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The Effect of Edible Plant Oils on Increasing the Viability of *Lactocaseibacillus rhamnosus* GG During the Microencapsulation by Spray Drying Process

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Abstract: This work concerns the spray drying of probiotic bacteria *Lactocaseibacillus rhamnosus* GG suspended in a solution of starch, whey protein concentrate, soy lecithin, and ascorbic acid, with additional selected natural plant-origin liquid oils. The aim of this study was to examine these oils and their concentrations (20% and 30%) on bacterial viability during the spray drying (inlet temperature was 180 °C, outlet temperature from 50 to 54 °C, feed rate around 9 mL/min) and storage for 4 weeks at 4 °C and 20 °C, with attempts to explain the protective mechanism in respect including their fatty acid composition. The viability of microencapsulated bacteria, moisture content, water activity, color properties, morphology, particle size of obtained powders, and thermal properties of encapsulated oils were evaluated. The highest viability of bacterial cells after spray drying 83.7% and 86.0%, was recorded with added borage oil respectively with 20% and 30% oil content. This oil has a lower content of oleic and linoleic acid compared to other applied oils, but a high content of both vitamin E and γ -linoleic acid. However, this study did not confirm unambiguously whether and which of the components present in natural plant oils specifically affect the overall viability of bacteria during spray drying.



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Keywords: spray drying; oils; fats; microencapsulation; *Lactocaseibacillus rhamnosus* GG; protection; viability; fatty acids

1. Introduction

The social awareness of the role of a properly balanced diet in maintaining good health has increased the demand for functional food that has an additional, beneficial effect on the specific functions of the human body [1]. Food products containing probiotic microorganisms are considered part of functional foods due to their health-promoting abilities. According to the Food and Agriculture Organization of the United Nation (FAO) and the World Health Organization (WHO), probiotic bacteria are living microorganisms that confer health benefits when consumed in adequate amounts only if their viability is well preserved (10^6 – 10^7 CFU/g) [2].

Probiotics may consist of one strain or a mixture of few strains. The most commercially applied probiotic bacteria are bacteria of the genus *Lactocaseibacillus* (*Lbs. casei*, *Lbs. paracasei*, *Lbs. rhamnosus*), *Lactobacillus* (*Lb. acidophilus*, *Lb. gasseri*, *Lb. johnsonii*) *Bifidobacterium* (*B. bifidum*, *B. breve*, *B. longum*, *B. lactis*) and *Lactococcus* (*L. lactis*) [3–5].

Probiotic bacteria play an important role in the host organism, among others: increase the population of beneficial bacteria in the intestines, fight pathogens, inactivate bacterial toxins, prevent infections of the genitourinary system, affect the immunity to intestinal

diseases, reduce the side effects of antibiotics, lowering the level of cholesterol in the blood and have also an impact on reducing lactose intolerance and other food allergies [6].

However, in most cases, probiotic bacteria are sensitive to environmental conditions such as type of gas environment, pH, humidity, temperature, and light. When these conditions are not properly controlled, their viability decreases during both processing and storage, which reduces their effectiveness. In addition, losses in the number of viable microorganisms also occur during transport through the digestive tract, where they encounter particularly unfavorable conditions, such as a drastic change in pH [7,8].

The viability and stability of probiotic bacteria pose technological and marketing challenges for industrial producers. Intensive research activities focus on the protection of probiotic microorganisms [3].

Encapsulation technologies were developed and applied successfully to protect probiotic bacteria from damage caused by external factors [9]. Microencapsulation refers to a process where bioactive ingredients or cells are surrounded/encapsulated by a protective continuous film of wall material [10]. Controlled release from microcapsules can occur due to changes in specific triggers such as pH, temperature, mechanical stress, and/or salt concentration [11]. This depends on both the type of encapsulation process and the applied protective substances [12].

The material inside the microcapsule (protected material) is called the core, active substance, filler, or inner phase. The material surrounding the core is defined as the wall, film-forming material, shell, membrane, matrix, or outer phase [13]. To microencapsulate probiotic bacteria are commonly used extrusion, emulsion, lyophilization, and spray drying techniques.

Spray drying is one of the most frequently applied methods to encapsulate probiotics [14]. Its first observation and process description is dated to 1860 and the first simplified spray dryer device was patented by Samuel Perry in the United States in 1872. Until 1920, the process wasn't used commercially in the production of powdered milk and detergents. Large-scale production of these products began in the eighties of the last century [15].

Spray drying is the dynamic phase transformation process of starting a system in a liquid state (emulsion, dispersion, solution) into a solid powder by spraying the feed into a hot drying gas [16]. This process takes place in four stages: atomization of the liquid into the form of fine droplets similar to a mist (1), contact of the resulting droplets with hot air (2), evaporation of water (3), and separation of the powder from the moist air stream (4) [14].

The liquid formulation is fed either in the form of an emulsion, suspension, or solution (aqueous or organic solvent-based). Then, the liquid feed is pumped and atomized at the entrance of the drying chamber, where it is converted into very small droplets, which form the so-called "fog" (droplets with a diameter in the range of 10–150 μm), then microdroplets are in contact with the hot drying medium. Most commonly an inlet drying air temperature is between 150 and 200 $^{\circ}\text{C}$ [17–19].

Inlet temperature determines the ability of the mobile gas phase to remove the solvent by evaporation. The temperature must be as high as possible to obtain the lowest moisture content in the product while avoiding the thermal degradation and decomposition of the active components [20]. Achieving a large spray surface contact area due to the small size of generated droplets during atomization facilitates heat transfer from the hot drying gas to the atomized liquid particles. In this way, the solvent evaporates in a very short time (on average 1–20 s). Therefore, the dried particles usually do not reach the inlet temperature of the drying gas [21,22].

Two stages of solvent evaporation and temperature profiles over time can be distinguished. During the first stage, as soon as the hot air contacts the sprayed liquid, the heat transfer causes to raise the temperature of the droplets to a constant value. With the progress of solvent evaporation, the diameter of the dried droplet decreases, and the concentration of solid substances on its surface increases. During the second stage, when the water content in the droplet reaches a critical value, a characteristic “crust” is forming on its surface, so that the rate of evaporation decreases rapidly [20,23].

Then, the dried particles are transported by the drying air to the cyclone, where the powder is separated from the humid air and transferred to the product collection vessel [20].

Both the viscosity of the liquid system and the type of atomization, droplet size, and the proper manipulation of the atomization conditions affect the final size of the powder. The particle size of powders obtained by spray drying may be classified into three categories: small size (1–5 µm), medium size (5–25 µm), and large size (10–60 µm) [24]. Depending on the size and efficiency of the dryer, it is possible to obtain the final powder from a few grams to several tons per hour [25].

As presented above, there are many factors that affect the percentage of viable probiotic bacteria remaining after the spray drying and storage. One of these is the type of protective substances added.

Adding oil to a solution containing lactic acid bacteria that has been spray-dried is one of the effective methods of protecting these bacteria [26]. By adding oils, closed spaces are created, thanks to which lactic acid bacteria are less exposed to the effects of unfavorable environmental factors, such as water and H⁺ ions. An additional benefit of oily substances is that they can only be digested by the lipase in the intestine, allowing the protected microorganisms to be released close to their optimal site of action [26].

To our knowledge, there are no reports explaining the mechanism of action of protective substances on probiotic bacteria. In this work, commercial natural plant-origin liquid oils were used to verify which of them improved the viability of *Lactocaseibacillus rhamnosus* GG during the spray drying and storage for 4 weeks at refrigerated temperature and room temperature.

In this study, we focused primarily on elucidation the influence of the type of liquid oils and their concentration on the viability of *Lbs. rhamnosus* during both spray drying and storage at 4 °C and 20 °C (±1 °C). We also attempted to confirm and explain the protective mechanism of oils and to determine the effect of fatty acid content on LGG viability.

2. Materials and Methods

2.1. Materials, Chemicals and Reagents

The probiotic bacteria *Lbs. rhamnosus* GG used in this study were isolated from a dietary supplement called Acidolac Baby (Polpharma, Starogard Gdanski, Poland), that contains only this one strain of bacteria. The carrier material of the capsules was Starch Capsul[®] HS (Ingredion, Hamburg, Germany), which, among others, meets all the standards of food microorganisms and maximizes the “dry content” of solids in the solution before spray drying with its own high solubility above 99%. Whey protein concentrate (WPC) 80 (Institute of Dairy Industry Innovation, Mragowo, Poland) was used as a bacteria-carrying agent. Soy lecithin Emulfluid E (Cargill, Wayzata, MN, USA) was employed as an emulsifying substance. Ascorbic acid (AQUANOVA AG, Darmstadt, Germany) was used as an antioxidant.

The following natural plant-origin liquid oils were used in this study: cedar tree, hemp (low content of saturated fatty acids), and nigella, pumpkin, borage, and milk thistle (relatively high content of saturated fatty acids). All oils had a similar total amount of

unsaturated fatty acids, ranging from 81 to 89 [g/100 g]. Cold-pressed plant-origin liquid oils were purchased from EkaMedica (Kozy, Poland).

MRS and MRS-agar were purchased from BTL (Lodz, Poland). NaCl aqueous solutions were used to resuspend spray-dried powder in serial dilution, NaOH was used as a pH regulator of emulsions, and glycerol was used as a protectant to freeze bacterial cultures—all of these substances were purchased from Chempur (Piekary Slaskie, Poland).

2.2. Bacterial Culture

For long-term storage, *Lbs. rhamnosus* GG was kept in a 4:1 20% (w/v) aqueous glycerol/MRS solution at $-32\text{ }^{\circ}\text{C}$. For each experiment the bacterial cell cultures were obtained as follows: a frozen culture of *Lbs. rhamnosus* was activated for 22–24 h using the MRS broth incubated at $37\text{ }^{\circ}\text{C}$. Then, the MRS broth was inoculated with 1% (v/v) of the bacterial cells and incubated at $37\text{ }^{\circ}\text{C}$ for 22–24 h [27]. Bacterial cells were concentrated by centrifugation at $\text{RCF (g force)} = 313 \times \text{g}$ for 10 min at $21\text{ }^{\circ}\text{C}$.

The supernatant was removed, and bacterial biomass was washed with 0.85% (w/v) saline solution [27].

2.3. Emulsions Characterization and Preparation

In this study, dispersion of *Lbs. rhamnosus* GG was prepared as multicomponent aqueous emulsion based on starch Capsul[®] HS, whey protein concentrate (WPC 80%), soy lecithin, vitamin C, and various oils, which were fed as liquid emulsion into the spray drying system to obtain microcapsules in form of dry powder.

Starch is widely available as a low-cost food product. The modified starches are widely used as encapsulating agents due to their unique properties such as stabilization of the emulsion, low viscosity of formed solutions, and film-forming capabilities [28]. Whey protein concentrates (35–85% protein) and whey protein isolates (of purity >95% wt. protein) have been used as encapsulating materials of probiotic bacteria [29]. Whey proteins are a suitable medium for encapsulation of bacteria due to their high nutritional composition and good film-forming capabilities. They also have an amphoteric character, good thermal stability, proper gelling, capabilities of both fast hydration/dehydration, and good emulsifying properties. Furthermore, they can interact, trap, and protect probiotic ingredients [30]. Soy lecithin is an amphiphilic substance with both lipophilic and hydrophilic properties that can be used to cover the surface of powder particles, constituting an interaction between the fatty substance and water, thanks to which the dispersion of the product in an aqueous environment is easier and final system is more stable [31]. Lipid matrices such as fatty acids, mono- and diglycerides, vegetable oils, waxes, and resins are used to encapsulate hydrophilic probiotics to protect them against water and moisture [32,33].

Since lipids have poor mechanical properties and are chemically unstable, they are often combined with other biopolymers such as polysaccharides or proteins to increase their efficiency in the encapsulation of probiotics [34].

In addition, oily substances have the advantage that they can only be digested by the lipase in the intestine so that the bacteria can be released close to their optimal site of action [26].

Fifteen percent (w/v) of aqueous starch solution was sterilized in the autoclave at $121\text{ }^{\circ}\text{C}$ for 15 min (Prestige Medical, Blackburn, UK), and after cooling down, an additional 5% wt. aqueous WPC was added while stirring on a magnetic stirrer (IKA, Warsaw, Poland) in the appropriate amount to obtain the final 20% (w/v) solution. Afterwards, lecithin and ascorbic acid were added in a ratio of 1:10 at a final concentration of 0.6% wt. to oil concentration, and pH was adjusted to 6.5 with 1 M NaOH. The bacterial biomass was dispersed in appropriate oil while stirring at 300 rpm using a magnetic stirrer (IKA) and

then was added to aqueous starch, WPC, soy lecithin, and ascorbic acid solution in the appropriate amount to obtain 10^9 CFU/mL and stirred at 300 rpm.

Subsequently, all the formed emulsions were spray-dried. Oils were applied in two different concentrations of 20% and 30% wt. to the dry mass of starting dispersion. A solution of starch, whey protein concentrate, lecithin, and ascorbic acid without oils was used as a control.

2.4. Spray Drying Process

Spray drying was carried out with a laboratory-scale spray dryer (B-290, Büchi, Flawil, Switzerland). Spray drying parameters were kept constant during all experiments: inlet temperature of 180 °C, feed rate of 30% (approximately 9 mL/min), spray air flow of 7.9 dm³/min, and the aspirator air flow of 80% (32 m³/h). The outlet air temperature ranged from 50 to 54 °C. The resulting powder was collected from a single cyclone separator [35].

2.5. Viability Rate of *Lactocaseibacillus rhamnosus* and Powder Storage

To check the number of viable bacterial cells in the inoculum and in emulsion form a 1:10 dilutions was prepared and plated on MRS agar in duplicate. To facilitate the release of bacteria from the oil 100 µL of Tween 80 was added to 9 mL 0.85% (w/v) NaCl, then the spray-dried powder was added in a ratio 1:9 and mixed by vortexing [27]. Afterwards a tenfold serial dilution was prepared and plated on MRS agar in duplicate. Then the Petri dishes were incubated at 37 °C. After 48 h of incubation, the grown bacterial colonies were counted, by expressing log colony-forming units per milliliter (log CFU/mL) [25].

The resulting powder was stored at 4 °C and 20 °C (± 1 °C) in 50 mL sterile centrifuge tubes. Then the probiotic cell viability was checked after 1, 2, and 4 weeks [27].

Colony-forming unit counts before and after spray drying were compared to obtain the bacterial viability rate.

$$\text{Percent viability [\%]} = N_f / N_r \times 100$$

where, N_r = log CFU/mL before spray drying, N_f = log CFU/mL after spray drying [11].

2.6. Differential Scanning Calorimetry (DSC)

The thermal properties of the oils were determined using a differential scanning calorimeter DSC 2500 (TA Instruments, New Castle, DE, USA). Approximately 5–6 mg of oils were placed on aluminum pans and heated from −90 °C to 100 °C at a rate of 10 °C/min. All DSC values are the average of two measurements.

The DSC measurements were carried out thanks to courtesy of the Department of Polymer and Biomaterials Science at the West Pomeranian University of Technology in Szczecin. The parameters of Differential Scanning Calorimetry measurements were established based on the experience of scientists from this Department.

2.7. Moisture Content

A sample of approximately 1 g was placed on an aluminum pan and dried. The moisture content value was measured using a moisture analyzer WPS 50SX/1 set at 120 ± 2 °C (Radwag, Radom, Poland).

2.8. Water Activity

The analysis of water activity of powders was carried out using the MS1 Set-aw, (Novasina, Lachen, Switzerland). Immediately after spray drying, the 1 g of powder was placed in a plastic container. Water activity was measured at 25 °C after 15 min of sample stabilization.

2.9. Particle Size of Powders

The particle size distribution of powders was measured using the particle size analyzer MasterSizer 2000 (Malvern Instruments, Malvern, UK) with a Scirocco 2000 dry sampling system (Malvern Instrument Ltd., Worcestershire, UK). All parameters were kept constant: refractive index: 1.52, vibration feed rate: 40–50%, measurement time: 15 s, 5 scans, dispersive air pressure: 4 bar. The size of particles was described as volume-weighted mean $D_{4,3}$. The final values are the average of 3 measurements.

2.10. Powders Morphology

The surface morphology of the powders was determined using a scanning electron microscope (SEM) (Vega 3 LMU, Tescan, Brno, Czech Republic). The samples of powders obtained by spray drying with an adhesive carbon tape were applied to special round metal tables. Then, using an automatic sputter coater (Quorum Q150R S, Lambda Photometrics, Harpenden, UK), the samples were covered with a thin layer of technical gold. The surface morphology of the sample was imaged at the magnification of $3000\times$ using a secondary electron detector (SE detector) with a voltage range of 10 kV. The parameters of the microscopic examination were determined based on our own experience.

2.11. Color Measurement

Color measurements have been conducted using a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan). Before each measurement, 1 g of powder was placed in special plastic cuvettes. The CIE Lab color scale was used to measure color variation ranging from 0–100 for the following parameters: L^* —black to white, a^* —red (+) to green (−), b^* —yellow (+) to blue (−) [36].

2.12. Statistical Analysis

All data are presented as mean $\pm$ standard deviation (SD). Statistical significance was tested by analysis of variance (one-way ANOVA) followed by Tukey's test. All statistical analyses were performed using Statistica version 10 StatSoft Poland, (Krakow, Poland). Compared values were considered significantly different when $p < 0.05$.

3. Results and Discussion

3.1. Viability Rate of *Lacticaseibacillus rhamnosus* After Spray Drying and During Powder Storage

Adding oil and other hydrophobic substances to the microcapsules is an effective method of protecting the lactic acid bacteria. Mandal et al. [28] have shown that the viability of bacteria in the soybean oil matrix is higher than that of free microorganisms [28].

Othmana et al. [37] spray-dried *Lactobacillus bulgaricus* using a mixture of gum Arabic, gelatin, and coconut oil in various concentrations as protective substances. The content of individual components of the protective composition was at different levels: gum Arabic between 30–40%, gelatin at 20–25%, and coconut oil at 40–55%. The highest percentage of viability after spray drying was observed when gum Arabic and gelatin reached the maximum limit and coconut oil the minimum limit in the feed composition [37].

The most frequently used liquid oils are fish oil, sunflower oil, and soybean oil. Eratte et al. [27] have shown that the presence of tuna oil (15 g, 6% w/v) increases the viability of *Lacticaseibacillus casei*. They obtained 37.62% and 56.19% bacterial cell viability without and with tuna oil, respectively [27].

In our study, specially prepared emulsions with the addition of various liquid oils (in two concentrations) were spray-dried to obtain microencapsulated LGG bacteria in the form of dry powders. We showed that the addition of oils (regardless of their content

in the emulsion) increased the viability of bacteria both during the spray drying process and during storage for 4 weeks at 4 °C and 20 °C (± 1 °C). In all samples, the number of probiotics before spray drying was approximately at a level of 10^9 CFU/mL. The results of performed tests are shown in Figure 1A–D.

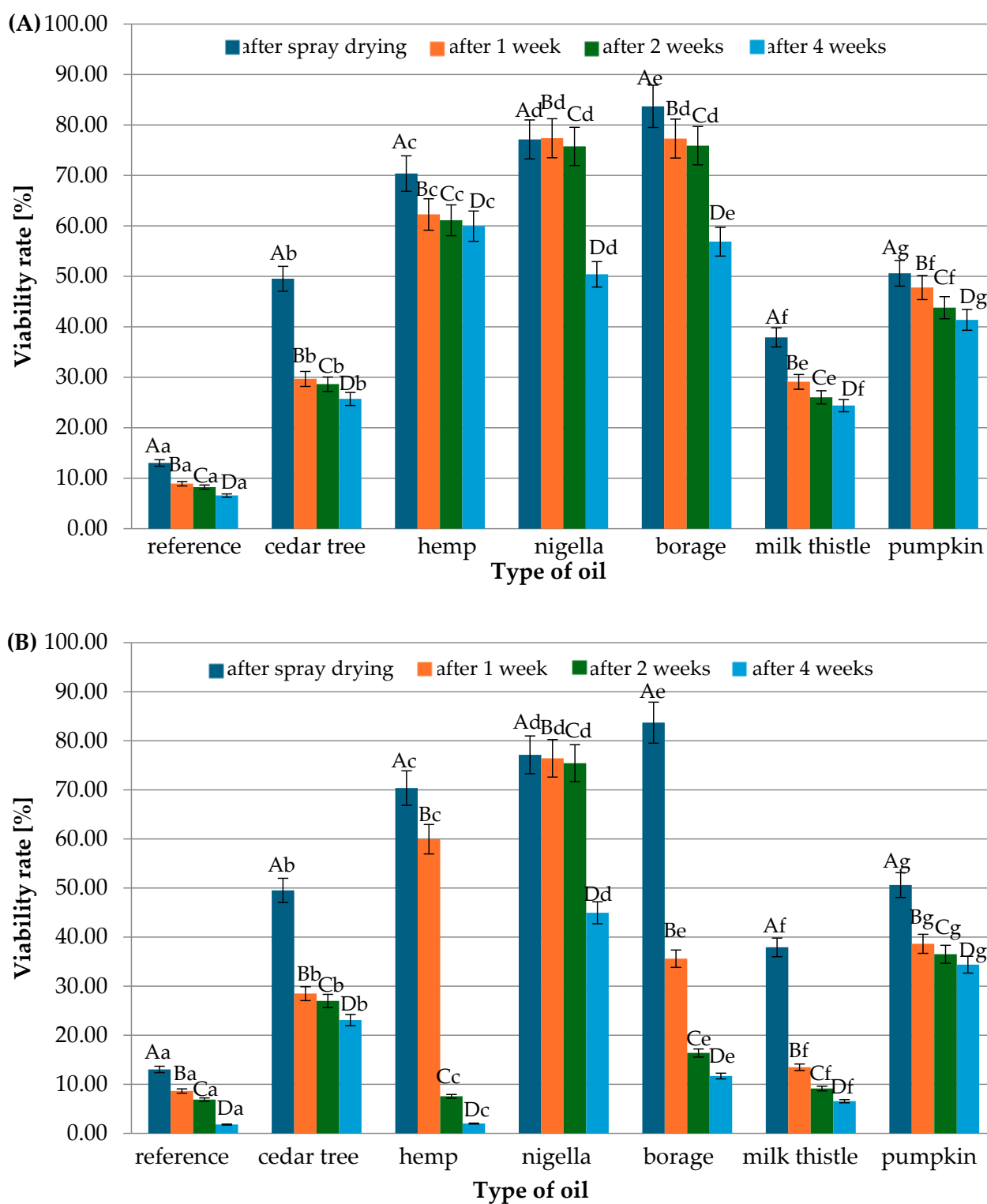


Figure 1. Cont.

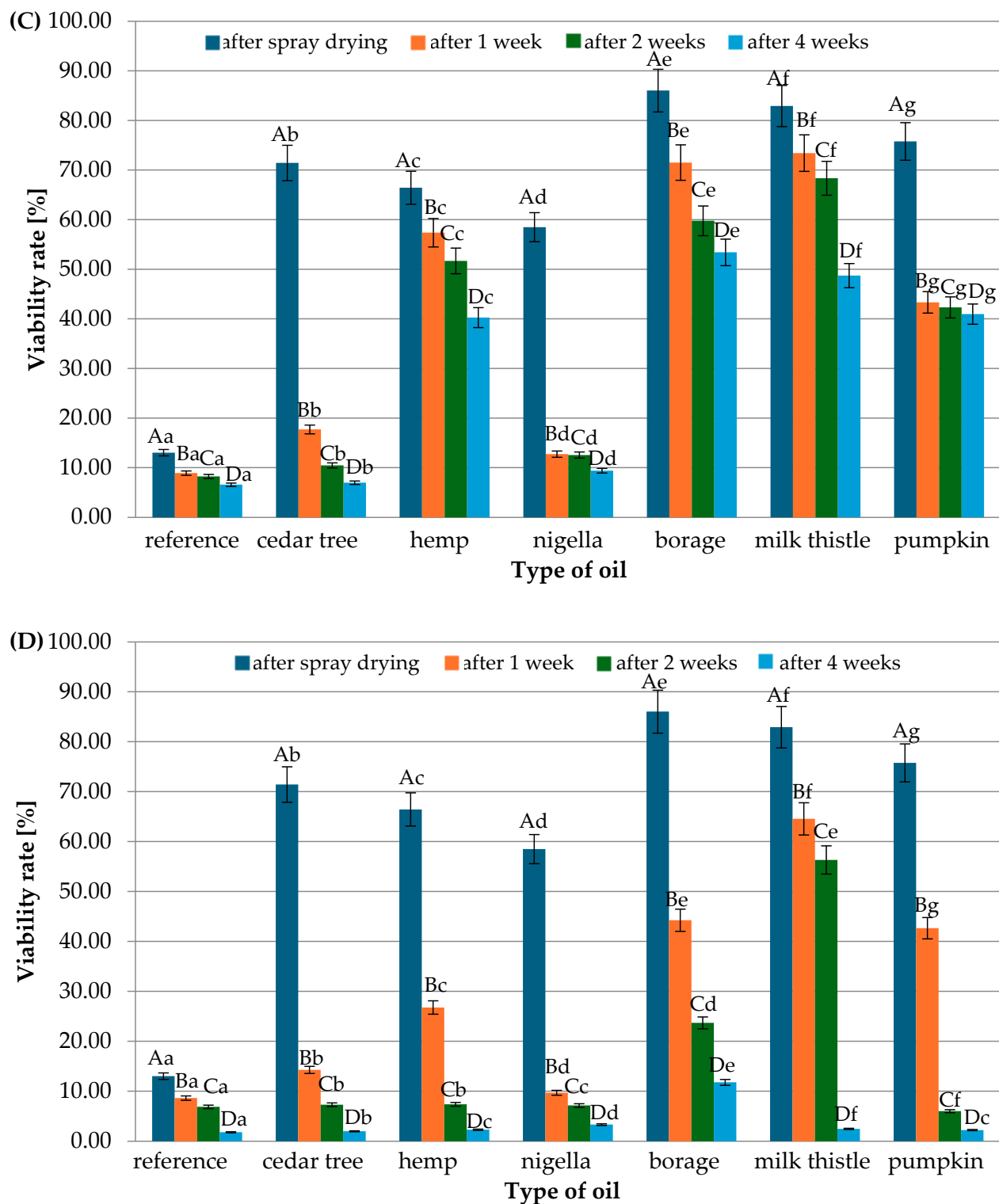


Figure 1. The effect of the concentration of various oils on the viability of LGG bacteria after the spray-drying and during the storage of various encapsulation systems (A) 20% of oils stored at 4 °C (B) 20% of oils stored at room temperature (C) 30% of oils stored at 4 °C (D) 30% of oils at room temperature. Values with different uppercase letters in the same column are significantly different at $p < 0.05$. Values with different lowercase letters in the same column are significantly different at $p < 0.05$. The statistical analyses were performed using Statistica version 10 StatSoft Poland, (Krakow, Poland).

The viability rate of LGG after spray drying in the control sample was only about 13% of the starting concentration. In the case of 20% oil content, the highest percentage

of bacterial cells that survived the drying process was observed when borage oil (83.69%) was added as a protective material, and the lowest when milk thistle oil (37.91%) was added (Figure 1A or Figure 1B). At a higher oil concentration of 30%, the best result was also obtained for borage oil (86.03%), where at the same time the lowest viability among all tested oils was observed for nigella oil (58.49%) (Figure 1C or Figure 1D). The above results show that the viability of bacteria after the drying process in the case of using each oil, regardless of its concentration, differed significantly from the control sample ($p < 0.05$) (Figure 1A–D).

The viability rate of the probiotic bacteria in final dry powders was determined after storage at 4 °C and 20 °C (± 1 °C) for 4 weeks. Higher bacterial viability was noted when the powder was stored in refrigeration conditions (regardless of the concentration of added oils) (Figure 1A–D). After 4 weeks of storage of the reference sample, at a temperature of 4 °C 6.57% of live bacteria remained, while respectively at room temperature only 1.80%. Regardless of the type and concentration of protective substance, in each case, a better result was obtained during storage in refrigerated conditions (Figure 1A–D). After 4 weeks of storage of the powders at room temperature (with 30% oil added in a ratio to the dry weight of the solution), viability in all samples was similar to the controls of 2–3.3% (except for the borage oil) (Figure 1D).

When the powders were stored the highest decrease of viable bacteria was observed for powders with 20% hemp oil stored at 20 °C from approx. 60% after 1 week to approx. 7.5% after 2 weeks (Figure 1B). Powders with 20% wt. added cedar tree oil and nigella oil stored for 4 weeks were characterized by a similar degree of bacterial viability at both refrigeration and room temperature conditions (Figure 1A or Figure 1B).

In the case of storage of the powder with the addition of 30% of cedar tree oil, a significant decrease in LGG viability was observed both during storage at 4 °C and 20 °C, with slight differences in viability during 4 weeks of storage at both temperatures. For powder with 30% nigella oil at both storage temperatures, the highest decrease in the percentage of viable bacteria was observed after 1 week, while with the addition of milk thistle oil after 4 weeks (Figure 1C or Figure 1D). These results show that bacterial survival after storage of the powders for 4 weeks with each oil (irrespective of its concentration and storage temperature) was significantly different from the reference sample ($p < 0.05$) (Figure 1A–D).

Liu et al. [38] spray-dried three lactobacilli isolates (LB1, S64, and K67) in 10% w/v sodium caseinate (NaCas) as a control, in 10% w/v NaCas in the presence of either low melting point fat (LMF) or vegetable oil. Among the LB1 isolate samples containing vegetable oil as the core material, viability after the spray drying process was almost the same (about 16%), not significantly different compared to the control sample ($p < 0.05$). In contrast, the addition of a low melting fat gradually increased the viability of LB1 from 16% to 63% as the LMF to wall ratio increased from 0.25 to 1.00 [38].

Agudelo et al. [39] observed, that the bacterial viability lasts longest at low temperatures when spray-dried *Lbs. rhamnosus* in the carrier based on the mixture of whey protein isolate and maltodextrin (WPI:MD), with the addition of sucrose or trehalose (S or T) as protective agents. Three formulations were spray-dried: WPI:MD 1:2, WPI:MD:S 1:1:1, and WPI:MD:T 1:1:1. They observed a decrease in cell viability ($-\log N/N_0$) of *Lbs. rhamnosus*, relative to the initial count, in probiotic systems stored for 3 months at 5 °C 4.5, 1.4, 1.6 and at 4 °C 3.9, 1.9, 1.9, respectively, for the following systems WPI:MD, WPI:MD:S and WPI:MD:T [39].

In general, the application of oils in this study results in higher viability. However, no direct relationship between a higher concentration of oils and the increase in the viability rate of *Lbs. rhamnosus* after the spray drying process was observed, both during the spray

drying and storage (Figure 1A–D). Figure 2 shows the viability rate of bacteria after spray drying as a function of the type and concentration of oil. When higher concentrations of oils such as cedar tree, borage, milk thistle, and pumpkin were applied, the viability of the bacteria after spray drying increased, while in the case of hemp and nigella oil, a decrease in viability was observed. Moreover, the effect of oil concentrations was relatively high, where one could observe the increase in viability for higher concentrations of cedar tree, milk thistle, and pumpkin oils, whereas in the case of nigella oil there is an opposite relation. Surprisingly in the case of hemp and borage oils, the changes were almost negligible (Figure 2).

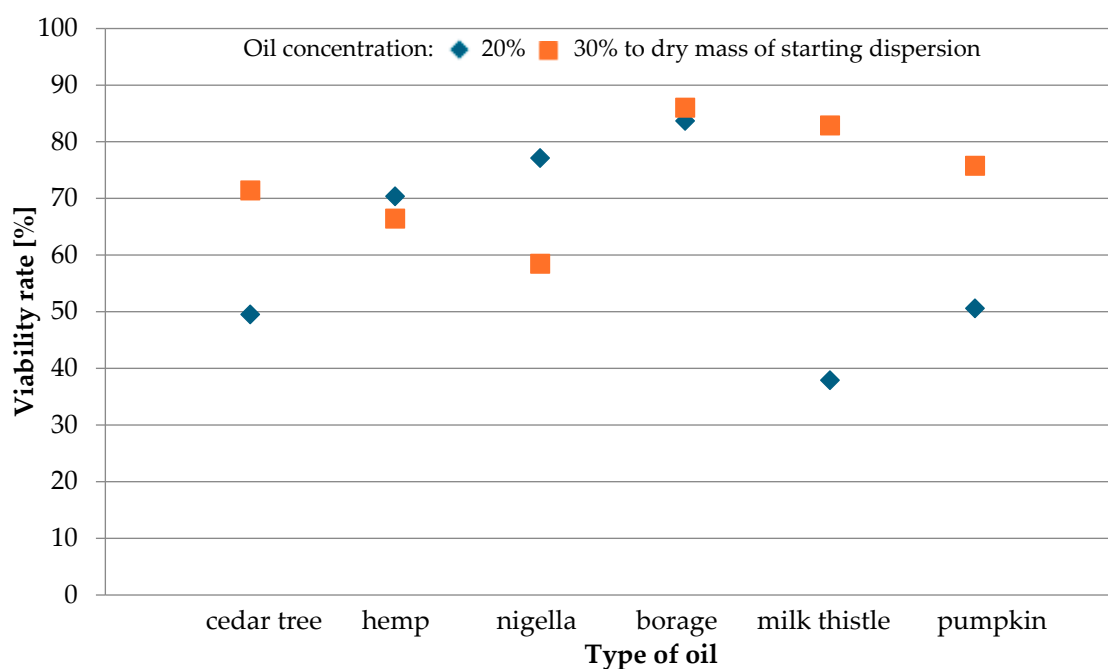


Figure 2. Viability rate of LGG bacteria after spray drying as a function of the type and concentration of oil.

3.2. The Composition of Oil and *Lactocaseibacillus rhamnosus* Viability

One of the assumptions of this study was to explain which fatty acids contained in the oils used to influence the viability of *Lbs. rhamnosus* during spray drying and during storage of the powder resulting from the drying process.

Oils containing more unsaturated fatty acids, due to their greater susceptibility to oxidation processes, are believed to reduce the oxidative stress of microorganisms during spray drying to a greater extent [40]. The content of saturated and unsaturated fatty acids in applied oils (information provided by the distributor) is presented in Table 1. Hemp oil contains the highest amount of both polyunsaturated and total unsaturated fatty acids, whereas pumpkin oil has the highest content of monounsaturated fatty acids.

Table 1. The content of saturated, mono-, poly-, and total unsaturated fatty acids in individual oils.

Type of Oil	Saturated Fatty Acids	Monounsaturated Fatty Acids	Polyunsaturated Fatty Acids	Total Unsaturated Fatty Acids
Cedar tree	7.0	25	63	88
Hemp	10	13	76	89
Nigella	16	24	59	83
Borage	16	27	57	84
Milk thistle	18	24	58	82
Pumpkin	19	28	53	81

In Figure 3 the effect of the polyunsaturated fatty acids content in tested oils on the viability of *Lbs. rhamnosus* GG bacteria during the spray drying process is presented. A better relationship between the content of polyunsaturated fatty acids and the viability of LGG during drying was observed for systems of higher 30% (Figure 3B) of oil in the dry mass of starting dispersion, compared to systems of 20% of oil (Figure 3A). However, in both cases, relatively low R-squared values show that the linear model does not fit and there have to be some additional factors besides polyunsaturated fatty acids content which influence the viability of bacteria cells during the spray drying process.

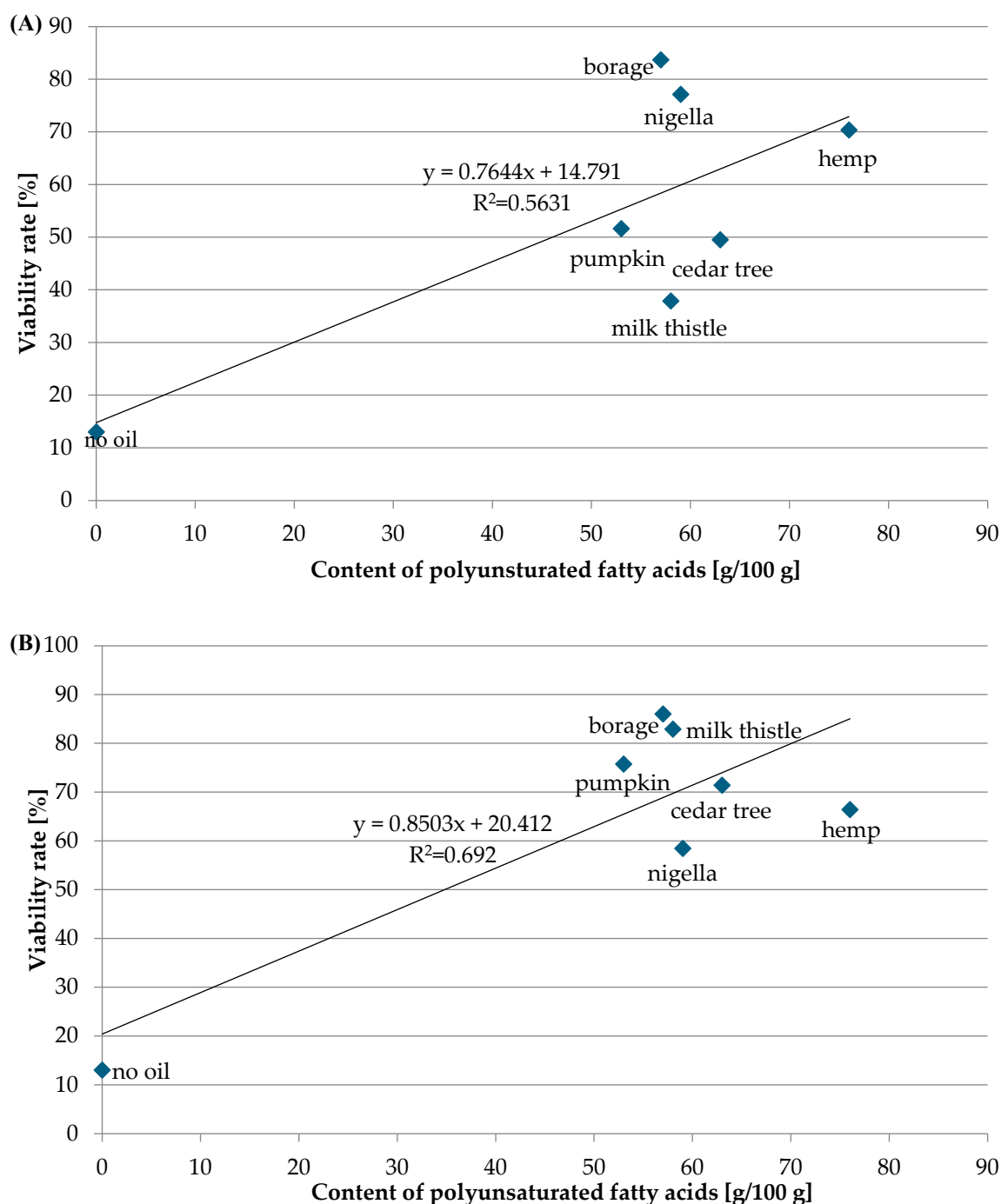


Figure 3. Viability rate as a function of polyunsaturated fatty acids content in oils used (A) systems of 20% of oil in the dry mass of starting dispersion (B) systems of 30% of oil in the dry mass of starting dispersion.

Additionally, it can be noticed that the values of the R-squared (R^2) coefficient of determination and the slope of line a for the equation $y = ax + b$ are higher with a higher share of unsaturated acids in the tested oils (30% by weight compared to 20%), which could confirm the beneficial effect of the content of unsaturated fatty acids on the increase in viability during the drying process.

In addition to unsaturated fatty acids, some polyphenols present in natural liquid oils can also act as antioxidants. For example, it was observed that sunflower seed oil, which is rich in polyunsaturated fatty acids and due to its high content of α -tocopherol, can reduce oxidative damage to bacteria during the spray drying process [41].

Due to some relationships between final viability bacteria during spray drying and the content of unsaturated fatty acids, the corresponding most common types of unsaturated fatty acids and their content, the amount of vitamin E present in applied oils, and the viability rate of LGG have been compiled in Table 2.

Table 2. The type of unsaturated fatty acids and their concentration, vitamin E content (information based on the distributor), and viability rate of *Lbs. rhammosus* GG after spray-drying both oil systems.

Type of Oil	Monounsaturated		Polyunsaturated		Vitamin E [mg]	Viability Rate of LGG [%]	
	Oleic Acid [g/100 g]	Linoleic Acid [g/100 g]	α -Linoleic Acid [g/100 g]	γ -Linolenic Acid [g/100 g]		20% of Oil Content	30% of Oil Content
Cedar tree	25	43	-	20	70	49.42	71.43
Hemp	-	55	16	-	78	70.37	66.44
Nigella	24	56	-	-	78	77.13	58.49
Borage	18	37	-	20	78	83.69	86.03
Milk thistle	24	56	-	-	48	37.91	82.91
Pumpkin	26	52	-	-	-	51.64	75.77

The main components of most oils are both oleic (except hemp oil) and linoleic acids. Cedar tree and borage oil also contain γ -linoleic acid and hemp oil—alpha-linoleic acid. All oils contain additionally vitamin E (except pumpkin oil).

Generally, the highest percentage of living bacterial cells, both after spray drying and after storage, was recorded when borage oil was added as a protective material, both in the case of their 20% and 30% content in the dry mass. The borage oil has the lowest content of oleic and linoleic acid and a relatively high content of vitamin E with the additional presence of γ -linoleic acid. In the case of 20% oil, the lowest percentage of bacterial cells that survived the drying process was recorded for milk thistle oil (37.9%), whereas for the 30% system, the lowest one was for nigella oil (58.5%). Both, nigella and milk thistle oils have higher content of oleic and linoleic acid than borage oil. Nigella oil has the same amount of vitamin E as borage oil (Table 2).

A relatively high viability rate of bacteria was also observed for systems with hemp oil (especially after adding 20% oil), which contains alpha-linoleic acid and doesn't contain oleic acid. Pumpkin oil has almost the same content of oleic acid and linoleic acid as that of milk thistle oil. Pumpkin oil hasn't vitamin E (while milk thistle has 48 mg/100 g), however, presents a stronger protective effect in the case of 20% oil (51.64% vs. 37.91%). Pumpkin oil (without vitamin E and without γ -linolenic acid content) demonstrated a stronger protective effect compared to cedar tree oil, which has vitamin E and γ -linolenic acid in its composition (Table 2).

Ngamekaue et al. [42] have spray-dried *Limosilactobacillus reuteri* KUB-AC5 bacteria using 10% (w/w) whey protein isolate (WPI) alone and WPI (91.14% protein, 3.14% carbohydrates) with 4% (w/w) and 8% (w/w) coconut oil (CO) added as an encapsulating agent. They observed that the addition of coconut oil protected the bacteria during the

drying process. Using the same drying conditions, 4.28% cell survival was obtained with the addition of WPI, 15.50% with the addition of WPI and 4% (w/w) CO, and 11.06% with WPI and 8% (w/w) CO. Coconut oil contained 92.85% saturated fatty acids [42].

The research results presented in this study refer to two of our other papers in which, among other things, we analyzed the fatty acid profile in the fatty substances used as protective additives and wanted to demonstrate their effect on the viability of *Lbs. rhamnosus* as a model probiotic strain during the spray drying process and during powder storage.

In our other study, in which we investigated the effect of different types of butter on the viability rate of *Lbs. rhamnosus* GG, analysis of the fatty acid profile showed that the high bacterial viability was influenced (among other things) by the lower content of palmitic acid (C16:0) as well as, the lowest amounts of monounsaturated and polyunsaturated fatty acids (compared to other butters) [43].

Our 2023 study, which investigated the effect of other solid fats on LGG viability, showed that C 18:1 oleic acid and C 18:2 linoleic acid, as well as total monounsaturated fatty acids and C:2 and C:3 polyunsaturated fatty acids, may determine the protective effect of the tested triacylglycerols on bacteria [35].

As the above shows, the type of fatty acids has an ambiguous effect on the viability of probiotic bacteria during the spray drying process.

3.3. The Thermal Characterization of Oils Using Differential Scanning Calorimetry (DSC) and the Effect of the Melting Temperature of Oils on the Viability Rate of Bacteria Cells After Spray Drying

The most widely used technique for the thermal characterization of oils is differential scanning calorimetry (DSC). To characterize the thermal behavior of various oil samples, commonly both melting and crystallization heat transfer parameters are used, which requires the intake or release of thermal energy that corresponds to process enthalpy [44].

For the oil samples used in this study, DSC measurements were made to compare the melting and crystallization transition temperatures. For all oil samples, one could observe that the first exothermic peaks are between -67.7 and -58.6 °C, except for borage oil. In this temperature range, these oils have a tendency to crystallize. No exothermic peak occurred for the borage oil sample as it doesn't release energy to the system, whereas an endothermic peak was obtained for it at a similar temperature at -63.70 °C. This oil is the only one that begins to melt at such a low temperature, which is due to the slightly different content of specific fatty acids. It has the lowest content of both oleic and linoleic acid (compared to other oils), secondly, it has a relatively high content of vitamin E and the additional presence of γ -linoleic acid (Table 3). As noted above, the highest bacterial cell viability during the spray drying process was observed for both 20% and 30% addition of borage oil (Figure 1). For all oil samples, there are endothermic peaks in the range between -31 °C and -24.7 °C which refers to their melting processes. The additional endothermic peaks were observed in other temperatures: for pumpkin oil -14.45 °C and -7.64 °C, for borage oil -9.61 °C and for milk thistle -3.89 °C (Table 3). Liu et al. [38] performed DSC analysis in the temperature range from 0 °C to 80 °C. There were not any endothermic and exothermic peaks for emulsions based on vegetable oils. Similarly in this study, the transition peaks in the temperature range 20 °C to 100 °C which is characteristic for the whole spray-drying process have been not observed [38].

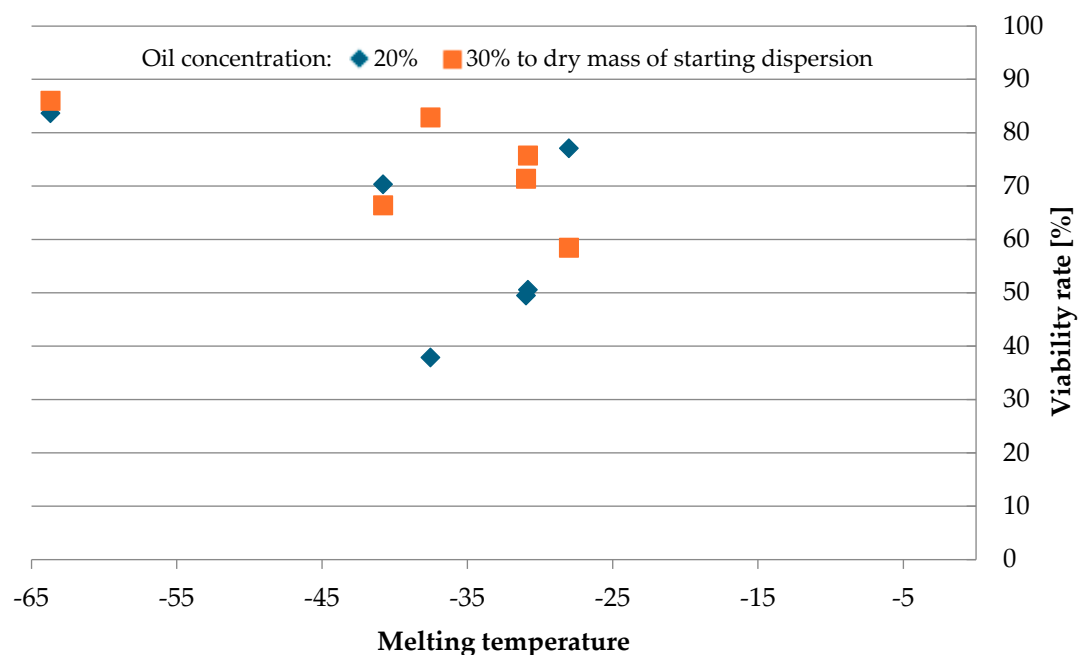
Yin et al. [45] spray dried LGG in coacervates formed by whey protein isolate-high melting point fat shortening oil (SO) and gum arabic through complex coacervation. DSC analysis showed that the addition of shortening oil reduced the temperature of particles by increasing the enthalpy of melting during the spray drying process [45].

Table 3. Comparison of transition temperatures for melting and crystallization of different oils based on DSC measurements.

Type of Oil	Transition Temperature [°C]			
	1	2 Melting	3	1 Crystallization
Cedar tree	−30.98			−58.64
Pumpkin	−30.85	−14.45	−7.64	−65.60
Borage	−63.70	−27.32	−9.61	−
Nigella	−28.03			−61.21
Hemp	−40.81	−29.91		−63.17
Milk thistle	−37.55	−24.71	−3.89	−67.10

In general, the thermal properties of different oils can be characterized by various transition temperatures. DSC provides useful information about thermodynamic changes that are associated with the oils transforming from one to another physical state.

We have also tried to verify whether the melting temperature of oils and their final content in the system correlate with the viability rate of bacteria cells after spray drying (Figure 4). It was observed that in the case of milk thistle, pumpkin, hemp, and nigella oil, the viability rate of LGG after the drying process with the addition of a smaller (20% wt.) amount of these oils increases with the increase of the melting temperature of these oils, while in the case of a larger amount (30% wt.) of these oils, there is an opposite relation. This indicates that during the spray-drying process, not only the type of oil but also the final content in the system influences the viability rate of bacteria.

**Figure 4.** Viability rate after spray drying as a function of the content of oil used and its relation to the melting temperature (in blue 20% and in red 30% of oil).

3.4. Moisture Content, Water Activity, and Particle Size $D_{4,3}$ Parameter of Powders

The values of moisture content, water activity, and particle size $D_{4,3}$ parameter of spray-dried microcapsules formulated by adding different oils with various oil/dry mass ratios (20 and 30%) are displayed in Table 4. The moisture content of the reference sample was $4.04\% \pm 0.04$. For powders with 20% of oil the moisture content was in a broad range from $3.28\% \pm 0.01$ (milk thistle oil) to 5.95 ± 0.02 (pumpkin oil), whereas for powders of 30% oil, the results range even broader from $2.72\% \pm 0.02$ (hemp oil) to $5.9\% \pm 0.01$ (cedar tree oil) (Table 4).

Table 4. Water content, water activity a_w , and particle size parameter $D_{4,3}$ of spray-dried powder.

Type of Oil	Water Content		a_w		$D_{4,3}$ [μm] of Powders	
	20% *	30% *	20% *	30% *	20% *	30% *
Reference sample	4.04 ± 0.035^a		0.120 ± 0.02^a		29.4 ± 2.1^a	
Pumpkin	5.95 ± 0.017^b	5.8 ± 0.026^b	0.277 ± 0.003^b	0.225 ± 0.002^b	415.7 ± 2.8^b	463.8 ± 0.8^b
Cedar tree	4.7 ± 0.026^c	5.9 ± 0.010^c	0.181 ± 0.002^a	0.224 ± 0.003^b	433.2 ± 3.3^c	724.5 ± 2.5^c
Nigella	5.35 ± 0.010^d	5.56 ± 0.017^d	0.373 ± 0.002^c	0.128 ± 0.001^c	175.9 ± 2.6^d	321.1 ± 1.9^d
Hemp	4.82 ± 0.010^c	2.72 ± 0.017^e	0.151 ± 0.001^a	0.118 ± 0.002^a	396.2 ± 5.2^e	498.8 ± 1.4^e
Borage	5.9 ± 0.017^b	5.41 ± 0.020^f	0.335 ± 0.002^c	0.258 ± 0.003^d	222.8 ± 7.5^f	321.5 ± 1.9^d
Milk thistle	3.28 ± 0.010^e	5.49 ± 0.026^g	0.230 ± 0.01^d	0.258 ± 0.001^d	54.6 ± 2.7^g	506.8 ± 2.0^f

Values are mean $\pm$ standard deviations of triplicate determinations. * a ratio of oils to dry mass of starting dispersion. Values with different lowercase letters in the same column are significantly different at $p < 0.05$. The statistical analyses were performed using Statistica version 10 StatSoft Poland, (Krakow, Poland).

The lowest water content was in the microcapsules formulated with the addition of hemp oil with a ratio of oil/dry mass of a solution of 30% ($2.72\% \pm 0.02$) and the highest with *pumpkin oil* in a ratio of 20% ($5.95\% \pm 0.02$). The moisture content in all samples, both with the addition of lower (20%) and higher (30%) content of oils, significantly differs from the reference sample ($p < 0.05$). The samples with a 20% addition of pumpkin and borage oil, as well as cedar and hemp oil, do not differ significantly from each other ($p > 0.05$), while all samples with a 30% addition of oils differ significantly from each other ($p < 0.05$) (Table 4).

Eratte et al. [27] spray-dried *Lactocaseibacillus casei* 431 using the system based on whey protein isolate, gum Arabic (WPI-GA), and in whey protein isolate, tuna oil, gum Arabic (WPI-O-GA), obtained the final moisture content of the powders 2.90 ± 0.2 and $3.19 \pm 0.2\%$, respectively. By adding tuna oil during the SD process, they obtained 56.19% of live bacteria, while without the addition of oil, only 37.62% [27].

According to Liao et al. [46], lower water amounts ($<6\%$) are required to prolong the shelf life of probiotic powders, which complies with the results presented in this study [7]. However, no relationship between the amount of oil added and the moisture content in the powder was observed. There was also no relationship between moisture (both using 20% and 30% oils) and viability immediately after spray drying (Table 4).

The water activity (a_w) of the reference sample was 0.120 ± 0.02 . The a_w value ranges from 0.118 ± 0.002 (hemp oil in a ratio of 30% to dry mass of solution) to 0.373 ± 0.002 (nigella oil in a ratio of 20%). In powders with 20% oil, the results range from 0.151 ± 0.001 (hemp oil) to 0.373 ± 0.002 (nigella oil), whereas in powders with 30% oil they are in the range from 0.118 ± 0.002 (hemp oil) to 0.258 ± 0.003 for both milk thistle and borage oils. In general, when a smaller amount of pumpkin, nigella, hemp, and borage oil was used, the water activity of the powders after drying was higher than when a larger amount of these oils was applied, while an opposite relation was observed for cedar tree and milk thistle oils. The a_w value of powders with the addition of hemp oil, both 20% and 30%, does not differ significantly from the reference sample ($p > 0.05$). The water activity of the powder with the addition of 20% cedar tree oil also does not differ significantly from the control sample ($p > 0.05$). In the case of other powders with lower (20%) and higher (30%) oil content, significant differences in a_w values are observed ($p < 0.05$). Higher water activity values have been observed to correlate with better viability when using cedar tree, nigella, hemp, and milk thistle oil, for both oil contents. When using pumpkin and borage oil, the relationship is reversed (a higher percentage of live *Lbs. rhamnosus* corresponds to lower a_w), however, in the case of borage oil, the difference in viability is relatively small, maybe due to already high viability rate of bacterial cells for systems with addition of this oil.

Picot and Lacroix spray-dried fresh cultures of *Bifidobacterium breve* R070 (BB R070) and *Bifidobacterium longum* R023 (BL R023) using milk fat and/or denatured whey proteins as immobilizing material. The water activity and moisture content of the obtained powders

were at an acceptable level for food powders. The same a_w value was obtained for BB R070 with both encapsulation systems, and for BL R023 with added milk fat and denatured whey proteins (0.16). For BL R023 with added milk fat, the water activity value was 0.18. The moisture content for *Bifidobacterium breve* R070 was 1.95 and 1.40, and for *Bifidobacterium longum* R023 it was 2.05 and 2.05. These results were obtained for powders with added both milk fat, and milk fat and denatured whey protein, respectively [11].

Eratte et al. [27] found out for microencapsulated *L. casei* 431 by spray drying in either whey protein isolate, gum Arabic (WPI-GA) system or whey protein isolate, tuna oil, gum Arabic (WPI-O-GA), that the water activity of final powder 0.23 ± 0.004 and 0.26 ± 0.014 %, respectively with final conclusion of positive effect of tuna oil on final number of active bacteria cells [27]. Liao et al. (2017) have reported that the best expected a_w values to store probiotic powders were between 0.07 and 0.11 [46]. According to Viernstein et al. [47], lower a_w (0.2–0.3) caused better cell viability during storage [47]. In the case when powders are stored at room temperature, water activity is a key factor for the high viability of lactic acid bacteria [48].

Another example would be spray drying of three *Lactobacillus* isolates in 10% w/v sodium caseinate (NaCas) with added LMF or vegetable oil, with a ratio of oil or LMF to NaCas of 1:1. In both types of microcapsules the moisture content of the powders was approximately 3.3 and 3.7% wt., and the water activity was 0.19 and 0.20 for oil + NaCas and LMF + NaCas, respectively [38].

In addition, it is known that the high a_w value contributes to the growth of other microorganisms including bacteria and fungi, causing undesirable chemical reactions and as a consequence the reduction of the number of probiotic bacteria and shorter shelf-life [49]. In this case, the selection of proper protective substances is important, because different substances have different capabilities of absorbing water molecules, both during the drying and post-storage process. These properties help to keep the dormant state of probiotics acquired in the drying process during the whole storage [50]. After 4 weeks of storage at room temperature, in the case of powders with the addition of more oils, one could observe a more rapid decrease in the percentage of live *Lbs. rhamnosus* in powders with higher a_w values. In the case of powders with an a_w in the range of 0.225–0.258, the reduction in viability was at the level of approx. 70–81.5%, while in the case of water activity of 0.118 and 0.128, it was approx. 64.5% and approx. 52%, respectively. No relationship was observed for powders with 20% wt. content of oil stored for 4 weeks at 20 °C.

The average size of powder particles may affect their final properties, including their stability and efficiency of microencapsulation. Microcapsules with bigger mean diameters due to a lower surface-to-volume ratio offer better protection against external factors than smaller ones, but at the same time, they have lower dispersibility in final products. However, sometimes too small particles may be so-called “hollow microcapsules” that have no core materials embedded [51].

The particle size parameter of the microcapsules presented as $D_{4,3}$ values are displayed in Table 4. The particle size $D_{4,3}$ parameter in all powders, both with the addition of lower (20%) and higher (30%) content of oils, significantly differs from the reference sample ($p < 0.05$).

The highest average particle size ($506.8 \pm 2.0 \mu\text{m}$) was observed for powder with the addition of milk thistle oil in the higher ratio (30%) to dry mass of solution, whereas the lowest ($54.5 \pm 2.7 \mu\text{m}$) was noticed for powder with the same oil but in a ratio of 20%. The average particle size of the reference sample was $29.4 \pm 2.1 \mu\text{m}$. It was observed that the average particle size of the powder rises with increasing the oil concentration. Most of the diameters of microcapsules in this study (Table 4) are very high, which may be due to the

fact that the powder particles have the tendency to form aggregates, especially in the case of higher oil load.

Zhao et al. [51] spray drying *L. acidophilus* using as wall materials β -cyclodextrin and acacia gum in a ratio of 9:1 (w/w), and microcapsules were prepared at four different concentrations of wall materials (15, 20, 25, and 30% (w/v)). They obtained the particle diameters of microcapsules that were mostly between 6.6 and 60.2 μm and varied with sizes ranging from 2.8 to 120.2 μm [51].

Khem et al. [52] microencapsulated by spray drying two strains of *Lpb. plantarum*, A17 and B21, with different WPI concentrations. Microcapsules prepared at lower concentrations (10%) resulted in smaller particles in the range of 1 to 7.8 μm , and at a higher concentration of 20% had with sizes ranging between 2 and 14 μm and those for 30% had diameters of up to 23 μm [52].

3.5. Particle Size Distribution and Surface Morphology of Powders

The viscosity of solutions subjected to drying is influenced by the type of ingredients used and their concentration. Depending on the viscosity of the solution the average size of powders can be modified. Where together with higher viscosity, one could observe the increase in both: sizes of formed liquid droplets and as a result of final dry powders. High molecular weight materials that promote gelation, can lead to an increase in the diameter of the spray-dried powders [53].

As shown in Figure 5, one could observe significant differences in the particle size distribution of analyzed powders with both 20% and 30% oil. Materials which are partially insoluble in water and therefore form a suspension tend to form agglomerates when dried [54]. Therefore, for final confirmation of their “real size” and examination of surface morphology, we used the additional microscopic observations.

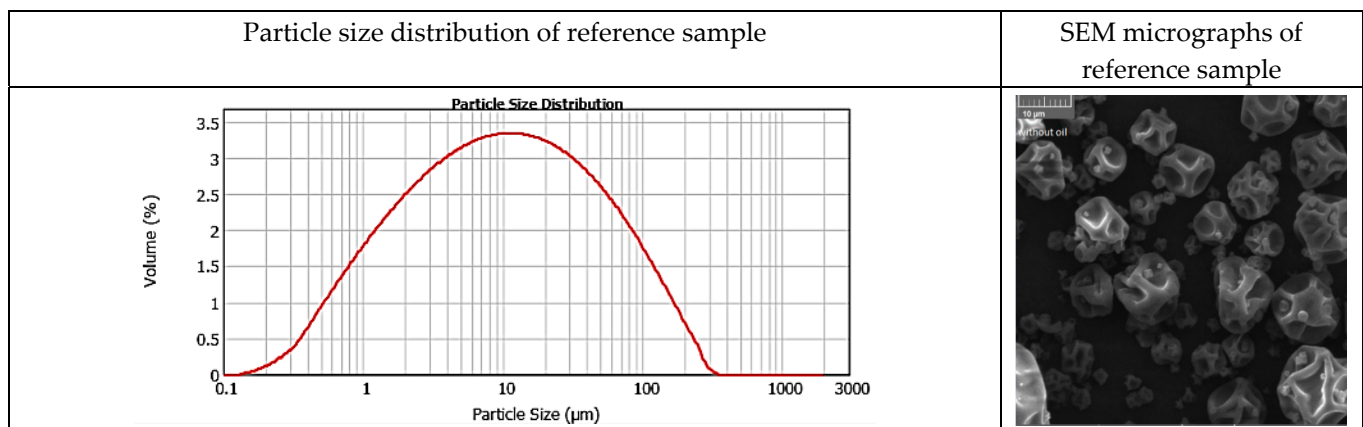


Figure 5. Cont.

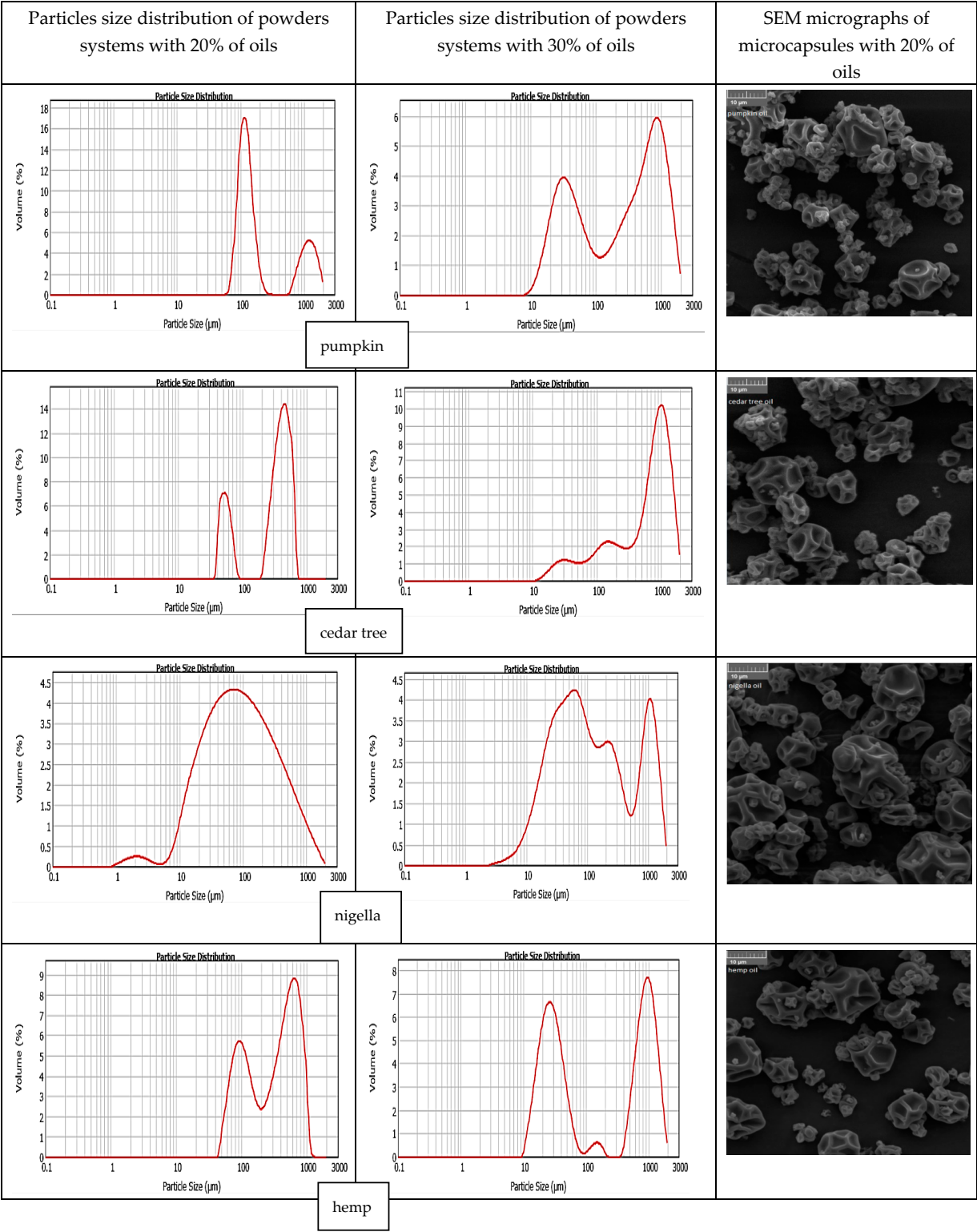


Figure 5. Cont.

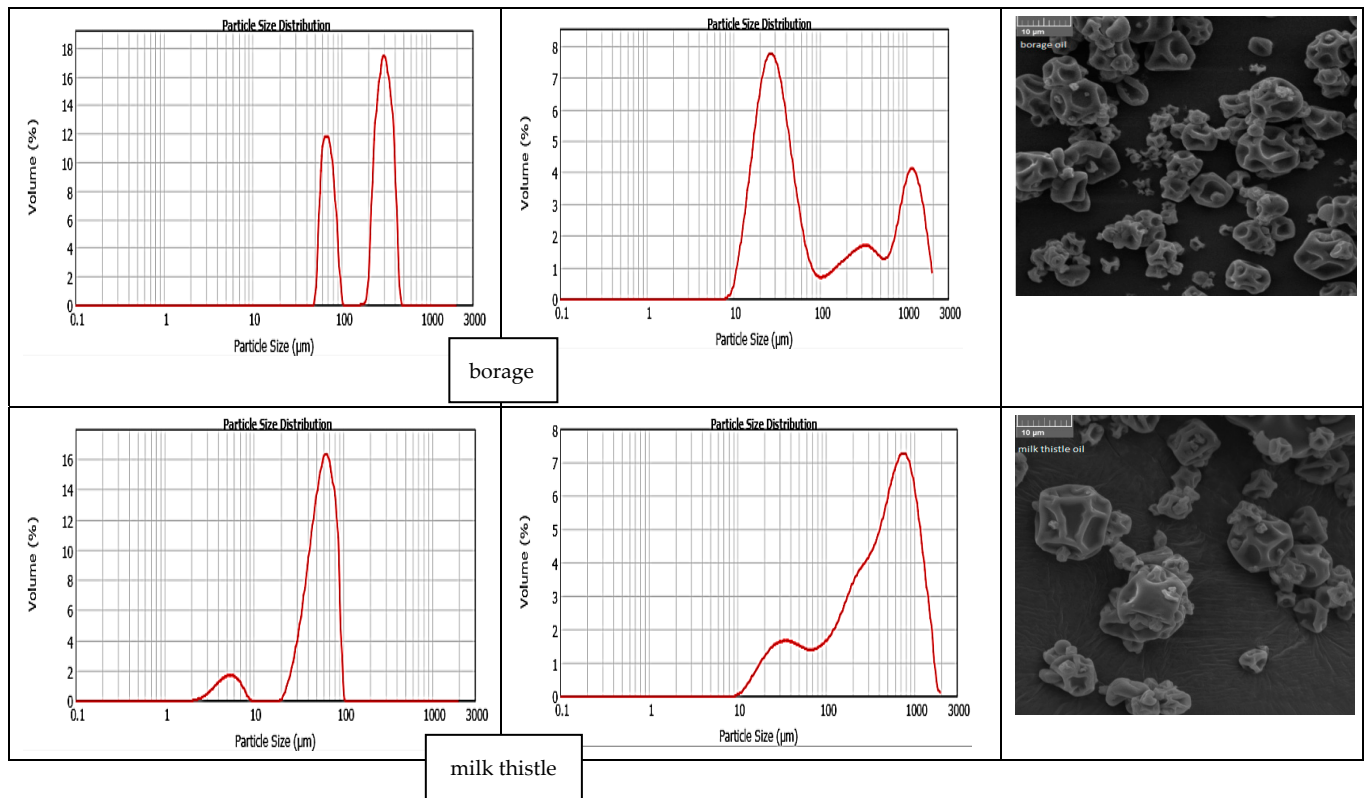


Figure 5. Particle size distribution of powders systems with 20% wt. and 30% wt. and SEM micrographs of spray-dried powders with 20% wt. of different oils.

The reference powder sample has a monomodal particle size distribution, while all powder systems with 20% oil have a bimodal distribution of particles, and powders with 30% oil are mostly trimodal (except pumpkin and milk thistle) (Figure 5). Therefore, such examination of particle size distribution is very important because it confirms the entire range of powder particle sizes.

The close analysis of powder particles by scanning electron microscopy confirms that the LSD results of the average particle size $D_{4,3}$ reflect various forms of agglomerates than to the average size of single particles.

The SEM micrographs in Figure 5 show the surface of various microcapsules with 20% of evaluated oils. The surface morphologies of microcapsules with a higher oil content (30%) were very similar and, therefore, they are not presented in this publication.

All collected photos generally indicate that capsules are significantly smaller in size than those measured with the LSD technique. Probably during these measurements, the dispersion energy of flow air was too low to overcome the interactions between the microcapsules in powder form. Therefore, it is important to use both $D_{4,3}$ parameters and analyze whole diagrams of particle size distributions and compare them with observations by scanning electron microscopy (SEM) for accurate and precise real particle size analysis.

No cracks were noticed on the external surfaces of various particles, which indicated good protection of immobilized probiotics against various gasses, including oxygen and moisture, resulting in high encapsulation efficiencies and good effectiveness of protection, both just directly after the drying process and during long-term storage [55].

Barajas-Alvarez et al. [50] encapsulated *Lbs. rhamnosus* cells by spray drying using 10% (w/w) gum Arabic, 5% (w/w) gum Arabic blended with 5% (w/w) agave fructans/maltodextrin/inulin or trehalose as wall materials. They also observed particles with a partially spherical shape did not show visual fractures or cracks on their surface [50].

Powder particles containing different oils had similar morphology, indicating that the type of oil had no effect on the appearance of the microcapsules. Most of the microcapsules were spherical in shape with dimpled surfaces and sizes in the range from 0.4 to 21 μm .

Similar SEM micrographs of the microcapsules containing *L. acidophilus* NCIMB701748 stabilized in a 3-component carrier based on maltodextrin: whey protein concentrate: D-glucose 60:20:20 (w/w) were obtained by Behboudi-Jobbehdar S. et al. [56]. They observed that the structure of the powder had a partially collapsed spherical shape [56]. According to Aniesrani Delfiya D.S. et al. [57] these irregularities were formed due to the slow film formation during SD of the atomized droplets and uneven shrinkage during the early stages of drying [57].

3.6. Color Measurement

The color of spray-dried powders can be influenced by their structure, packing density, color of individual carrier components, and processing conditions, e.g., overheating during the spray drying process or long residence times in the drying chamber [27]. The color of spray-dried powders can be also affected by the type and content of coating materials [58].

Table 5 shows the main color parameters of the obtained powders. In general, the L^* value corresponds to the lightness in the range from black (0) to white (100), the positive a^* value refers to a red color, and a positive b^* value indicates to yellow coloration of the powder. All results are average values of 5 measurements with standard deviation. The values of the L^* , a^* , and b^* parameters of all samples, both with the addition of 20% and 30% oils, differ significantly from the control sample ($p < 0.05$) (only for the powder with the addition of 20% pumpkin oil, there are no significant differences in the parameter a^* , compared to the reference sample ($p > 0.05$)).

Table 5. Effect of the type of oils and their concentrations (20 and 30% in a ratio to dry mass of starting dispersion) on color characteristics of the spray-dried powders.

Type of Oil	L^*		a^*		b^*		ΔE	
	20% **	30% **	20% **	30% **	20% **	30% **	20% **	30% **
Reference sample	89.64 $\pm$ 0.01 ^a		0.26 $\pm$ 0.01 ^a		13.70 $\pm$ 0.01 ^a		20% **	30% **
Milk thistle	88.35 ^b	87.36 ^b	0.85 ^b	0.54 $\pm$ 0.01 ^b	14.56 ^b	13.70 $\pm$ 0.01 ^a	1.20	1.49
Pumpkin	86.82 ^c	87.26 ^c	0.24 $\pm$ 0.01 ^a	0.07 ^c	12.08 ^c	14.03 ^b	2.07	1.55
Cedar tree	89.31 ^d	86.62 ^d	0.79 ^c	0.63 ^d	12.02 ^d	12.92 ^c	1.31	2.05
Nigella	87.08 ^e	86.38 ^e	0.50 $\pm$ 0.01 ^d	0.57 ^e	13.73 ^e	13.20 $\pm$ 0.01 ^d	1.66	2.14
Hemp	88.96 ^f	82.63 ^f	0.82 ^e	0.13 $\pm$ 0.01 ^f	13.02 ^f	13.36 $\pm$ 0.01 ^e	0.95	4.57
Borage	88.53 ^g	75.61 ^g	0.75 $\pm$ 0.01 ^f	0.17 ^g	12.46 ^g	11.13 ^f	1.24	9.59

The values presented are the average of three determinations $\pm$ standard deviation. Values with different lowercase letters in the same column are significantly different at $p < 0.05$. ** oil content in relation to the dry mass of the initial dispersion. L^* for the lightness from black (0) to white (100). a^* from green (−) to red (+). b^* from blue (−) to yellow (+). The statistical analyses were performed using Statistica version 10 StatSoft Poland, (Krakow, Poland). $0 < \Delta E < 1$ The difference is unnoticeable. $1 < \Delta E < 2$ The difference is only noticed by an experienced observer. $2 < \Delta E < 3.5$ The difference is also noticed by an inexperienced observer. $3.5 < \Delta E < 5$ The difference is clearly noticeable. $\Delta E > 5$ Gives the impression that these are two different colors.

The control sample was the brightest. Regardless of the type and concentration of oil, the powders were relatively light (L^* values in a range from 76 to 89). Powders with higher oil content were observed to be lighter (L^* value) in color (except for pumpkin oil) towards a shade of green (a^* value) (except for nigella oil). No significant influence of the oil type and their concentration on the b^* value was observed.

Bhagwat et al. [59] had microencapsulated probiotic *Enterococcus* strains using whey protein and maltodextrin as an encapsulating matrix. They obtained L^* values of 70.0 ± 0.2 , 71.3 ± 0.5 , and 70.6 ± 0.5 for different bacterial strains. All samples were characterized by a

positive a^* value suggesting a tendency to red color hues and positive b^* values indicating a yellowish hue color [59].

Whereas De Castro-Cislaghi et al. [60] microencapsulated *Bifidobacterium* Bb-12 by spray-drying using whey as a carrier material. They received the high-value L^* (85.96 ± 1.81), indicating that the powder showed light color, the high parameters of a^* (0.95 ± 0.05) and b^* (11.42 ± 0.44), indicating shades tending to red and yellow, respectively. This may be because of the Maillard reaction between reducing sugars and proteins [60].

Also provided in Table 5 are the ΔE (DeltaE or dE) values, which are a measure of the change in visual perception between two colors when comparing a reference sample with a given sample [61]. The color difference between two colors is described as the distance between the involved locations ($L1$, $a1$, and $b1$ as well as $L2$, $a2$, and $b2$) and is calculated using the Euclidean distance Formulas [62]:

$$\Delta E_{ab} = \sqrt{(L1 - L2)^2 + (a1 - a2)^2 + (b1 - b2)^2}$$

The delta E values are described in the legend of Table 5 [63]. The difference in color of just one powder (with an additional 20% of hemp oil) compared to the control sample is unnoticeable. The delta E value of most samples is in the range of 1–2 (The difference is only noticed by an experienced observer) or slightly above 2 (the difference is also noticed by an inexperienced observer). Only the powder with 30% borage oil added gives the impression of two different colors compared to the control sample ($\Delta E = 9.59$).

4. Conclusions

Probiotic bacteria of *Lbs. rhamnosus* GG can be protected during both the spray drying and storage processes by the proper selection of an encapsulation system.

The addition of plant oils to the spray-dried encapsulation systems has been shown to have a protective effect on *Lbs. rhamnosus* GG during storage both at room and refrigerated temperature (compared to the control sample). Stored powders are more stable at 4 °C (viability in all samples was higher). However, no direct correlation was observed between the increase in bacterial viability and the concentration of the applied oils. The LGG microcapsules with matrix based on starch, WPC, soy lecithin, ascorbic acid, and various plant oils at two 20% and 30% wt. concentrations revealed properties of acceptable standards. The moisture content value in all samples is within acceptable limits for food powders, but no relationship between moisture (both using 20% and 30% of oils) and viability immediately after spray drying. Higher water activity values were observed to correlate with higher bacterial viability immediately after drying after using four oils (cedar tree, nigella, hemp, and milk thistle, for both oil contents). When using the other two (pumpkin and borage oil) the relationship is reversed (a higher percentage of live *Lbs. rhamnosus* corresponds to a lower a_w). After 4 weeks of storage at room temperature, in the case of powders with higher amounts of oils added, a faster decrease in the percentage of live *Lbs. rhamnosus* was observed in powders with higher a_w values. No relationship was observed for powders with 20% wt. oil content stored for 4 weeks at 20 °C.

The highest viability of bacterial cells after spray drying 83.7% and 86.0%, was recorded for systems with borage oil respectively for systems with 20% and 30% oil content. DSC measurements of this oil sample did not show an exothermic peak because it does not release energy into the system. It is the only oil that starts to melt at such a low temperature, which is due to slightly different contents of specific fatty acids. This oil is characterized by a high content of vitamin E and γ -linolenic acid (compared to other applied oils), which may indicate a protective effect of these ingredients on the *Lactocaseibacillus rhamnosus* GG bacteria during spray drying and storage.

The obtained results indicate that even a relatively small addition of natural cold-pressed oils as protective additives can be used in industrial processes to increase the durability of products using powders with probiotic bacteria obtained by spray drying.

In addition, the process is practically no different from the classic spray drying, and by adding oils, products with a longer shelf life can be created.

However, this study hasn't demonstrated whether and which of the components present in natural plant oils affecting directly the overall viability of bacteria during the spray drying process.

There is still a need for additional research to confirm the protective mechanism of other substances present in natural oils on LGG and other probiotic bacteria. It is important to clarify the protective effect of various types of natural hydrophobic substances, including triacylglycerols and their derivatives plus the presence of other bioactive ingredients which are naturally occurring in biological oils both as individual molecules and as their mixtures. Additional analyses are also needed to verify that the obtained results are universal for other probiotic bacteria.

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