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Coencapsulation of a probiotic ***Lactobacillus brevis* KBM2** with flaxseed and ***Pulicaria dysenterica*** extract

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.

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Congratulations 2023

وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ

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Gratitude

Alhamdoulillah ,

Well, we did it we all accomplished one of the major early milestones of our lives, university graduation.

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Benmohammed fadia ines

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Abbreviation List

BLis: Blind spot information system.

CFU: Colony forming unit.

CMC: Carboxymethyl

DNA: Deoxyribonucleic acid.

EPS: Extracellular polymeric substance.

GIT: Gastric intestinal trac.

GRAS: Generally recognized as safe.

FAO: Food and agriculture organization

LAB: Lactic acid bacteria.

Lb: Lactobacillus.

MRS: Man-Rogosa-Sharp.

NS: Normal saline.

PBS: Phosphate Buffer Saline.

PCR: Polymerase chain reaction.

pH: Potential of hydrogen.

rpm: rotation per minute.

rRNA: Ribosomal RNA.

SGI: simulated gastro-intestinal

W/O: Water-in-oil.

WHO: World health organization.

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Introduction

Recently, research on probiotics as nutraceuticals and functional food has received increasing attention globally **(Siang et al., 2019)** as well as the development of novel food products containing probiotics with potential health benefits is currently gaining more attention **(Li et al., 2019)**. Probiotic as defined by the Food and Agriculture Association of the United Nations (FAO) and the World Health Organization (WHO) are "live microorganisms (bacteria or yeasts), that provide health benefits to the host when administered in adequate amounts" **(WHO, 2001, p. 34).**

Lactic acid bacteria (LAB), is an important group of probiotics, well known by their health effects, the vast majority of marketed probiotics belong to the genera *Lactobacillus*. The beneficial effects of probiotics on the human gut flora include anti-microbial activity, prevention and treatment of diarrhea, relief of symptoms caused by lactose intolerance, anti-mutagenic and anti-carcinogenic activities, and immunostimulatory effect **(Bron et al., 2012; Amine et al., 2014).**

However, various factors might contribute to a reduction in probiotic viable cell counts such as gastrointestinal conditions, food matrices, food production, and storage conditions **(Silva et al., 2018)**. In order to overcome these challenges, microencapsulation of probiotics could protect probiotics against these harsh conditions. In addition, it also allows the controlled release of probiotics in the gastrointestinal tract **(Ozyurt & Otles, 2014; Shori, 2017).**

Probiotics are currently encapsulated in polymer matrices for various applications. It is crucial to choose the right encapsulation technique. Given that probiotics are living cells, the settings for implementing this technology are intended to keep them viable, and the solvents used in the encapsulating method must be non-toxic. **(Gbassi & Vandamme, 2012).**

Microencapsulation is a process by which individual particles/droplets of solid/liquid material (the core) are entrapped or coated with a continuous film of polymeric material (the shell) to produce microcapsules **(Paula et al., 2020).** Different biopolymers such as starch, cellulose, and whey protein have been previously used as encapsulating agents for microorganisms. However, since now, alginate (ALG) has remained the most commonly used hydrocolloid for microencapsulation due to its non-toxicity and low cost. As well for being biocompatible with other polymers, they are often combined with other materials to obtain the most stable structures. In addition, alginates are compatible to almost all encapsulation processes, moreover, the

alginates are the most used polymeric material in extrusion **(Seth et al., 2017; Arepally & Goswami, 2019; Shafzadeh et al., 2019)**.

Microencapsulation has been a prominent method for the protection of probiotics from harsh conditions, since it offers greater viability to the strains and boosts the efficiency of probiotic action. Furthermore, stabilizing probiotics with a carrier may boost their survival in goods through both processing and GIT transition, where they may withstand harsh gastric and intestinal conditions (such as acid, bile, and enzymes) and bind to the gut epithelium **(Naklong et al., 2023).**

Probiotics are often encapsulated with prebiotics and plant extracts, as prebiotics act as a nutrient for probiotics and give a synergistic therapeutic effect towards humans and so does plant extracts for their contain of fiber, vitamins and minerals. In fact, these two (Prebiotics and plant extracts) make a suitable material to promote bacterial growth **(Sathyabama & Vijayabharathi, 2014; Chan & Pui, 2020; Yong et al., 2020)**.

Via this work, we aim to study the effect of encapsulating *Lactobacillus brevis* KBM2 using alginate in addition to flaxseed and *Pulicaria dysenterica* extract on the cells viability during exposition to simulated gastrointestinal stresses and during cold storage.

Chapter I.

Literature Review

I.1. Lactic Acid Bacteria definition

Lactic acid bacteria (LAB) are a type of microorganism uses the metabolism of externally present carbohydrates to produce lactic acid **(Wang et al., 2021)**. They are abundant in nature and can be found in a wide range of settings. They are extremely biodiverse, strongly tied to human life and production, and have significant social and economic value **(Probst et al., 2013).**

Lactic acid bacteria are non-spore-forming, Gram-positive, catalase negative, anaerobic or aerotolerant bacteria that produce lactic acid as the major end-product of sugar fermentation **(George et al., 2018)**. They are usually rods or cocci that tolerate low pH well. Additionally, they are involved in food production and health industries. Although LAB includes more than 60 genera, in general, the most common genera in food fermentation include *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, Weissella* **(Mokoena., 2017)**.

I.1.2. Classification

LAB classification is largely based on morphology (cocci ,tetrads, rods), mode of glucose fermentation, growth at different temperatures (15 and 45 ^{o}C), configuration of lactic acid produced, ability to grow at high salt concentrations (6.5% NaCl; 18% NaCl) substrate spectrum, and acid, alkaline or ethanol tolerance, as well as fatty acid composition and cell wall composition, lactic acid isomers from glucose, behavior against oxygen (anaerobic or microaerophilic growth), bile tolerance, type of hemolysis, arginine hydrolysis, acetoin formation, production of extracellular polysaccharides, presence of certain enzymes, growth factor requirement, growth characteristics in milk, murein, teichoic acid, serological typing, menaquinone type, fatty acid composition, electrophoretic mobility of the lactate dehydrogenases, PCR-based fingerprinting techniques and DNA base composition (Specific amplified polymorphic DNA-PCR)

(Pfannebecker & Freohlich, 2008; Sebastian et al., 2011; Petri et al.,2013).

I.1.3. Taxonomy

LAB are members of the phylum Firmicutes, a kind of Gram-positive bacteria, and their DNA contains "low" (55 mol%) G+C. They are included in the Firmicutes' third class, known as Class III, or the Bacilli, together with the Clostridia (Class I) and Mollicutes (Class II).

The Firmicutes are distinguishable from the other Gram-positive phylum, the Actinobacteria, by having a high mol% G+C (55 mol%) in the DNA, according to comparative sequence analysis of the 16S rRNA gene **(Galperin., 2016)**.

I.1.4. LAB Metabolites

The production of one or more active metabolites such as organic acids (acetic, lactic, propionic formic, and butyric acids) by LABs is the reason for their preservative effect, that intensify their action by reducing the pH of the media, and other substances, such as fatty acids, ethanol, hydrogen , peroxide, antifungal, diacetyl, compounds (phenyl-lactate, , hydroxyphenyl-lactate, propionate, cyclic dipeptides and 3-hydroxy fatty acids), bacteriocins (reuterin, nisin, lacticin, pediocin, reutericyclin, enterocin and others) and bacteriocin-like inhibitory substances (BLIS), exopolysaccharides (EPS) during fermentation **(De Vuyst et al., 2007; Sobrino-Lopez et al., 2008; Deepak et al., 2015).** Certain LABs are enzyme producers for protease, amylase, glucoamylase and lipase during fermentation and which assist in simplifying complex inedible substrates, thereby facilitating increased nutrient absorption **(Farhad et al., 2015; Tamang et al., 2016; Ho et al., 2018)**.

I.1.5. Ecology

Although the total load and relative abundance of the LAB in microbial ecosystems are incredibly diverse and dependent on the particular environment, they can be found almost everywhere **(McAuliffe, 2018)**. They might be obtained from diverse sources such vegetables meat, fruits and dairy products. They can also be found in intestinal, mucous membranes of the respiratory, and other anatomical sites of man and animals, they are also found in plants, manure, soil, and wastewater **(Jager et al., 2018)**.

I.1.6. LAB as Probiotics

According to the **WHO. (2001)** which known to be the directing and coordinating authority on international health work within the United Nations system confirms that live microorganisms that, when given in sufficient proportions, provide a health benefit on the host are known as probiotics **(Hill et al., 2014)**. The majority of probiotics that have been researched thus far are LABs (order *Lactobacillales*), with some of the most promising probiotic strains being members

of the recently reclassified Lactobacillaceae family and they are GRAS (generally recognized as safe) **(Boeck et al., 2021)**.

The probiotic LAB could be present in the spontaneous fermentation of different food, they have become widespread in the manufacture of fermented dairy, meat and vegetables products **(Devlieghere et al., 2007, Lozo et al., 2007)**. Due to their characteristics, certain probiotic LAB present the ability to resist bile salts and acidic conditions, and additionally they produce bacteriocins that are active against food pathogens and spoilage microorganisms, and contribute to a probiotic culture that may have potential applications for improving the safety of food products **(Oh et al., 2000; Ahmed et al., 2010)**. Various studies have shown that LAB degrade mycotoxins, inhibit pathogenic microorganisms' growth and have a probiotic effect **(Landete., 2017)**. They possess various health benefits to the host, such as improving digestion **(Jager et al.,2018)**, enhancement of immune function, management of inflammatory bowel diseases **(Isolauri et al., 2001)**, alleviation of constipation **(Miller et al., 2017)**, and strengthening the mucosal barrier **(Alard et al., 2018)**. Some LAB isolates even hold antidiabetic or anticancer effects **(Lakritz et al., 2014; Niibo et al., 2019)**.

I.1.7. *lactobacillus brevis* generalities

Levilactobacillus brevis, often known as *lactobacillus brevis* it is a member of the lactic acid bacteria (LAB), which are cocci or rod-shaped Gram-positive bacteria, catalase-negative, non-motile and non-sporulating. *L. brevis* grows optimally within a pH range of 4 to 6 and in a temperature of at 30 °C (**Felis et al., 2007, Vos et al., 2011).** It is an obligatory hetero-fermentative bacterium producing carbon dioxide, lactic acid, acetic acid and/or ethanol **(Feyereisen et al., 2019)**. Duar et al assigned lactobacilli species into three main lifestyle categories: host adapted or as "nomadic" and free living (environmental and plant isolates), **(Duar et al., 2017).** *Lb. brevis* has been isolated from fermented cabbage as well as from silage and other fermented foods **(Makarovaet al., 2006, Fukao et al., 2013)**. *Lb. brevis* strains, consequently have been widely used in the production of fermented foods among other lactobacilli because they have been granted Qualified Presumption of Safety (QPS) status **(Feyereisen et al.,2019)**. In addition to their application in food fermentations they are purported to have potential as health-promoting or probiotic bacteria **(Vos et al., 2011, Salvetti et al., 2012)**.

I .2. Flaxseed

Flaxseed also known as linseed is the seed of the flax plant, which is an annual herb that grows to about 2 feet tall and belongs to the *Linaceae* family which is of Mesopotamic origin. The species name of flax, *ustitatissimum*, means "very useful" **(Sumara et al., 2022).** Flaxseed has been cultivated for 5,000 years; It's usually eaten in one of three ways: ground powder, oil, or whole seed. Flaxseed is relatively flat with a mostly oval shape and a pointed tip. Seed color varies from dark to yellow while seed texture is smooth and shiny with commercial seed measuring approximately 2.5 × 5.0 × 1.5 mm as presented in (Figure 1) **(Singh et al., 2011, Carraro et al., 2012)**.

Flaxseed is widely distributed in Mediterranean and temperate climate zone **(Czemplik et al., 2012)**. It grows all over the world particularly in Ethiopia, China, India, Canada and United States. But it was most probably first cultivated in Egypt. Commercially, it was used in the manufacture of papers as well as clothes such as linen until the 1990s, though flaxseed oil and its by-products were utilized in livestock feeds **(Singh et al., 2011)**. Flaxseed has been consumed by humans since the dawn of time and from a health point of view, Flaxseed is highly valued by the food and pharmaceutical industries for valuable nutrients such as flaxseed gum, protein, linolenic acid and lignin **(Bouaziz et al., 2016)**. Global flaxseed production estimated at over 1.2 million tons **(Drozłowska et al., 2020)** and is produced in large quantities worldwide, with great potential for high value-added phytochemicals and biopolymers **(Samborska et al., 2021)**. The average composition of flaxseed is 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash **(Shim et al., 2014)**. Flaxseed is used for their antioxidant properties which contained in their phenolic acids such as ferulic, p-coumaric and caffeic acid **(Waszkowiak, et al., 2015)**. Natural mucilages and gums derived from seeds are relatively high molecular weight hydrocolloids formed intracellularly or extracellularly as pathological or metabolite products. Mucilages and gums have shown various pharmaceutical uses such as viscosity enhancers, disintegrants, binders, emulsifiers and release modifiers **(Choudhary., 2014)**.

Figure I.1. Flaxseed (*Linum usitatissimum*).

I.2.1. Flaxseed Gum

Flaxseed gum (FG) is an important polysaccharide gum derived from the seeds hull of the flaxseed plant (*Linum usitatissimum*). It has shown promising effects as immunological characteristics resulting in antitumor, antiviral, anti-infective effects, antioxidant, antimutation, and hematopoietic activities **(Zhang et al., 2010)**. Moreover, the gum is nontoxic and do not cause considerable side effects. In addition, since polysaccharides can provide the prevention and treatment of numerous diseases allied to oxidative damages it can be applicable to improve the shelf life of food commodities **(Zhao et al., 2014)**. These natural polymers have advantages over synthetic polymers, including their environmental sustainability, low cost, biocompatibility, biodegradability, biosafety, as well as their versatile physical and chemical properties. This gum is mainly confined to the region of the outer layer of flaxseed shell (approx. 8% of the seed weight) and comprises about 50%-80% of carbohydrates and 4%-20% protein. Water-soluble flaxseed polysaccharides commonly referred to as flaxseed gum (FG) can be easily released upon soaking the seeds in water. FG is a heterogeneous polysaccharide that comprises of two polysaccharide constituents, a neutral arabinoxylan (75%) including l-arabinose, d-xylose, and d-galactose and the acidic rhamnogalacturonan (25%) containing l-rhamnose, l-fucose, l-galactose, and d-galacturonic acid **(Liu et al., 2016; Devi & Bhatia, 2019)**. FG has several applications in the food and by-product's preparation. Because of its excellent functional properties, FG can be used as an effective thickener, stabilizer, and emulsifier in the food industry **(Kaushik et al., 2017)**.

Another feature of FG is increases the resistance of multiphase systems to environmental stresses because of the formation of a multiform structure **(Khalloufi et al., 2009; Liu et al., 2018; Drozłowska et al., 2020)**.

I.3. *Pulicaria dysenterica*

Plants are considered as a repository of bioactive molecules, produced as secondary metabolites, known for being traditionally used for medical purposes since immemorial time. These bioactive compounds are often differentially distributed among groups of plants and only present in very low quantities in plants **(Zullaikah et al., 2018)**. Natural bioactive compounds from plants extracts, either as pure compounds or as standardized extracts, are of increasing interest for their versatile applications in pharmaceutical, nutraceutical and cosmetic industry **(Dhanani et al., 2017; Zengin et al., 2017)**. The genus Pulicaria is an herbaceous plant of the Asteraceae family and consists of approximately 100 species **(Salleh et al., 2021)**. *Pulicaria dysenterica (L.) Bernh*. syn. *Inula dysenterica L., Asteraceae*, is a perennial plant, up to 100 cm height with yellow flowers, growing on damp places. It is found in South, West and Central Europe, Iran, Iraq, Anatolia, Pakistan, Afghanistan and the Maghreb. The aerial parts of the *P. dysenterica* are used in the treatment of dysentery and diarrhea in Iranian traditional medicine. They are also used for the treatment of dysentery in the United Kingdom. The plant has an insecticidal property **(Kozarević et al., 2022)**. *Pulicaria* species are used in the treatment of several diseases such as cancers, fever, hypoglycemia, microbial, inflammation, and spasmodic diseases; Meanwhile, they have different biological properties such as cytotoxic, antibacterial, anti-inflammatory, antihistaminic, antifungal and insecticide **(Salleh et al., 2021)**

I.4. Encapsulation technology

I.4.1. Definition of encapsulation

Encapsulation is a physicochemical or mechanical process that involves enveloping a substance in a material to form/ or store individual particles of an active substance, with sizes ranging from a few nanometers to a few millimeters for protection and/or subsequent release **(Chavarri et al., 2012; Salaün, 2016)**. Encapsulation shell materials include a variety of polymers, carbohydrates, fats and waxes, depending of the core material to be protected **(Paula et al., 2022)**.

I.4.2. Materials for encapsulation

An important step in the microencapsulation process is the selection of the appropriate encapsulating materials. The polymers should be chemically compatible, non-reactive with the component to encapsulate and provide the desired coating properties such as strength, flexibility, impermeability and stability **(De prisco & Mauriello, 2016)**. Carrier materials should serve as protection for probiotics and also be safe for consumption or GRAS (Generally Recognized as Safe) and cost effective **(De Vos et al., 2010)**. Materials used in microencapsulation as film coating or matrix-forming components include several categories shown in (Table 01)

Table I 1: Class and different types of coating materials **(Mandal et al., 2013; Lakkis, 2016; Akinci & Holthaus, 2023)**

Class of coating material	Specific types of coatings
Gums	Arabic, xanthan, tragacanth, seed gum
Carbohydrates	Alginate, starches, maltodextrins, chitosan, sucrose, glucose, carrageenans.
Cellulose	Carboxymethyl(CMC) cellulose, ethylcellulose, acetylcellulose
Lipids	Wax,stearic acid,fats,beeswax
Proteins	Casein, gelatin, albumin, whey/soybean proteins
Food-grade polymers	polypropylene, polyvinyl acetate, polystyrene, polybutadiene

Researchers have employed a wide variety of polymers for encapsulation; thus, it is critical to choose the right polymer based on the goal of encapsulation. The most commonly used food-grade biopolymers include proteins (whey proteins and caseins and gelatin) and carbohydrates (alginate, starch, gums, chitosan, carrageenan and xanthan) **(Amira, 2020)**, following we cited the most used ones

I.4.2.1. Alginate

Alginate is a naturally derived extract from brown seaweed. The ability to form gels at ambient temperatures (i.e., without heating) makes alginate gels an attractive option for the microencapsulation of thermosensitive bioactives and viable cells **(Akinci & Holthaus, 2023)**.
It is mainly consisted of the sodium salt of alginic acid, or that is, a mixture of polyuronic acids composed of residues of D-mannuronic and L-guluronic acid.

As alginates are biocompatible with other polymers, they are often combined with other materials to obtain the most stable structures **(Pop et al, 2017)**.

I.4.2.2. Gum Arabic

Gum Arabic (also known as gum acacia) is the dried sap from two species of tree native to the Sahel region of Africa: *Acacia senegal* and *Acacia seyal*. For many decades, it has been used to spray dry microencapsulate various active substances and probiotics due to its branched structure, good water solubility, emulsifying properties, and low viscosity. However, to optimize the microencapsulation of probiotic bacteria, it must be stabilized by materials such as gelatin, creating a sufficiently hard coating **(Zhao et al., 2018; Sillick & Gregson, 2023)**.

I.4.2.3. Chitosan

Chitosan is a linear polysaccharide polycation composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylatedunit) and N-acetyl-D-glucosamine (acetylated unit). It is extracted by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, it can't be used alone **(Estevinho et al., 2013).** Although chitosan cannot be used alone for the microencapsulation of bacterial cells, it is a very good additive that can increase the survival of bacteria in the final product **(Kowalska et al., 2022)**.

I.4.2.4. Whey Proteins

Whey proteins were the first to be used in the microencapsulation of probiotics. Due to their chemical and physical properties, whey proteins isolate obtained as a by-product of cheese production, are readily used in the production of probiotic microcapsules **(Kowalska et al., 2022)**. In fact, these properties allow for intermolecular cross-linking with other polymers **(Carvalho et al., 2013)**. Whey proteins are often added to carbohydrate biopolymers to increase the stability of microcapsules **(Devi et al., 2017)**.

I.4.2.5. Soy proteins

Soy protein isolates are widely available, have proven health and nutritional benefits, and are characterized by low immunogenicity and similarity to components of extracellular matrix tissue **(Tansaz & Boccaccini, 2016).** Most often, probiotic bacterial cells are microencapsulated in soy protein concentrate, which is produced from high-quality, clean, and husk-free soybeans **(Nesterenko et al., 2013)**.

I.4.2.6. Carrageenans

Carrageenans, a type of polysaccharide derived from red seaweed and microalgae, they come in three different shapes: kappa, lambda, and iota, with kappa being the most common. Carrageenans cannot be employed separately in microcapsules, similar to chitosan **(Shi et al., 2013)**. It has been established that carrageenans, in the form of gels, beads and films, can efficiently encapsulate flavors, fragrances, probiotics, and enzymes **(Chakraborty, 2017)**.

I.4.2.7. Ethylcellulose

Ethylcellulose is a linear polysaccharide formed when the hydroxyl groups of cellulose are replaced with ethyl groups. Its unique properties allow for the effective microencapsulation of probiotic bacteria. The most important of these properties are water insolubility; hydrophobicity; physiological indifference; lack of odor; lack of taste; low number of calories; stability during storage **(Niu et al., 2020).**

I.4.2.8 Gelatin

Gelatin is frequently used in the food and pharmaceutical industries. It is a protein derived by partial hydrolysis of collagen of animal origin. Gelatin has a very special structure and versatile functional properties, and forms a solution of high viscosity in water, which sets to a gel during cooling. It does not form beads but could still be considered as material for microencapsulation **(Rokka & Rantamäki, 2010).**

I.5. Methods of microencapsulation

Numerous encapsulation techniques have been developed and the most reported are:

I.5.1. Extrusion

Widely used to encapsulate bacterial cells, the extrusion technique is simple, easy to use and presents a low cost, besides being a relatively mild process, which ensures high viability of the encapsulated cells. Essentially, this approach includes the use of hydrocolloid solutions containing microbial cultures (probiotics). The resulting mixture is fed into an extruder, typically a syringe (a nozzle). Pressure exerted on the syringe plunger drops its contents into a gelling solution, with gentle stirring.

The size and shape of the drops depend on the diameter of the needle, and the distance between the needle and the gelling solution **(Gbassi et al., 2012; Etchepare et al., 2016; Rodrigues et al., 2017)**.

I.5.2. Spray-drying

It is the traditional first technological approach and is most used where a powdered form of flavor or ingredient is desired **(Gharsallaouiet al., 2007**). A heated stream is sprayed into contact with a solution that has the proper viscosity or consistency, quickly creating powdered microcapsules **(Gopi &Balakrishnan, 2022; Meyers et al., 2023;)**. To encapsulate bacterial cells by the spray drying technique, various natural polymers can be used, especially gum Arabic and starches, due to their recognized ability to form spherical particles after the drying process. However, other materials, such as inulin, alginates, gums and mucilages have also been used **(Bustamante et al., 2017; Hadzieva et al., 2017; Sarao & Arora, 2017)**.

I.5.3. Emulsion

An emulsion is the dispersion of two immiscible liquids with a stabilizing agent, which usually presents a greater affinity to the continuous phase than to the dispersed phase **(Alemzadeh et al., 2020)**. In addition, a solidifying agent can be used to separate the dispersed phase droplets from the continuous phase **(Zhang et al., 2016**). If the dispersed phase is aqueous, the emulsion is named a water-in-oil (W/O) emulsion, whereas the opposite is known as oil-in-water (O/W) emulsion or reverse phase **(Goibier et al., 2020)**.

I.5.4. Freeze Drying

The fundamental idea behind freeze-drying is the sublimation of water that is already present in the material. When compared to other methods, the freeze-drying method for microencapsulating probiotics stands out because it boosts the survival rate of probiotic cells. Additionally, items that have been freeze-dried may be readily reconstituted and have a long shelf life **(Joye & McClements, 2014; Li et al., 2019)**.

I.5.5. Coacervation

Coacervation is a straightforward procedure that entails forming a homogenous layer of the polymeric wall material around the core material and it is accomplished by modifying the

physicochemical characteristics of the wall material through temperature, pH, or ionic strength changes**. (Choudhury et al., 2021)**.

I.5.6. Fluidized bed

The fluidized bed technique is used for coating, granulation or drying, in which a coating is atomized over solid particles in suspension. This technique is a fast process, which presents a low cost and high production. Moreover, several encapsulating materials, such as lipids, proteins and polysaccharides can be used **(Rodrigues et al., 2020)**. Here, the core material is fluidized by application of air, onto which a coating material is sprayed**. Figure I.2** shows diagrammatic representation of different microencapsulation techniques

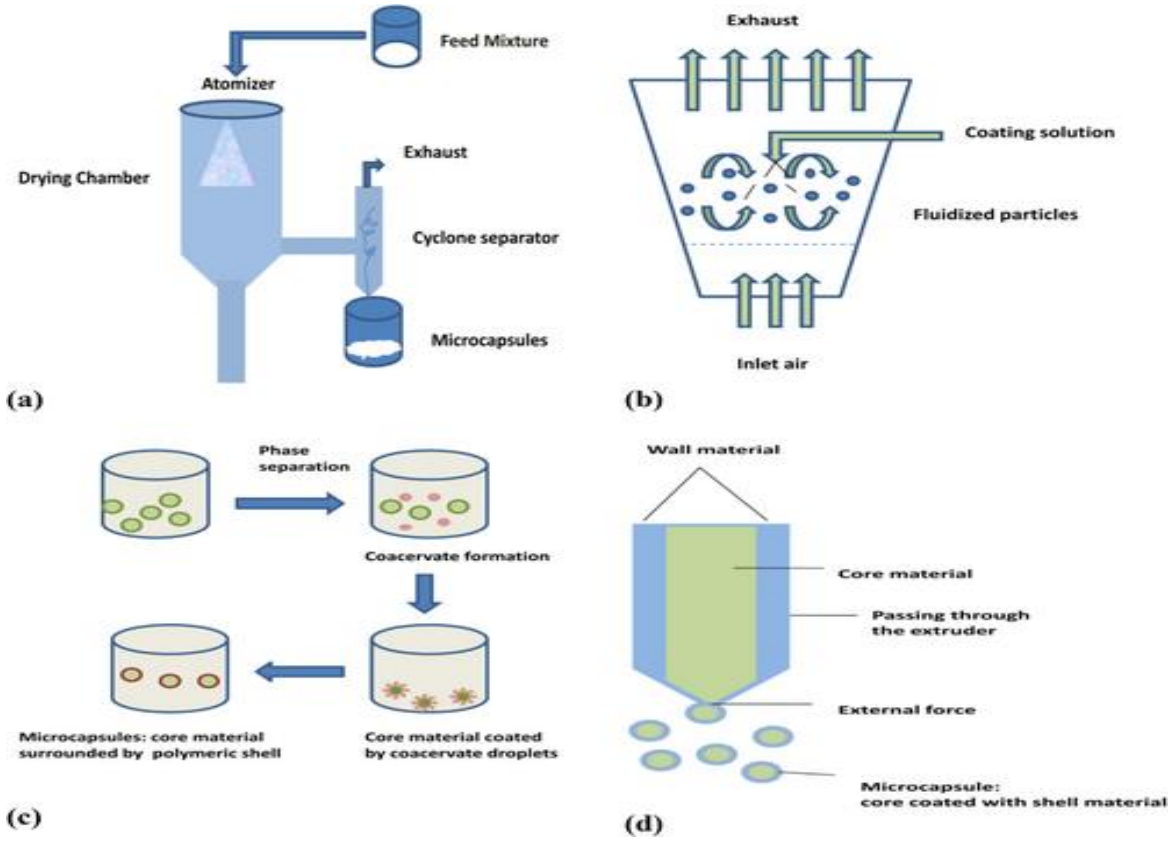

Figure I. 2. Different techniques of microencapsulation: (a) spray drying; (b) fluidized bed coating; (c) coacervation; (d) extrusion **(Choudhury et al., 2021).**

Chapter II.
Materials & Methods

This work was achieved in the Laboratory of Microbiology, Department of Applied Microbiology and Food Sciences in Mohammed Seddik BenYahia University (Jijel) during May and June 2023.

II.1. Material

II.1.1. Biological Material: The biological material used to perform this work is as follow:

- ✓ **Bacterial strain studied**: During this study, we used a strain of lactic acid bacteria previously isolated and identified by Dr. Samiya AMIRA (Department of Applied Microbiology and Food Sciences, Mohammed Seddik Ben Yahia University/Jijel) as *Lb. brevis* KBM2. The strain origin is" Klila"; an Algerian traditional cheese. The purity of the strain was confirmed by streaking on MRS agar plates and Gram stain.
- ✓ **Bioactive compounds**: flaxseed was purchased from a local grocer in the Wilaya of Jijel for the extraction of gum and preparation of an extract out of these seeds.

  Pulicaria dysenterica extract was previously extracted from the aerial part of the plant which was provided by Dr. Asma Cherbal (Department of Department of Cellular and Molecular Biology, Mohammed Seddik Ben Yahia University/Jijel).

II.1. 2. Chemicals and reagents (see appendices): For the realization of our work, we used the following:

- ✓ **Acids and alcohol**: HCl (0.1M), Ethanol (95%).
- ✓ **Buffers**: (Phosphate Buffer Saline) pH 7,4.
- ✓ **Culture media**: A single culture medium was used MRS (de Man-Rogosa-Sharp provided by the university laboratory) with the two forms, broth and agar.
- ✓ **Polymers**: Sodium alginate polymer (2%), flaxseed gel, and flaxseed extract (mucilage).

II.1.3. Equipment and devices: The performance of the work required the following:

- ✓ Autoclave (PbiBrand)
- ✓ Bunzen burner
- ✓ Colony counter (Funke gerber)
- ✓ Incubator (Memmert)
- ✓ Magnetic stirrer
- ✓ Microtube centrifuge (Sigma Laborzentrifugen l-14K)

- ✓ Optical microscope (Paralux)
- ✓ Oven (Memmert)
- ✓ pH meter with digital display (Hanna instrument)
- ✓ Precision balance (Denvar)
- ✓ Refrigerator (ENIEM)
- ✓ Sterile syringe filter (0.2µm,VWR)
- ✓ Vortex (VWR, VV3 S40)
- ✓ Water bath (Memmert)

II.2. Methods

II.2.1. Flaxseed mucilage extraction (polysaccharide)

Flaxseed mucilage (FM) was extracted based on the method reported by **Elboutachfaiti et al. (2019);** 10 grams of flaxseeds were put in 250 grams of distilled water with agitation over an increased temperature up to 90°Cfor two consecutive hours.

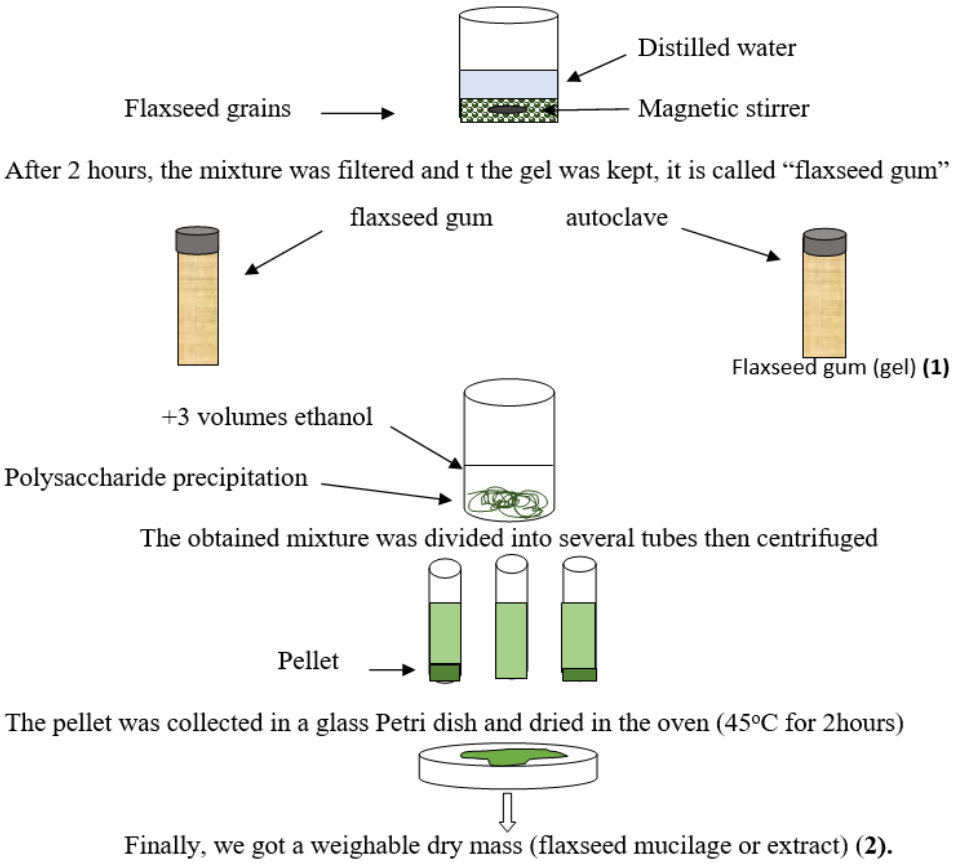

Figure II 4. Steps of flaxseed mucilage preparation.

The yield (%) of linseed polysaccharide was calculated by the method below according to **Mohanta et al. (2023).**

$$\text{Yield (\%)}: \frac{Mass\ (g) of\ dried\ linseed\ polysacharide\ (extracted)}{MMass\ (g) of\ linseed\ poder} \times 100$$

II.2.2. Bacterial preparation

Two successive overnight culture of the bacterium (2%) was used to prepare bacterial suspension for encapsulation. After centrifugation at 6000rpm and washing, the obtained bacterial suspension containing around 10 Log cells was used for encapsulation.

II.2.3. Encapsulation of bacterial strain in different matrices

The extrusion method reported by **De prisco et al. (2015)** was used with slight modifications in the encapsulation of *Lb*. *brevis* KBM2 strain. The encapsulation was performed using the prepared sterile solutions of sodium alginate, sodium alginate (2%)+ FS gum (1%), sodium alginate (2%)+ FS mucilage (1%) , sodium alginate (2%)+ plant extract (1%). In a 50ml flask, the previously obtained cell suspension was aseptically encapsulated by mixing it until homogeneity with polymer solution. These mixtures were introduced separately into a sterile syringe (2,5) and dropped in a sterile and cooled (4°C) 0.1M calcium chloride solution. The resulting beads were left subsequently rotated from 30 to 45min to harden, then they were filtered and conserved in sterile normal saline at 4°C for future use.

II.2.4. Encapsulation efficiency (EE%)

To enumerate the entrapped bacteria, 1 g of the encapsulated beads was added to 9 ml of sterile PBS solution (pH 7.4) and vortexed till dissolving. During this process, the alginate beads were destroyed and the bacterial cells were released. After this step, different dilutions were provided to attain the number of countable cells by pour plate method using standard plate counts on MRS agar. The plates were incubated for 48h at 37 °C **(Shafizadeh et al., 2020)**. The encapsulation

efficiency (EE) (%) of *Lb. brevis* was calculated using Eq. 1 and expressed as the percent of the log CFU per ml.

EE (%) = (log10 (N1)/log10 (N0)) × 100…… (1)

In this equation, N0 was the number of bacterial cells caught inside the capsules, and N1 was the number of free cells added to the hydrocolloids during encapsulation **(Darjani et al., 2016)**.

II.2.5. Evaluation of cell viability during storage for different periods

The viability of microencapsulated bacteria was also investigated throughout various storage times. The procedure was performed using the method described by **Deprisco et al. (2015)**. Bacterial cells were stored in in (NS). Microcapsules were stored for 4 weeks at 4°C in NS and viable counting was performed on MRS agar at 0, 1, 2, 3 and 4 weeks, free cells were managed in the same way and used as control sample. The survival of the probiotics was then determined using the plate count method with two repetitions **(Wang et al., 2019)**.

II.2.6. Simulated gastrointestinal (GI) conditions

The survival of free (10 Log/CFU) and (1g) of encapsulated probiotic bacteria was evaluated in simulated intestinal solution after incubation in simulated gastric solution. Both simulated solutions used in the current study were provided according to the method reported by **Darjani et al. (2016)**. The pH was adjusted to 2 for gastric solution and to 7.5 for the intestinal one. One g of the encapsulated beads was mixed with 9 ml of simulated gastric solution contained pepsin and incubated for 90 min at 37 °C rotator shaker. Then, the beads were incubated in 9 ml of sterile simulated intestinal solution with bile salts and pancreatin. The beads were also incubated at 37 °C in the incubator shaker for 180 min **(Darjani et al., 2016)**.

The viability was calculated according to the following equation with two repetitions (**2)**

Viability (survival) = Log CFUt/Log CFUi ×100 ……. (2)

CFUt: Colony Forming Unit after the given period of storage or incubation.

CFUi: Colony Forming Unit at t=0h.

Chapter III.
Results & Discussion

III. 1. *Lb. brevis* KBM2 revivification

A conserved copy of *Lb. brevi*s KBM2 bacterium was revivified in MRS media; the growth was firstly verified in the MRS broth medium, after that the purity was checked on MRS agar and by Gram stain. In fact, the macroscopic aspect after streaking on the Petri dish showed colonies with the same aspect, color, shape and size, they are white with creamy.

As for the microscopic aspect of the bacterium, all colonies are rods, purple colored, they are gram positive and the culture is pure.

III.2. Yield of extraction

According to **Aspé** and **Fernandez. (2011)**, the extraction yield is the measure of the solvent efficiency to extract specific components from the original material and it can be expressed as a percentage of the material's mass or dry weight. In our study, the yield was 5% which is similar to the result obtained by **Paynel et al. (2013)** 5.3% and near to the value reported by **Shafizadeh et al. (2020)** 7.6%. Certain parameters such as pH of the extraction medium, the solvent of extraction, and the extraction temperature were the major factors affecting the extraction yield of flaxseed gum. The optimal extraction conditions were a water-to-seed ratio of 1:25, and a temperature of 80-90°C according to **Kaushik et al. (2017)**; moreover, according to **Puligundla & Lim. (2022)** high-quality macromolecules were obtained via the extraction of flaxseed in neutral to basic medium (7.5-9.69).

III.3. Microcapsules characteristic

As mentioned in the part of materials and methods, sodium alginate was used separately, combined to flaxseed extract or to flaxseed gel or combined to *Pulicaria* extract. By consequence 4 types of beads were obtained (figure III.2. (a, b, c, d)), and they differed by their color, shape and their size.

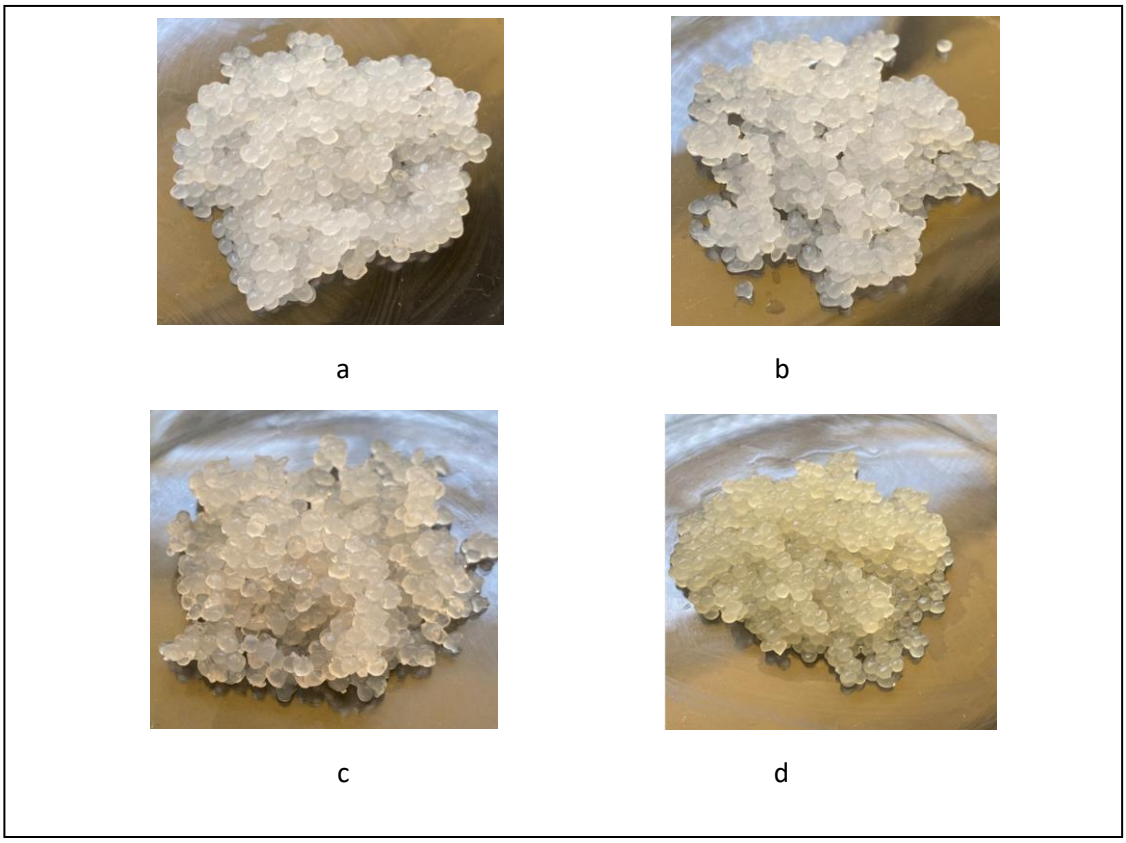

Figure III.2. Characteristics of different beads (a: beads of sodium alginate,b: beads of alginate-flaxseed extract, c:beads of flaxseed gum,d: beads of alginate-*Pulicaria* extract).

As shown in the figure above, all beads were smooth and exhibited a spherical and regular shape, however ALG+PE, ALG+FE and ALG+FG beads were slightly elliptic in shape and bigger. Beads color was dependent to the color of the mixture of the polymers used; ALG beads were white and opaque; FE beads were also white but transparent, FG beads were light brown whereas PE beads color was a light green according to the amount of the polymer or extract present in the mixtures, this is convinced by **Osorio et al. (2011)** and **Reyes et al. (2023)** publications where they mentioned that the nature and concentration of the materials played an important role in the color of the microcapsules.

The diameters of the beads were measured using a calliper, and the average weight of four (4) beads of each polymer was obtained using a calibrated micrometer scale; the results are displayed in **Table III.1**

Table III.1. Microcapsules diameters and weight

Parameters	PE	FE	FG	ALG
Diameter (mm)	2.17	2.6	2.46	2.24
Weight (mg)	54.9	103.2	45.1	49.55

ALG-FE beads followed by ALG-FG beads had slightly larger diameters of 2.6mm and 2.46mm in order, ALG beads 2.24mm that was similar to **Dallabona et al. (2020)** with a diameter ranging from 2.0–2.5 mm and 2.17mm for PE beads. However, the *Lb.brevis* KBM2 microbeads in this study were bigger in sizes as compared to the encapsulated *L. plantarum* ATCC 8014 enriched with inulin (1.18–1.19 mm) as reported by **Pop et al. (2016)**. As for the weight, there were differences in distribution among the four polymer materials. The highest value 103mg was registered for FE beads that exhibited a broader range of particle size distributions in comparison to ALG+PE 54.9mg followed by ALG 49.55mg and the lowest value was registered for ALG+FG = 45.1 mg.

The droplet size and hence, the final beads size and shape, are influenced by the needle tip diameter of the dripping system, viscosity, the distance between the origin of extruding needle and the gelation bath, flow rate of the feed material, the applied polymer, concentration and homogeneity of the gel and the period of time waited for the beads to harden under rotation **(Dallabona et al., 2020; Rodrigues et al., 2020).**

III.4. Encapsulation efficiency (EE%)

The yield of encapsulated probiotics was calculated according to equation (2) in materials and methods and the results are shown in **figure III.3.** Alginate had an EE% of 74% that is lower in comparison to the results obtained by **Darjani et al. (2016)** and other studies that had an alginate (2%) EE% of 89%. However, the EE% was improved by 18% by incorporating the PE extract 1% (w/v) to equal 92%.

EE% of (70%) was registered for ALG-FE 1% (w/v) beads and the lowest percentage of 58% was registered for ALG-FG 1% (w/v). These results implies that the rest of the non-encapsulated probiotic were either inactivated (not encapsulated) or lost into the collector solution during the extrusion process; whereas, incorporating ALG with PE improved the EE of the cells in comparison to ALG+FG which had a negative effect on the EE. According to **Krasaekoopt & Watcharapoka. (2014)** and **Shafizadeh et al. (2020)** a further increase in the flaxseed mucilage concentration (0.8% w/v) did not improve the encapsulation efficiency and the retention *of L. rhamnosus* GG cells was hindered at high concentrations of mucilage due to the increase in the viscosity, therefore, lowering the probiotics microencapsulation efficiency.

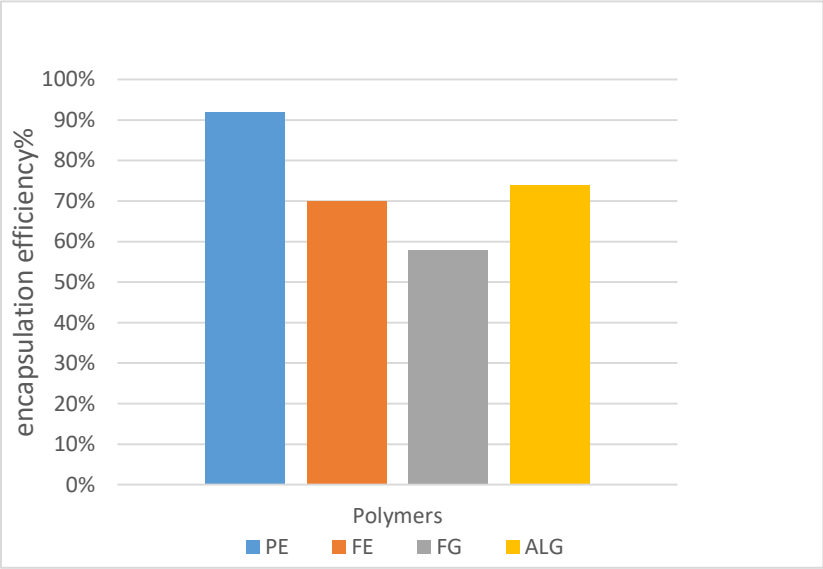

Figure III.3. Encapsulation efficiency of *Lb.brevis* KMB2 with alginate and alginate +*Pulicaria* extract(PE), alginate+flaxseed extract(FE), alginate+ flaxseed gum (FG).

III.5. Viability in simulated gastro-intestinal solution

To employ probiotics as functional components in food items and get all of their health benefits, they must survive passage through the gastrointestinal tract **(Darjani et al., 2016)**. The viability of free, encapsulated and co-encapsulated *Lb.brevis* KBM2 with ALG and with ALG-PE/ALG-FE/ ALG-FG incorporation have been investigated after a 90 min incubation time in simulated gastric solution, followed by 180 min incubation time in simulated intestinal solution. The results are presented in the **figure III.4.**

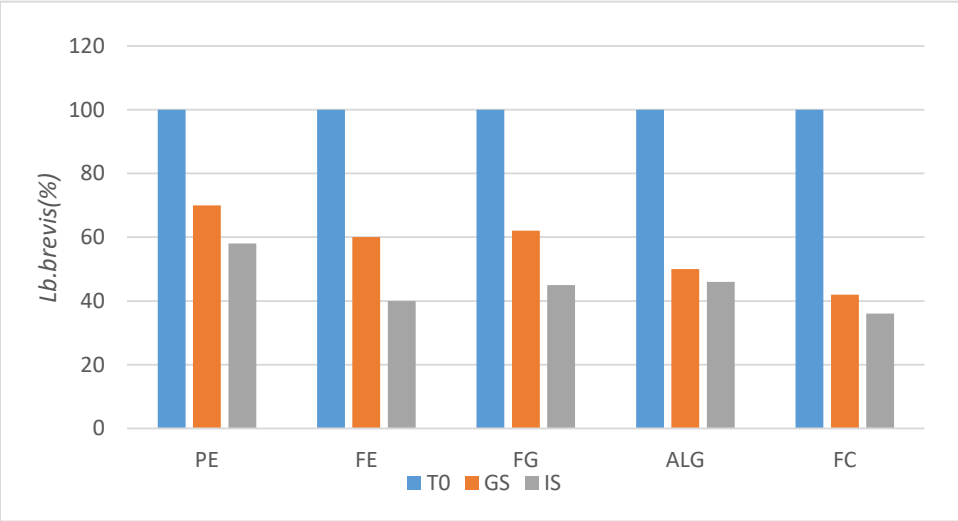

Figure III.4. Viability of free and encapsulated cells of *Lb.brevis* KBM2 subjected to SGS (1.5h, pH 2.5) and SIS (3h, pH 7.5).

According to the results in **Figure III.4**; the viable count of cells showed that free cells lost 58% of their initial count after 90min incubation in SGS and decreased again after exposure in SIS for 180min to reach 36% of lost. As observed, the microencapsulated cells in alginate alone had a 10% increase of the survival rate after incubation in SGS and SIS was 51% and 46% in order. The best survival rate percentages to the exposure to SGS belongs to the co-encapsulated cells, where ALG-PE had a percentage of 71, followed by close viability rates for ALG+FG and ALG+FE of 63% and 61% in order. On the other hand, the viability of co-encapsulated cells that survived the SGS showed a remarkable decrease after a 3h incubation in SIS. Where PE co-encapsulated cells had the best survival percentage of 58% in comparison to the FE and FG co-encapsulated cells (41% and 45%) which was approximately equal to that of the ALG encapsulated cells.

As for the encapsulated cells, the viability enhanced considerably using different polymers. In all cases, the survival of microencapsulated cells under simulated gastrointestinal conditions was better than that of the free cells showing the protection provided by these polymers with good resistance and tolerance to low pH level and bile salts **(Wang et al., 2019).**

The reduction in the viable numbers of both free and encapsulated probiotic strains on exposure to bile salt solution for the mentioned time was probably due to the breakdown of cell wall **(Shi et al., 2013).**

Our results are close to those by **Amira et al. (2020)** realized on free and encapsulated *Lb. casei* in sodium alginate 2% and showed that the encapsulated *Lb. casei* cells showed high survival rates in the acidic environment (pH 2,0). These results are also in accordance with **Ouled-Haddar et al. (2016)** results that indicated that the microencapsulation using sodium alginate (2%) protected efficiently the probiotic cells in conditions similar to those of GIT, since an improvement of about 19% was recorded after 4h and 30min of exposure to simulated GIC. Similar to our results, **Shafizadeh et al., (2020)** reported that the microencapsulation of *Lb.casei* with a combination of ALG (2%) and FM (0.9%) provides a relatively good protection of probiotic bacteria against gastrointestinal simulated conditions and enhances their viability. As reported by **Peredo et al., (2016)** due to the electrostatic interactions that occur between ALG and FM, leading to the formation of a strong wall on the surface of the microcapsules protecting them against harmful conditions, the incorporation of FM into the structure of ALG-based capsules can improve the stability of encapsulated bacteria during passing the gastrointestinal tract.

Based on the research, flaxseed mucilage showed high prebiotic potential as probiotics could utilize it for growth **(Lai et al., 2020)**. Mucoadhesive polymers have been discovered to aid probiotic adherence in the GIT, making them suitable as encapsulating polymers for probiotic delivery methods. **(Salachna, Pietrak & Łopusiewicz,. 2021)**

In addition, according to **Bustamante et al. (2015)** the encapsulated LAB with FM showed a decreased in relative viability which was almost constant in the last 3 h of the test. The LAB encapsulated with FM and flaxseed polysaccharides reached a relative viability of 0.47 after 6 h of incubation. The results obtained in other studies have shown mixed results, depending on the methodology, composition of reagents and strains evaluated.

To the best of our knowledge, no work has been found concerning the effect of co-encapsulation of LABs with *Pulicaria* extract, the viability values obtained with the mixture of PE was better

than with alginate alone, which suggests that PE may even have stimulated the growth of *Lb.brevis* KBM2 and it has proved to be biocompatible with ALG and its application in microencapsulation. However, a few studies exist on the encapsulation of LABs with other plant extracts.

An example study, **Shehata et al. (2019)'s** that consists on studying the survival in SGS conditions of *Lb. plantarum* DSM 20205 and *P.acidilactici* DSM 20238 control encapsulated probiotic cells (CC) that contains alginate only and novel encapsulated probiotic cells (NC) with herbal extracts such as fennel (*Foeniculum vulgare*), moringa (*Moringa oleifera*), sage (*Salvia officinalis*) and green tea (*Camellia sinensis L.*), their results showed significantly better survivability and enhancement in viability of probiotics with (NC) than was seen with (CC),

Neuenfeldt et al. (2022) experience of co-encapsulating a *Lb. rhamnosus* bacterium with blueberry extract showed that the addition of the extract improves the viability of the microcapsules and provides much more protection when passing into the gastric fluid than the free bacteria encapsulated alone. In this case, co-encapsulation of blueberry extract has provided positive effects on the survival *of Lb. rhamnosus* and demonstrates its promising application in food.

Moreover, **Rodrigues et al. (2022)** in their study to use mucilaginous solutions obtained from tamarind, mutamba, *cassia tora*, psyllium and konjac powdered to encapsulate *Limosilactobacillus reuteri* in alginate beads by extrusion technique evidence that the use of gums and mucilages combined with alginate improved the survival of probiotic *L.reuteri* after exposure to gastric and enteric environments.

Gebara et al. (2013) and **Raddatz et al. (2020)** studies have reported higher microencapsulated probiotic microorganism viability compared to free cells, especially in conditions simulating the gastrointestinal tract. Therefore, Greater viability and bioavailability can be attained by combining various active compounds as compared to solo encapsulated components **(Raddatz & Menezes, 2021).**

Therefore, our results strongly support the improvement of the survival of probiotic bacteria in the human digestive system by co-encapsulating probiotic bacteria with flaxseed mucilage or plant extract.

III.6. Effect of storage on the survival of free and encapsulated cells

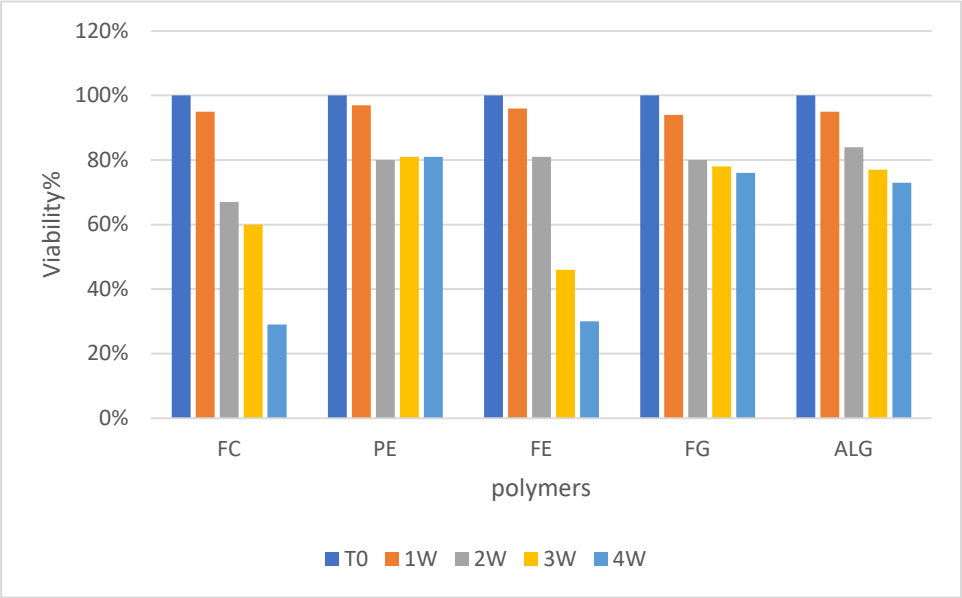

Figure III.5. Viability of *Lb.brevis* KBM2 during Storage.

As shown in **figure III. 5**, the encapsulated *Lb.brevis* KBM2 had a higher viability percentages than the free cells within 4 weeks of storage in 9% NS at a refrigeration temperature of 4°C. However, the decrease in viability is clear with increasing storage period. *Lb.brevis* KBM2 free cells viability was reduced to reach 29% after 28 days of storage at 4°C. Whereas, the highest survival percentage was registered with ALG-PE microcapsules (1W: 97% to 82% after 4 weeks) that remained higher than 80% Additionally, ALG-FG and ALG showed close results (76%, 73% on week4) respectively, The least promising result was registered for flaxseed extract encapsulated cells that decreased on week 3 to reach 46% and 29% by the end of the experience. According to **Cruz et al. (2015)**. The decrease in survival and resistance seen during storage might be related to a decrease in water activity and an increase in osmolarity **(Jorgensen et al., 1994)**.

Regardless, the sodium salt of alginic acid, sodium alginate, is typically used for microencapsulation, during which free calcium ions induce ionotropic gelation to form cross-linked calcium alginate, it is favored given that it contains more G-block, which provides mechanical stability, high porosity, and cell tolerance **(Seth et al., 2017; Reineccius et al., 2023).** Defects can induce oxidative or hydrolytic breakdown over extended periods of time **(Vasisht, 2014).**

Phytochemical studies done by **De la Luz et al. (2019)** concluded that the *P. dysenterica* extract has high levels of total phenolic, flavonoid, phenolic acid and flavonol content, in addition to considerable important biologically active compounds that could justify its remarkable antioxidant activity that is in accordance with **Qin et al. (2020)** results for free radical scavenging, which is the capacity of antioxidants to promote bacterial growth by eliminating free radicals. Consequently, our results support the improve of probiotic stability by encapsulation with the PE.

For the effect of the storage on the beads that were affected in normal saline shows that water can diffuse through the polymeric matrix altering the volume of the hydrogel leading to bead contraction or expansion. Since water generally moves between gaps in the polymer network, factors altering the space between polymer chains, such as hydrogel charge, composition and density, are expected to have an impact on the water diffusion coefficient **(Caccavo et al., 2018).**

Conclusion

Microencapsulation of probiotics is important to ensure that the cells are protected under harsh environmental conditions. Our work interests to demonstrate the effect of extrusion method on probiotic viability during storage under the temperature of 4°C in 0.9% normal saline, as well as for their gastro-intestinal resistance. The strain used was *Lb. brevis* KBM2, which was encapsulated in different matrices: alginate, flaxseed extract, flaxseed gum and *Pulicaria dysenterica* extract. The results illustrated that:

Encapsulation of *Lb. brevis* KBM2 with a combination of ALG (2%) and PE provides the best protection of probiotic bacteria against gastrointestinal simulated conditions and enhances their viability. It also, showed the highest cells number during storage.

Pulicaria dysenterica and flaxseed, due to their nutritive components, can promote the growth of *Lb. brevis* KBM2. Using these extracts and mucilage in combination with ALG as a wall material for microencapsulating this bacterium improves their resistance against simulated gastrointestinal conditions.

Therefore, the applied approach in this experiment might prove beneficial for the delivery of probiotic cultures to the simulated human gastro-intestinal tract. Of the four types of microcapsules in this research, PE coating provided the best protection of cells and there was low survival of free *Lb.brevis* KBM2 in the presence of gastric solution due to its low acid resistance.

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Appendices

1- **MRS agar :** 100ml of MRS broth was added to 12.4 g of agar.

2- **Normal saline 0.9%:** prepared by dissolving 9g of NaCl in 1000ml of distilled water.

3- **Sodium Alginate 2%:** 2 grams of Alginate were dissolved in 100ml of distilled water.

4- **Buffer solution:** It was prepared using two separate solutions for (700ml):

4-1.solution A (NaH_2PO_4) : 13.8g of NaH_2PO_4 has been dissolved in 200ml of distilled water.

4-2.solution B (Na_2HPO_4) : 63.6g of Na_2HPO_4 has been dissolved in 200ml of distilled water.(pH was adjusted to 7,4)

5- **$CaCl_2$ solution**: This solution was used as a hardening solution for beads. It was prepared at concentration of 0.1M and sterilized and kept cold until use.

6- **Gastric solution**

Here, four different components were mixed (5g of Na Cl_2, 0,22g of Ca Cl_2,2,2g of K Cl_2 and 1,2g of $NaHCO_3$) and dissolved in distilled water to obtain a volume of 100ml; the pH was adjusted to 2 and the sterilization of the solution was completed via autoclaving.

7- **Intestinal solution**

Three different components were mixed: (0.12g of NaCl, 0,023g of KCl and 0,64g of ($NaHCO_3$) which were dissolved in distilled water and the volume was completed to 100ml; the pH was adjusted to 7.5 and the solution was autoclaved.

Realized by:	**Examiners:**
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Co-encapsulation of *Lb.brevis* KBM2 with flaxseed and *Pulicaria dysentirica* extract

Abstract

The aim beyond this work is the study of the effect of the co-encapsulation of *Lactobacillus brevis* KBM2 with sodium alginate with multiple polymers such as flaxseed gum/extract and *Pulicaria dysentrica* extract. The encapsulation was performed by the extrusion method; and the effectiveness was evaluated via several steps. The results showed that the co-encapsulation with Alginate and *Pulicaria* extract gave remarkable and promising numbers compared to the other polymers. This study confirms the effectiveness of bacterial encapsulation in 2% sodium alginate- flaxseed and *Pulicaria* extract.

Key words: Co-encapsulation**,** Encapsulation, *Lactobacillus brevis* KBM2, flaxseed, *Pulicaria dysentirica,* gastrointestinal conditions.

Résumé

L'objectif au-delà de ce travail est l'étude de l'efficacité de la co-encapsulation de *Lb. brevis* KBM2 avec de l'alginate de sodium avec de multiples polymères tels que la graine de lin gomme/extrait et l'extrait de *Pulicaria dysentirica*. L'encapsulation a été réalisée par la méthode d'extrusion ; et l'efficacité a été évaluée en plusieurs étapes. Les résultats ont montré que la co-encapsulation avec l'alginate et l'extrait de *Pulicaria dysentirica* donnait des résultats remarquables et prometteurs par rapport aux autres polymères. Cette étude confirme l'efficacité de l'encapsulation bactérienne dans 2% d'alginate de sodium-extrait de graines de lin et de *Pulicaria dysentirica*.

Mots clés : Co-encapsulation**,** Encapsulation, *Lactobacillus brevis* KBM2, grin de lin, *Pulicaria dysentirica,* conditions gastrointestinales, storage.

ملخص

الهدف من هذا العمل هو دراسة فعالية الكبسلة للبكتيريا اللبنية *Lactobacillus brevis* KBM2 ، باستخدام ألجينات الصوديوم وعدة مركبات عضوية هي مستخلص نبتة رعراع أيوب وبذور الكتان تم تنفيذ عملية الكبسلة باستخدام طريقة البثق، حيث تم تقييم فعاليتها على عدة مستويات. تظهر النتائج أن عملية الكبسلة مع ألجينات الصوديوم ومستخلص رعراع أيوب نتج عنه نتائج ملحوظة وواعدة مقارنة بمركبات العضوية الأخرى. تؤكد هذه الدراسة فعالية الكبسلة البكتيرية في 2% من ألجينات الصوديوم المستخلص من بذور الكتان ورعراع أيوب.

الكلمات المفتاحية: الكبسلة, الكبسلة المشتركة, البكتيريا اللبنية, بذورالكتان, مستخلص رعراع أيوب, ظروف الجهاز الهضمي.