UFT Academic Publishing House, Plovdiv

FSAB Food Science and Applied Biotechnology

Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2020.xx.xx

Review

Biopreservation of emulsified food and cosmetic products by synergistic action of probiotics and plant extracts: a Franco-Bulgarian perspective

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Abstract

Simultaneous addition of probiotics and plant extracts in emulsified food or cosmetic products, such as dressings or lotions might not only be convenient to deliver probiotics and plant extracts with interesting biological (e. g. anti-inflammatory) properties but also for their preservation. Franco-Bulgarian ESCAPE project ambition is to identify synergistic combinations of probiotic bioprotective lactic acid bacteria (LAB) and antimicrobial plant extracts for the preservation of food or cosmetic emulsions. In line with this objective, the present review will successively present (i) examples of factors acting synergistically with plant or microbial origin antimicrobial metabolites consistently with hurdle technology principles and the states of the art regarding (ii) antimicrobial plant extracts or (iii) LAB for emulsified products preservation, (iv) the selection of probiotic LAB and plant extracts associations, and (v) the distribution of LAB and antimicrobial metabolites in the different phases of emulsions. Indeed, the respective distributions of LAB, antimicrobial metabolites and unwanted microorganisms in such biphasic systems have been identified as a key factor for the efficiency of antimicrobial metabolites or LAB against unwanted microorganisms. Finally, the respective effects of plant extracts (vi) and LAB (vii) on the stability of emulsions are reviewed before concluding on the promises of the proposed approach.

Keywords: cosmetic and food emulsions, biopreservation, antimicrobial plant extracts, lactic acid bacteria, distribution of antimicrobial metabolites in emulsions, emulsions stability

Abbreviations:

LAB - Lactic Acid Bacteria; ESCAPE - Biopr**E**servation by **S**ynergisti**C** **A**ction of **P**robiotics and plant **E**xtracts

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Article history:
Received 23 September 2019
Reviewed 4 November 2019
Accepted 13 December 2019
Available on-line 01 June 2020

https://doi.org/10.30721/fsab2020.xx.xx

Introduction

The daily consumption/use of emulsified food/cosmetic products makes them good candidates for the delivery of health-promoting ingredients such as plant extracts and probiotics. Since the aqueous phase of many emulsified products is susceptible to microbial growth, their microbial contamination is usually prevented by a thermal treatment (e. g. pasteurization) and the addition of organic acids to decrease pH or preservatives such as potassium sorbate in foods or parabens in cosmetic products in order to extend their shelf life after opening. Despite its efficiency, this combination of a thermal treatment with the addition of antimicrobial additives in the formulation of emulsified products cannot be applied to probiotics and to a lesser extent to most of plant extracts since it would result in their thermal inactivation.

Moreover, besides their interest in functional foods and cosmetics, consumers are increasingly concerned with the safety of synthetic ingredients. This can be illustrated by controversies regarding the innocuity of some preservatives. For instance, sulfites are traditionally used as antioxidants and preservatives in fruit and vegetable products but reduce vitamin B_1 uptake and are listed as allergens due to the fact they could promote asthma attacks of asthma patients. Although there is less evidence regarding the toxicity of parabens (Kirchhof and De Gannes 2013), users of cosmetic products are reluctant to their use, namely in France. These concerns stimulated the demand for organic or "clean label" (i. e. with natural and familiar ingredients) foods and cosmetics (Fan et al. 2018).

In this context the antagonistic activity against unwanted micro-organisms exerted by probiotic LAB as well as phytoconstituents offers an opportunity to design innovative food or cosmetic emulsions which besides their health-promoting properties would prevent contamination of these products by pathogenic or spoiling microorganisms. This analysis led University of Food Technologies Plovdiv, PAM (University of Bourgogne Franche-Comté - AgroSup Dijon) and BioDyMIA (Université Lyon 1 - ISARA Lyon) research units to build a Franco-Bulgarian scientific cooperation project (ESCAPE: bioprEservation by SynergistiC Action of Probiotics and plant Extracts).

The applicative aim of ESCAPE project is to achieve biopreservation of food (mayonnaise, salad dressings, etc.) and non-food (cosmetics - face, body, hand creams, scrubs, hair conditioners, etc.) emulsions by the synergistic action of plant extracts (including essential oils) and probiotic lactic and propionic acid bacteria. Indeed, the shelf-life of many emulsified food and cosmetic products is often limited by oxidation and/or microbial spoilage. In this context, the *in vitro* antioxidant and antimicrobial activities of plant extracts are promising (namely of phenolic-rich plant extracts as recently reviewed by Bouarab-Chibane et al. (2019)). Lactic and propionic acid bacteria strains that besides their probiotic properties inhibit the growth of unwanted microorganisms (and/or possess interesting antioxidant activities) are also of interest (Nedelcheva et al. 2010; Léonard et al. 2015; Teneva et al. 2015; Zhang et al. 2017). The objective and the originality of ESCAPE project is to select probiotic strains of lactic acid and propionic acid bacteria that can survive in emulsions matrices in the presence of selected plant extracts (including essential oils), that do not destabilize or affect negatively the organoleptic properties of emulsions, and which have a synergistic action on the control of unwanted microorganisms and of oxidation in such products.

Methodology

The methodology proposed by this research consortium to develop food or cosmetic emulsions preserved by relevant associations of probiotics and phytoconstituents acting synergistically to control unwanted microorganisms (Fig.1) as well as the corresponding state of the art are presented in this review. Besides the good manufacturing practice and hygiene pillars, hurdle technology principles will be exploited to effectively control the growth of unwanted microorganisms. The activity against unwanted microorganisms of plant extracts and probiotics chosen based on a state of the art will be screened *in vitro*. Following this primary screening, compatible plant extracts - probiotic LAB

combinations choice will be based on a secondary *in vitro* screening of the antibacterial activity of selected plant extracts against selected probiotic LAB. Finally, the distribution in emulsions of probiotic LAB, their antimicrobial metabolites and those present in plant extracts will be investigated as well as the effect of probiotic LAB and plant extracts on emulsions stability and quality. Indeed, food and cosmetic emulsions are generally biphasic systems with an aqueous and an oily phase stabilized namely by emulsifiers. Distribution of bacteria and antimicrobial metabolites depend thus on their physico-chemical properties and on the structure and constituents of the emulsion and can affect (positively or negatively) the stability of emulsions.

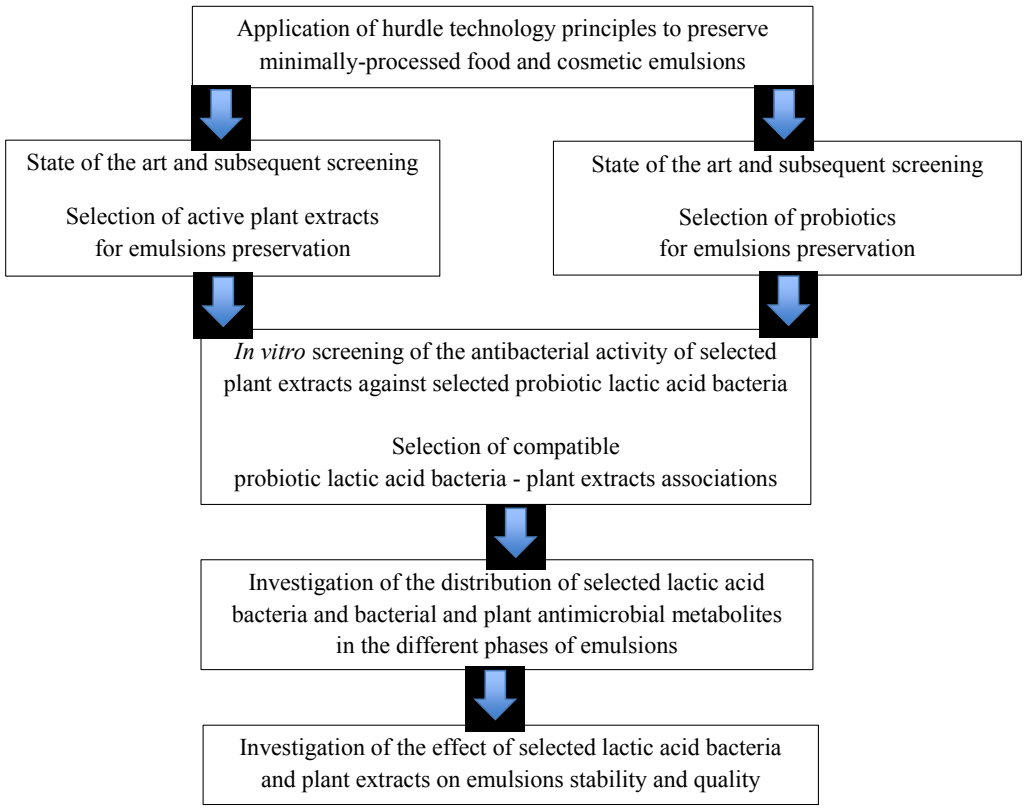

Figure 1. Methodology for the biopreservation of food and cosmetic emulsions by synergistic combinations of probiotics and plant extracts.

Hurdle technology principles

Hurdle technology combines different preservative factors ("hurdles") in order to get a series of such factors that any microorganisms present should not be able to overcome (Leistner and Gorris 1995). In this context, ESCAPE project's ambition is not only to identify associations of probiotics and phytoconstituents acting synergistically to control unwanted microorganisms but to combine these 2 "hurdles" with other factors inhibiting the growth of unwanted microorganisms in a given minimally-processed emulsified food or cosmetic product. Examples of such factors are refrigeration temperature, modified atmosphere packaging (which can affect redox potential depending on oxygen concentration), and pH. Combined factors can interact synergistically to favor inhibition/inactivation of unwanted microorganisms resulting in a greater level of protection as proposed by Leistner (1978). Alternatively, the gamma hypothesis states that environmental factors combine in a multiplicative manner without any antagonistic or synergistic activity (Zwietering et al.

1992). Finally, some factors have been reported to be antagonistic (e. g. Casey and Condon (2002) reported that sodium chloride decreases the bactericidal effect of acid pH on *E. coli* O157:H7). Synergy tests of antimicrobial combinations, such as checkerboard method, can be used to estimate whether factors have interactions or not. When antagonistic effect is due to a stress response of target microorganisms following application of a first factor, it is advisable to apply the combination of factors simultaneously. Generally speaking, the order and the kinetics of application of each stress factor is conditioning their efficiency.

In this context, it is interesting to note that several authors investigated the combined effects of several factors inhibiting the growth of unwanted microorganisms in emulsified food products (Table 1). Most of investigations and corresponding models were focused on the control of foodborne pathogenic bacteria (namely *L. monocytogenes* strains). However, one must keep in mind that the shelf life of many foods including emulsions such as acidified sauces, salad dressings, spreads and dips is controlled by the growth of food-spoilage microorganisms including some LAB strains as underlined by Manios et al. (2014). In this context, another interest of the combination of factors inhibiting the growth of unwanted microorganisms might be the broadening of antimicrobial activity spectrum.

Table 1. Examples of factors combinations inhibiting the growth of unwanted microorganisms in emulsified food products

Emulsified product	Unwanted microorganism(s)	Preservative factors	Reference
salad dressing	*Escherichia coli*	oregano essential oil and salt	Gonçalves Cattelan et al. (2018)
emulsified, vegetable-based spreads of low pH (3.90 - 4.15) adjusted with acetic acid	food-spoiling microorganisms	pH, storage temperature, and acetic acid concentration	Manios et al. (2014)
béarnaise sauce	*L. monocytogenes*	a_w, pH, organic acids (acetic or propionic acid)	Nyhan et al. (2018)
mayonnaise-based deli salads	*L. monocytogenes*	enterocin AS-48 in combination with essential oils, natural bioactive compounds and chemical preservatives	Cobo-Molinos et al. (2009)

State of the art regarding antimicrobial plant extracts for emulsified products preservation

As a result of their metabolism, plants produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins. The antimicrobial activity of plant extracts is namely due to the antimicrobial activity of some of these plant secondary metabolites. They are the source of plant-derived antimicrobial substances. The detailed understanding of the antimicrobial action mechanism of medicinal plants extracts is the first and foremost step in their optimal utilization as natural antimicrobials to extend food shelf life and maintain food quality (Elisha et al. 2017).

In the last years, the preference of foods preserved with natural additives have led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity. To meet this consumer demand, essential oils (EOs) have shown promising results as natural antimicrobials against a wide range of spoilage and pathogenic bacteria. Originally added to change or improve taste, nowadays it is well known that EOs can enhance the shelf life of unprocessed or processed foods because of their antimicrobial nature (Speranza and Corbo 2010).

Due to the high degree of microbial insemination of spices, food emulsions like dressings and salad dressings have a very short shelf life without the addition of preservatives. To avoid this, various extracts and EOs of spices can be obtained and applied, which provides for an extended shelf life of the emulsified products without the addition of preservatives. Spice EOs and extracts have pronounced antimicrobial and antioxidant properties and can be applied in small amounts. Their antimicrobial activity allows their application as natural preservatives to help increase food safety (Chatsisvili et al. 2012). Several authors investigated the use of antimicrobial plant extracts for emulsified products preservation (Singh et al. 2003; Chang et al. 2012; Terjung et al. 2012; Trinh et al. 2013; Chen et al. 2015; Garcia-Diez et al. 2017; Bouarab-Chibane et al. 2018b; Kočevar Glavač and Lunder 2018; Pernin et al. 2019). However, most of them reported a low efficiency (as revealed by higher minimal inhibitory concentrations), because of the presence of both emulsifiers and lipid phase, which are causing entrapped antimicrobial phytoconstituents, making them less available in the aqueous phase where target microorganisms are present (Chang et al. 2012; Terjung et al. 2012; Trinh et al. 2013; Bouarab-Chibane et al. 2018b; Pernin et al. 2019).

Some authors suggest that the location of plant antimicrobial phytoconstituents in emulsified products according to their solubility (Bouarab-Chibane et al. 2018a,b; Pernin et al. 2019), the surrounding pH (Smith-Palmer et al., 2001; Gaysinsky et al. 2005b) and temperature (ter Steeg et al. 2001; Davidson et al. 2005; Gaysinsky et al. 2005b; Bouarab-Chibane et al. 2018a), the surface properties of the targeted bacteria (Uhart et al. 2006; Negi 2012) should be taken into account in estimating their efficacy as antimicrobial preservatives in emulsified products. Smith-Palmer et al. (2001) reported that low pH has enhanced the antimicrobial activity of hydrophobic plant secondary metabolites in food by making them more hydrophobic, thereby favoring their interaction with cell membranes of microorganisms (Negi 2012; Perricone et al. 2015). Therefore, the antimicrobial activity of plant extracts should be retained in low pH and low fat products, like vegetables and fruit juice (Smith-Palmer et al. 2001) since interactions between the hydrophobic antimicrobials and lipids on one hand and between targeted microorganisms and lipid phase on the other hand are avoided. Indeed, an opposite effect could be encountered for emulsified products at low pH, where the hydrophobic active agents can also interact with the lipid phase at the expense of microorganisms. In the same way, low temperature may improve the activity of plant antimicrobials in emulsified products only if hydrogen bonds between the antimicrobials and the bacterial surface are favored at the expense of van der Waals and hydrophobic interactions between the biomolecules and lipids (Bouarab-Chibane et al. 2018a). The decrease in temperature has been reported to improve the activity of EOs in vegetables (Smith-Palmer et al. 2001). In addition, predictive models of the effect of temperature on the antimicrobial activity of phenolic compounds in water-in-oil emulsions indicate a loss of efficiency at high temperatures (ter Steeg et al. 2001).

Moreover, emulsifiers and lipids can interact with bacterial surfaces and form fat coatings protecting them from antimicrobial agents (Uhart et al. 2006; Ly-Chatain et al. 2010). In contrast, some emulsifiers may improve the efficacy of hydrophobic plant antimicrobials thanks to their dispersion in the aqueous phase, after conveying the biomolecules in contact with the targeted microorganisms (Blaszyk and Holley 1998; Gaysinsky et al. 2005a, b; Donsì and Ferrari 2016). In this sense, the use of nanoemulsions as a delivery system of poorly water-soluble plant antimicrobial metabolites may be an interesting approach to ensure their antimicrobial activity in emulsified products (Gaysinsky et al. 2005a, b; Donsí et al. 2011; Mahmood et al. 2017).

In some studies, the octanol-water partition coefficient ($\log P_{oct/wat}$) of antimicrobial phytochemicals has been considered as a key factor. Pernin *et al*. (2019) found a loss in eugenol ($\log P_{oct/wat} = 2.61$) anti-listerial activity compared to ferulic acid ($\log P_{oct/wat} = 1.67$), in an oil-in-water emulsion, which may be related to the difference in their $\log P_{oct/wat}$. The decrease of the antimicrobial activity of phenolics in high-fat products seems to be closely related to their high octanol-water

partition coefficient ($logP_{oct/\ wat}$) (Bouarab-Chibane et al. 2018a,b). However, physical structure of the product (Speranza and Corbo 2010), the type of emulsion (Fig. 2): oil-in-water (Terjung et al. 2012; Kočevar Glavač and Lunder 2018 Pernin et al., 2019) or water-in-oil emulsion (ter Steeg *et al*., 2001) (which is determined according to preferential distribution of the emulsifier), and the size of emulsion droplets (ter Steeg et al. 2001; Terjung et al. 2012) are other important factors which may affect the antimicrobial activity of plant secondary metabolites in emulsified products.

Because of its preferential location in the oil-water interfaces, carvacrol was found more effective in coarse lipid droplets in oil-in-water emulsion (Terjung et al. 2012) whereas it was less effective for spreads with high size distribution in water-in-oil emulsion (ter Steeg et al. 2001). In the first case, the high entrapment of carvacrol in emulsion interfaces and its low solubility in excess emulsifier micelles leads to the loss of its antimicrobial activity in small emulsions (Terjung et al. 2012). The high energy sources contained in large spread droplets was implicated in the low antimicrobial activity of carvacrol compared to smaller droplets in water-in-oil emulsion (ter Steeg et al. 2001). Due to differences of solubility and thus of distribution of antimicrobial molecules in the different phases of emulsions (oily and aqueous phases, oil-water interfaces, and micelles of emulsifiers if emulsifiers are in excess), it has been proposed by Terjung et al. (2014) to combine antimicrobials of different solubilities to enhance shelf life of structurally-complex products such as "emulsion-type" sausages.

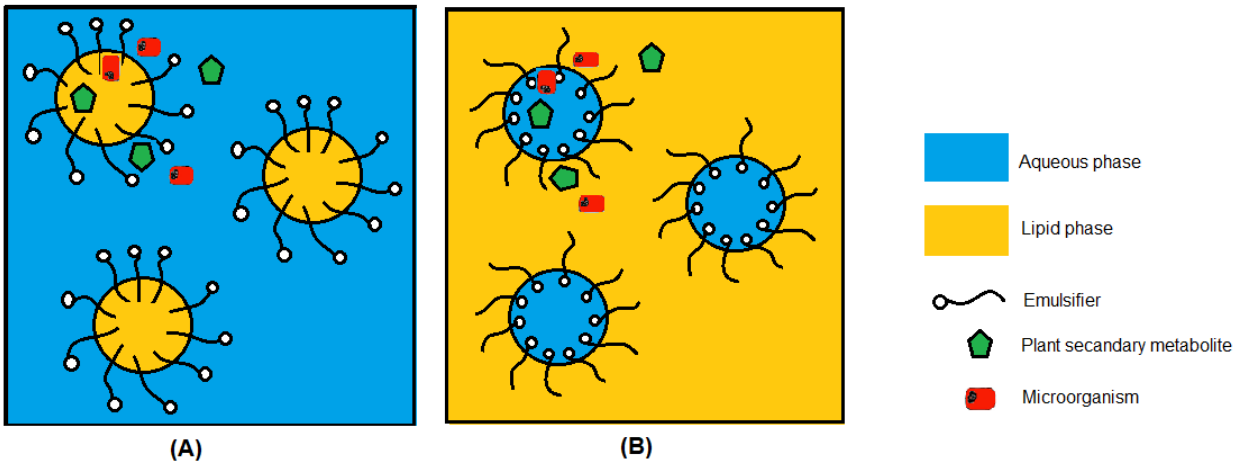

Figure 2. Scheme presenting possible distribution of both plant secondary metabolites and microorganisms with respect to emulsion droplets (oil-in-water (A) and water-in-oil emulsions (B)), without excess emulsifier, which may affect the antimicrobial activity of plant extracts in emulsified products.

Until now, based on the several limitations of plant extracts cited above, microbial safety cannot be ensured by the plant secondary metabolites, alone. In practical use, they have always been combined with other microbial barriers (like modified atmosphere and cold storage (Djenane et al. 2011; Pesavento et al. 2015; Bouarab-Chibane et al. 2017) for emulsified products preservation. Antimicrobial plant extracts and probiotics combination deserves to be evaluated for emulsified products preservation. The lack of broad-spectrum activity of plant extracts, reported in the literature (Taguri et al. 2006) may be used positively, by selecting potential probiotics-plant extracts combinations with synergistic action, to be used for preserving emulsified products.

State of the art regarding LAB for emulsified products preservation

Mayonnaises are finely dispersed food emulsions of the oil-in-water type, the main components of which are generally plant oil, egg powder, dry milk, and auxiliary materials (flavor enhancers, flavorings, acidifiers, etc.). The egg yolk is used as emulsifier

in a mixture with the dry milk. In the modern technology, modified starches and hydrocolloids are used as emulsifiers and stabilizers. The pH of the product ranges from 3.5 to 4.5, with higher values being typical of low-calorie mayonnaise types that contain less fat and acids.

Mayonnaise contains various microorganisms that usually enter by air, equipment and raw materials. Acid-resistant bacteria from the genus *Lactobacillus* (*Lactobacillus brevis*) or from the genus *Bacillus* (*Bacillus subtilis, Bacillus mesentericus*), yeast (*Saccharomyces* sp.) and molds (*Aspergillus* sp.) can survive in this type of emulsion (Mun et al. 2009).

In practice, the term dressing refers primarily to salad dressings based on mayonnaise or cream, as well as to those made from yogurt. There are four main groups of salad dressings: acetic, mayonnaise, sweet-acid and dairy. The microflora of salad dressings includes LAB, mainly of the genera *Lactobacillus* and *Leuconostoc*; yeasts - *Zigosaccharomyces*, *Saccharomyces*, and various fungi (Hunsakul et al. 2016).

Thus, lactobacilli and/or bifidobacteria are being introduced in these emulsions in combinations with EOs and extracts that replace the highly contaminated spices used for their extraction in order to prevent the growth of undesirable microorganisms and to provide extended shelf-life of the products without the use of preservatives.

Probiotics are defined as "living microorganisms which when administered in adequate amounts, confer beneficial effect to the host" (Charalampopoulos et al. 2002, 2003; Stanton et al. 2005). LAB and bifidobacteria are the major bacterial species in the production of probiotics and are part of the natural beneficial intestinal microflora. They are traditional cultures in the production of fermented foods (Kociubinski and Salminen 2006; Siro et al. 2008). Some strains of the genera *Lactobacillus*, *Bifidobacterium*, and some *Propionibacterium* species are included in the composition of probiotics and probiotic foods because of their health effects (FAO-WHO 2002; Collado et al. 2007). Probiotic microorganisms contribute to the restoration of the balance of the gastrointestinal microflora, play an important role in maintaining health and improve the quality of certain foods, obtained with their inclusion (Soccol et al. 2010; Lopez de Lacey et al. 2014). The basic requirements for probiotic strains are to be part of the human microflora, to be able to adhere to epithelial cells or cell lines, to survive and grow in the gastrointestinal tract, to allow for industrial cultivation and accumulation of high concentrations of cells, to have antimicrobial activity and to produce substances with antimicrobial nature, as well as to modulate the immune response and, last but not least, to be safe for clinical and food application.

Probiotic foods are one of the newest trends in nutrition science. This group of foods contains, along with their nutritional ingredients, a high concentration of probiotic bacteria and substrates that favor their growth in the gastrointestinal tract. In the form of foods and beverages, they play a role similar to that of probiotics, having primarily preventative action.

LAB (primarily *Streptococcus, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Weissella* species) play pivotal roles in a broad spectrum of food fermentation processes (Chumchuere et al. 2000; Peyer et al. 2016; Tamang et al. 2016). One of the most important applications of LAB is their use as starter cultures in the production of fermented dairy products. Furthermore, lactic fermentation also acts as a low-cost method for food preservation, since LAB inhibit pathogenic and/or undesirable spoilage microbiota like *Listeria*, *Clostridium*, *Staphylococcus* and *Bacillus*. These food preservation activities mainly result from acidification of the matrix, competition for nutrients, and the production of antimicrobial compounds such as bacteriocins. (Tamang et al. 2009; Ben-Harb et al. 2019).

In connection with the proposed ESCAPE project, the team from the University of Food Technologies, Plovdiv has explored various methods for the biopreservation of food and cosmetic emulsions. Teneva (2017) examined the possibilities for the biological preservation of three different types of mayonnaise without the addition of preservatives. Two of the batches were prepared with sunflower oil and the third batch was prepared with sesame oil. Each batch was biopreserved using the probiotic

strain *Lactobacillus plantarum* D1. Mayonnaises were stored under refrigeration conditions for 30 days (storage temperature of 0-4 °C), monitoring the changes in the pH, concentration of viable cells of LAB, and standardized microbiological and organoleptic characteristics typical of this type of food emulsion. The probiotic strain applied for the biopreservation was selected on the basis of its resistance to the plant extracts and EOs of cardamom, coriander, pepper and cumin which can be used as biopreservatives in the same food emulsions.

One of the mayonnaise batches was prepared with sunflower oil and was preserved with free *Lb. plantarum* D1 cells. The addition of the cells led to a slight change in pH in the first days of storage and the culture remained active throughout the whole refrigeration storage period (Fig.3). The microbiological studies conducted periodically on standard mayonnaise parameters included in the Bulgarian Standard for such food products (total aerobic bacteria, cfu (colony forming units)/g; *E. coli*, *Enterobacteriaceae*, cfu/g; *S. aureus*, cfu/g; *Salmonella*, cfu/g; fungi and yeasts, cfu/g) concluded that the biopreserved product fully met the requirements of the standards in force in the Republic of Bulgaria. Similar tests were carried out with immobilized cells of the same probiotic strain in Ca-alginate emulsion (data not shown). The data presented by Teneva (2017) show that the immobilization process positively affected not only the microbiological characteristics but also the emulsifying characteristics of the prepared mayonnaise. The addition of probiotic cells to the food emulsion allowed biopreservation of the product by meeting the standard microbiological parameters for up to 30 days storage at 4 °C. Furthermore, the viable probiotic cells contained in the product would carry out their regulatory and preventative role after consumption.

Dimbareva (2016) explored the possibilities for biopreservation of cosmetic emulsions. She investigated the preservation of creamy masses with the addition of two probiotic LAB strains: *Lactobacillus acidophilus* Ac and *Lactobacillus delbrueckii* ssp. *bulgaricus* GB, as well as a combination of the two strains. The changes in the pH and the standard microbiological parameters for this type of cosmetic products were monitored. The cosmetic creams were stored for 60 days at room temperature (18-20 °C) (Table 2A). Experimental data show that the pH of the creamy mass changed slightly and the number of viable probiotic cells decreased by 2-3 log units along the storage process of up to 60 days. With regard to pathogenic microorganisms, the creamy mass met the requirements set in the cosmetic product standards, but the number of living cells of mesophilic aerobic and facultative anaerobic microorganisms (TBA) was high, which was more pronounced in the control variant. Data obtained from Dimbareva (2016) show the successful biopreservation of the cosmetic emulsion over a period of 60 days using probiotic LAB.

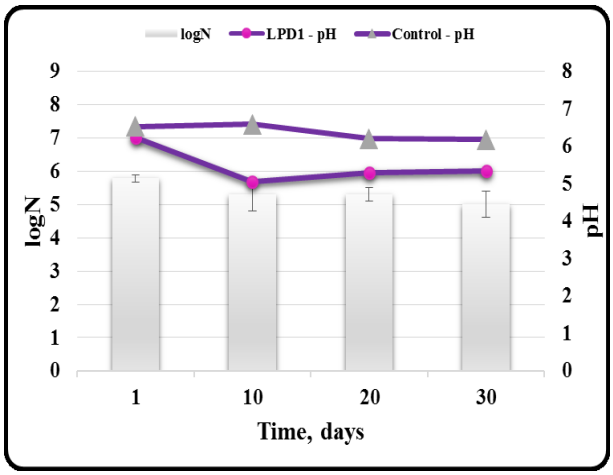

Figure 3. Biopreservation of mayonnaise with the probiotic strain *Lactobacillus plantarum* D1 (Teneva 2017).

Denkova et al. (2013) and Dimbareva (2016) also investigated creamy cosmetic emulsions biopreserved using the probiotic *Lactobacillus* strains *Lactobacillus reuteri* D, *Lactobacillus acidohilus* 5R, *Lactobacillus plantarum* NBIMCC 2415, and *Propionibacterium freudenreichii* ssp. *shermanii* NBIMCC 328. The creamy cosmetic emulsions were stored at room temperature for 12 months and the changes in the pH, the concentration of viable cells of probiotic bacteria and the microbiological safety according to the permissible levels in the BNAEOPC Handbook were monitored. Experimental data given in Table 2B show a slight

change in the pH of the creamy cosmetic emulsions. The most profound changes were observed in the control sample - the number of mesophilic aerobic and facultative anaerobic bacteria (TBA, cfu/g) was high (10^5cfu/g); fungi and *Staphylococcus aureus* were found as well. In all test samples the concentration of viable cells of probiotic bacteria decreased by 2-3 log N but 100 - 1000 cfu/g of viable probiotic cells remained up to the end of the storage period in all cosmetic creams. Only creamy cosmetic emulsions preserved using *P. freudenreichii* ssp. *shermanii* NBIMCC 328 and the combination of *L. acidophilus* 5R and *P. freudenreichii* ssp. *shermanii* NBIMCC 328 were microbiologically safe for use (Table 2B).

Table 2A. Biopreservation of creamy cosmetic emulsions (CCEs) with probiotic lactic acid bacteria (Dimbareva 2016)

	I batch				II batch			
	pH		TAB, cfu/g		pH		TAB, cfu/g	
	1 day	60 days	1 day	60 days	1 day	60 day	1 day	60 day
Control	5.1	5.4	<10	6.10^5	6.0	5.8	<10	5.10^5
CCE + GB	4.5	5.0	<10	8.10^3	4.3	4.5	<10	70
CCE + Ac	4.3	4.5	<10	1.10^3	4.2	4.2	<10	50
CCE + GB + Ac	4.5	4.5	<10	20	4.2	4.5	<10	10

***** *Lactobacillus acidophilus* Ac – Ac; *Lactobacillus delbrueckii* ssp. *bulgaricus* GB - GB

Table 2B. Changes in the pH and microbiological parameters of the creamy cosmetic emulsions (CCEs) biopreserved using probiotic bacteria during 12 months of storage at room temperature (Denkova et al., 2013; Dimbareva, 2016)

№	pH		TBA, cfu/g		*E.coli*, cfu/g		*S. aureus*, cfu/g	
	1 day	12 months	1 day	12 months	1 day	12 months	1 day	12 months
Control	5.5	5.4	<10	$1.0x10^5$	<10	<10	<10	$4.0x10^3$
CCE + D	5.4	5.3	<10	$1.0x10^5$	<10	<10	<10	<10
CCE + PFS	5.6	5.5	<10	$2.0x10^2$	<10	<10	<10	<10
CCE + LP2415	5.5	5.2	<10	$1.3x10^4$	<10	<10	<10	<10
CCE + LA5R+ PFS	5.4	5.2	<10	$2.0x10^3$	<10	<10	<10	<10
CCE + LA5R	5.6	5.0	<10	$1.2x10^4$	<10	<10	<10	<10

№	*P. aeruginosa*, cfu/g		Fungi and yeast, cfu/g	
	1 day	12 months	1 day	12 months
Control	<10	<10	<10	$2.1x10^2$
CCE + D	<10	<10	<10	$2.6x10^2$
CCE + PFS	<10	<10	<10	<10
CCE + LP2415	<10	<10	<10	<10
CCE + LA5R+ PFS	<10	<10	<10	<10
CCE + LA5R	<10	<10	<10	<10

*****_Lactobacillus reuteri_ D - D, _Lactobacillus acidohilus_ 5R – LA5R, _Lactobacillus plantarum_ NBIMCC 2415 – LP2415 and the _Propionibacterium_ strain _Propionibacterium freudenreichii_ ssp. _shermanii_ NBIMCC 328 – PFS

Having in mind the results and conclusions from the previous work of the project team members and other authors, during the implementation of the ESCAPE project, the international team will focus its research on the isolation and identification of new probiotic LAB strains, focusing research

efforts on the investigation of their antimicrobial activity against pathogenic and saprophytic microorganisms and on the possibilities of their application for the biopreservation of a wide range of food and cosmetic emulsions.

Selection of probiotic LAB - plant extracts associations

Adding both plant extracts and probiotic LAB in emulsified food or cosmetic products requires that there exist plant extracts concentrations affecting unwanted microorganisms without (or to a lesser extent) affecting probiotic LAB. This is likely one of the reasons why only a few authors considered the possibility to combine antimicrobial plant extracts and LAB for the preservation of foods. However, plant phenolics and phenolic-rich plant extracts were recently reported to differentially affect common food-borne pathogenic bacteria and LAB (Pacheco-Ordaz et al. 2017; Chan et al. 2018). Trinh (2015) compared the antibacterial activity against 2 LAB strains (*Lactococcus lactis* subsp. *lactis* ATCC 11454 and *Lactobacillus plantarum* ATCC 43199) and 3 food-borne pathogenic bacteria or their surrogates (*L. innocua*, *E. coli*, *S. aureus*) of 5 Vietnamese EOs which were selected base on their activity against *L. innocua*. While MIC of *Cymbopogon citratus* EO against *L. innocua* LRGIA 01 (350 $mg.L^{-1}$) was lower than against *Lb. plantarum* ATCC 43199 (1500 $mg.L^{-1}$) (in that case *Lb. plantarum* strain was less sensitive to EO than *L. innocua* strain), this was not always the case (e. g. *Cymbopogon winteratnius* EO had a lower MIC against *Lc. lactis* ATCC 11454 (250 $mg.L^{-1}$) than against *L. innocua* LRGIA 01 (500 $\mu g.mL^{-1}$)). However, *Lb. plantarum* strain was more resistant to EOs than *Lc. lactis* strain. A preliminary *in vitro* screening of the effect of plant extracts on probiotic LAB and unwanted microoorganisms will thus remain necessary.

More authors investigated the combination of plant secondary metabolites with metabolites produced by LAB (namely bacteriocins). Interestingly, several authors reported a synergistic effect of bacteriocins and antimicrobial phytocomponents: nisin or pediocin PA1 with wine polyphenols (Knoll *et al.*, 2008), divergicin M35 and garlic extract (Zouhir et al. 2008), enterocin AS-48 and several EOs (Cobo-Molinos et al. 2009), and enterocin A and thyme EO (Ghrairi and Hani 2015).

The possibility to combine a plant extract and *Carnobacterium maltaromaticum* cultures in fish model systems was examined by dos Reis et al. (2011). They observed that the respective contributions of plant extract and *C. maltaromaticum* to the control of *L. monocytogenes* was influenced by food matrix. Despite the promises of this approach, no systematic examination of the possibility to combine plant extracts and LAB to control unwanted bacteria in food and cosmetic emulsions was conducted to our knowledge.

Distribution of LAB and antimicrobial metabolites in the different phases of emulsions

Emulsions are non-homogeneous polyphasic systems. This means that every component of the system will be located in a specific phase. The presence of components may modify to some extent the emulsion characteristics but also, this will drive the activity of the component. Indeed, the first step in the action of an active compound is the contact between the antimicrobial active and its target.

As stated above, plant extracts will locate in the emulsion depending on the respective LogP of their constituents. For bacteria, localization will also occur driven by the surface properties of cells (Ly et al. 2006b, Naïtali et al. 2009). Several forces are involved in the localization of bacteria but they follow the DLVO (Derjaguin, Landau, Vervey, and Overbeek) theory in its revisited version taking into account the Lewis interactions (Bellon-Fontaine et al. 1996) with often a high importance of electrostatic interactions when the ionic strength is low (Ly et al. 2006a, Ly et al. 2008b). These forces are coming from components of the surface of bacteria like proteinase, permease or teichoic acid (Germond et al. 2003, Habimana et al. 2007, Ly et al. 2007). Purely physical forces are also involved depending on the form of bacteria (Ly-Chatain et al. 2010). As a result, when bacteria have an electrostatic affinity for lipids, they can contribute to coalescence by bridging droplets, whereas if their hydrophobicity put them at the interface, they can stabilize emulsions (Li et al. 2001; Ly et al. 2008a). Chain of bacteria or long bacillus can also act as a

net to catch droplets, whereas cells adhering to lipids can be lifted to the top layer during coalescence (Ly-Chatain et al. 2010). Bacteria were thus added, according to their surface properties, to emulsions for stabilization and texturing purposes (Firoozmand and Rousseau 2015). Some authors modified the surface properties by a chemical treatment (Jiang et al. 2019), inspired by the experiments by the group of Rosenberg (Goldberg et al. 1990) or by overexpressing genes coding for surface components (Tarazanova et al. 2018). The effect of cell growth on emulsion properties had to be carefully monitored (Ly-Chatain et al. 2010; Nia and Raikos 2019).

An important point when using active cells in an emulsion is to keep cell activity despite the presence of antimicrobial compounds in some phases. Several decades ago, some authors proposed to immobilize microbial cells in biotechnological processes to increase activity and stability (Siess and Diviès 1981). From that time, encapsulation of microbial cells has developed with various techniques (Anal and Singh 2007; Rathore et al. 2013). Some techniques to make small capsules loaded with cells were based on emulsification (Qi et al. 2019). Authors took inspiration in the various techniques to propose encapsulation or protection of cells in emulsions. A very simple way to preserve activity has been investigated by adding oat flour, which not only improved the texture properties of the salad dressing emulsion but also increased the life of a probiotic strain of *Lactobacillus paracasei* subsp. *paracasei* (Mantzouridou et al. 2013). Another strategy was based on the use of fat replacers (β-glucan and phytosterol) that could decrease the amount of fat in cream cheese, improve texture and encapsulate and protect bacteria (Ningtyas et al. 2019). A structural strategy was also attempted to increase the oil phase in the emulsion to improve protection and activity of *Lactobacillus plantarum* strains. However, all parameters are related and modifying the composition of the emulsion will have an impact on its structure, stability, on the activity of its antimicrobial components and of course, on the activity of probiotic cells (Su et al. 2018). The formulation step has thus to be finely tuned to get the expected results.

Effect of plant extracts on emulsions stability

Food and cosmetic emulsions are thermodynamically unstable biphasic mixtures that aim at rapid separation through different mechanisms - coalescence, flocculation, creaming or sedimentation, and Ostwald ripening. Thus, they need stabilization by using different stabilizers. One of the most promising directions is the use of biopolymers to stabilize emulsions. One of the new trends in this regard is the use of natural components to replace synthetic stabilizers and preservatives, since in most cases they also possess significant biological activity and may be beneficial to human health (McClements and Jafari 2018; Jarzebski et al. 2018). Since in most cases inorganic particles or chemical components serve as emulsion stabilizers, replacing them with plant raw materials / extracts, resulting in a new added value of the product obtained is the most often applied strategy. Various studies reveal the possibilities of applying different types of biopolymers from plant raw materials as agents to improve the colloidal stability of emulsions. Polysaccharides (cellulose, starch, lignin) (Nasrabadi et al. 2019; Tenorio et al. 2017; Silmore et al. 2016; Song et al. 2015; Timgren et al. 2011), various types of proteins (Hu et al. 2016; Liang and Tang 2014; Liu and Tang 2016; Jiao et al. 2018) can be used as stabilizers. Data show that the interaction of polysaccharides and proteins by various mechanisms results in complexes having surface-active properties and improving the stabilizing properties of both types of biopolymers (Nasrabadi et al. 2019). In some cases, other stabilizing agents are found in plant extracts, such as saponins, which are complex glycosides. They contain carbohydrates and related steroids or triterpenoids in their chain. This type of compounds is used in the food industry to stabilize the foam in some beverages as well as in the formation of stable macro- and micro-emulsions. Since they can be found in some plants, their use may be in the form of extracts which stabilize food or cosmetic emulsions (Bai et al. 2016; Jarzebski et al. 2018). The formation of stabilizing properties of the extracts can be achieved by combining the conditions of extraction and modeling conditions such as pH and ionic strength as well as the content

of proteins and polysaccharides, their total concentration, particle size, etc. (Nasrabadi et al. 2019; Dickinson 2008; Guclu-Ustundag and Mazza 2007). Fructans are another potential stabilizer of plant origin. In recent years, data have been reported that some of the fructans have surface-active properties (Bravo-Núñez et al. 2019; Sosa-Herrera et al. 2016).

Effect of LAB on emulsions stability

In recent years, some studies have shown the potential applications of bacterial cells including LAB to stabilize different types of emulsions (Jiang et al. 2019; Falco et al. 2017; Firoozmand and Rousseau 2016).

The emulsifying properties of various groups of substances (glycolipids, lipopeptides, protein-like substances, phospholipids, fatty acids and lipopolysaccharides) produced by various lactobacilli species have been investigated. LAB produce different types of metabolites including the so-called bio-surfactants (SURFace ACTive AgeNTS or SURFACTANTS). This group of substances produced by some LAB is related to the ability of microorganisms to attach to the different compartments of the gastrointestinal tract and to form a biofilm. By producing different types of substances from this group, LAB have the potential to prevent pathogen adhesion and the subsequent biofilm formation (Satpute et al. 2016; Gan et al. 2002). "Biosurfactants" reduce surface tension and interfacial tension and have activity at interfaces. There are a number of biosurfactant classes described so far according to their chemical structure, producing strain and mode of action (Satpute et al., 2010, 2016). Madhu et al. (2014) explored the possibilities for production and characterization of biosurfactants in the cultivation of *Lactobacillus plantarum* CFR 2194 isolated from kanjika, a rice-based ayurvedic fermented product. It has been found that the strain produced substances having a glycoprotein-like structure that possesses emulsifying properties and greatly improve the emulsification index, emulsification activity, and emulsion stability. In addition, the produced surfactant had antimicrobial activity against food pathogens such as *Escherichia coli* ATCC 31705, *E. coli* MTCC 108, *Salmonella typhi*, *Yersinia enterocolitica* MTCC 859, and *Staphylococcus aureus* F 722.

Currently, there is growing interest in the application of natural biosurfactants and bioemulsifiers, mainly in the cosmetic, pharmaceutical, and food industries. However, there are no studies on the optimization of the extraction conditions of cell-bound biosurfactants.

LAB are Gram-positive, so their cell wall consists of a layer of peptidoglycan covered by lipoteichoic acid, polysaccharides and surface-bound proteins. The concentration of these substances in the cell wall is the cause for the hydrophilic nature of LAB, making them unsuitable for incorporation in some cosmetic emulsions (Boonaert and Rouxhet 2000). In this connection, various authors examined cell treatment with octenyl succinic anhydride (OSA) aiming at improving their emulsifying ability and making them fit for incorporation in food and cosmetic emulsions. Jiang et al. (2019) chemically modified the cell hydrophobicity of a LAB strain, *Lactobacillus acidophilus* (La5), using OSA and investigated foams and emulsions produced with both the unmodified and modified cells in terms of their foamability, foam stability, emulsion storage stability, and the microstructure. The OSA modification increased effectively cell hydrophobicity and the modified bacteria adsorbed well on the oil-water or air-water interface, thus stabilizing the foams or emulsions. The greater the OSA modification was, the better the foamability and foam stability were.

Ly et al. (2007) examined the effects of the surface properties of LAB on the stability of model food emulsions. The bacteria were added to oil-in-water (o/w) emulsions stabilized by milk proteins (sodium caseinate, whey proteins concentrate or whey proteins isolate) at different pH values (from 3 to 7.5). The effect of bacteria on the emulsions' stability depended both on the surface properties of the strains and on the emulsion characteristics. Flocculation and aggregation were observed in emulsions at pHs for which the bacterial surface charge was opposite to the one of the proteins. Furthermore, the effects of bacteria on emulsion stability depended also on the concentration of cations present in the media such as Ca^{2+}. The bacteria could interact with other compounds in

matrices through the bacterial surface properties, consequently affecting the emulsion stability. The effect was notably due to the surface charges of bacteria. If negatively charged bacteria are added to an emulsion containing positively charged droplets, the bacteria can absorb directly to the emulsion droplets by electrostatic interactions decreasing the repulsion between the emulsion droplets and resulting in an aggregation of the oil globules. The choice of a bacterial strain according to its surface properties can have an important impact on the stability and characteristics of an emulsion. The negatively charged bacteria could also interact with the negatively charged droplets by the intermediate help of cations. The design of new food products with specific surfactants and functional ingredients has also to take into account properties of the surface of bacteria. Properties such as isoelectric point and hydrophobicity are of great interest in many food processes. In the case of fermented products or in fermentation media containing lipids, proteins or minerals, the presence of bacteria could affect the organization of matrices and also the localization of bacteria (Sireswar et al. 2017).

Conclusion and perspectives

Daily consumption of food emulsions such as sauces, dips or spreads and daily use of topical lotions makes them good candidates for the delivery of probiotics and phytoconstituents with interesting biological properties. Moreover phytoconstituents and probiotics can be combined to control unwanted microorganisms. While this approach has not been applied to food or cosmetic emulsions to date, it has recently been applied to apple and seabuckthorn juices (Sireswar *et al.*, 2017): seabuckthorn juice supplemented with malt extract to favour probiotic growth successfully eliminated enteropathogenic bacteria.

Acknowledgements

This work was supported by the Bulgarian National Fund "Scientific Research" under the project КП-06-Rila/2 from 20.12.2018 "Bio-pr**E**servation by the **S**ynergisti**C** Action of **P**robiotics and plant **E**xtracts (ESCAPE)". Cooperation between University of Food Technologies Plovdiv (Bulgaria), BioDyMIA research unit (EA n°3733, Université Claude Bernard Lyon 1 - ISARA Lyon, France), and PAM (Université de Bourgogne - AgroSup Dijon Joined Research Unit) was supported by Franco-Bulgarian cooperation Hubert Curien Programme (PHC Rila) (ESCAPE project). The authors wish to express their gratitude for the supports of the Ministry of Education and Science (Bulgaria), the Embassy of France to Bulgaria in Sofia ("Ministère des Affaires Etrangères", France), Campus France and "Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation" (France).

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