.

1.1 "Physiologically functional substances in soybeans [6]"

S.N.	Components in soybeans	Physiological functions
1.	Soybean proteins	Reduction of serum cholesterol, prevention of cardiovascular diseases, reduction of body fat and promotion of serum insulin
2.	Peptide from proteins	Antioxidant activities, inhibition of angiotensin-converting enzymes and promoting action of phagosytosis
3.	Isoflavones	Anti-carcinogenetic activities, prevention of cardiovascular diseases, prevention of osteoporosis, antioxidant activities and alleviation of menopausal symptoms
4.	Saponins	Anticarcinogenic activities, hypocholesterolemic effects, inhibition of platelet aggregation, HIV preventing effects and antioxidant activities
5.	Phytosterol	Anti-carcinogenic activities
6.	Phytic acid	Anti-carcinogenic activities
7.	Lectin (Hemagglutinin)	Activation of lymphocytes (T cell) and aggregating action of tumor cells
8.	Nicotianamine	Inhibitor of angiotensin-converting enzyme
9.	Protease inhibitors	Anti-carcinogenic activities

1.2 "Consumption of soybeans in various countries [7]"

S.N.	Countries Soybean	Consumption (g/d)
1.	Japan	29.5
2.	Korea	19.9
3.	Hong Kong	10.3
4.	China	9.3
5.	United States minor	224

In the United State, Soybean products such as soymilk and tofu are highly consumed. Asians consume 9 to 30g soybeans per day, with individual and regional variation.

1.3 "Proximate Composition of Soybean and tofu [8]"

S.N.	Components	Soybean	Tofu
1.	Protein	40%	8g
2.	Carbohydrate	35%	1.5g
3.	Lipid	20%	3.5g
4.	Calcium	277mg	130mg
5.	Magnesium	228mg	1.10mg

In addition, it contains no cholesterol or lactose and only a small quantity of saturated fatty acid, and is suitable for those who are lactase deficient. Soybean seeds have a protein content of 35–40% on a dry weight basis, which makes them a relatively inexpensive source of protein for human consumption [9]. Soybeans have been transformed into various forms of soybean foods, tofu being the one most widely accepted throughout the world.

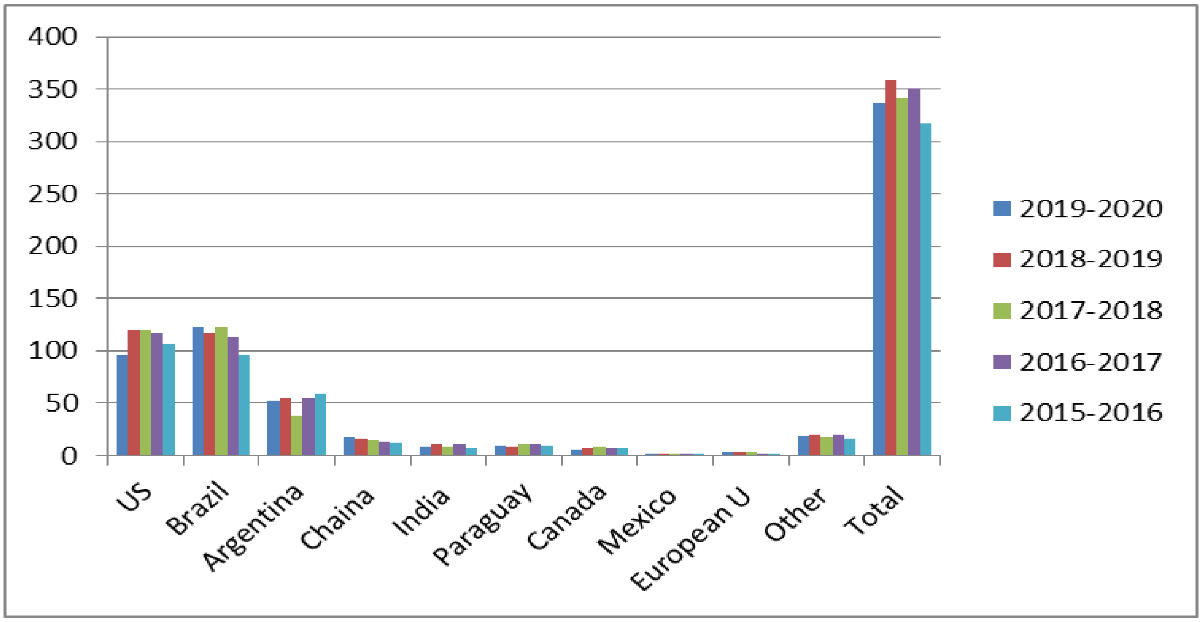

Fig-1 World soybean production (The soybean processors association of India)

Tofu is popular as bean curd or soy paneer, prepared by soy milk after coagulation and pressing the resulting curds into white blocks of varying softness; it can be silken, soft, firm, or extra firm. Beyond these broad categories, there are many varieties of tofu. It has a subtle flavour and spongy texture, so it can absorbs flavours very well.

It is a traditional component of East Asian and Southeast Asian cuisines and has been consumed in China for over 2,000 years. US, Brazil and Argentina are highest producer of soybeans in the world. Nutritionally, tofu is low in calories, while containing a relatively large amount of protein. It is high in iron, nutritional chat and can have a high calcium or magnesium content depending on the coagulants (e.g. calcium chloride, calcium sulphate, and magnesium sulphate) used in manufacturing. Tofu is usually recognized as a salt or acid coagulated soy protein gel containing water, soy lipid, and other constituents entrapped inside its network. *Glycine* (11S globulin) and β-conglycinin (7S globulin) are two major proteins in soybeans, which account for about 65-80% (by weight) of the total seed proteins present [4]. Western countries have started to take an increasing interest in tofu due to its good nutrition and health benefits to human. The increase consumption of soy-based products leads to the incentive for more sustainable soybean processing, by reducing the carbon footprint and/or greenhouse gas emissions compared to current processes.

1.4 "State wise varieties of Soybeans"(ICAR-IISR website)

S.N.	**State**	**Suitable verities**
1.	Andhra Pradesh	LSb-1, Pratikar (MAUS 61), Pooja (MAUS 2), MACS 450, Pant Soybean 1029, MACS 124, Monetta and Bragg
2.	Arunachal Pradesh	1 (NRC 2), JS 80-21, Samrudhi (MAUS 71), Pratap Soya (RAUS 5), Bragg, Indira Soya 9
3.	Bihar	PK 416, Pusa 16, Pusa, 24, Pant Soybean 564, Pant Soybean 1024, Pant Soybean 1042, Bragg and SL 525
4.	Chhattisgarh	Ahilya 1 (NRC 2), Ahilya 3 (NRC 7), Ahilya 2 (NRC 12), Ahilya 4 (NRC 37), JS 71-05, JS 335, JS 80-21, JS 75-46, MACS 58, JS 9041, Indira Soya 9, JS 93-05, Kalitur, Parbhani Sona (MACS 47), Pratishta (MAUS 61-2), Monetta, Punjab-1, PK 472, Shakti (MAUS 81), Samrudhi (MAUS 71) and Pratap Soya (RAUS 5)
5.	Delhi	PK 416, Pusa 9712, Pant Soybean 564, Pant Soybean 1024, Pant Soybean 1042, Bragg and SL 525
6.	Gujarat	Gujarat Soybean 1, Gujarat Soybean 2, JS 93-05, JS 335, JS 80-21, JS 75-46, MACS 58, Monetta, Parbhani Sona (MAUS 47), Pratishta (MAUS 61-2), Shakti (MAUS 81) and PK 472
7.	Harayan	Punjab-1, Pk 416, Pusa 16, Pant Soybean 564, Pant Soybean 1024, Pant Soybean 1042 and SL 525
8.	Himachal Pradesh	VL Soya 2, VL Soya 47, Shilajeet, Shivalik, Bragg, Pusa 16, Hara Soya and Palam Soya
9.	Jharkhand	Birsa Soybean 1, Ahilya 1 (NRC 2), JS 80-21, Samrudhi (MAUS 71), Pratap Soya (RAUS 5) and Bragg
10.	Karnataka	Hardee, Bragg, Sneh (KB 79), KHSb-2, Pratikar (MAUS 61), Pant Soybean 1029, MACS 124, MACS 450 and Pooja (MAUS 2)
11.	Madhya Pradesh	Ahilya 1 (NRC 2), Ahilya 3 (NRC 7), Ahilya 2 (NRC 12), Ahilya 4 (NRC 37), JS 71-05, JS 335, JS 80-21, JS 75-46, MACS 58, JS 90-41, Indira Soy 9, JS 93-05, Kalitur, Parbhani Sona (MAUS 47), Pratishta (MAUS 61-2),Monetta, Punjab-1, PK 472 and Shakti (MAUS 81)

12.	Marathwada region of Maharashtra	Ahilya 1 (NRC 2), JS 335, JS 93-05, JS 80-21, MACS 58, Parbhani Sona (MAUS 47), Pratishta (MAUS 61-2), Shakti (MAUS 81), MACS 13, Monetta, Prasad (MAUS 32) PK 472, Shakti (MAUS 81), TAMS-38 and Phule Kalyani (DS-228)
	Southern Maharashtra	MACS 124, MACS 450, Pant Soybean 1029, Pooja (MAUS 2), Pratikar (MAUS 61), Prasad (MAUS 32), MACS 13, Monetta and Phule Kalyani (DS-228)
13.	Nagaland	Ahilya 1 (NRC 2), JS 80-21, Samrudhi (MAUS 71), Pratap Soya (RAUS 5), Bragg, Indira Soya 9
14.	Orissa	JS 80-21, Pusa 24, Indira Soya 9, Ahilya 1 (NRC 2), Ahilya 2 (NRC 12), Ahilya 3 (NRC 7), Ahilya 4 (NRC 37) and Pusa
15.	Punjab	PK 416, Pusa 16, Pant Soybean 564, Pant Soybean 1024, Pant Soybean 1042, SL 295, Bragg and SL 525 16.
16.	Rajasthan	Pratap Soya (RAUS), Bragg, Punjab-1, PK 472, MACS 58, JS 80-21, JS 335, Ahilya 4 (NRC 37), Parbhani Sona (MAUS 47), JS 93-05, Pratishta (MAUS 61-2) and Shakti (MAUS 81)
17.	Sikkim	NRC 2, JS 80-21, MAUS 124, MAUS 71, RAUS 5, Pusa 16, Bragg and Indira Soya 9
18.	Tamil Nadu	Co 1, Co Soya 2, ADT-1, MACS 124, MACS, 450, Pooja (MAUS 2), Pratikar (MAUS 61) Hardee, Pant Soybean 1029, and Bragg
19.	West Bengal	Ahilya 1 (NRC 2), JS 80-21, Samrudhi (MAUS 71), Pratap Soya (RAUS 5), Bragg and Indira Soy 9
20.	Uttar Pradesh	Pusa 16, Pant Soybean 1092, Pant Soybean 1042, Pant Soybean 1024, Pant soybean 564, PK 472, PK 472, PK 416, Pratishta (MAUS 61-2), JS 93-05, Ahilya 4 (NRC 37), JS 335, SL 525, PS 1241, PK 262 and PK 327
21.	Uttrakhand	Hara Soya, Palam Soya, Punjab-1, Pusa 16, PS 1241, VL Soya 1, VL Soya 2, VL Soya 21, VL Soya 47, Shilajeet and Pant Soybean 1092

Soybean varieties, soybean quality (growth and storage environment dependent), and processing conditions, methods: Bench scale and production scale [10, 11]. Coagulation of the protein and oil (emulsion) suspended in the boiled soymilk is the most important step in the production of tofu making process but is complicated as the process depends on complex interactions. Tofu is thought to be a good medium for the growth of lactic acid bacteria due to a high protein content and pH close to neutral. During fermentation and growth of probiotic cultures a soy product becomes a product with functional properties [6]. This process is accomplished with the aid of coagulants.

Probiotics are live microorganisms that can be found in fermented foods and cultured milk, and are widely used for the preparation of infant food. They are well-known as “health friendly bacteria”, which exhibit potential health benefits and positively influencing the intestinal microbial balance' [12]. They boost up the micro flora of the gastrointestinal tract and helpful to enhance digestive system and immunity. Another benefit of the consumption of probiotics is their ability to reduce cholesterol absorption [13]. Probiotic bacteria are useful to mitigate lactose intolerance, treatment of diarrhea, anticarcinogenic properties, and reduction in blood cholesterol and improvement in immunity [14]. Consumption of high concentration of probiotic bacteria per gram of product is required to confer health benefits [15].

Probiotic bacteria are more and more often used in industrial production of fermented foods. Fermentation is used to conserve food in dairy, meat and fruit-vegetable industries. Probiotics are used in plant-derived products; include high nutritional and sensory values in these raw materials. Probiotics enhance better assimilation of individual nutritional components; they synthesize some vitamins of B group and K. They increase absorption of minerals and vitamins.

Texture and yield of tofu are important determinants of product acceptance by consumers and producers, respectively. Factors such as variety of soybean, processing method and type and concentration of coagulant have been reported to influence the yield, quality, and texture of tofu [16] and his colleagues have reported that the varietal differences in seed protein content, seed size and soy milk solids were a significant determinants of tofu yield. The quality of tofu is affected by soybean variety due to differences in chemical compositions [17]. In addition to the total protein content, the quality of tofu is also affected by the compositions and content of the stored proteins [18]. For example, glycinin (11S) and β-conglycinin (7S) have different contributions to the functional properties of soybean protein, while the 11S/7S ratio influences the quality of tofu.

Tofu has a very short shelf-life, and it is usually consumed freshly. In order to obtain a longer shelf-life for tofu, considerable attention has been given to the development of methods for extending the shelf life of tofu. Some researchers have studied quality improvement and (preservation period) shelf-life extension of tofu. Extend the shelf-life of tofu, physical methods include low temperature storage, high pressure processing [19], the control of microbial growth is necessary to extend the shelf-life and improve the quality and safety of tofu, microwave treatment [20], coagulation with organic acid and pH adjustment of immersion solutions have been tried [21].

In addition, antimicrobial chemicals or herb extracts with bactericidal activities, such as chitosan is use as an additive to tofu for the purpose of shelf-life extension because it has antimicrobial activity [22]. However, none of these methods have been employed by commercial tofu manufacturers. Thus, there is need for a more practical and efficient method [23], isothiocyanates from horseradish [24]. CaO from oyster shell power [25], and *Ocimum sanctum* extracts [26] have been added to tofu as coagulants or as preservatives. Although these methods could delay the spoilage of tofu in different extents, they would probably affect organoleptic properties and increase the cost. Therefore, few of them have been applied in the commercial tofu factories yet. Bio preservation has substantially attracted attentions to improve the shelf-life and the safety of food [27]. Lactic acid bacteria (LAB) have been exploited as biopreservative microorganisms and have played a vital role in a diversity of food

2. METHOD

2.1 *Extraction of Soymilk from Soybean seeds*

Soymilk is produce by the method described by [28, 29, 30] with some modifications. One hundred grams of clean soybeans is first rinse and soaked in 1000 mL of distilled water for 16 h at room temperature. Soaked soybeans seeds is drain, rinse, and grind in blender/ grinder for 3 min on high speed with boiling water. The water is added to make a total of 1100 g of soybean slurry. The ratios of dry soybean to water will 1:10 on weight basis. The slurry is filter through 4 layers cheesecloth to separate the 880 mL soymilk from the hulls and other insoluble materials. Seven hundred and fifty millilitres of soymilk is heat to boiling for 10 min and then boiling water will be added to maintain 750 mL of soymilk.

2.2 Preparation of fermented and unfermented tofu

2.2.1 Fermented tofu

Tofu is preparing follow pervious methods with some modificatios [31, 32]. Production of tofu by using different strains of *Lactobacillus acidophillous, L. Casei, L. paracasei*, The 750 ml soymilk is pasteurizing for 10 minutes at the temperature of 80-85°C. Tofu is prepared by coagulating the soymilk using calcium sulphate, calcium chloride, magnesium sulphate and shell powder, coagulated soymilk is inoculated with different strains of LAB. The soybean curd is transfer to a tofu-forming box line with 2 layers of cheesecloth and pressed stored at 4°C.

2.2.2 Unfermented tofu

Tofu is preparing follow pervious methods with some modifications [33, 31]. The 750 ml soymilk is pasteurizing for 10 minutes at the temperature of 80-85°C. Tofu is prepared by coagulating the soymilk using calcium chloride, calcium sulphate, magnesium sulphate, shell powder and lemon juice etc. The whole mixture is mildly mix and left for 10 minutes so as protein could coagulate. The soybean curd is transfer to a tofu-forming box line with 2 layers of cheesecloth and pressed and stored at 4°C.

3. PHYSIOCHEMICAL ANALYSES

Texture analysis of tofu is determining by texture profile analyser [34, 35]. Yield of tofu is record and express as weight (g.) of tofu obtain from 100 g dry soybean seeds [36]. Colour of tofu, expressed in L, a, and b values, according to the method described by [37]. Microstructure of tofu is determined by using Scanning Electron Microscope [38].

Protein content of tofu is analysing by the micro Kjeldahl method described by [39]. Fat content of tofu is determined by the Soxhlet extraction method described by [39]. Total soluble solids content of tofu is determine using refractometer described by [39]. Minerals content of tofu is determined described by [39]. The moisture content of tofu is analysing by the vacuum oven method described by method [39]. The pH of tofu is determined by pH meter [39]. The ash content of tofu is determined using the method of AOAC, 1970.[39].

4. SENSORY ANALYSIS

Descriptive sensory tests are amongst the most sophisticated tools in the arsenal of the sensory scientist [40] and involve the detection (discrimination) and description of both the qualitative and quantitative sensory components of a consumer product by trained panels of judges [41]. The qualitative aspects of a product include all aroma, appearance, flavour, texture, aftertaste and sound properties of a product, which distinguish it from others. Sensory judges then quantify these product aspects in order to facilitate description of the perceived product attributes. Descriptive sensory analyses are also used for quality control, for the comparison of product prototypes to understand consumer responses in relation to products' sensory attributes, and for sensory mapping and product matching.

It may also be used to track product changes over time with respect to understanding shelf-life and packaging effects, to investigate the effects of ingredients or processing variables on the final sensory quality of a product, and to investigate consumer perceptions of products [e.g. Free-Choice Profiling (FCP)]. There are several different methods of descriptive analysis, including the Flavour Profile Method [42], Texture Profile Method [43], Quantitative Descriptive Analysis TM [44], the Spectrum TM method [41], Quantitative Flavour Profiling [45], Free-choice Profiling [46] and generic descriptive analysis.

Fresh tofu is subjected to sensory evaluation based on the method proposed by [47]. Tofu is into cubes and place on a plastic plate with a random for sensory evaluation is body and texture, flavour, taste, colour and appearance, sweetness, and overall acceptability. The result is recorded using with a 9 point hedonic scale where, 1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much and 9= like extremely for each sample.

5. EXTENSION OF SHELF LIFE

The shelf life of food is defined as the duration of time from its date of manufacture to the date it is no longer acceptable for a consumer. Factors that affect food quality and shelf life of the product may vary depend on: processing, storage, delivery, chemical reactions, and physical and biological changes that occur naturally in the food. The factors that contribute to food shelf life are normally classified as intrinsic and extrinsic specific purpose. These factors are referred to as intrinsic and extrinsic factors: Intrinsic factors are an inherent part of the food and include water activity (aw), pH, oxidation-reduction potential (Eh), oxygen content, and nutrients, Extrinsic factors that control the rates of reaction include temperature, relative humidity (RH), gas atmosphere, light, and packaging that can influence on the rate of reactions, depending on the specific packaging material. The Institute of Food Science and Technology (IFST) in the United Kingdom has defined the “shelf life” as “the period of time during which the food product is remain safe; certain to retain desired sensory, chemical, physical, microbiological and functional characteristics; and comply which any label declaration of nutritional data when stored under the recommended conditions” [48].

Recently, the applications of naturally occurring extracts have become the preferred preservation method due to their potential health benefits and safety [49]. A new tendency in food preservation is emerging in the use of natural preservatives combined with minimal processing techniques that can synergistically improve food quality and safety [50]. Many researchers have reported the antimicrobial effects of plant essential oils, including rosemary, sage, thyme, clove, oregano, garlic, and basil [51, 52, 53, 54, 55, 56, 57].

To increases the shelf life of tofu, Oyster shell powder is being used at different levels 0.05%, 0.1%, and 0.2%. The tofu place in a polypropylene container containing 100 mL of sterilize distilled water as an immersion solution can be stored for 6 months at 4°C. The shelf life of tofu is determined described by [25].

Chitosan (deacetylated chitin), a polycationic biopolymer is commercially prepared from shellfish-processing waste and is non-toxic, biodegradable and biocompatible [58]. It exhibits antibacterial and antifungal activity and has therefore received attention as a potential food preservative of natural origin [59, 60, 61]. Several researchers have developed methods to improve the properties of chitosan using chemical and enzymatic modifications. However, chemical modifications are generally not preferred for food applications because of the formation of potential detrimental products. Chitosan– lysozyme conjugates have been reported to have better emulsifying properties and bactericidal action [62]. Chitosan has an amino group which can react with the carbonyl group of a reducing sugar.

Nisin is currently industrially produced and is used for specific applications such as prevention of spore germination and growth of pathogenic bacteria contaminating the surface of food products. It has been commercialized for the first time in the 50’s to inhibit the outgrowth of *Clostridium tyrobutyricum* responsible for late cheese blowing [63]. Lactic acid bacteria (LAB) naturally produce nisin in raw milk and dairy products [64], however LAB growth for nisin industrial production requires complex nutrition conditions which increases production costs and complicates the purification steps. Nisin is the only bacteriocin approved as a food preservative, which explains its increasingly common use in food industry. This use is governed by the "FAO / WHO Codex Committee on Milk and Milk Products" which has accepted the use of nisin as a food additive in a concentration not exceeding 12.5 mg of pure nisin per kilogram [65]. Nisin has been approved as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) since 1988 because it has long been used in food preservation without being involved in health problems. Nisin is effective against several pathogenic Gram positive bacteria such as Listeria monocytogenes and C. botulinum, but also against some Gram negative pathogens such as Escherichia coli and Salmonella spp.

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