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Research paper

Elastic recovery of filler-binders to safeguard viability of *Lactobacillus rhamnosus* GG during direct compression

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ABSTRACT

Tablets are increasingly explored as dosage form for oral probiotics, especially for applications such as pharyngitis and dental health. In such tablets, the dry form increases the stability and the shelf life of the product. In addition, the probiotic cells are entrapped in the tablet matrix, which protects them against the environmental factors in the human body. However, the development of a probiotic tablet with an adequate number of viable cells remains a challenge due to the stress of the compression process. The adverse conditions during production can damage the cells, which leads to a loss of viability and a failure of the therapy. This study aimed to investigate the effect of the compression behavior of filler-binders on the survival of *Lactobacillus rhamnosus* GG during tablet production. The probiotic tablets were manufactured by direct compression of a freeze-dried mixture of the model *L. rhamnosus* GG, a filler-binder and a suitable amount of lubricant. The compression behavior was determined by analyzing Heckel and force-displacement plots. The results demonstrated that the elastic recovery of the filler-binder during decompression played a protective role in bacterial survival, reducing the compression stress during manufacturing. Consequently, the bacterial cells were less damaged, which resulted in a higher survival rate and a better stability during long-term storage. In conclusion, the elastic recovery of a filler-binder showed to be an important key in safeguarding probiotic cells during direct compression and storage.

1. Introduction

Probiotics are defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as viable live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host [1]. Documented beneficial effects of probiotics include prevention and treatment of enteric infections, urogenital diseases, bacterial vaginosis and skin disorders, and these effects have to be probiotic strain dependent. In the pharmaceutical industry, the most commonly used probiotic strains belong to the genus *Lactobacillus* and *Bifidobacterium*. During this study, the focus was on the genus *Lactobacillus* and more specifically on *Lactobacillus rhamnosus* GG. *L. rhamnosus* GG is one of the best documented probiotic strains in the world [2]. By definition, probiotics should be alive, so that the maintenance of the viability during production up to the time of consumption is crucial [3–7]. The number of viable bacterial cells that is needed to confer the health benefit is depending on the biological target and on pharmaceutical dosage form [8,9]. Commercialized probiotic products are available in different forms such as capsules, tablets and ovules. The ease of administration, accurate dosage, good patient acceptance, suitability of large-scale production and the stability make tablets the dosage form of choice [10,11]. The development of a successful probiotic tablet formulation is a substantial challenge due to the stress of the compression process. The mechanical stress can cause morphological, biochemical and genetic modifications. This can lead to sublethal and lethal injuries and a loss of viability of the probiotic strain, which can significantly reduce probiotic efficacy. The determination of the ideal compression and formulation conditions is therefore necessary to limit the loss of viability.

The effect of compression on bacterial survival depends on the applied pressure, the type of bacterial strain and the excipient used to form the tablet matrix [12–14]. For example, pressures up to 30 MPa have been shown to cause damage to the bacterial cell wall of *L. acidophilus* ATTC 4356 and a little loss of viability. When the pressure

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exceeded 90 MPa, the cell wall and membrane were damaged and the bacterial cell survival decreased linearly with the compression pressure [14]. However, another study reported that the genetic profile and the pathogen inhibition of *L. rhamnosus* Lcr35® were maintained after compression and the gastric resistance was improved [9]. The bacterial survival during compression depends also on tablet porosity [12,13]. For example, an increased densification process of *L. fermentum* CECT 5716 resulted in lower tablet porosity and higher level of mortality [15]. However, literature on tableting of probiotic bacteria is limited and little research has been performed to relate the loss of viability of probiotic bacteria to the compression behavior of direct compression filler-binders. Therefore, the focus of this study was on the relation between the compression properties of filler-binders and the viability and stability of *L. rhamnosus* GG in the tablets.

The reduction in complexity, risk and cost of processing makes direct compression the preferred tablet production method in the pharmaceutical industry. Direct compression requires that the major excipients of the powder blend have adequate flow and compaction properties. The compaction properties are described by compactibility and compressibility. The compactibility is the ability of the powder to be compressed into a stable and strong compact, whereas the compressibility is the ability of the powder to deform under pressure [11,16,17]. The three following deformation methods are proposed: a) elastic recovery as a spontaneously reversible deformation b) plastic deformation as irreversible deformation c) fragmentation as an irreversible process of breakage of big particles into smaller ones [10]. The study of the compression behavior of powders has become an important role in the design and development of tablets. The compression behavior can be defined by several compression equations, which relates a property of the state of consolidation of a powder bed as a function of the compaction pressure [18]. One of the most widely used mathematical models for the measurement of the compressibility is the Heckel analysis. This model is based on the assumption that powder compression follows first-order kinetics with the interparticulate voids as reactants and the densification of the powder as the product. Therefore, the porosity of the tablet is directly proportional to the degree of the compact densification with increasing compression pressure [19,20]. The compression behavior can also be modeled by work equations. During direct compression, mechanical energy is consumed by particle rearrangement, interparticulate friction, die-wall friction, plastic deformation and elastic deformation. The consumed mechanical energy can be calculated from the force-displacement plot, which demonstrates the relationship between upper punch displacement and upper punch force (Fig. 1) [18]. The deformation of perfectly elastic materials is completely reversible and no energy dissipates, which means that any energy put into the material during loading will be entirely returned. Contrary, the energy is stored by plastic deforming materials. Therefore, the calculation of the expansion work ($work_{exp}$) could be used to characterize the mechanical properties of the powder [10,21,22].

Because the compression behavior of direct compression filler-binders could play an important role in bacterial survival during tablet production, ten commonly used filler-binders were analyzed for their compression behavior by examining the Heckel and force-displacement plot. These results were linked with the survival of *L. rhamnosus* GG during tablet production and storage.

Due to the increasing interest in the respiratory microbiome as a target for acute respiratory prophylaxis and treatment, the probiotic cells were processed in a throat lozenge. Flow cytometry in combination with fluorescent staining was applied to monitor the physical state of the bacterial cells.

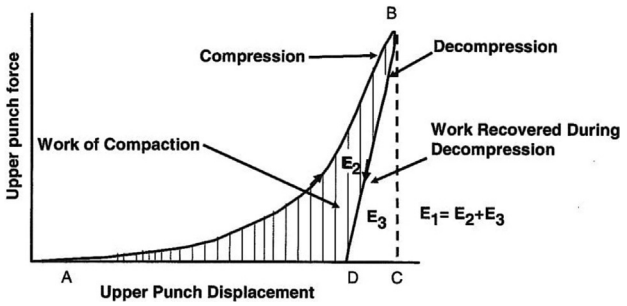

Fig. 1. Force-displacement plot illustrating E_1: the total work done on the tablet ($work_{total}$) E_2: the energy used in the formation of the compact ($work_{net}$) and E_3: the recovered energy during decompression ($work_{exp}$) [18].

2. Materials and methods

2.1. Probiotic strain and excipients

Freeze-dried *Lactobacillus rhamnosus* GG (ATCC31103) was provided to us as THT 030903 by THT (Gembloux, Belgium). Subsequently, probiotic tablets were made as powder blends of freeze-dried LGG, a direct compression filler-binder and a suitable amount of lubricant to void sticking to punches and die (magnesium stearate; Fagron, Nazareth, Belgium). To target the probiotic bacteria at the pharyngeal microbiome, the probiotic bacteria were processed into a throat lozenge and an orally disintegrating tablet. Direct compression filler-binders that are suitable for these pharmaceutical dosage forms were selected and analyzed. The studied filler-binders, which are listed in Table 1, were chosen to represent a range of materials with different compression behaviors (plastically or elastically deformation, fragmenting).

2.2. Compression of probiotic powder

The probiotic tablets were prepared by direct compression using a single punch tablet press (MCC Corporation, NJ, US) fitted with flat-faced bevel-edged punches with a diameter of 8 mm. Homogeneous mixtures of 10% (w/w) freeze-dried *L. rhamnosus* GG, 85% (w/w) filler-binder and 5% (w/w) magnesium stearate were compacted at a compression pressure of 80 MPa and a dwell time of 62 ms. The compression pressure of 80 MPa was selected according to screening experiments which were in line with the investigations of Chan and Zhang [14]. Three batches of each filler-binder were produced.

2.3. Evaluation of direct compression filler-binders

2.3.1. Modulated differential scanning calorimetry (mDSC)

The solid state transitions in the filler-binders were determined by using modulated differential scanning calorimetry (MDSC, Discovery 25, TA Instruments, Zellik, Belgium). This technique separates the total heat flow into reversing and non-reversing signals. Heat capacity related events like glass transition and melting point are displayed in the reversing signals. However, kinetic events like reorganization, enthalpic recovery, water evaporation, decomposition, crystallization and crystal perfection are displayed in the non-reversing signals. A mass between 4,0 and 10,0 mg of each filler-binder was placed in preweighed Tzero pans (TA Instruments, Zellik, Belgium). An empty Tzero pan was used as reference. The heat run was specific for each filler-binder, started at −40 °C and ended at their degradation temperature, which was analyzed by using thermogravimetric analysis (TGA Q50, TA Instruments, Zellik, Belgium). The heat run was set at 10 °C min^{-1} and the modulation period and amplitude were rated at 1 min and 1,60 °C, respectively. The heat flow signals were obtained and analyzed by Trios software (TA Instruments).

2.3.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA Q50, TA Instruments, Zellik, Belgium) was carried out to investigate the thermal performance of the filler-binders. A mass between 10,0 and 20,0 mg was placed in a preweighed platinum TGA pan (TA Instruments, Zellik, Belgium). Each

Table 1
Studied direct compression filler-binders.

Trade name	Excipient	Manufacturer supplier
Neosorb® XTAB 200S	Sorbitol	Roquette Pharma
Pearlitol® 200 SD	Mannitol	Roquette Pharma
Xylisorb® XTAB 240	Xylitol	Roquette Pharma
GalenIQ® 720	Isomalt	Beneo GmbH
GalenIQ® 721	Isomalt	Beneo GmbH
Emdex®	Dextrates	JRS Pharma GmbH & Co
Tablettose® 100	Lactose	Meggle Pharma
Microcelac® 100	Lactose + MCC[a]	Meggle Pharma
Combilac®	Lactose + MCC[a] + native corn starch	Meggle Pharma
Prosolv® ODT G2	MCC + CSD[b] + mannitol + fructose + crospovidone	JRS Pharma GmbH & Co

[a] MCC = microcrystalline cellulose.
[b] CSD = colloidal silicon dioxide.

filler-binder was heated up from 50 to 400 °C at a constant heating rate of 10 °C min^{-1}. Purified nitrogen was used as the carrier gas to provide an inert atmosphere for pyrolysis and to remove the gaseous and condensable product. The TGA thermographs were obtained and analyzed by Trios software (TA Instruments).

2.3.3. Analysis of filler-binder compression behavior

The physical behavior of the filler-binders under pressure, namely brittle fracturing and - or plastic deformation, was investigated by the Heckel analysis, according the equation:

$$\ln(1/\varepsilon) = KP + A \quad (1)$$

where ε is the porosity of the tablet, P the compression pressure (MPa) and K and A are analysis parameters. The filler-binders were lubricated with 5% (w/w) magnesium stearate and compacted at a compression pressure of 80 MPa. The compression pressure and displacement of the punches were measured with strain gauges and linear variable differential transformers (LVDT) respectively and analyzed with Advanced Instrumentation monitor software (MCC Corporation, NJ, US). After compaction, ten tablets of each mixture were weighed (Mettler Toledo, Zaventem, Belgium). The true density (ρ_T) of the filler-binders, which are listed in Table 2 [23], was used for the calculation of the in-die porosity, according the formula:

$$\varepsilon = 1 - D \quad (2)$$

$$D = \rho_A/\rho_T \quad (3)$$

In each equation, ε is the porosity, D is the relative density, ρA is the apparent density and ρT is the true density. The slope of the Heckel plots (K) was converted to the yield pressure (P_y), which is a measurement of the powder compressibility. Filler-binders with high P_y are brittle and consolidate via fragmentation. Low P_y indicates that the filler-binder undergo plastic deformation during compression.

Table 2
True density (P_T) of the studied filler-binders.

Filler-binder	P_T (g cm^{-3})
Neosorb® XTAB 200S	1,51
Pearlitol® 200 SD	1,51
Xylisorb® XTAB 240	1,52
GalenIQ® 720	1,52
GalenIQ® 721	1,47
Emdex®	1,54
Tablettose® 100	1,55
Microcelac® 100	1,55
Combilac®	1,55
Prosolv® ODT G2	1,51

2.3.4. Calculation of $work_{exp}$ of compaction from the force-displacement profile

The force-displacement profile was generated by Advanced Instrumentation monitor software (MCC Corpration, NJ, US). The area under the unloading curve of this profile represents the mechanical energy that was stored or recovered during decompression. Negative values for $work_{exp}$ indicate that the mechanical energy was recovered during decompression as expansion work applied to the punches, reflecting elastic recovery. Positive values indicate that the mechanical energy was stored in the compact, representative for plastically deforming filler-binders.

2.4. Test of bacterial viability in tablets

2.4.1. Plate counting

Three batches of each filler-binder were produced. Three samples before and three tablets after compression were evaluated for their bacterial viability. The powder blends and tablets were diluted 10 times in purified water. Afterwards, a tenfold serial dilution of these suspensions was made in triplicate and spread onto MRS agar plates (Carl Roth, Belgium) in duplo. The plates were incubated aerobically at 37 °C for 72 h. After incubation, the colony forming units (CFU) per plate were enumerated. CFU counts of the powder blends before and the tablets after compression were compared to obtain the bacterial survival rate. Each filler-binder was tested in triplicate.

2.4.2. Flow cytometry

The probiotic tablets were analyzed by flow cytometry for the quantification and distinguish of viable cells from irreversible damaged ones. The probiotic tablets were diluted 10 times in purified water, following by a fourfold serial dilution. 900 µL of the bacterial suspension was subjected to fluorescence staining by incubating with 2 µL of a combination of SYTO 9 with propidium iodide (PI) (ThermoFisher, Oregon, VS) on ice for 15 min. The combination of SYTO 9 and PI fluorescent dyes determines bacterial viability based on membrane integrity. SYTO 9 is a cell membrane-permeable green fluorescent nucleic-acid, which labels all bacteria green regardless of their membrane status. PI, which is a red fluoresecent nucleic-acid, can penetrates only bacteria with compromised membranes. The binding of PI to dsDNA is stronger than that of SYTO 9 [24,25]. The experiments were run on an Attune NxT Acoustic Focusing Cytometer (Model AFC2, Thermo Fisher Scientific, Woodlands, Singapore), equipped with a blue laser (488 nm) as excitation source and six detection channels including FSC, SSC and four fluorescent channels (BL1-BL4). For each sample, 100 µL of bacterial suspension was carried out. The data analysis was performed with Attune NxT software version 2.7.

2.4.3. Tablet evaluation

Ten probiotic tablets were weighed (Mettler Toledo, Zaventem, Belgium) and evaluated for their diameter, thickness and water activity

(LabTouch Aw Novasine, Lachen, Switzerland). The tablet hardness was measured with a tablet hardness tester (PTB-411; Pharmatest, Hainburg, Germany) and the tensile strength was calculated according to Fell and Newton's method [26], in which the radial tensile strength (σ) is given by:

$$\sigma = 2F/\pi Dh \quad (4)$$

where σ is the tensile strength (MPa), F is the applied load (N), D is the diameter of the tablet (mm) and h is the thickness of the tablet (mm).

2.4.4. Stability of the probiotic tablets

For stability testing, the probiotic tablets were stored in heat-sealed light resistant aluminum bags (LamiPouch; DaklaPack, Lelystad, The Netherlands) at room temperature (20–25 °C) and refrigerated conditions (4–8 °C) for 3 and 12 months respectively. The bacterial cell viability in the tablet was investigated monthly. The number of viable bacterial cells was determined by the plate procedure as previously described.

2.4.5. Statistics

The data were analyzed with the statistical software GraphPad Prism 7.04 (GraphPad software inc, La Jolla, CA, USA). One-way analysis of variance (ANOVA) and the false discovery rate of Benjamini, Krieger and Yekutieli were used to determine whether or not data were significantly different. The statistical tests were performed at a significance level p = 0,05. The relationship between different parameters were studied by linear regression and one phase exponential association.

3. Results

3.1. Evaluation of direct compression filler-binders

3.1.1. Modulated differential scanning calorimetry (mDSC)

The water content of the filler-binders was analyzed by TGA and listed in Table 3. The water content of Neosorb® XTAB 200S, Pearlitol® 200SD and Xylisorb® XTAB 240 was negligible. A weight loss event was observed for Prosolv® ODT G2, GalenIQ® 720, GalenIQ® 721 and Emdex® around 100 °C, which reflected the evaporation of residual moisture in the powder matrix. This kinetic event was also detectible as an endothermic peak in the non-reversing heat flow of the mDSC graphs. The water in Tablettose® 100, Microcelac® 100 and Combilac® originated from the monohydrate form of lactose, which is released at a temperature of 150 °C. The reversing heat flow of the mDSC graphs of all the filler-binders showed a single melting event. The corresponding melting points (T_m) are listed in Table 3. The lowest T_m was found by Neosorb® XTAB 200S and Xylisorb® XTAB 240, whereas Tablettose® 100, Microcelac® 100 and Combilac® showed the highest T_m. The degradation temperature ($T_{degradation}$) was also detected by the TGA measurements. Emdex® started to undergo thermal degradation at a temperature of 205,20 °C. Tablettose® 100, Neosorb® XTAB 200S, GalenIQ® 720 and GalenIQ® 721 occurred at higher temperatures (~300 °C).

Table 3
Thermal analysis of the studied filler-binders.

Filler-binder	Water content (%)	T_m (°C)	$T_{degradation}$ (°C)
Neosorb® XTAB 200S	0,30	98	306,07
Pearlitol® 200SD	0,30	166	292,31
Xylisorb® XTAB 240	0,30	97	272,36
GalenIQ® 720	4,99	151	307,86
GalenIQ® 721	2,51	155	300,34
Emdex®	7,68	149	205,20
Tablettose® 100	5,14	222	296,58
Prosolv® ODT G2	1,20	162	284,78
Microcelac® 100	3,96	222	286,17
Combilac®	4,10	221	286,95

Table 4
Filler-binders ranked according to their yield pressure (P_Y).

Filler-binder	P_Y (MPa)	R^2
Xylisorb® XTAB 240	178,56	0,998
Emdex®	161,29	0,993
Pearlitol® 200SD	147,06	0,999
GalenIQ® 721	133,33	0,998
Microcelac® 100	131,58	0,999
Combilac®	123,46	0,998
Prosolv® ODT G2	113,64	0,993
GalenIQ® 720	102,04	0,999
Tablettose® 100	90,09	0,998
Neosorb® XTAB 200S	50,50	0,994

3.1.2. Analysis of filler-binder compression behaviour

The yield pressure (P_y) of the studied direct compression filler-binders and the R^2 of the linear regions of the Heckel plots are displayed in Table 4. Generally, the yield pressure decreased in the following order: Xylisorb® XTAB 240 > Emdex® > Pearlitol® 200SD > GalenIQ® 721 > Microcelac® 100 > Combilac® > Prosolv® ODT G2 > GalenIQ® 720 > Tablettose® 100 > Neosorb® XTAB 200S.

3.1.3. Calculation of $work_{exp}$ of compaction from the force-displacement profile

$Work_{exp}$ was determined from force-displacement measurements of the unloading curve and is considered a measure of the elasticity of the studied filler-binders. As earlier explained, negative values for $work_{exp}$ indicate that the filler-binders undergo elastic deformation during decompression, whereas positive values indicate that the mechanical energy was stored. The values for $work_{exp}$ are shown in Table 5. The most elastic filler-binders were Tablettose® 100, Microcelac® 100 and Combilac®. The lowest degree of elastic recovery was determined with the filler-binders Emdex®, GalenIQ® 721, Neosorb® XTAB 200S and Xylisorb® XTAB 240.

3.2. Impact of direct compression binders on the viability of probiotic bacteria

3.2.1. Plate counting

The average viability at time-zero of the analyzed probiotic tablets of three batches of each filler-binder (n = 9) is shown in Fig. 2(B). The error bars denotes the standard deviation between the different tablets. The lowest bacterial survival rate was found in the probiotic tablet formulations based on the filler-binders Xylisorb® XTAB 240 and Emdex®. The difference in log-reduction between the tablet formulations was low. However, the survival rate (%) within the tablets consisted of Xylisorb® XTAB 240 and Emdex® was significantly lower than in the formulations consisted of the filler-binders Pearlitol® 200SD, Tablettose® 100, Microcelac®100 and Combilac® (One-way ANOVA;

Table 5
Filler-binders ranked according to their $work_{exp}$ (kN s).

Filler-binder	$Work_{exp}$ (kN s)	Energy (J)
Tablettose® 100	−0,047	2054
Microcelac® 100	−0,029	2760
Combilac®	−0,025	2831
GalenIQ® 720	−0,014	2333
Prosolv® ODT G2	−0,009	3807
Pearlitol® 200SD	0,005	2137
Emdex®	0,027	2427
GalenIQ® 721	0,033	2191
Neosorb® XTAB 200S	0,041	3274
Xylisorb® XTAB 240	0,126	2116

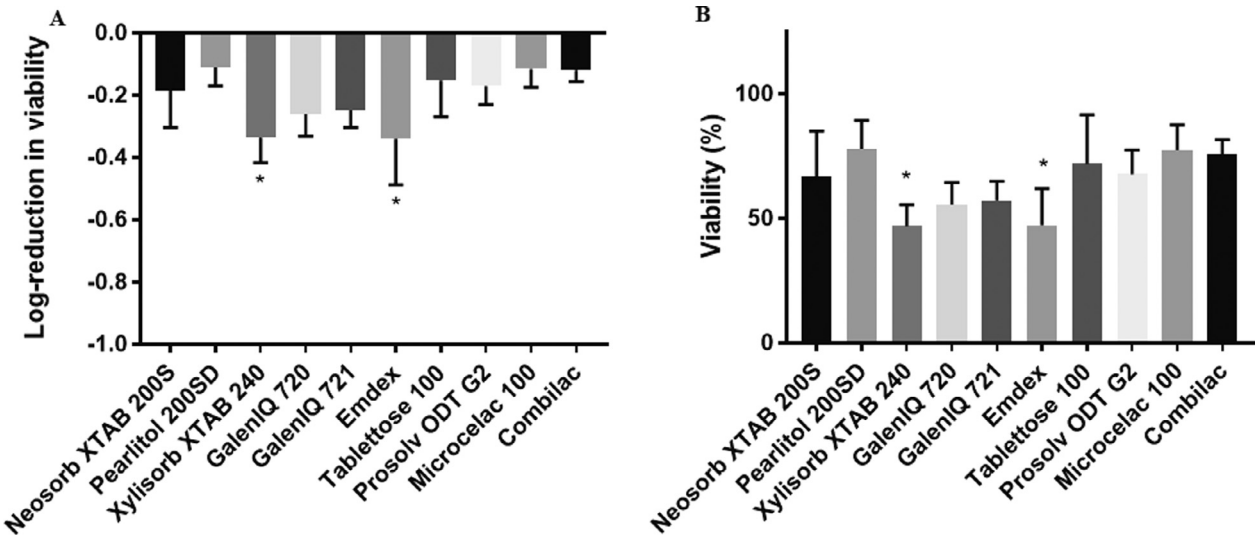

Fig. 2. Difference in log-reduction (A) and viability (B) between the filler-binders after compression. The filler-binders with a significant lower survival rate are indicated with a star (*) (One-way ANOVA, false discovery rate of Benjamini, Krieger and Yekutieli).

False discovery rate of Benjamini, Krieger and Yekutieli).

3.2.2. Flow cytometry

Table 6 displays the cell counts of the SYTO 9 and PI positive populations of each filler-binder, ranked according their percentage of injured cells. The SYTO 9 positive groups of the filler-binders Tablettose® 100, Microcelac® 100 and Combilac® were densely populated. These bacterial cells were alive and intact and consequently were not stained by PI. The bacterial cells of the other formulations were mainly located in the PI positive group. This group represent viable cells with a damaged membrane. The density plots of the filler-binders Tablettose® 100 and GalenIQ® 721 are shown in Fig. 3.

3.3. Tablet evaluation

Table 7 shows the weight, diameter, thickness, crushing strength and tensile strength of the filler-binders. At comparable compression forces, Neosorb® XTAB 200S produced the hardest tablets, whereas Tablettose® 100 produced the softest tablets. The tablets made of Combilac®, Prosolv® ODT G2 and Microcelac® 100 exhibited similar tensile strength to Tablettose® 100. The tensile strength of Xylisorb® XTAB 240, Emdex®, Pearlitol® 200SD and GalenIQ® 721 and GalenIQ® 720 fell in between.

3.4. Stability of the probiotic tablets

Fig. 4 illustrates the evolution of the viability (%) during storage at room (A) and refrigerated temperature (B). When stored at refrigerated temperatures (4–8 °C) the survival of *L. rhamnosus* GG within the tablets was significantly better ($p < 0,05$) than when stored at room temperature (20–25 °C). No significant differences ($p > 0,05$) were observed between the number of viable cells at time zero and after 12 months of storage at a refrigerated temperature. None of the stored tablets showed more than 1 log-reduction in cell viability. Meanwhile, storage at room temperature resulted in a significant decrease of viability ($p < 0,05$). Also, a difference in survival rate between the filler-binders was noticed. When the tablets were stored at room temperature, an average log-reduction of 4 was determined for the filler-binders Prosolv® ODT G2, Combilac®, Tablettose® 100, Neosorb® XTAB 200S and Microcelac® 100. Whereas an average log-reduction of 6 was determined for the filler-binders GalenIQ® 720, GalenIQ® 721, Emdex®, Xylisorb® XTAB 240 and Pearlitol® 200SD. Nevertheless, little difference in water activity was observed between the tablet formulations and the storage conditions (Table 8).

Table 6
Cell counts of SYTO 9 and PI positive populations, ranked according to their percentage of injured cells.

Filler-binder	SYTO 9+	PI+	Injured (%)
GalenIQ® 721	2087	6294	75
Xylisorb® XTAB 240	4449	13,312	75
Neosorb® XTAB 200S	1735	4643	73
GalenIQ® 720	1525	4037	73
Pearlitol® 200SD	2790	7048	72
Emdex®	2094	4416	68
Prosolv® ODT G2	2642	4338	62
Combilac®	3651	2761	43
Microcelac® 100	3857	2661	41
Tablettose® 100	4148	1900	31

4. Discussion

The probiotic tablets consisted of the filler-binders Xylisorb® XTAB 240 and Emdex® contained a significant lower number of viable cells than the tablets based on the filler-binders Pearlitol® 200SD, Tablettose® 100, Microcelac® 100 and Combilac® (Fig. 2). It was previously reported that the loss of viability during compression was influenced by the size of the powder particles and their physical behavior under high pressure, namely brittle fracturing and - or plastic deformation [12,13]. Moreover, the initial mortality would be attributable to the shearing forces caused by interparticulate movement and the pore size reduction. Plumpton et al. reported how mortality increased with decreasing porosity [12]. In this study, the compression behavior was determined by analyzing the Heckel and force-displacement plot. However, no correlation could be found between the yield pressure of the filler-binder and the survival rate of *L. rhamnosus* GG (Fig. 5(A)) since data were randomly distributed (R^2 of 0,2172 (linear regression; Graphpad software inc, La Jolla, CA, USA)). On the other hand, a relationship between $work_{exp}$ and bacterial viability was observed (R^2 of 0,5089). More specifically, the higher the degree of elastic recovery, the better the survival rate during direct compression (Fig. 5(B)). To validate these results, the compressibility and viability of the filler-binders Emcompress® (JRS Pharma GmbH & co) and Avicel® PH 101 (FMC corp) were studied in more detail. The values of $work_{exp}$ were 0,064 and −0,043 respectively. 32,05% of *L. rhamosus* GG survived using Emcompress®, whereas 73,89% survived with Avicel® PH

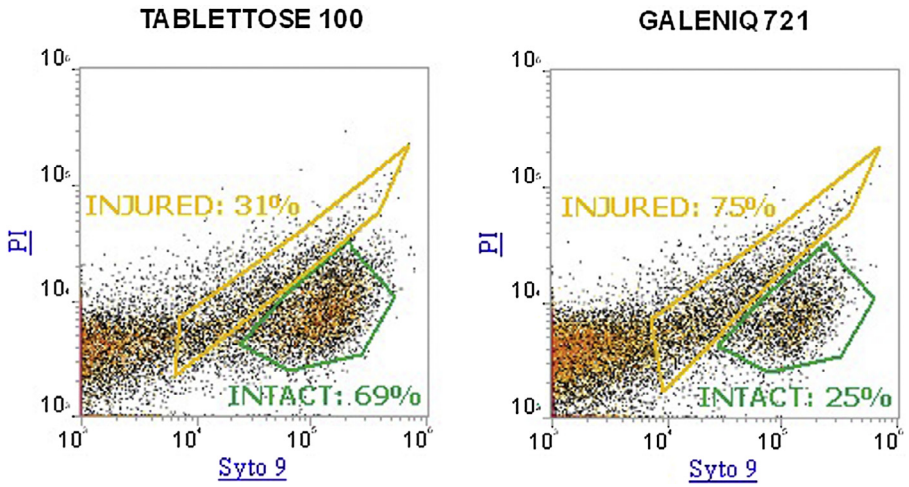

Fig. 3. Density plots of the filler-binders Tablettose® 100 and GalenIQ® 721. 31% of the bacterial cells in the tablet formulation with Tablettose® 100 was populated in the PI positive group. This percentage increased to 75% in the tablet formulation with GalenIQ® 721. Therefore, the probiotic bacteria are better guard from harm conditions during direct compression by the filler-binder Tablettose® 100 in comparison with GalenIQ® 721.

Table 7
Weight, diameter, thickness crushing strength and tensile strength of the filler-binders.

Filler-binder	Weigth (mg)	Diameter (mm)	Thickness (mm)	Crushing strength (N)	Tensile strength (MPa)
Tablettose® 100	301	9,98	7,59	28,29	0,24
Combilac®	263	9,98	7,13	32,15	0,29
Prosolv® ODT G2	292	9,98	7,62	34,77	0,29
Microcelac® 100	273	9,98	7,21	37,03	0,33
Xylisorb® XTAB 240	322	9,97	7,54	50,52	0,43
Emdex®	351	9,96	8,10	56,11	0,44
Pearlitol® 200SD	260	9,97	7,09	58,80	0,53
GalenIQ® 721	230	9,96	6,86	67,97	0,63
GalenIQ® 720	256	9,96	6,96	88,99	0,82
Neosorb® XTAB 200S	261	9,92	6,92	169,44	1,57

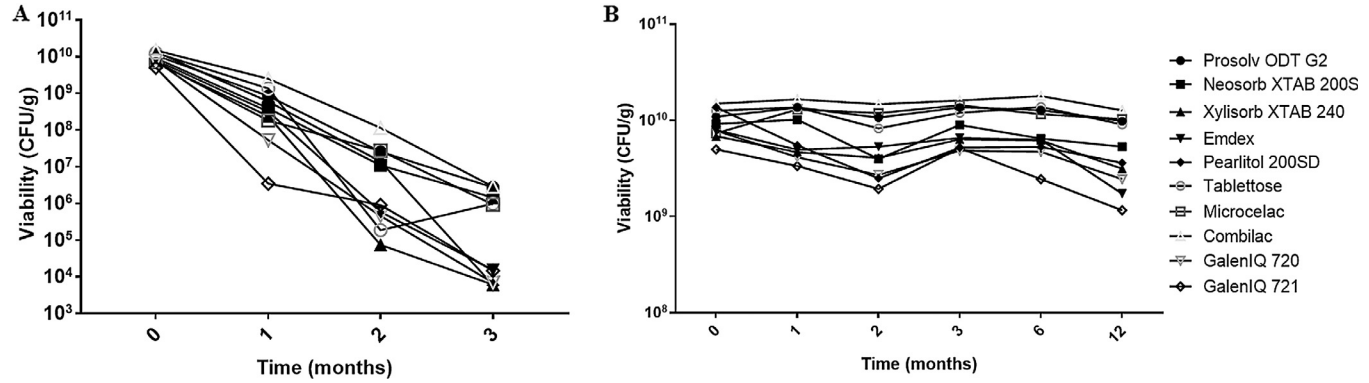

Fig. 4. Evolution of the viability (%) of *L. rhamnosus* GG during storage at room (A) and refrigerated temperature (B) for 3 and 12 months respectively.

Table 8
The water activity of the tablets after three months of storage (n = 3).

Filler-binder	Room temperature	Refrigerated temperature
Tablettose® 100	0,383	0,354
Microcelac® 100	0,368	0,359
Combilac®	0,346	0,332
GalenIQ® 720	0,397	0,375
Prosolv® ODT G2	0,387	0,376
Pearlitol® 200SD	0,377	0,354
Emdex®	0,442	0,393
GalenIQ® 721	0,375	0,366
Neosorb® XTAB 200S	0,345	0,330
Xylisorb® XTAB 240	0,405	0,416

101. These results indicate that the degree of elastic recovery during decompression is more important than the mechanism of densification with regarding to bacterial viability. The filler-binders Emcompress® and Avicel® PH 101 are not suitable for a throat lozenge or an orally disintegrating tablet. Therefore, they were not further characterized.

To prove the protective role of elastic recovery during direct compression, the vitality of the bacterial cells was examined by first staining the probiotic cells with SYTO 9 in combination with PI and then analyzing by flow cytometry. Usually 38% of the bacterial cells in the tablets consisted of the filler-binders Tablettose® 100, Microcelac® 100 and Combilac® were populated in the PI positive group. The membrane integrity of these cells was affected. In the other tablet formulations, the percentage of injured cells increased to 71%, which means that just 29% of the bacterial cells were viable and intact. These results assume that the probiotic bacteria are better guard from harm conditions during direct compression by the filler-binders Tablettose® 100,

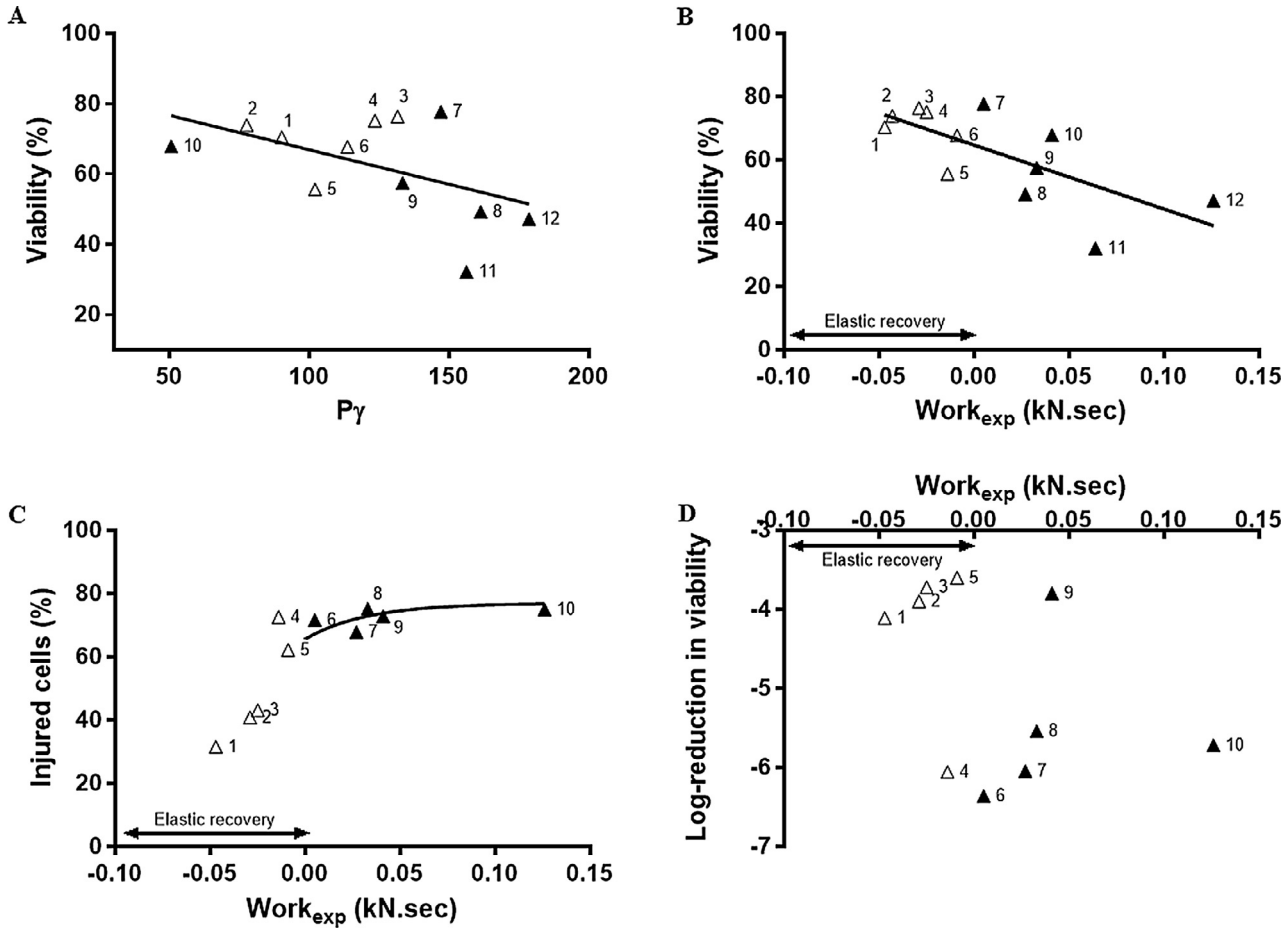

Fig. 5. (A) relationship between P_Y and viability (%) (B) relationship between work$_{exp}$ and viability (%) (C) relationship between work$_{exp}$ and percentage of injured cells (D) relationship between work$_{exp}$ and log-reduction during storage at room temperature for 3 months. Elastic filler-binder are presented with the symbol Δ, plastic filler-binders are presented with the symbol ▲ (A-B) 1. Tablettose® 100 – 2. Avicel® PH 101 – 3. Microcelac® 100 – 4. Combilac® – 5. GalenIQ® 720 – 6. Prosolv® ODT G2 – 7. Pearlitol® 200SD – 8. Emdex® – 9. GalenIQ® 721 – 10. Neosorb® XTAB 200S – 11. Emcompress® – 12. Xylisorb® XTAB 240 (C-D) 1. Tablettose® 100 – 2. Microcelac® 100 – 3. Combilac® – 4. GalenIQ® 720 – 5. Prosolv® ODT G2 – 6. Pearlitol® 200SD – 7. Emdex® – 8. GalenIQ® 721 – 9. Neosorb® XTAB 200S – 10. Xylisorb® XTAB 240.

Microcelac® 100 and Combilac®. These powders were elastic and the mechanical energy was recovered during decompression. The relationship between work$_{exp}$ and the percentage of injured cells is showed in Fig. 5(C). An exponential relationship was observed, reaching a plateau at 76,93% (One phase exponential association; Graphpad software inc, La Jolla, CA, USA)). More specifically, the percentage of injured cells increases with work$_{exp}$ but reached a maximum of 76,93% at 0,07 kN s. This means that higher values for work$_{exp}$ will not lead to a higher percentage of injured cells. These results prove that the elastic recovery of the filler-binder plays a protective role in bacterial survival during direct compression.

As shown in Fig. 4, the survival of *L. rhamnosus* GG within the tablets stored at refrigerated temperature was statistically significant better ($p < 0,05$) than when stored at room temperature. These results confirm the dependency of viability on storage temperature as reported by Villena et al. and Klayraung et al. [15,27]. A refrigerated temperature reduces the rates of negative chemical reactions like membrane lipid oxidation [28], which improves bacterial survival during long-term storage. Consequently, storage at room temperature resulted in a statistically significant decrease of viable cells. The observable difference in log-reduction between the tablet formulations can be identified as the result of the elastic recovery during direct compression. More in detail, survival of *L. rhamnosus* GG within the tablets consisted of a filler-binder with a negative value for work$_{exp}$ was better than in the tablet formulation consisted of a filler-binder with a positive value for work$_{exp}$ (Fig. 5(D)). The filler-binders GalenIQ® 720 and Neosorb® XTAB 200S seem to be an exception to this rule.

In conclusion, the degree of elastic recovery plays an important role in the protection of probiotic bacteria during direct compression. When the probiotic cells will be compressed in combination with an elastic filler-binder, a higher number of bacterial cells remains intact, what makes them less vulnerable to lethal injury during storage.

Acknowledgments

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Disclosure of interest

None.

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