Journal Pre-proofs

Influence of multiple compression phases during tableting of spray dried *Saccharomyces cerevisiae* on microbial survival and physical–mechanical tablet properties

Karl Vorländer, Arno Kwade, Jan Henrik Finke, Ingo Kampen

PII: S0378-5173(24)01182-7
DOI: https://doi.org/10.1016/j.ijpharm.2024.124948
Reference: IJP 124948

To appear in: *International Journal of Pharmaceutics*

Received Date: 5 August 2024
Revised Date: 25 October 2024
Accepted Date: 10 November 2024

Please cite this article as: K. Vorländer, A. Kwade, J.H. Finke, I. Kampen, Influence of multiple compression phases during tableting of spray dried *Saccharomyces cerevisiae* on microbial survival and physical–mechanical tablet properties, *International Journal of Pharmaceutics* (2024), doi: https://doi.org/10.1016/j.ijpharm.2024.124948

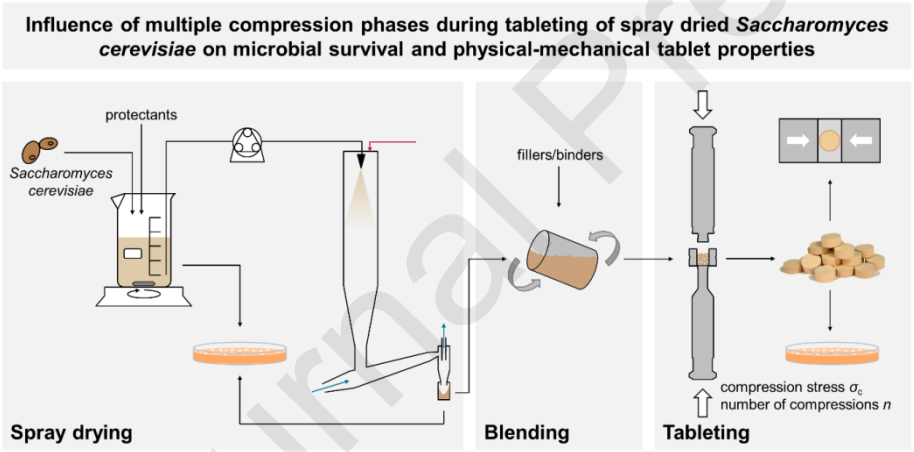
Influence of multiple compression phases during tableting of spray dried *Saccharomyces cerevisiae* on microbial survival and physical-mechanical tablet properties

Karl Vorländer[a,b,*], Arno Kwade[a,b], Jan Henrik Finke[a,b], Ingo Kampen[a,b]

[a] Institute for Particle Technology (iPAT), Technische Universität Braunschweig, Volkmaroder Straße 5, 38104 Braunschweig, Germany

[b] Center of Pharmaceutical Engineering (PVZ), Technische Universität Braunschweig, Franz-Liszt-Straße 35A, 38106 Braunschweig, Germany

Graphical Abstract



* Corresponding author.

E-Mail addresses: k.vorlaender@tu-braunschweig.de (K. Vorländer), a.kwade@tu-braunschweig.de (A. Kwade), jan.finke@tu-braunschweig.de (J.H. Finke), i.kampen@tu-braunschweig.de (I. Kampen)

Abbreviations: **CDW**, cell dry weight; **CFU**, colony forming units; **DCP**, dicalcium phosphate; **ISO**, isomalt; **LAC**, lactose; **MCC**, microcrystalline cellulose; **MgSt**, magnesium stearate; **PBS**, phosphate buffered saline

Commented [KaV1]: Reviewer 1: The article addresses the multiple compression of powders containing microorganisms. The authors have already published several articles on this topic, with four of them being published in 2023. All these articles are closely related (some even share nearly the same graphical abstract). Considering that some of them (e.g., the one on speed and this one) primarily present negative results, we believe that some of these studies should have been published together, as they share most of their results and methodologies. We are not convinced that the multiplication of small publications, rather than more substantial ones, is beneficial for the research itself.

Commented [KaV2R1]: The authors would like to thank reviewer 1 for the time and effort reviewing the manuscript. It is true that several publications have already emerged from the research project in the last year. The similarity of the graphical abstracts is intentional on our part. It allows potential readers to quickly see that essentially the same process chain is always the subject of research here, but different aspects are examined in detail.
Of course we also discussed to combine different investigations in one manuscript, especially since this would also mean less work for us. However, other reviewers told us in earlier reviews, that we should focus in order to create manuscripts of a reasonable complexity. In particular, the proposed combination of the kinetic investigations and the investigations presented here could hardly be meaningfully accommodated in a combined publication, since different methods were used to dry the microorganisms. Furthermore, we always strive to make our research results available to the community as quickly as possible, and the aforementioned investigations were carried out with a corresponding time lag. At the time of publication of the kinetic investigations, multiple stress had not yet been investigated.

Commented [KaV3]: Reviewer 2: Assessing the impact of multiple compression events upon the microorganism viability is an interesting study and it was outlined and discussed clearly and concisely.

Commented [KaV4R3]: We thank reviewer 2 for the time and effort to read and comment on our manuscript. We appreciate the positive overall assessment and the comments for further improvement of our manuscript.

Abstract

The viability of probiotic microorganisms is essential for their health-promoting effects and must be preserved in the best possible way during the production of the final dosage form, such as tablets. This applies to both drying and tableting. *Saccharomyces cerevisiae* is spray-dried with suitable protective additives, which were identified in a previous study in which also the influence of the formulation during tableting was investigated. One aspect that has not yet been addressed is the effect of multiple compression, as it is typical with pre- and main compression when using rotary tablet presses. To investigate this, tablets are compressed up to five times. It is shown that when tablet strength and survival are considered together, the application of a pre- and main pressure does not have a significant effect. This facilitates the transferability of findings of compaction studies with a single compression phase. In addition, the data allow to consolidate the mechanism of inactivation of microorganisms during tableting found in previous studies by the same authors. This is based on the porosity reduction, whereby it is shown in the present study that it is irrelevant how this reduction is achieved (change in compression stress or the number of compression cycles).

Keywords

Probiotics

Viability

Spray drying

Compaction

Porosity

Tensile strength

1. Introduction

The processing of viable microorganisms into tablets is of particular importance in the context of probiotic microorganisms (Klayraung et al., 2009). These microorganisms provide the patient with health benefits when taken in viable form and in sufficient doses (Joint FAO/WHO Working Group, 2002). Dry formulations are preferred due to their better storage stability in unrefrigerated storage and handling (Santivarangkna, 2016). However, the further processing of dried microorganisms into tablets is a challenging process step due to the compressive and shear stresses involved (Vorländer et al., 2020). Nevertheless, it is usually favored over the administration of loose powders or powders filled into capsules, as microorganisms in loose powders are more exposed to the harsh conditions in the stomach (Klayraung et al., 2009) and the production of capsules is more cost-intensive than tableting.

Gentle processes and suitable formulations are required to dry microorganisms in a life-preserving manner, typically by freeze drying, fluidized bed drying or spray drying (Broeckx et al., 2016). Despite process parameters that are as gentle as possible (especially low temperatures), high losses in the viability of microorganisms are sometimes observed since water molecules enable the correct conformation of various biological structures (Ananta et al., 2005; Crowe et al., 1987; Oliver et al., 1998). To counteract this, protective additives are added to the cell suspension before drying. These stabilize essential biological structures (theories of vitrification, water replacement and preferential hydration), whose conformational change would otherwise be accompanied by irreversible inactivation of the microorganisms, in particular through the loss of the integrity of the cell membrane (Belton and Gil, 1994; Broeckx et al., 2016; Cordone et al., 2007; Wolkers and Oldenhof, 2021). Numerous studies have dealt with the identification of effective protective additive formulations in the drying of microorganisms (Broeckx et al., 2016). A universal formulation does not yet exist. However, studies have shown that a combination of trehalose and skimmed milk powder can protect *Saccharomyces cerevisiae* cells from lethal damage during freeze drying, fluidized bed spray granulation and spray drying (Vorländer et al., 2023a; Vorländer et al., 2023d; Vorländer et al., 2020).

The further processing of dried microorganisms into tablets has also already been investigated in numerous studies. In some cases, aspects such as protection against bile juices or storage stability have already been addressed (Klayraung et al., 2009). However, it is first necessary to know the damage mechanisms during densification in order to be able to counteract these in a targeted manner (Vorländer et al., 2023b). Survival during tablet production has already been investigated in several studies. Various aspects have been brought into focus. These include, in particular, the formulation and its deformation behavior (Ayorinde et al., 2011; Blair et al., 1991; Byl et al., 2018; Fassihi and Parker, 1987; Plumpton et al., 1986a), but also kinetic factors of compression such as dwell time and consolidation time (Fassihi and Parker, 1987; Vorländer et al., 2023c) or the geometry of the tablets produced. The damage to the microorganisms can be of a thermal or mechanical nature (Chesworth et al., 1977). Studies with cells of different sizes indicate that the mechanical component is predominant, as larger microorganisms show lower survival (Plumpton et al., 1986b). Earlier studies by the authors of the present publication have recently shown that tablet porosity is essentially decisive for the inactivation of microorganisms, with the change in tablet porosity showing a correlation across formulations (Vorländer et al., 2023d; Vorländer et al., 2023b).

Previous studies on the tableting of viable microorganisms have generally used hydraulic presses or compaction simulators, applying compression profiles with a single main pressure. An important aspect for the transfer of the results of these studies to an industrial scale has not yet been taken into account. The high production speeds when using rotary tablet presses usually require the use of a pre-pressure and a main pressure in order to prevent the formation of tablet defects in the best possible way (Hansen and Kleinebudde, 2021; Mazel and Tchoreloff, 2020; Patel et al., 2006). In the production of tablets with probiotic microorganisms, the microorganisms contained are subjected to multiple stresses in this case. The applied pre-

compression pressure is typically significantly lower than the main pressure (Patel et al., 2006). With a low pre-compression stress, densification is significantly lower there. Nevertheless, the question remains to what extent this first densification is associated with additional damage to the microorganisms.

In order to answer this question, spray-dried yeast cells are mixed with various ~~dry binding agents~~ fillers in the present study. The mixtures are densified with a compaction simulator at different compression stresses. The same compression stress is applied once, twice or five times. This extremization compared to a low initial pressure and the actual main pressure in rotary tablet presses is intended to reveal influences that would otherwise remain undetected. At the same time, the findings should further strengthen the understanding of the physical damage mechanisms during tableting.

Commented [KaV5]: Reviewer 2: Line 118: These materials are more commonly considered as fillers whereas other excipients are specified for only their binder capabilities. This comment also applies to line 97.

Commented [KaV6R5]: Following your suggestion, we have changed this in the manuscript.

Commented [KaV7]: Reviewer 2: Line 97: What is meant by damage? Biological or physical damage?

Commented [KaV8R7]: That is a good question. What is meant here is physical damage, which subsequently may lead to biological damage (e.g., physical damage of cell membrane results in leakage of cytosol).

2. Materials and methods

2.1. Spray drying

Baker's yeast *Saccharomyces cerevisiae* (Lallemand-DHW GmbH, Vienna, Austria) as a model organism was spray dried as established elsewhere (Vorländer et al., 2023d). In brief, a suspension with a cell dry weight concentration (CDW) of 50 g/L and a concentration of 50 g/L trehalose dihydrate (FormMed HealthCare AG, Frankfurt am Main, Germany) and 50 g/L skimmed milk powder (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was prepared and spray-dried after one hour of incubation at room temperature. Spray drying was conducted in co-current (ProCepT 4 M8-TriX, PROCEPT nv, Zele, Belgium). Before the cell suspension was sprayed, water was sprayed for at least 15 minutes to bring the entire system to a state of equilibrium (temperature and humidity). The flow rate was adjusted so that the same mass flow of vaporizable water was sprayed as during the spraying of the cell suspension. In order to limit the thermal stress on the product, the product was removed after every 60 minutes of drying time. The inlet temperature was 100 °C, the volume flow of the drying air 0.3 m^3/min, the mass flow of the cell suspension 2 g/min, the nozzle diameter 1.2 mm and the nozzle pressure 1.5 bar. In addition, 0.12 m^3/min air was supplied to the cyclone to reduce the separation limit.

2.2. Preparation of powder blends

The spray-dried product was mixed (3D shaker mixer TURBULA, Willi A. Bachofen AG at 49 min^{-1}, 5 min) in a mass ratio of 1:3 with the ~~dry binders~~fillers dicalcium phosphate (DCP, DI-CAFOS A150, kindly provided by Chemische Fabrik Budenheim KG, Budenheim, Germany), isomalt (ISO, GalenIQ 721, kindly provided by BENEO GmbH, Mannheim, Germany), lactose (LAC, Granulac 70, kindly provided by MEGGLE GmbH & Co. KG, Wasserburg am Inn, Germany) or microcrystalline cellulose (MCC, Vivapur 102, kindly provided by J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany). In the case of DCP, ISO and LAC, 1 wt.-% of magnesium stearate (MgSt, MAGNESIA GmbH, Lüneburg, Germany) was added as a lubricant and mixed for a further 2 min. MCC required no lubrication as ejection forces were low and tool wear is correspondingly low even without addition of MgSt, which is known to negatively affect the tensile strength of MCC tablets (Puckhaber et al., 2022).

2.3. Preparation of tablets

A compaction simulator (Styl'One evolution, Medelpharm, Beynost, France) was used to produce the tablets. This is instrumented with force and displacement sensors and enabling the calculation of in-die porosity data during compaction. It was equipped with flat, round punches with a diameter of 11.28 mm. The die was filled manually in order to keep mass fluctuations as low as possible. The target mass of the tablets was 450 mg. The compression was displacement-controlled with a generic, symmetrical trapezoidal compression profile with a constant ~~punch~~ speed of 45 mm s^{-1} of the upper and lower punch and dwell times between 30 and 40 ms. The compression height was adjusted so that compression stresses in the range of 25 to 300 MPa were applied. Initially, tableting was carried out with a singular compression with and without pre-compression. The pre-compression stress was 10% of the main compression stress. For another batch, ~~Compression~~ compression was performed once in one test series and twice or five times in the two other test series. In the case of multiple compressions, the tablet remained in the die between the compression repetitions and was only ejected after the last compression. In order to achieve the respective target compression stress with an increasing number of compression repetitions, the compression height was reduced accordingly with each compression repetition. In contrast to multiple compression, the results of compaction with and without pre-compression show hardly any differences and are therefore not considered in more detail in this manuscript. For reference purposes, most diagrams from results and discussion section are shown in the appendix analogously for compaction with and without pre-compression (Suppl. 1 – Suppl. 6).

Commented [KaV9]: Reviewer 2: Line 118: These materials are more commonly considered as fillers whereas other excipients are specified for only their binder capabilities. This comment also applies to line 97.

Commented [KaV10R9]: Following your suggestion, we have changed this in the manuscript.

Commented [KaV11]: Reviewer 1: L124 : could the authors precise the origin of MgSt?

Commented [KaV12R11]: We apologize for our negligence. The supplier has been added to the manuscript.

Commented [KaV13]: Reviewer 2: Line 124: Why was MgSt not added to MCC?

Commented [KaV14R13]: With MCC, the ejection forces were low enough to not require lubrication, which would have unnecessarily reduced the tablet tensile strength otherwise. We have added this.

Commented [KaV15]: Reviewer 1: Our first comment is that the authors state that multiple compression is studied because precompression is often used. To emphasize the effect, they use multiple compressions at the same pressure instead of one small and one large pressure, as is typically done when precompression is used. However, we regret that the authors did not present, in at least one case, a scenario closer to a real precompression + compression cycle, where plastic deformation occurs in two steps. In the cycles used, only the first compression results in significant plastic deformation, as only minimal changes in porosity are observed in the subsequent compressions. The authors should at least demonstrate that their hypothesis—that their conditions are more drastic—is indeed valid.

Commented [KaV16R15]: We apologize for omitting this data. We now address this in the manuscript: The diagrams were added to the supplementary material and the performance of these experiments is described in section 2.3. There we directly point out the significantly smaller differences in the data compared to the multiple application of the same compression stress, which is why further consideration is omitted in the manuscript. For comparison purposes we designed the new diagrams (with and without pre-compression) analogously to the diagrams in the main manuscript. A joint presentation with the other data in one diagram in the main manuscript would be too confusing due to the tableting of different batches and the associated deviations. In our opinion, a separate presentation in the manuscript contributes too little to the manuscript to justify almost doubling the number of diagrams.

Commented [KaV17]: Reviewer 1: L130 : could the authors give the speed?

Commented [KaV18R17]: We have added the speed of 45 mm/s.

Commented [KaV19]: Reviewer 2: Line 130-134: Was the upper and the lower punches moving at the same time? Or, was compression one-sided (only one punch moving)?

Commented [KaV20R19]: With the compaction simulator used, both punches move simultaneously. We have clarified this in the manuscript.

2.4. Analysis of spray dried powder and blends

2.4.1. Residual moisture

The residual moisture of the spray-dried product was determined thermogravimetrically (120 °C, DBS 60-3, KERN & SOHN GmbH, Balingen-Frommern, Germany).

2.4.2. Viability and survival

The viability was determined by counting the number of colony forming units (CFU). Samples of the spray-dried product were suspended in a phosphate-buffered saline solution (PBS, 1.6 g/L NaCl, 0.04 g/L KCl, 0.284 g/L Na_2HPO_4, 0.054 g/L KH_2PO_4, pH 7.4; Sigma-Aldrich Chemie GmbH, Munich, Germany) and serially diluted with PBS in the same way as samples of the sprayed cell suspension. Appropriate concentrations were spread on agar plates (10 g/L yeast extract, 20 g/L peptone ex casein, 22 g/L glucose monohydrate, 15 g/L Agar-Agar Kobe 1; all from Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Colonies were counted automatically after 30 hours of incubation at 30 °C (ProtoCOL3 Plus, Synbiosis, Cambridge, United Kingdom). On this basis, the viability (Eq. 1) and survival rate (Eq. 2) were calculated. For further details, please refer to other publications (Vorländer et al., 2023a; Vorländer et al., 2020)

$$\text{viability}\ [\mathrm{CFU/g_{CDW}}] = \frac{\text{count of colonies [CFU]}}{\text{plated concentration [g}_{\mathrm{CDW}}\text{/L]} \cdot \text{plated volume [L]}} \quad (1)$$

$$\text{survival rate}\ [\%] = \frac{\text{viability after process step [CFU/g}_{\mathrm{CDW}}\text{]}}{\text{viability before process step [CFU/g}_{\mathrm{CDW}}\text{]}} \quad (2)$$

The viability of the spray-dried product was (2.52 ± 0.7) · 10^{10} CFU/g_{CDW}, which corresponds to a survival rate of 65 % during spray drying. In the following, survival rates are given exclusively for the compaction step.

2.4.3. True density of powder blends

The true solid density ρ_{true} of the powder mixtures was determined using a helium gas pycnometer (Ultrapyc 1200e, Quantachrome Instruments, Boynton Beach, FL, United States). The measurement was carried out at room temperature, with a single weighing and 10-fold measurement of the powder volume.

2.5. Analysis of tablets

2.5.1. Storage

After production, the tablets were stored in polyethylene bags in the refrigerator at 4 °C until physical-mechanical and microbiological characterization. The analysis was carried out 24 to 30 hours after tableting.

2.5.2. Tablet porosity

For every test, 10 tablets were weighed (m_t). In addition, the tablet height h and the tablet diameter d were determined using a breaking strength tester (MultiTest 50, Sotax AG, Aesch, Switzerland). Together with the true density ρ_{true}, the porosity ε of the tablets can be calculated:

$$\varepsilon = 1 - \frac{4 \cdot m_t}{\rho_{true} \cdot \pi \cdot d^2 \cdot h} \quad (3)$$

In addition, the minimum in-die porosity $\varepsilon_{in\text{-}die}$ can be calculated based on the measurement of the punch distance during compaction, where h_{min} is the minimum punch distance and d_{die} is the inner diameter of the die:

$$\varepsilon_{in-die} = 1 - \frac{4 \cdot m_t}{\rho_{true} \cdot \pi \cdot d_{die}^2 \cdot h_{min}} \quad (4)$$

2.5.3. Tensile strength

The breaking strength tester was also used to determine the breaking force F of the tablets by diametrical loading between two jaws until failure, analogous to the European Pharmacopoeia (Ph. Eur. 9.2 2.9.8). This and the dimensions of the tablets were used to calculate the tensile strength σ_t (Fell and Newton, 1970):

$$\sigma_t = \frac{2 \cdot F}{\pi \cdot d \cdot h} \quad (5)$$

3. Results and discussion

3.1. Influence of compression ~~stress~~ on survival

Mixtures of spray-dried yeast cells with DCP, ISO, LAC or MCC were compacted into tablets using compression stresses σ_c in the range of 25 to 300 MPa. The compression was not only carried out once with the respective compression stress, but also twice or five times with the same compression stress. The compression stress of the up to five compression phases per tablet showed unavoidable slight fluctuations. In the following, the maximum applied compression stress $\sigma_{c,\,max}$ is considered. This determines the survival of the yeast cells during tableting (Fig. 1). Regardless of what the spray-dried yeast cells are mixed with, after tableting a steady decrease in survival can be seen as the compression stress increases. This is consistent with the results of earlier studies by the same authors, when tableting the same spray-dried organism (Vorländer et al., 2023d). The same also applies to the tableting of freeze-dried yeast cells (Vorländer et al., 2020) and in the case of fluidized bed spray-granulated yeast cells (Vorländer et al., 2023a; Vorländer et al., 2023c; Vorländer et al., 2023b), but also to the tableting of various (probiotic) bacteria (Ayorinde et al., 2011; Blair et al., 1991; Chan and Zhang, 2002; e Silva et al., 2013; Fassihi and Parker, 1987; Muller et al., 2014; Nagashima et al., 2013; Plumpton et al., 1986a, 1986b; Poulin et al., 2011; Stadler and Viernstein, 2001) and (probiotic) yeasts (Nagashima et al., 2013; Plumpton et al., 1986b). Nevertheless, the formulation also seems to influences the survival. In general, ~~with the highest occurring in the case of LAC, followed by DCP, MCC and finally ISO. T~~the same ranking was

Commented [KaV21]: Reviewer 2: Line 173: Equation 3 uses the tablet diameter to measure the volume when the radius should be used.

Commented [KaV22R21]: This is not correct. To calculate the volume here, the diameter must be used in the formula. You have probably overlooked the 4 in the numerator. Since the diameter of tablets is always measured, we would prefer to use the diameter in the formulas for the sake of simplicity and not make any changes. However, there was another mistake in the equation that we have corrected.

Commented [KaV23]: Reviewer 1: In general, we found the present paper a little t superficial. The discussion of the results is very broad and mainly try to prove a point instead of discussing really what is on the graphs. We present several examples thereafter.

Commented [KaV24R23]: To keep the manuscript in a reasonable length, we focused on the main outcomes of the data, which might seam to be a litte superficial. However, this approach was positively interpreted by Reviewer 2 ("was outlined and discussed clearly and concisely").
In order to take account of the different points of view, we have included additions at certain points to adequately address deviations from our overarching statements. This is indicated at the appropriate places in the manuscript.

~~also~~ observed as in an earlier study by the same authors, in which the mixing ratio was also varied (Vorländer et al., 2023d): The highest survival was observed with LAC, followed by DCP, MCC and finally ISO. In deviation from this, the curve for single compression of ISO shows an unexpected kink and survival rate is higher than for MCC at 150 MPa and higher than for MCC and DCP at 300 MPa. The present study also shows an influence of the number of compression repetitions on survival. Survival is essentially lower the more often compression is performed. An exception to this is the single and double compression in the case of DCP, where the same survival rate was obtained, and in the case of MCC, the curve for double compression is above the curve for single compression. In addition, the survival rate in the case of ISO at 150 MPa at 5-fold compression is slightly higher than at double compression, which is attributed to the great uncertainty of the data point at double compression. In order to explain ~~this effect~~the effect of multiple compression on survival, the porosity as a structural parameter of the tablets resulting from the compression must be considered.

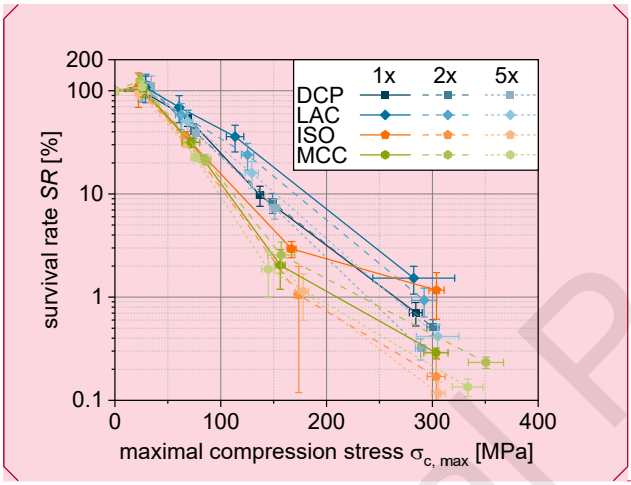


Fig. 1: Survival as a function of single, dual or quintuple applied compression stress on survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. Data points represent mean and standard deviation ($n = 3$).

3.2. Compressibility

The porosity can be used as a structural parameter, as it is a measure of the densification of the material. The compressibility describes the porosity as a function of the applied compression stress (Fig. 2a). The higher the compression stress, the lower the tablet porosity. Compressibility also depends on the formulation and is lowest when DCP is used, followed by MCC, ISO and finally LAC. However, there is only a particularly clear difference at the lowest compression stress. At higher compression stresses, the compressibility profiles of MCC, ISO and LAC cross over, sometimes even several times, and are much closer to each other. This behavior was also shown in a previous study by the same authors (Vorländer et al., 2023d). In addition, an influence of the number of compressions can be observed over the entire compression stress range and for all formulations. The porosity is lower with multiple compressions. This effect increases with higher applied compression stresses. To illustrate this even more clearly, the experimental data were fitted according to the compressibility model of Gurnham and Reynolds (Gurnham and Masson, 1946; Reynolds et al., 2017; Zhao et al., 2006):

Commented [KaV25]: Reviewer 1: The comments on Figure 1 are an example of the superficial nature of some of the authors' interpretations. The authors state: "the formulation also influences the survival, with the highest occurring in the case of LAC, followed by DCP, MCC, and finally ISO." If this was previously proven in another publication, it is not clearly evident from the graph: for single compression, ISO is above MCC at 150 and 300 MPa and is even above DCP at the last data point. Authors should describe what is actually shown in the figure, not what they want to see. They further state: "The present study also shows an influence of the number of compression repetitions on survival." The trends are far from clear for all products: for MCC, two compressions are above single compression; for DCP, 1 and 2 compressions are superimposed; for ISO, 2 and 5 compressions are superimposed; and for LAC, the survival rate decreases with the number of compressions. We understand that there is variability, but again, authors should describe what is shown in the figure, not what they want to see.

Commented [KaV26R25]: As mentioned above, we understand that deviations from general trends should be named, even if they cannot be explained at this stage and are presumably a consequence of the variability of the data. Accordingly, we have made the indicated changes in the manuscript.

Commented [KaV27]: Reviewer 1: L219-220 authors state: " the porosity is lower with multiple compression". Again, this is far from obvisous on figure 2 and 3 for all the products…

Commented [KaV28R27]: Since the differences in the porosity are indeed small, we created a figure in Suppl. No. 1 (Suppl. No. 7 in the revision), where the systematic changes can be seen clearly. Only for one curve (DCP at 50 MPa), a slightly higher porosity at 2-fold and 5-fold compression can be observed. Considering this, it can be seen that the statements made can be regarded as generally valid. In addition we have modified Figure 3 to better emphasize the formulation-specific common trend. It can now be seen more clearly that the data points for the most part follow a common trend and that, depending on the number of compressions, systematically different porosities and survival rates result for the same compression stress.

$$\varepsilon = -\frac{1}{K} \cdot \ln \frac{\sigma_{c,\,max}}{\sigma_{c,\,\varepsilon=0}} \qquad 6$$

The parameter K is associated with the intrinsic resistance of the material to deformation. The compression stress $\sigma_{c,\,\varepsilon=0}$ is the stress required to produce tablets with zero porosity. With these parameters, porosity values were calculated for defined compression stress values to improve comparability (Suppl. 7). The fit parameter K is unaffected by the number of compressions (Suppl. 8) but the more often the tablets are compressed, the lower $\sigma_{c,\,\varepsilon=0}$ (Fig. 2b). The further densification of the tablets and thus reduction in tablet porosity is in line with expectations. As the number of compression repetitions increases, the ~~tablet~~ punches have to be moved closer together in order to achieve the target compression ~~force~~stress. The multiple application of force favors further densification through additional particle fragmentation and (visco)plastic deformation. At the same time, it is obvious that the microorganisms are also exposed to further stresses, which reduce survival accordingly. This is also shown by the plot of survival as a function of tablet porosity (Fig. 3). Although there are different curves depending on the formulation, it is also evident that it is largely irrelevant whether a lower porosity was achieved by a higher compression stress or by applying the same compression stress several times. The data points of the different curves of a formulation fall on a common line for the different compression repetitions (shown by exponential regression ($SR(\varepsilon) = a + b \cdot e^{-k_{SR,\varepsilon} \cdot \varepsilon}$ with $k_{SR,\,\varepsilon}$ as tablet porosity-related inactivation rate and a and b as empirical fit parameters) and confidence interval in Fig. 3). In earlier studies by the same authors, it was found analogously that the same porosity-related survival rates are obtained regardless of whether high compression stresses and short dwell times or vice versa have contributed to achieving this porosity state (Vorländer et al., 2023c). The survival of the microorganisms is therefore not determined by the applied compression stress itself, but the compression stress determines the porosity in the tablets in a formulation-specific process function, on which the survival of the microorganisms depends as a property function. The lower the porosity of a formulation, the greater the mechanical stress on the microorganisms due to compressive and shear stress and the lower their survival.

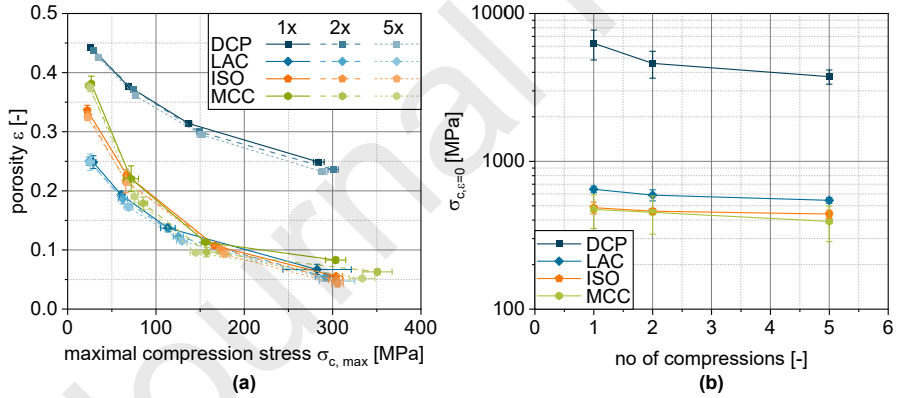


Fig. 2: (a) Compressibility of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. The compression was carried out 1, 2 or 5 times. Data points represent mean and standard deviation ($n = 10$). (b) Fit parameter $\sigma_{c,\,\varepsilon=0}$ according to equation 5.

Commented [KaV29]: Reviewer 1: L231 : Pleas emodify the expression "tablet punch"

Commented [KaV30R29]: We have removed "tablet" to get the correct expression.

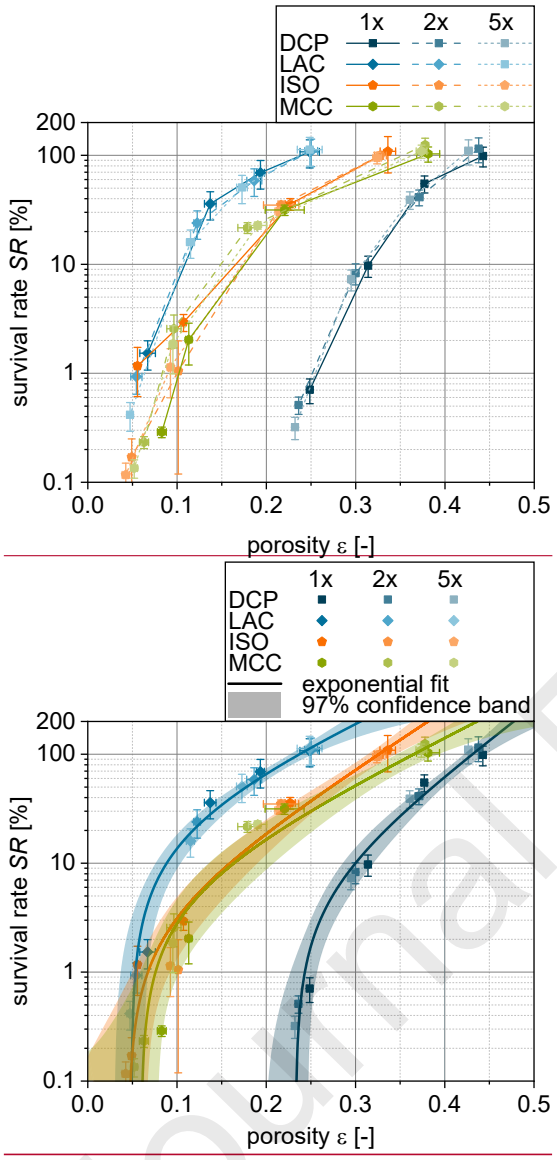


Fig. 3: Survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively, as a function of tablet porosity. Data points represent mean and standard deviation ($n = 3$). All data points of one formulation were fitted together using an exponential function. The 97% confidence interval is also shown.

The different positions of the curves were attributed to the different deformation mechanisms of the excipients DCP, LAC, ISO and MCC in earlier work by the same authors (Vorländer et al., 2023b). Formulations with DCP are generally characterized by high porosity, which is due to the high stiffness and associated brittle fragmentation during compression. In contrast to plastic deformation, more voids remain. If the formulation contains particles that have a lower mean yield pressure than DCP, this other material is more likely to undergo compression and

is compacted more than the DCP fraction, resulting in different porosity distributions. Therefore, the densification of the spray-dried yeast cells is greater than the global tablet porosity would suggest. In previous studies, however, the change in tablet porosity was identified as a scaling variable across formulations (Vorländer et al., 2023d; Vorländer et al., 2023b), with the porosity of tablets tableted with a compression stress of 25 MPa serving as a reference. The analogous observation of the survival as a function of the tablet porosity change also shows a better correlation across formulations in this study than the observation of the absolute porosity, even if there is a stronger scatter in the range of high porosity changes (Fig. 4a). There is no systematic effect of the number of compression repetitions. In combination with the analogous calculation for the reduction of the minimum in-die porosity during compaction (Fig. 4b), it is also possible to interpret the significance of plastic and elastic deformation for the survival of the microorganisms during tableting. If not only the plastic deformation is considered (Fig. 4a), but the entirety of plastic and elastic deformation (Fig. 4b), a poorer correlation across formulations and the separation into 3 groups (DCP + LAC; ISO; MCC) can be seen. MCC shows the greatest deviation because this formulation shows the greatest proportion of elastic deformation. The elastic deformation of the formulation is mainly caused by the excipients. The data suggest that the elastic deformation of the excipient particles has a negligible influence on the survival of the microorganisms, even if this could be associated with shearing of the microorganisms (during both compaction and relaxation). However, the lack of energy for the plastic deformation of the yeast cells due to the elastic deformation of the excipients seems to outweigh this effect, which is why the consideration of the reduction of the out-die porosity and thus the plastic deformation enables a better correlation across formulations.

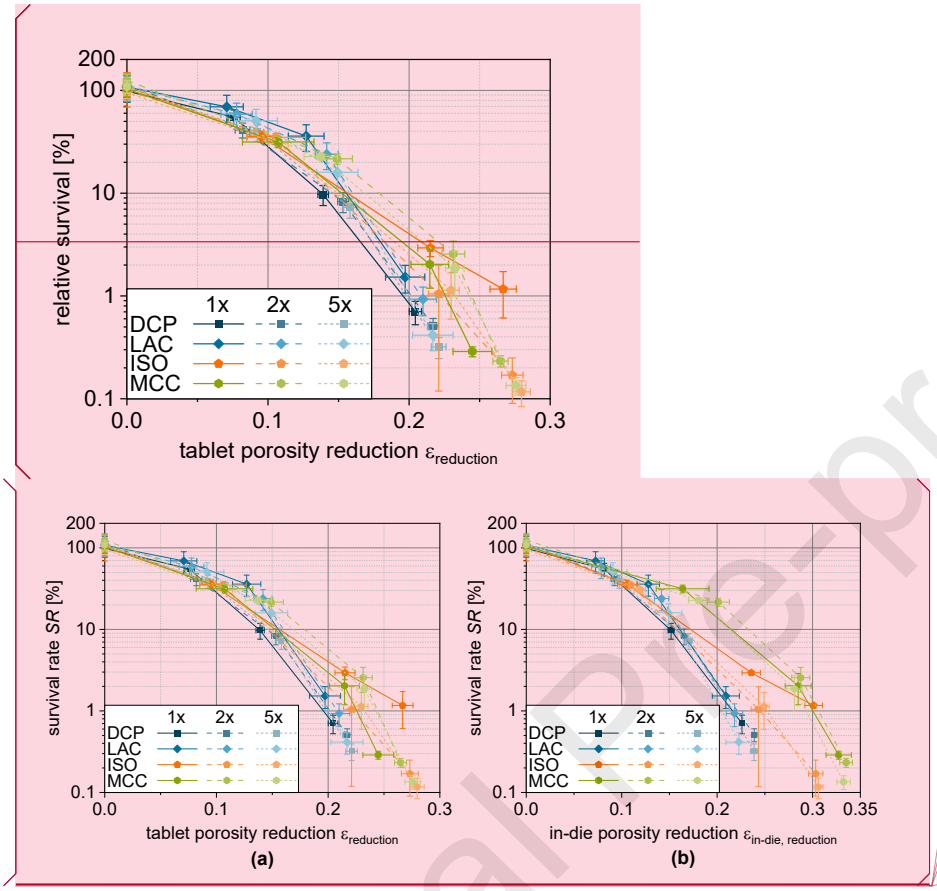


Fig. 4: Survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively, as a function of tablet porosity reduction (a) and reduction of minimal in-die porosity (b). Data points represent mean and standard deviation ($n = 3$).

3.3. Compactibility

In addition to the survival of the microorganisms during compression, the tablet strength achieved is crucial, as probiotic tablets must have sufficient strength to ensure safe handling in addition to the highest possible dose of viable microorganisms. The compactibility describes the relationship between the tablet porosity and the tensile strength of the tablets. In general, the strength of the tablets increases with decreasing porosity (Fig. 5). The compactibility is highest for the formulation with DCP, followed by MCC, ISO and finally LAC. This is consistent with findings in an earlier study by the same authors on the tableting of spray-dried microorganisms and reflects the ranking of the compactibility of the pure substances (Vorländer et al., 2023d). In this case, no influence of the compression repetitions can be determined, either. The tablet strength results exclusively from the tablet porosity and the resulting attractive interactions between the particles, whereby it is irrelevant whether densification is achieved by increasing the compression stress or multiple compressions. This is consistent with results from studies in which different tableting speeds were used and for the majority of the formulations tested, altered compressibilities but unaffected compactibilities were observed (Mizunaga et al., 2021; Tye et al., 2005).

Commented [KaV31]: Reviewer 2: Line 263 (Figure 4): The y-axis is labeled relative survival. The survival rates in all graphs need to be consistently labeled unless relative survival is different to survival rate (as labeled in the other graphs). If there is a difference, then it needs to be explained.

Commented [KaV32R31]: We apologize for the error. Both formulations refer to the same thing. In order to avoid confusion, we have standardized the naming on the axis.

Commented [KaV33]: Reviewer 1: Figure 4 is impossible to describe as the survival rate go in one or the other direction with the number of compressions. Are the authors sure that: "The analogous observation of the survival as a function of the tablet porosity change also shows a better correlation in this study than the observation of the absolute porosity".

Commented [KaV34R33]: It is absolutely correct that shifts and crossings of the curves occur here. However, we fear that we have formulated our statement here in a somewhat misleading way. In fact, we do not want to rule the multiple crossings and shifts out at all and wrote ourselves that there is no systematic effect of the number of compressions. Instead, our statement refers to the fact that the totality of all data points is significantly closer together than in Figure 3. To clarify this in the manuscript, we reiterate that the correlation is meant across formulations. It is obvious that the correlation of all data points in Figure 4 with a common fit function is higher than in Figure 3.

Commented [KaV35]: Reviewer 1: L279-281: the reference cited is very recent for something known for years. Consider citing Tye et al JPS 94 2005

Commented [KaV36R35]: We have followed the recommendation and also cite the publication by Tye et al. In view of the fit of the formulations and results used, we have not omitted the more recent publication.

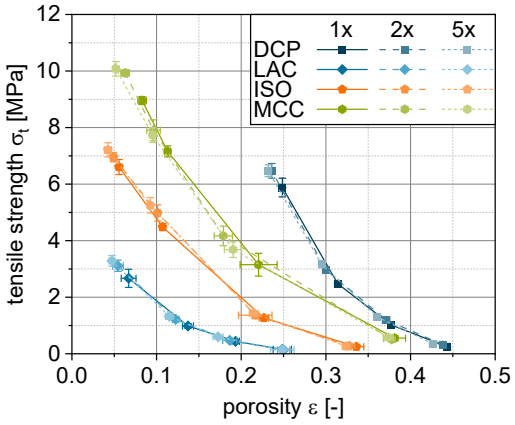


Fig. 5: Compactibility of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. The compression was carried out 1, 2 or 5 times. Data points represent mean and standard deviation ($n = 10$).

3.4. Tabletability

Without porosity as a structural parameter, tabletability correlates the relationship between compression stress and tablet strength. Tabletability results as a direct consequence of combining compressibility as a process function and compactibility as a property function. Accordingly, the compressibility curves are not surprising: The formulation with MCC shows the best tabletability and is followed by ISO, DCP and finally LAC (Fig. 6). Multiple compression is associated with an improvement in tabletability in line with the additional densification and unchanged compactibility. Similarly, this mechanism is present when the dwell time is varied (Wünsch et al., 2020).

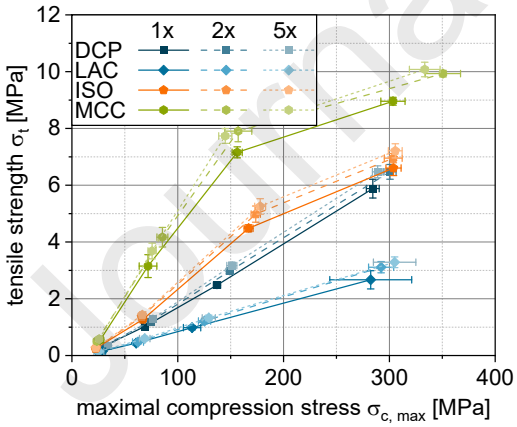


Fig. 6: Tabletability of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. The compression was carried out 1, 2 or 5 times. Data points represent mean and standard deviation ($n = 10$).

However, the question now is to what extent increased tabletability and reduced survival during multiple compression affect the overall performance of the tablets. For this purpose, the survival is considered as a function of the tensile strength of the tablets. It can be seen that both effects largely compensate each other. Double compression tends to be slightly advantageous. In the typical target tensile strength range of 1 to 1.7 MPa (Pitt and Heasley, 2013), the effect is less pronounced than at high compression stress and is therefore hardly relevant in the production of probiotic tablets.

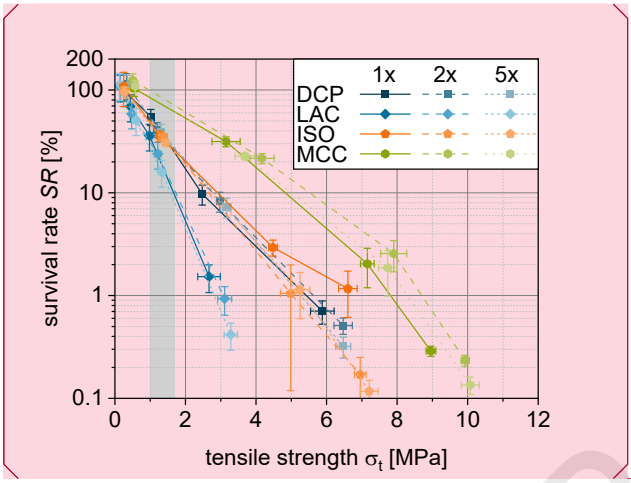


Fig. 7: Survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively, as a function of the tablet tensile strength. The compression was carried out 1, 2 or 5 times. Data points represent mean and standard deviation ($n = 3$). The gray-colored area marks the typical target tensile strength range of pharmaceutical tablets.

4. Conclusions

The survival of yeast cells during tableting was shown to be dependent on the formulation and the compression stress, which is consistent with current knowledge. In addition, a negative effect of compression repetition on survival related to the applied compression stress was identified. This could be linked to the additional densification and thus lower porosity of the tablets associated with multiple compression. The additional densification exposes the microorganisms to further pressure and shear stresses, whereby further lethal damage reduces survival. It was found that it is irrelevant to the extent of damage whether lower porosity is achieved by increasing the compression stress or applying the same compression stress multiple times. The same applies to the tensile strength of the tablets, i.e., the compactibility is unaffected by the number of compression repetitions. With multiple compressions, survival is reduced, whereby the porosity reduction could be confirmed as a scaling variable across formulations. At the same time, however, tabletability is improved due to the greater densification with multiple compression. Essentially, both effects, the lower survival and the higher tensile strength, compensate each other in this conflict of objectives, especially in the target tensile strength range of probiotic tablets. The present study thus contributes to strengthening the previously propagated mechanism of damage to viable microorganisms during tableting by reducing porosity and the associated intensification of compressive and shear stresses. At the same time, it becomes clear that formulation studies can be carried out with simple compression profiles without pre-compression, as tablet porosity determines survival and tensile strength regardless of the method used to achieve this structure.

Commented [KaV37]: Reviewer 1: Finally, the article misses the opportunity of discussing what really matters for the survival rate, in terms of plastic vs elastic deformations. Results seems to indicate that elastic deformation doesn't really matter as a change in porosity is necessary (i.e. plastic deformation) to see an impact on survival rate. This could be further discussed

Commented [KaV38R37]: Thank you for pointing this out. We have looked at this again in more detail and shown the survival as a function of the reduction of the minimum in-die porosity in order to illustrate the difference between the plastic deformation and the total deformation (plastic + elastic). It becomes clear that the cross-formulation correlation is lower when considering the reduction of the in-die porosity than when considering the reduction of the out-die porosity. This applies in particular to the formulations with MCC, which have a pronounced proportion of elastic deformation. The elastic deformation of the formulation is essentially caused by the excipients. The elastic deformation of the excipient particles appears to have a negligible influence on the survival of the microorganisms, even if this could certainly be

Commented [KaV39]: Reviewer 2: Line 303 (Figure 7): What does the grey area mean?

Commented [KaV40R39]: The gray area marks the typical target tensile strength range. We had forgotten to mention this in the caption and have now added it.

Commented [KaV41]: Reviewer 2: My main concern with the conclusions (shown in both the abstract and the conclusion sections) of the study is that the authors draw incorrect final conclusions about microorganism viability. Compression causes decrease survival (Fig 1). Compression also causes a decrease in porosity

Commented [KaV42R41]: We understand the basic idea behind this criticism. Figure 1 gives the impression that survival is directly dependent on the maximum compression stress. However, the figure does not show that there is a causal relationship here. To demonstrate such relationships, the interaction between process,

Commented [KaV43]: Reviewer 1: The first sentence of the conclusion supports our initial point: it is the same conclusion as in reference 2023d, which had already been demonstrated in 2023b and 2023a.

Commented [KaV44R43]: It is true that this first part is not a new insight. However, not all readers of the publication will have this deep insight in the literature as Rewier 1. Therefore, this short statement may help other readers to easier understand the whole subject of compression on microorganisms. Therefore, we would

Commented [KaV45]: Reviewer 1: Another point said by the authors is : "It was found that it is irrelevant to the extent of damage whether lower porosity is achieved by increasing the compression stress or applying the same compression stress multiple times". This point was not really demonstrated as the effect of

Commented [KaV46R45]: As mentioned above, we have modified Fig. 3 to make the effect more visible, even if the extend of this effect is admittedly limited. However, independently on the applied number of compression, the data points follow on a common curve for one formulation. However, for a higher

CRediT author statement

Karl Vorländer: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Funding acquisition. **Arno Kwade:** Conceptualization, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Jan Henrik Finke:** Conceptualization, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Ingo Kampen:** Conceptualization, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

This research was funded by the German Research Foundation (grant no. 457022725).

Declaration of competing interest

The authors declare no conflicts of interest. The funding organization and manufacturers who provided the excipients had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data availability

Data will be made available on request.

Acknowledgments

The authors acknowledge the funding of this research by the German Research Foundation (grant no. 457022725). The authors thank Budenheim KG, BENEO GmbH, J. Rettenmaier & Söhne GmbH + Co KG and MEGGLE GmbH & Co. KG for providing DCP, ISO, MCC and LAC, respectively.

References

Ananta, E., Volkert, M., Knorr, D., 2005. Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. International Dairy Journal 15, 399–409. https://doi.org/10.1016/j.idairyj.2004.08.004.

Ayorinde, J.O., Itiola, O.A., Odeku, O.A., Odeniyi, M.A., 2011. Influence of binder type and process parameters on the compression properties and microbial survival in diclofenac tablet formulations. Journal of Pharmaceutical Sciences 47, 845–854. https://doi.org/10.1590/S1984-82502011000400022.

Belton, P.S., Gil, A.M., 1994. IR and Raman spectroscopic studies of the interaction of trehalose with hen egg white lysozyme. Biopolymers 34, 957–961. https://doi.org/10.1002/bip.360340713.

Blair, T.C., Buckton, G., Bloomfield, S.F., 1991. On the mechanism of kill of microbial contaminants during tablet compression. International journal of pharmaceutics 72, 111–115. https://doi.org/10.1016/0378-5173(91)90048-S.

Broeckx, G., Vandenheuvel, D., Claes, I.J.J., Lebeer, S., Kiekens, F., 2016. Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. International journal of pharmaceutics 505, 303–318. https://doi.org/10.1016/j.ijpharm.2016.04.002.

Byl, E., Lebeer, S., Kiekens, F., 2018. Elastic recovery of filler-binders to safeguard viability of Lactobacillus rhamnosus GG during direct compression. European journal of pharmaceutics and biopharmaceutics. https://doi.org/10.1016/j.ejpb.2018.12.005.

Chan, E.S., Zhang, Z., 2002. Encapsulation of Probiotic Bacteria Lactobacillus Acidophilus by Direct Compression. Food and Bioproducts Processing 80, 78–82. https://doi.org/10.1205/09603080252938708.

Chesworth, K.A.C., Sinclair, A., Stretton, R.J., Hayes, W.P., 1977. Effect of tablet compression on the microbial content of granule ingredients. Microbios Letters 4, 41–45.

Cordone, L., Cottone, G., Giuffrida, S., 2007. Role of residual water hydrogen bonding in sugar/water/biomolecule systems: a possible explanation for trehalose peculiarity. Journal of Physics: Condensed Matter 19, 205110. https://doi.org/10.1088/0953-8984/19/20/205110.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Aurell Wistrom, C., 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. The Biochemical journal 242, 1–10. https://doi.org/10.1042/bj2420001.

e Silva, J.P.S., Sousa, S.C., Costa, P., Cerdeira, E., Amaral, M.H., Lobo, J.S., Gomes, A.M., Pintado, M.M., Rodrigues, D., Rocha-Santos, T., Freitas, A.C., 2013. Development of probiotic tablets using microparticles: viability studies and stability studies. AAPS PharmSciTech 14, 121–127. https://doi.org/10.1208/s12249-012-9898-9.

Fassihi, A.R., Parker, M.S., 1987. Inimical Effects of Compaction Speed on Microorganisms in Powder Systems with Dissimilar Compaction Mechanisms. Journal of Pharmaceutical Sciences 76, 466–470. https://doi.org/10.1002/jps.2600760611.

Fell, J.T., Newton, J.M., 1970. Determination of Tablet Strength by the Diametral-Compression Test. Journal of Pharmaceutical Sciences 59, 688–691. https://doi.org/10.1002/jps.2600590523.

Gurnham, C.F., Masson, H.J., 1946. Expression of Liquids from Fibrous Materials. Ind. Eng. Chem. 38, 1309–1315. https://doi.org/10.1021/ie50444a026.

Hansen, J., Kleinebudde, P., 2021. Enabling the direct compression of metformin hydrochloride through QESD crystallization. International journal of pharmaceutics 605, 120796. https://doi.org/10.1016/j.ijpharm.2021.120796.

Joint FAO/WHO Working Group, 2002. Guidelines for the Evaluation of Probiotics in Food: Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London Ontario, Kanada, 11 pp.

Klayraung, S., Viernstein, H., Okonogi, S., 2009. Development of tablets containing probiotics: Effects of formulation and processing parameters on bacterial viability. International journal of pharmaceutics 370, 54–60. https://doi.org/10.1016/j.ijpharm.2008.11.004.

Mazel, V., Tchoreloff, P., 2020. Role of Precompression in the Mitigation of Capping: A Case Study. J. Pharm. Sci. 109, 3210–3213. https://doi.org/10.1016/j.xphs.2020.07.021.

Mizunaga, D., Koseki, M., Kamemoto, N., Watano, S., 2021. Characterization of Tableting Speed-Dependent Deformation Properties of Active Pharmaceutical Ingredients in Powder Mixtures Using Out-of-Die Method. Chemical & pharmaceutical bulletin 69, 1184–1194. https://doi.org/10.1248/cpb.c21-00665.

Muller, C., Mazel, V., Dausset, C., Busignies, V., Bornes, S., Nivoliez, A., Tchoreloff, P., 2014. Study of the Lactobacillus rhamnosus Lcr35® properties after compression and proposition of a model to predict tablet stability. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 88, 787–794. https://doi.org/10.1016/j.ejpb.2014.07.014.

Nagashima, A.I., Pansiera, P.E., Baracat, M.M., Gómez, R.J.H.C., 2013. Development of effervescent products, in powder and tablet form, supplemented with probiotics Lactobacillus acidophilus and Saccharomyces boulardii. Food Science and Technology 33, 605–611. https://doi.org/10.1590/S0101-20612013000400002.

Oliver, A.E., Crowe, L.M., Crowe, J.H., 1998. Methods for dehydration-tolerance: Depression of the phase transition temperature in dry membranes and carbohydrate vitrification. Seed Sci. Res. 8, 211–221. https://doi.org/10.1017/S0960258500004128.

Patel, S., Kaushal, A.M., Bansal, A.K., 2006. Compression physics in the formulation development of tablets. Critical reviews in therapeutic drug carrier systems 23, 1–65. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v23.i1.10.

Pitt, K.G., Heasley, M.G., 2013. Determination of the tensile strength of elongated tablets. Powder Technology 238, 169–175. https://doi.org/10.1016/j.powtec.2011.12.060.

Plumpton, E.J., Gilbert, P., Fell, J.T., 1986a. Effect of spatial distribution of contaminant microorganisms within tablet formulations on subsequent inactivation through compaction. International journal of pharmaceutics 30, 237–240. https://doi.org/10.1016/0378-5173(86)90085-2.

Plumpton, E.J., Gilbert, P., Fell, J.T., 1986b. The survival of microorganisms during tabletting. International journal of pharmaceutics 30, 241–246. https://doi.org/10.1016/0378-5173(86)90086-4.

Poulin, J.-F., Caillard, R., Subirade, M., 2011. β-Lactoglobulin tablets as a suitable vehicle for protection and intestinal delivery of probiotic bacteria. International journal of pharmaceutics 405, 47–54. https://doi.org/10.1016/j.ijpharm.2010.11.041.

Puckhaber, D., Finke, J.H., David, S., Serratoni, M., Zafar, U., John, E., Juhnke, M., Kwade, A., 2022. Prediction of the impact of lubrication on tablet compactibility. International journal of pharmaceutics 617, 121557. https://doi.org/10.1016/j.ijpharm.2022.121557.

Reynolds, G.K., Campbell, J.I., Roberts, R.J., 2017. A compressibility based model for predicting the tensile strength of directly compressed pharmaceutical powder mixtures. International journal of pharmaceutics 531, 215–224. https://doi.org/10.1016/j.ijpharm.2017.08.075.

Santivarangkna, C., 2016. Storage Stability of Probiotic Powder, in: Petra Foerst, Chalat Santivarangkna (Eds.), Advances in Probiotic Technology.

Stadler, M., Viernstein, H., 2001. Tablet Formulations of Viable Lactic Acid Bacteria. Sci. Pharm. 69, 249–255. https://doi.org/10.3797/scipharm.aut-01-195.

Tye, C.K., Sun, C.C., Amidon, G.E., 2005. Evaluation of the effects of tableting speed on the relationships between compaction pressure, tablet tensile strength, and tablet solid fraction. Journal of Pharmaceutical Sciences 94, 465–472. https://doi.org/10.1002/jps.20262.

Vorländer, K., Bahlmann, L., Kwade, A., Finke, J.H., Kampen, I., 2023a. Effect of Process Parameters, Protectants and Carrier Materials on the Survival of Yeast Cells during

Fluidized Bed Granulation for Tableting. Pharmaceutics 15, 884. https://doi.org/10.3390/pharmaceutics15030884.

Vorländer, K., Bahlmann, L., Kwade, A., Finke, J.H., Kampen, I., 2023b. Tableting of fluidized bed granules containing living microorganisms. European journal of pharmaceutics and biopharmaceutics. https://doi.org/10.1016/j.ejpb.2023.03.011.

Vorländer, K., Bahlmann, L., Kwade, A., Henrik Finke, J., Kampen, I., 2023c. Influence of compression kinetics during tableting of fluidized bed-granulated microorganisms on microbiological and physical-mechanical tablet properties. European journal of pharmaceutics and biopharmaceutics. https://doi.org/10.1016/j.ejpb.2023.05.012.

Vorländer, K., Kampen, I., Finke, J.H., Kwade, A., 2020. Along the Process Chain to Probiotic Tablets: Evaluation of Mechanical Impacts on Microbial Viability. Pharmaceutics 12. https://doi.org/10.3390/pharmaceutics12010066.

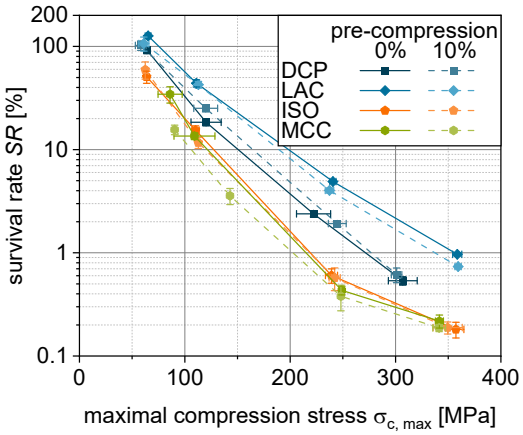
Vorländer, K., Pramann, P., Kwade, A., Finke, J.H., Kampen, I., 2023d. Process and formulation parameters influencing the survival of Saccharomyces cerevisiae during spray drying and tableting. International journal of pharmaceutics 642, 123100. https://doi.org/10.1016/j.ijpharm.2023.123100.

Wolkers, W.F., Oldenhof, H. (Eds.), 2021. Cryopreservation and Freeze-Drying Protocols, 4th ed. Springer US, New York, NY.

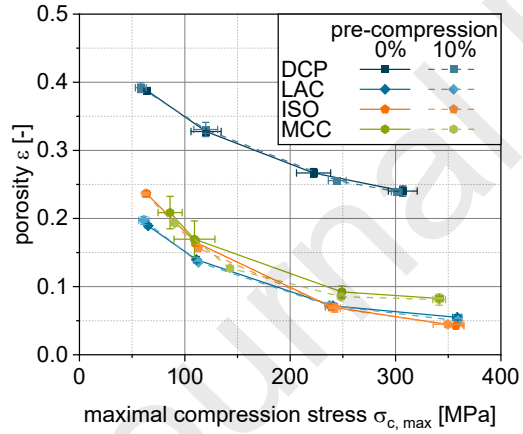
Wünsch, I., Friesen, I., Puckhaber, D., Schlegel, T., Finke, J.H., 2020. Scaling Tableting Processes from Compaction Simulator to Rotary Presses-Mind the Sub-Processes. Pharmaceutics 12. https://doi.org/10.3390/pharmaceutics12040310.

Zhao, J., Burt, H.M., Miller, R.A., 2006. The Gurnham equation in characterizing the compressibility of pharmaceutical materials. International journal of pharmaceutics 317, 109–113. https://doi.org/10.1016/j.ijpharm.2006.02.054.

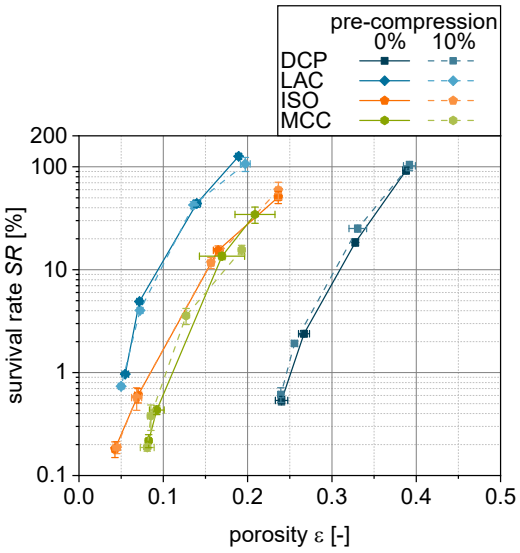
Supplementary Information



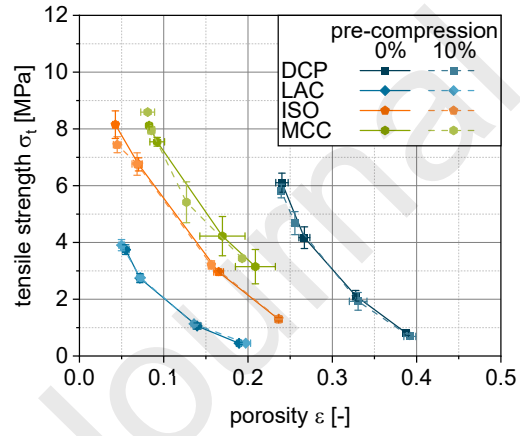
Suppl. 1: Survival as a function applied compression stress on survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 3$).



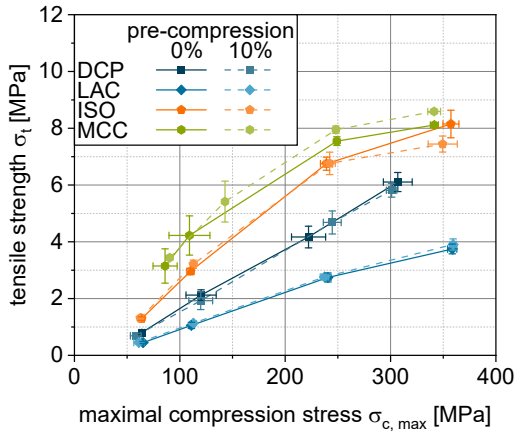
Suppl. 2: Compressibility of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 5$).



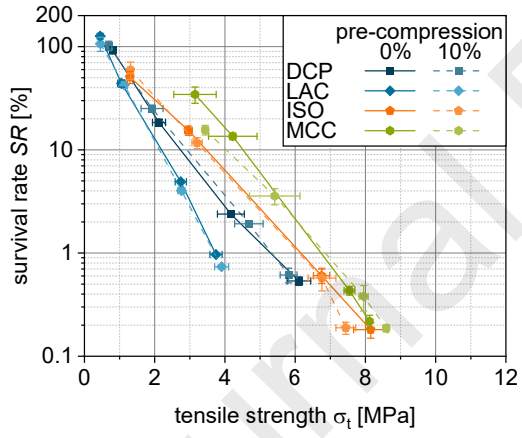
Suppl. 3: Survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively, as a function of tablet porosity. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 3$).



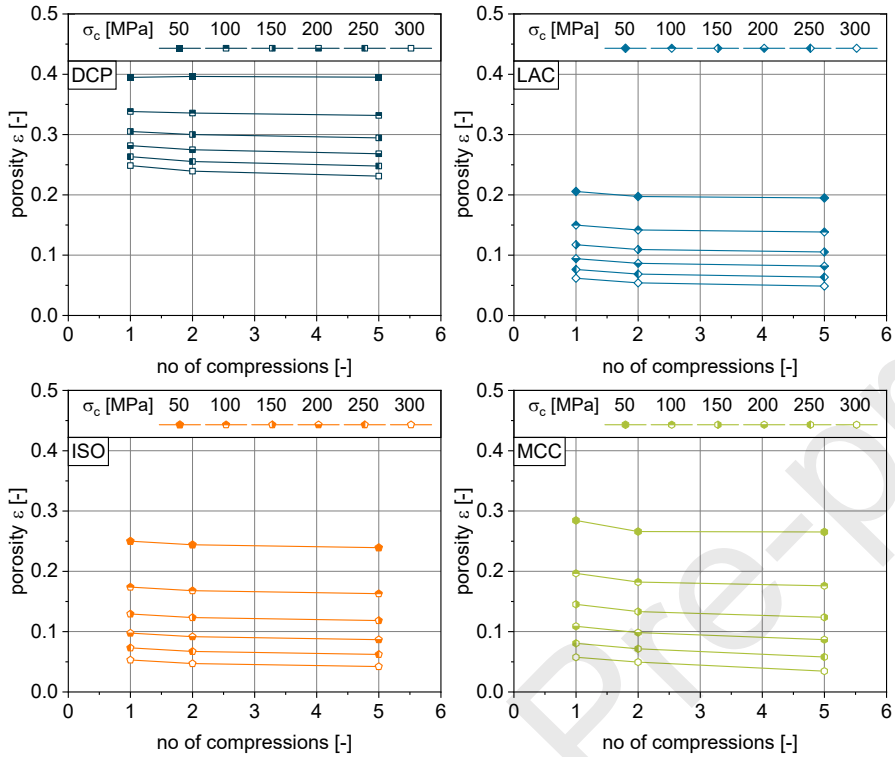
Suppl. 4: Compactibility of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 5$).



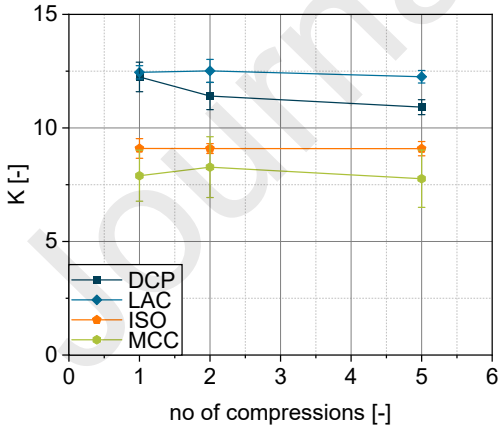
Suppl. 5: Tabletability of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 5$).



Suppl. 6: Survival of spray dried yeast cells mixed with DCP, LAC, ISO and MCC, respectively, as a function of the tablet tensile strength. Compression was either without pre-compression or with pre-compression of 10% of the main compression stress. Data points represent mean and standard deviation ($n = 3$).



Suppl. 7: Porosity values calculated with fit parameters of compressibility model for defined compression stresses depend on the number of compressions. Error bars are not shown to ensure the clarity of the illustration.



Suppl. 8: Fit parameter K of compressibility model dependent on the number of applied compression phases.