Survival of *Lactobacillus Rhamnosus* in a Tableting Process

April 26th 2023

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Short introduction into PROBIOTICS

BIOGRUND

Short introduction into PROBIOTICS

- Probiotics are live, apathogenic microorganisms (e.g. bacteria or yeasts) that are considered / proven to bring about distinct health benefits in humans or animals:

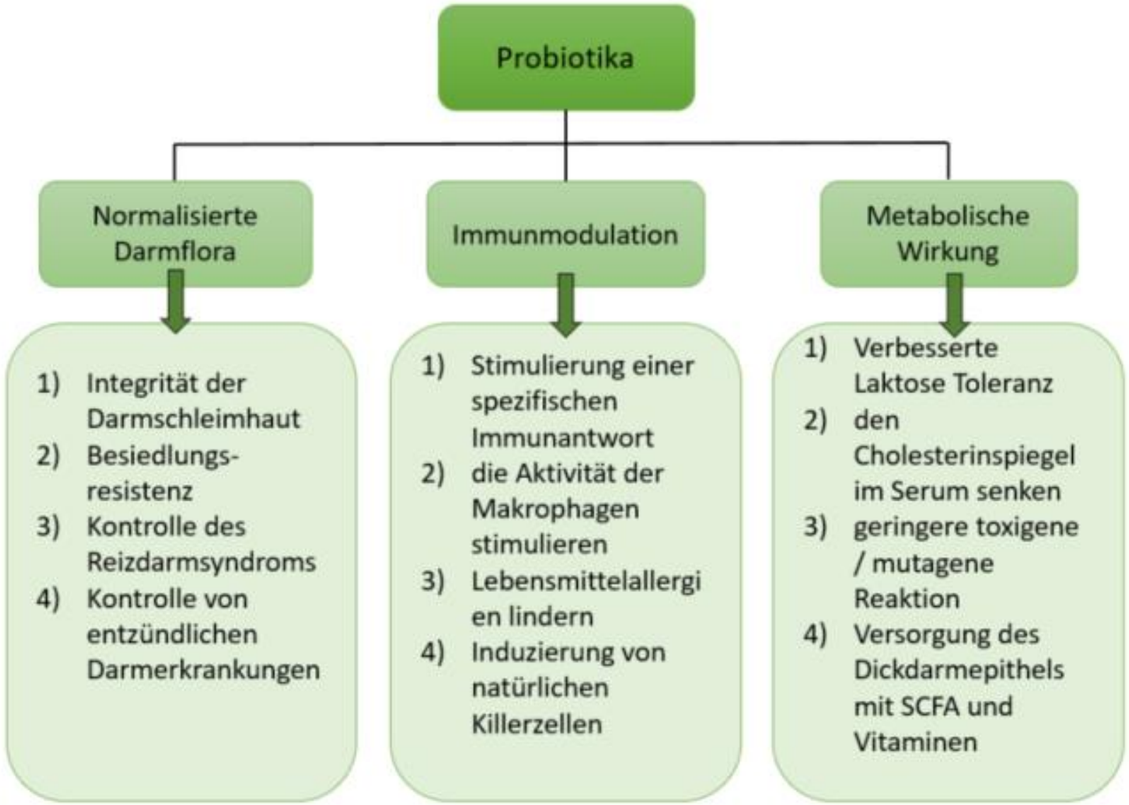


Abbildung 2: Schematische Darstellung über die verschieden Funktionen von Probiotika (in Anlehnung an [20])

Influences on survival of probiotic bacteria

Influences on survival of probiotic bacteria

- From their cultivation to consumption probiotic microorganisms are exposed to various type of stress that can reduce viability of the strains:

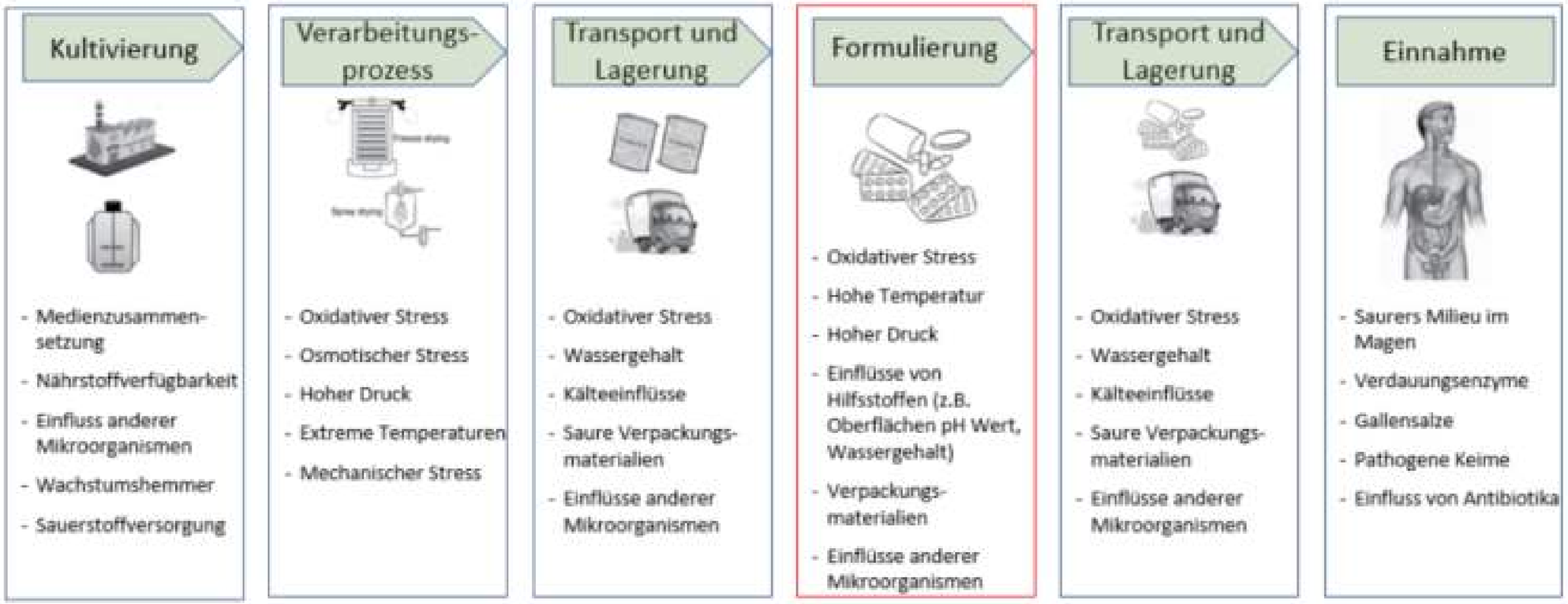


Abbildung 1: negative Einflüsse auf die Vitalität von probiotischen Kulturen von der Kultivierung bis zur Einnahme Probiotika (in Anlehnung an [1, 6])

Influences of tableting temperature and compression force on the survival of *L. rhamnosus*

BIOGRUND

Influences of tableting temperature and compression force on the survival of *L. rhamnosus*

Aims: Evaluation of machine and formulation influences on the survival of *L. rhamnosus*

Tablet Compositions: 200 mg Tablets, RoTab T rotary lab press; convex punches; ∅ = 7 mm; feeding speed 20 rpm

Materialien	M_LGG1	M_LGG2	M_LGG3	M_LGG4	M_LGG5	6M_LGG6	M_LGG7
DCPA	57,6	15,4	57,6	61,6	14,4	14,4	59,6
MCC	14,4	61,6	14,4	15,4	57,6	57,6	14,9
DIS	2,0	2,0	2,0	2,0	2	2	2,0
LUB	1,0	1,0	1,0	1,0	1	1	1,0
LGG	10,0	10,0	10,0	10,0	10	10	10,0
MAB	10,0	10,0	10,0	10,0	10	10	10,0
L-HPC	5,0	-	-	-	-	5	-
HPC	-	-	5,0	-	5	-	2,5

Influences of tableting temperature and compression force on the survival of *L. rhamnosus*

Tablet Compositions:

Tabelle 3: Rezeptur von CompactCel® F 900.17 DIS [

Flohsamenschalen
Hafer Fasern
Bio-Kartoffelstärke
Agave-Inulin-Pulver

Tabelle 4: Rezeptur von CompactCel® F 200.28 LUB

Mikrokristalline Cellulose (Typ 105)
Hafer Fasern
Sonnenblumenöl

Tabelle 5: Rezeptur von CompactCel® F 200.25 MAB

Mikrokristalline Cellulose (Typ 200)
Isomalt
Calcium Carbonat
Vorgelatinierte Stärke

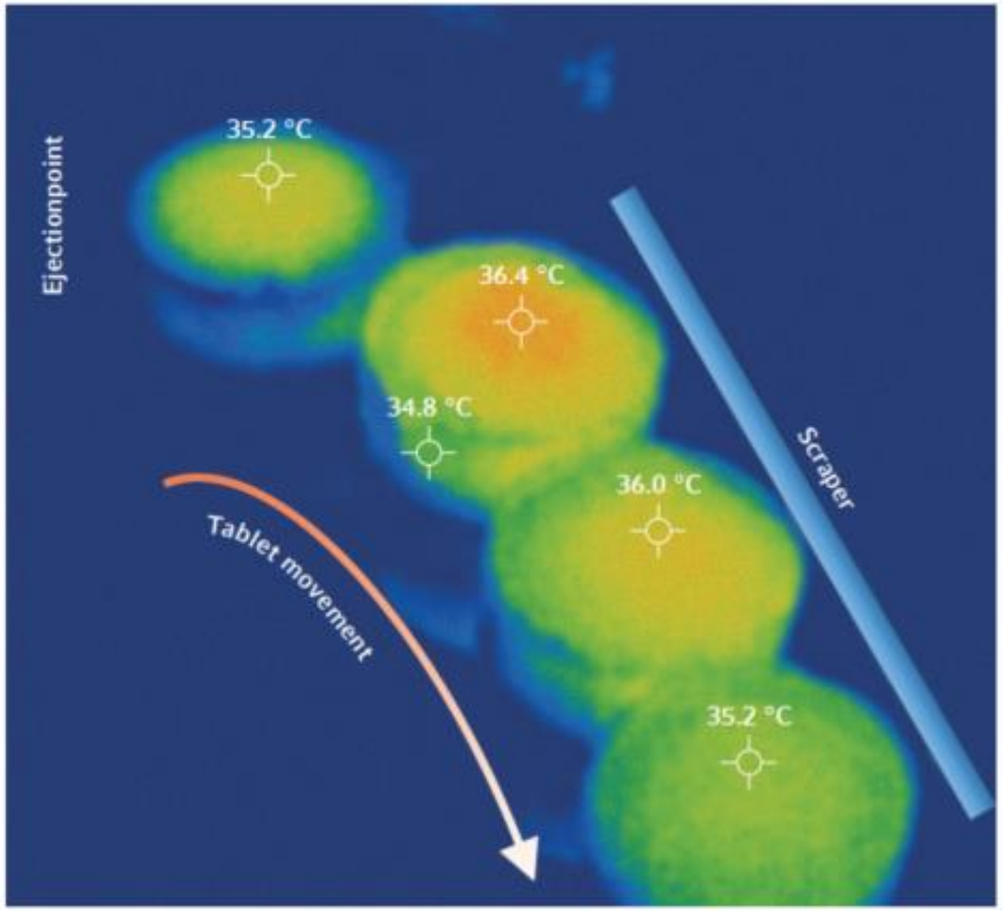
Influences of tableting temperature and compression force on the survival of *L. rhamnosus*

Tableting Parameters: DOE settings for the tablet press with variations of MCF and rotational speed

Name	Rezeptur	Hauptpresskraft [kN]	Umdrehungszahl [rpm]
N-PLA1 v N-LGG1	M_PLA1 v M_LGG1	4	10
N-PLA2 v N-LGG2	M_PLA2 v M_LGG2	4	10
N-PLA3 v N-LGG3	M_PLA3 v M_LGG3	12	10
N-PLA4 v N-LGG4	M_PLA2 v M_LGG2	12	40
N-PLA5 v N-LGG5	M_PLA4 v M_LGG4	4	40
N-PLA6v N-LGG6	M_PLA5v M_LGG5	4	40
N-PLA7 v N-LGG7	M_PLA4 v M_LGG4	12	40
N-PLA8 v N-LGG8	M_PLA6v M_LGG6	12	40
N-PLA9 v N-LGG9	M_PLA7 v M_LGG7	8	25
N-PLA10 v N-LGG10	M_PLA7 v M_LGG7	8	25
N-PLA11 v N-LGG11	M_PLA7 v M_LGG7	8	25

Influences of tableting temperature and compression force on the survival of *L. rhamnosus*

Tablet Analysis : Breaking Force, Friability, Ejection Force, Tablet Temerature, CFU count



https://www.optris.global/optris-xi-400

Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

- Results of the 11 rund within the DOE framework

Batch	Bruchkraft	SD	RSD	Zugfestigkeit	SD	RSD	Zerfallszeit	SD	RSD	Ausstoßkraft	SD	RSD	Temperatur	SD	RSD
	[N]	[N]	[%]	[N/mm²]	[N/mm²]	[%]	[s]	[s]	[%]	[N]	[N]	[%]	[°C]	[°C]	[%]
N-LGG1	51,6	2,7	5,2	1,1	0,1	4,6	40	11	26,7	279	28	10,0	29,9	0,3	1,0
N-LGG2	114,3	8,4	7,3	2,2	0,2	7,1	120	35	29,6	235	47	20,0	31,6	0,1	0,4
N-LGG3	175,6	14,5	8,3	4,6	0,4	8,5	3804	530	13,9	457	55	12,0	36,3	0,2	0,4
N-LGG4	256,2	15,9	6,2	6,0	0,4	6,2	3480	439	12,6	466	90	19,3	36,1	0,2	0,6
N-LGG5	35,0	3,7	10,6	0,7	0,1	9,8	29	4	13,7	271	21	7,7	31,2	0,2	0,5
N-LGG6	103,9	16,8	16,2	2,0	0,3	16,2	564	275	48,7	213	21	9,9	32,7	0,4	1,3
N-LGG7	129,7	8,5	6,6	3,3	0,2	6,6	678	157	23,1	438	45	10,2	39,7	0,1	0,4
N-LGG8	250,6	13,7	5,5	5,6	0,3	6,0	2021	170	8,4	341	67	19,7	40,6	0,2	0,6
N-LGG9	96,4	14,1	14,7	2,3	0,3	14,9	799	211	26,5	381	39	10,0	34,5	0,1	0,3
N-LGG10	97,3	6,0	6,2	2,4	0,2	7,6	976	223	22,9	391	51	13,0	35,2	0,2	0,7
N-LGG11	101,5	10,9	10,7	2,5	0,3	10,7	1024	230	22,5	375	41	10,9	35,2	0,2	0,5

Materialien	M_LGG1	M_LGG2	M_LGG3	M_LGG4	M_LGG5	6M_LGG6	M_LGG7
DCPA	57,6	15,4	57,6	61,6	14,4	14,4	59,6
MCC	14,4	61,6	14,4	15,4	57,6	57,6	14,9
DIS	2,0	2,0	2,0	2,0	2	2	2,0
LUB	1,0	1,0	1,0	1,0	1	1	1,0
LGG	10,0	10,0	10,0	10,0	10	10	10,0
MAB	10,0	10,0	10,0	10,0	10	10	10,0
L-HPC	5,0	-	-	-	-	5	-
HPC	-	-	5,0	-	5	-	2,5

Name	Rezeptur	Hauptpresskraft [kN]	Umdrehungszahl [rpm]
N-PLA1 v N-LGG1	M_PLA1 v M_LGG1	4	10
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N-PLA3 v N-LGG3	M_PLA3 v M_LGG3	12	10
N-PLA4 v N-LGG4	M_PLA2 v M_LGG2	12	40
N-PLA5 v N-LGG5	M_PLA4 v M_LGG4	4	40
N-PLA6v N-LGG6	M_PLA5v M_LGG5	4	40
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N-PLA8 v N-LGG8	M_PLA6v M_LGG6	12	40
N-PLA9 v N-LGG9	M_PLA7 v M_LGG7	8	25
N-PLA10 v N-LGG10	M_PLA7 v M_LGG7	8	25
N-PLA11 v N-LGG11	M_PLA7 v M_LGG7	8	25

Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

- Survival (viability) of L. rhamnosus within the DOE framework

Batch	Keimzahl	SD	RSD
	[10^11 KBE/g]	[KBE/g]	[%]
N-LGG1	1,15	$6,92*10^{10}$	60,4
N-LGG2	2,77	$5,49*10^{10}$	19,8
N-LGG3	1,13	$3,39*10^{10}$	30,0
N-LGG4	1,84	$8,92*10^{10}$	48,6
N-LGG5	2,12	$8,25*10^{10}$	38,9
N-LGG6	2,58	$4,12*10^{10}$	16,0
N-LGG7	1,38	$2,55*10^{10}$	18,5
N-LGG8	1,29	$1,26*10^{10}$	9,8
N-LGG9	1,51	$4,93*10^{10}$	32,6
N-LGG10	1,24	$8,28*10^{9}$	6,7
N-LGG11	1,15	$2,60*10^{10}$	22,5

Materialien	M_LGG1	M_LGG2	M_LGG3	M_LGG4	M_LGG5	6M_LGG6	M_LGG7
DCPA	57,6	15,4	57,6	61,6	14,4	14,4	59,6
MCC	14,4	61,6	14,4	15,4	57,6	57,6	14,9
DIS	2,0	2,0	2,0	2,0	2	2	2,0
LUB	1,0	1,0	1,0	1,0	1	1	1,0
LGG	10,0	10,0	10,0	10,0	10	10	10,0
MAB	10,0	10,0	10,0	10,0	10	10	10,0
L-HPC	5,0	-	-	-	-	5	-
HPC	-	-	5,0	-	5	-	2,5

Name	Rezeptur	Hauptpresskraft [kN]	Umdrehungszahl [rpm]
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N-PLA5 v N-LGG5	M_PLA4 v M_LGG4	4	40
N-PLA6v N-LGG6	M_PLA5v M_LGG5	4	40
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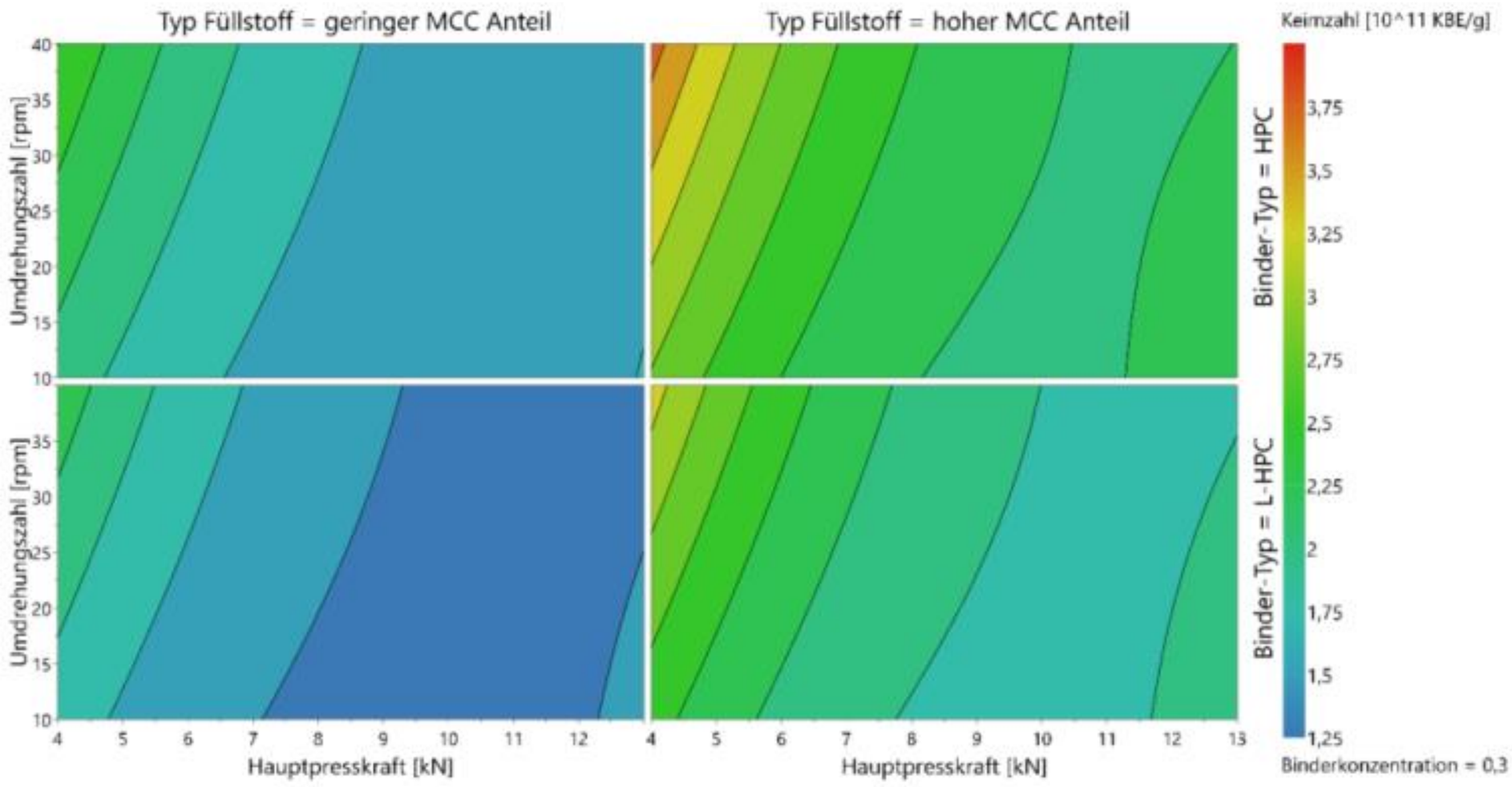
N-PLA8 v N-LGG8	M_PLA6v M_LGG6	12	40
N-PLA9 v N-LGG9	M_PLA7 v M_LGG7	8	25
N-PLA10 v N-LGG10	M_PLA7 v M_LGG7	8	25
N-PLA11 v N-LGG11	M_PLA7 v M_LGG7	8	25

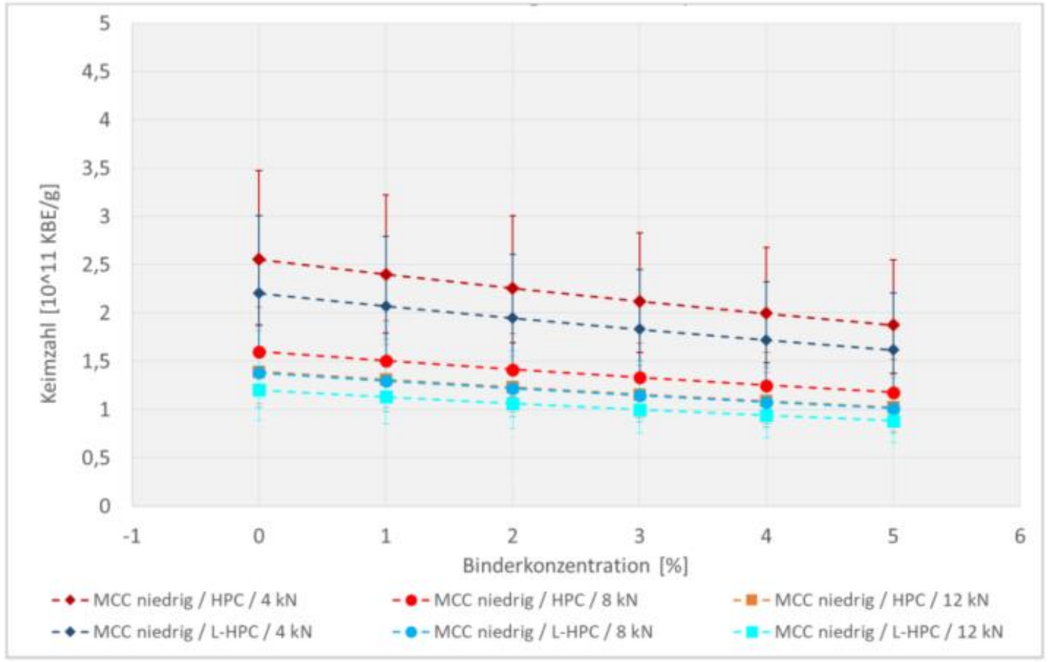
Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

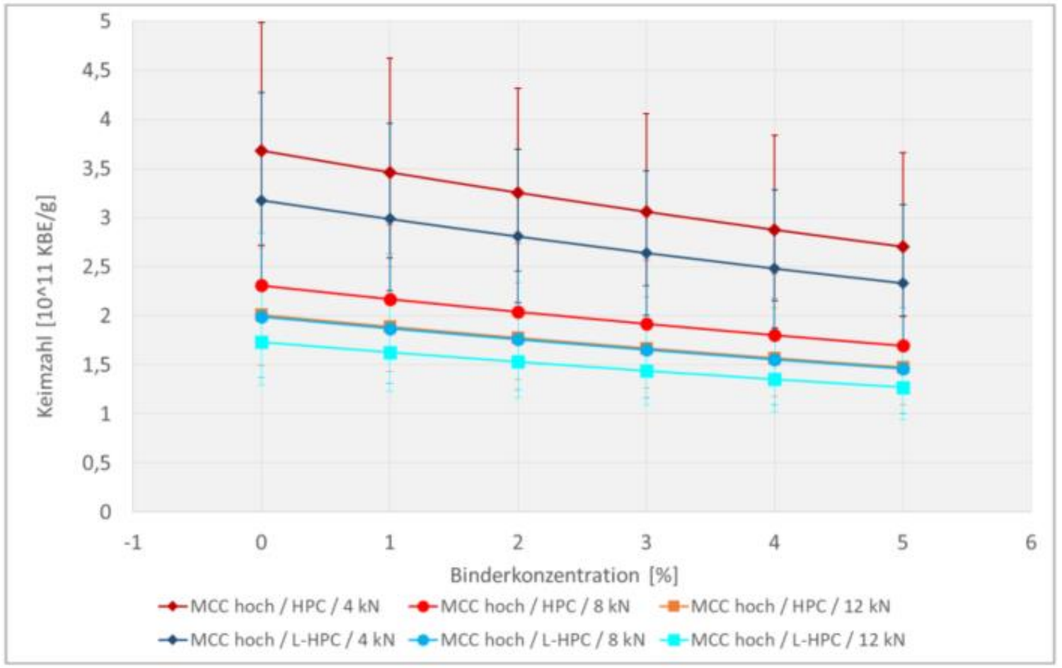
- Modelling of the data showed that the formulations with low concenctrations of MCC resulted in decreased number of viable bacteria
- Addtionally the compaction force plays a pivotal role
- L-HPC was found to be the less favourable binder of the two candidates



Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

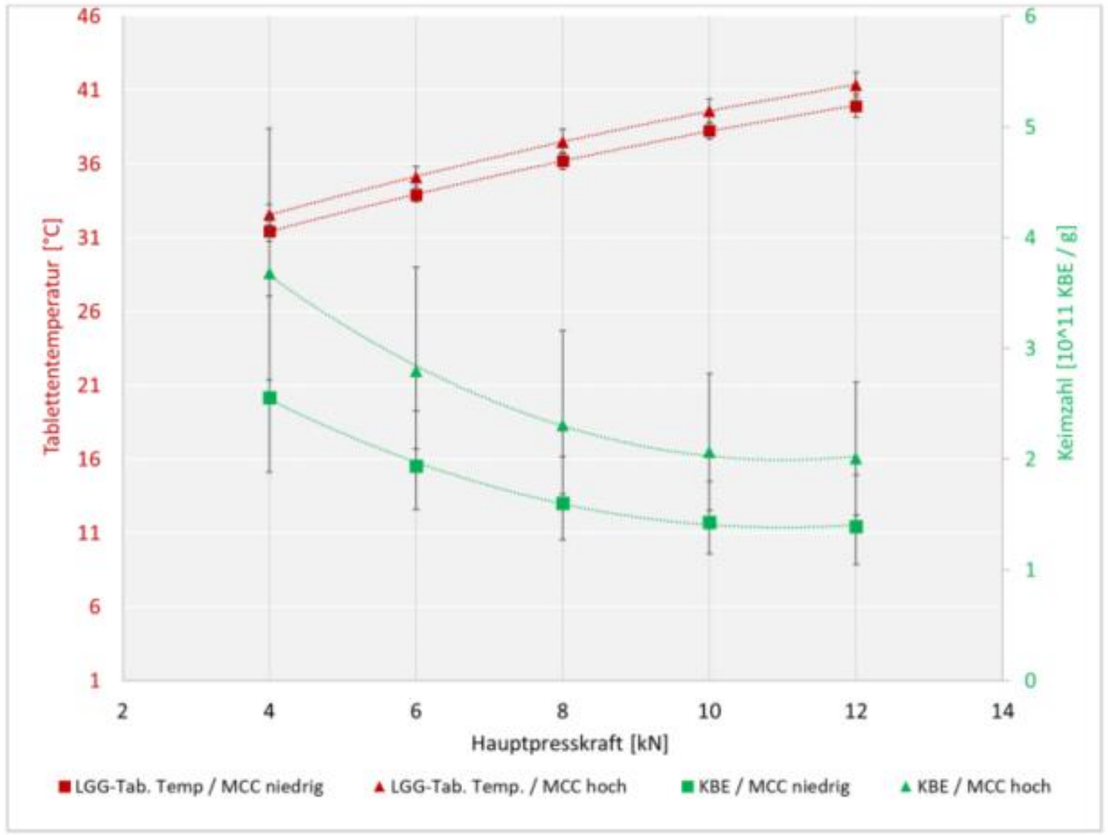
- The influences of the binders were not shown in the previous graph and are summarised below (data generated from data modelling)





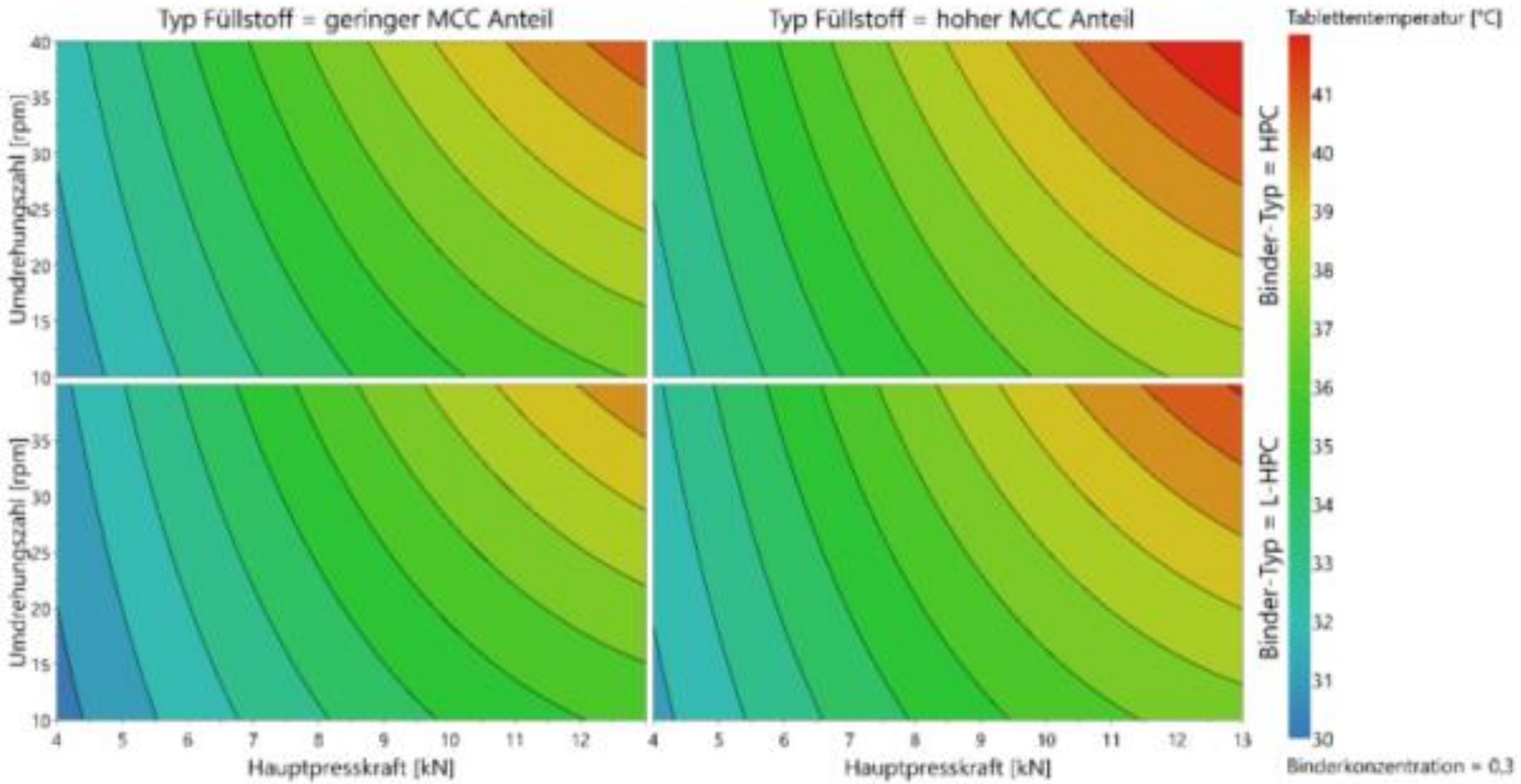
Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

- Looking closer at the influence of tablet temperature we see that there is a correlation between viability and compaction force but little correlation between tablet temperature and viability.



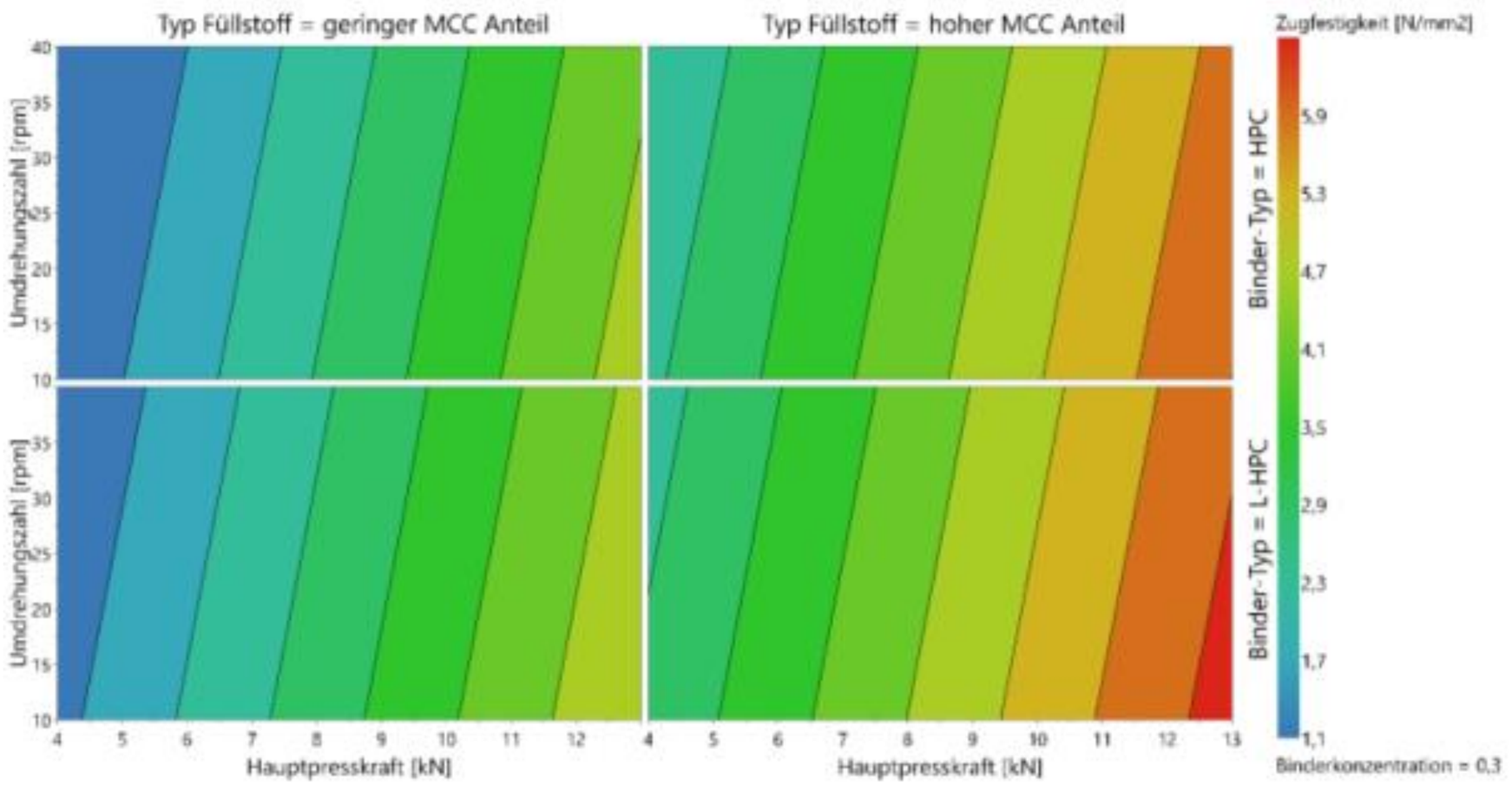
Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

- Tablet temperatures were higher with the plastically deforming (high MCC) mixtures
- This is congruent with the literature and is correlated to the deformation behaviour and heat capacity of the used materials
- As shown on the previous slide the higher temperature of the tablets with the high MCC content did not have a negative effect on bacteria survival



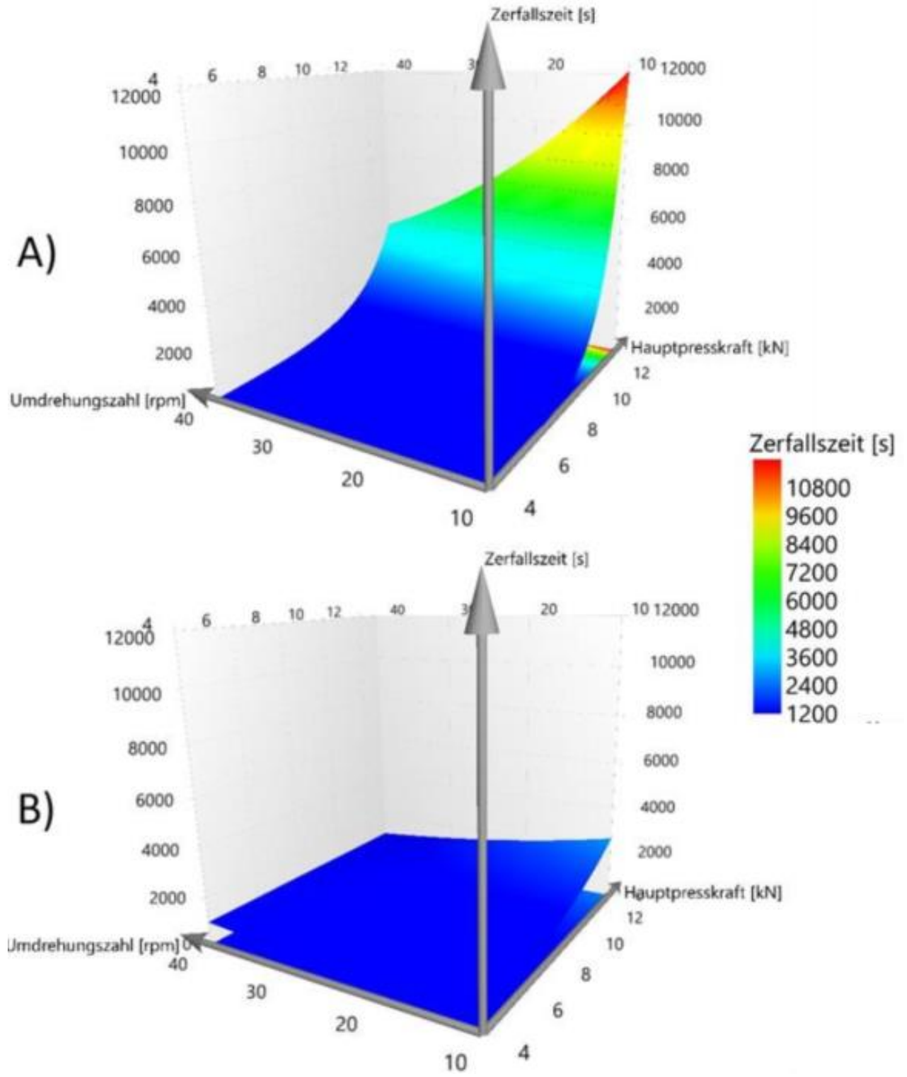
Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

- As excpected the plastically deforming mixtures yielded a higher tablet hardness than those made with brittle components
- The binder had little effect at the shown concentration of 0,3 %
- Increasing the tableting speed resulted in decreased tablet hardness



Influences of tableting temperature and compression force on the survival of *L. Rhamnosus: Results*

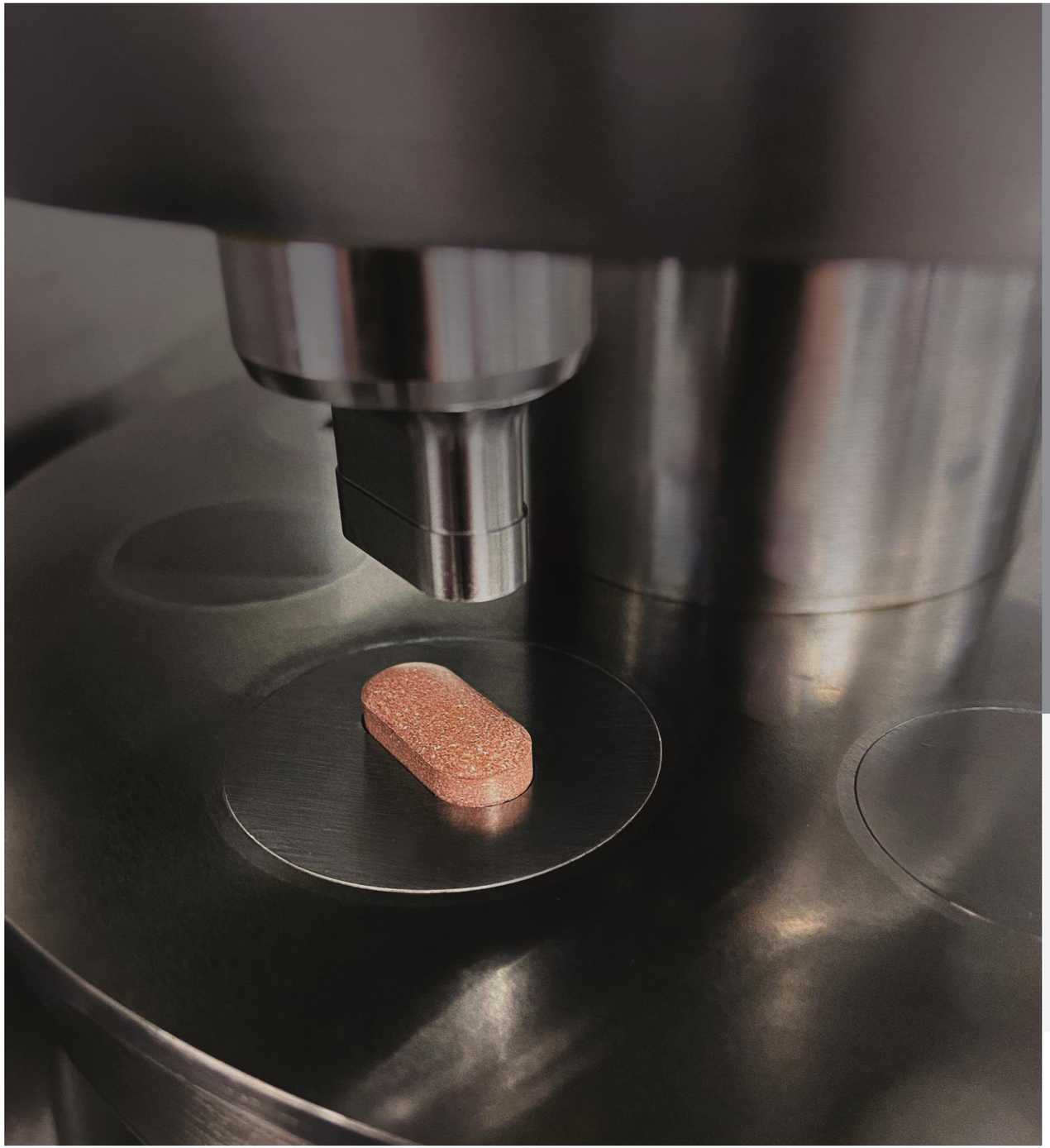
- Surprisingly the use of L-HPC (A) resulted in a strong delay of disintegration times at high compaction forces in comparison to the use of HPC (B)
- At lower compaction forces the use of the binders had little effect on the disintegration times



Influences of tableting temperature and compression force on the survival of *L. Rhamnosus*

- It was found that the main compression force plays the most important role regarding the viability of the bacteria
- However, formulation factors were of great significance too
- Brittle (hard) materials seem to be very unfavorable for use with live microorganisms
- It can be assumed that the microorganisms get ripped apart during the compaction with such materials due to sharp particle edges
- Higher compaction speeds had a positive impact by reducing the load impact time on the bacteria
- Increasing the binder concentrations had an adverse effect on the viability

LGG-Tabletten	Keimzahl
Typ Füllstoff	+
Hauptpresskraft	+++
Umdrehungszahl	+
Bindertyp	+
Binderkonzentration	++
HPK^2	+++
Interaktion HPK+ UDZ	++



Formulation Optimization

Formulation Optimization

Aims: Improve compaction behaviour of mixture at low compaction force

Control: Tablet Tensile Strength, Friability, Disintegration Time, Powder Flow

Tablet Compositions:

Compaction Force	MCC	Magnesium Carbonate	Isomalt	preg Starch	Psylium Husk	Oat Fibre	Inulin Powder	LGG	Mg-Stearate	HPC
kN	%	%	%	%	%	%	%	%	%	%
4	50 - 90	0-5	0 - 25	0- 25	0-5	0 - 5	0 - 5	10	1	0,5 - 2,5

Tableting Parameters: 200 mg Tablets, RoTab T rotary lab press; convex punches; ∅ = 7 mm; feeding speed 20 rpm, turret speed 25 rpm

Formulation Optimization

- Powder flow was sufficient for all mixtures with only minor differences between the mixtures
- Friability was also low for most tablets
- The Ejection force was negligible for all formulations

Exp Name	AOR [°]	Funnel Flow [g/min]
N1	30,1	4,95
N2	30,5	4,75
N3	29,2	4,70
N4	30,5	4,75
N5	31,0	4,05
N6	30,5	5,75
N7	30,5	4,75
N8	30,5	5,45
N9	30,5	4,50
N10	30,1	4,55
N11	30,1	4,40
N12	29,7	3,35
N13	30,5	4,15
N14	30,5	4,05
N15	30,1	3,95

Exp Name	Friability [%]	Ejection Force [N]
N1	0,003051	0,01
N2	0,411795	0,01
N3	0,045326	0,01
N4	0,001	4
N5	0,156867	0,27
N6	0,001	0,01
N7	0,001	0,01
N8	0,001	0,01
N9	0,319969	0,01
N10	0,236438	0,01
N11	0,001	0,01
N12	0,097356	0,01
N13	0,001	0,01
N14	0,001	0,01
N15	0,001	0,01

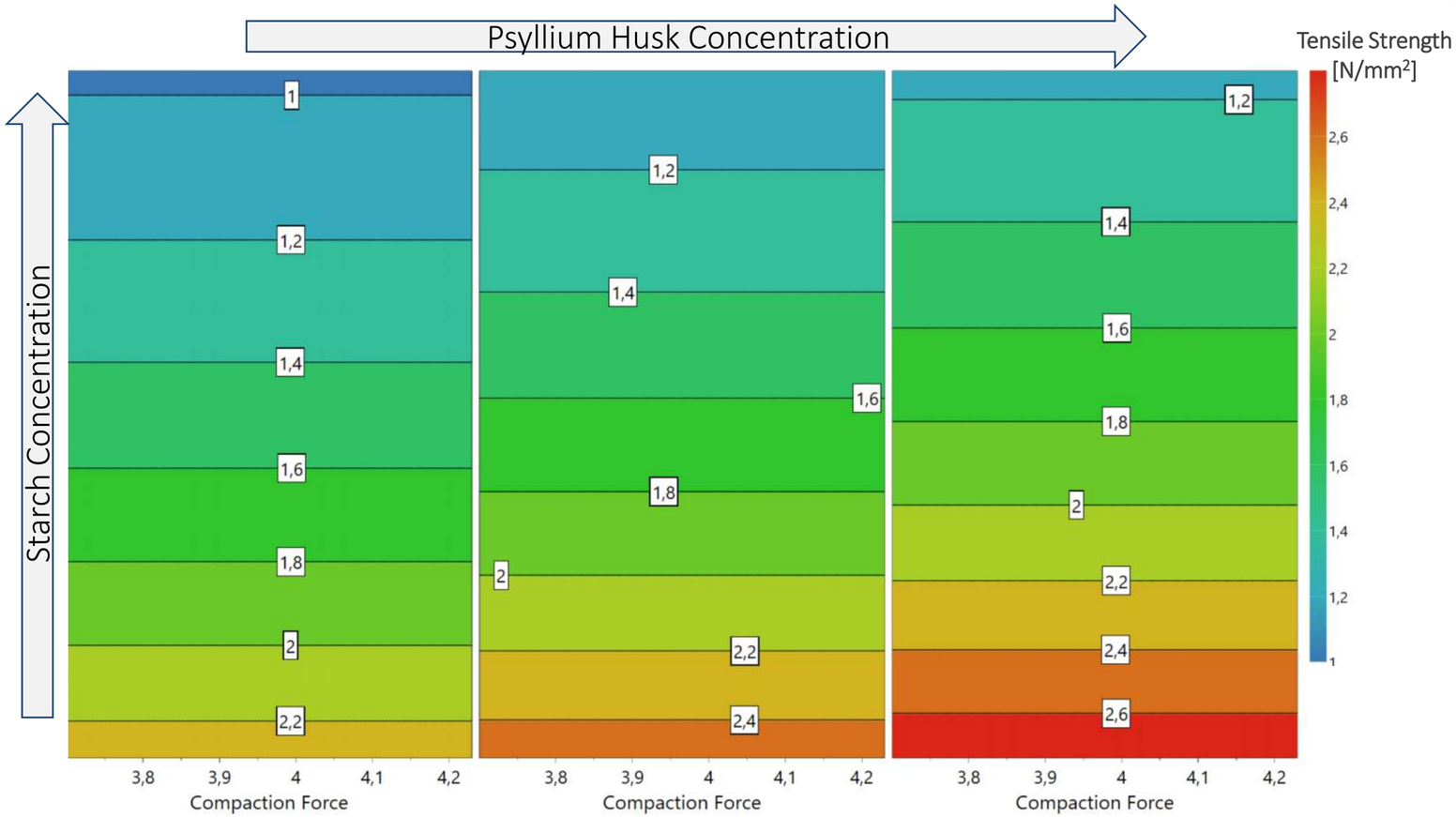
Formulation Optimization

- While little variation was observed for EJF, powder flow and friability large differences were observed for the tablet hardness and disintegration times
- Therefore, these were subjected to further data modelling and optimization

Exp Name	Tensile Strength [N/mm^2]	Disintegration time [min]
N1	2,031	2,110
N2	0,732	11,790
N3	2,476	11,170
N4	2,536	14,900
N5	1,214	4,483
N6	2,441	15,833
N7	2,915	8,372
N8	2,548	19,906
N9	0,825	6,944
N10	0,882	13,144
N11	2,555	6,006
N12	0,936	18,256
N13	1,831	8,833
N14	1,572	11,294
N15	1,522	10,944

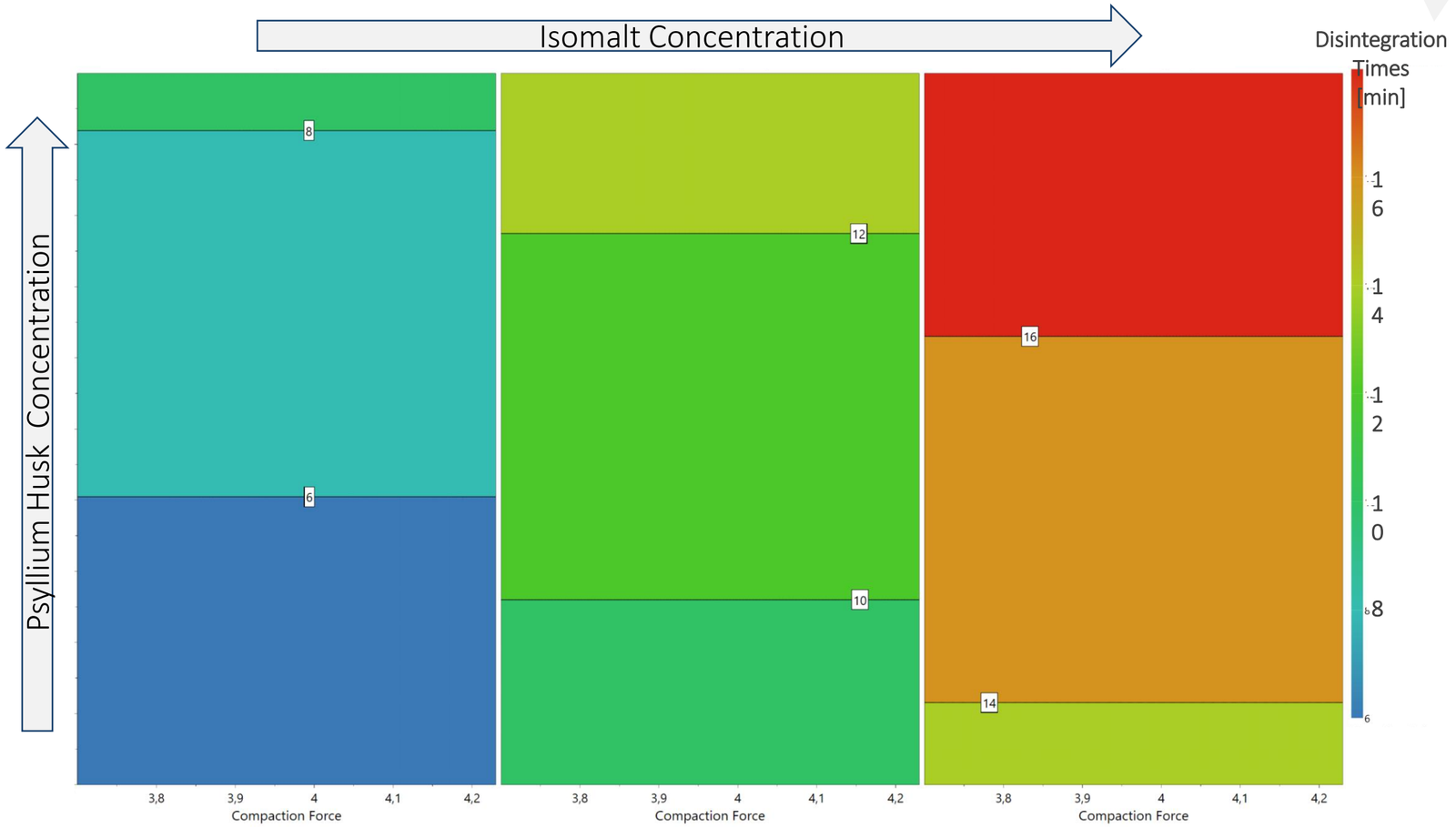
Formulation Optimization

- It was found that the tensile strength of the tablets depends mostly on the isomalt, starch and psyllium husk concentrations



Formulation Optimization

- It was found that the disintegration time of the tablets depends mostly on the isomalt and psyllium husk concentrations



Compaction Simulation

Compaction Simulation

Aims:

Checking if the last two optimization candidates work well in high speed industrial tableting processes

Evaluate if high speed tableting processes have unexpected / un-modelled effects on bacteria viability

Tablet Compositions:

Two formulations that contain MCC, Magnesium Carbonate, Isomalt, pregelatinized starch, psyllium husk, oat fibre, inulin powder, L. rhamnosus, Mg-St and HPC super fine powder

Tableting Parameters:

StylOne Evolution compaction simulator ; convex punches; ⌀ = 7,0 mm; Main Compression Force 1, 2, 3, 4, 5 kN, Pre Compression Force 20% of MCF ; 200 mg tablets

Compaction Simulation

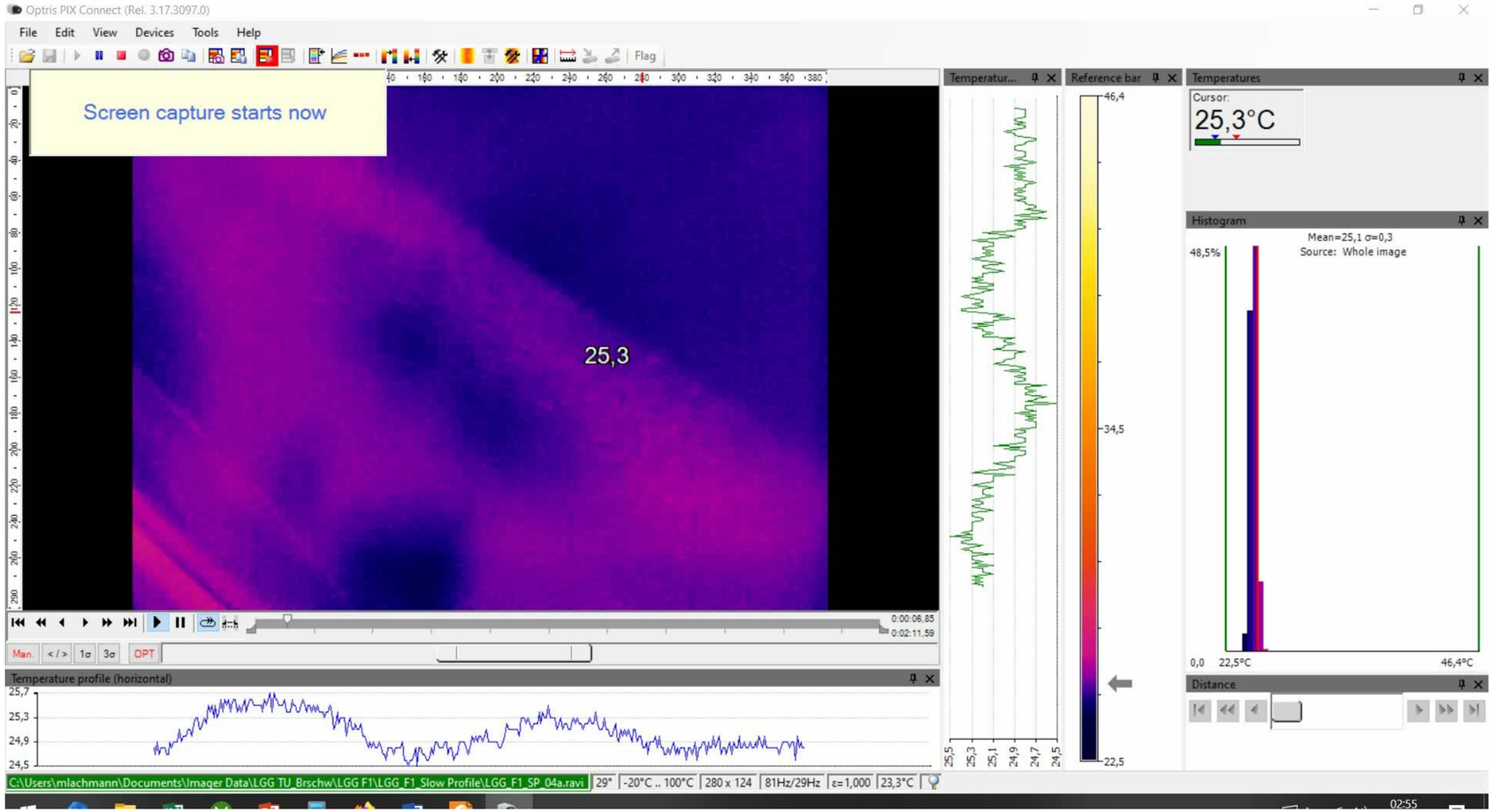
- A StylOne Evolution compaction simulator was used to simulate the compaction behaviour on a Romaco – Kilian KTP 720X 85 high speed press

Technical Data	KTP 720X			
Machine configurations	Mono-layer; Bi-layer; Core			
Number of press stations	85	77	63	51
Tool type* (EU and TSM)	B	B	B	D
Die type	BBS	BB	B	D
Maximum tablet diameter (mm)	11	13	16	25
Maximum output** (tablets/hour)	1,020,000	924,000	753,000	550,000
Maximum die filling (mm)	18			20
Maximum pre-compression force (kN)	100***			
Maximum main compression force (kN)	100***			
Power (kW)	38			
Standard voltage (V), frequency (Hz)	400, 50/60			
Compressed air (bar)	6			
Weight (kg)	5,600			

file:///C:/Users/mlachmann/Downloads/Romaco_DB_Kilian_KTP_720X-EN.pdf

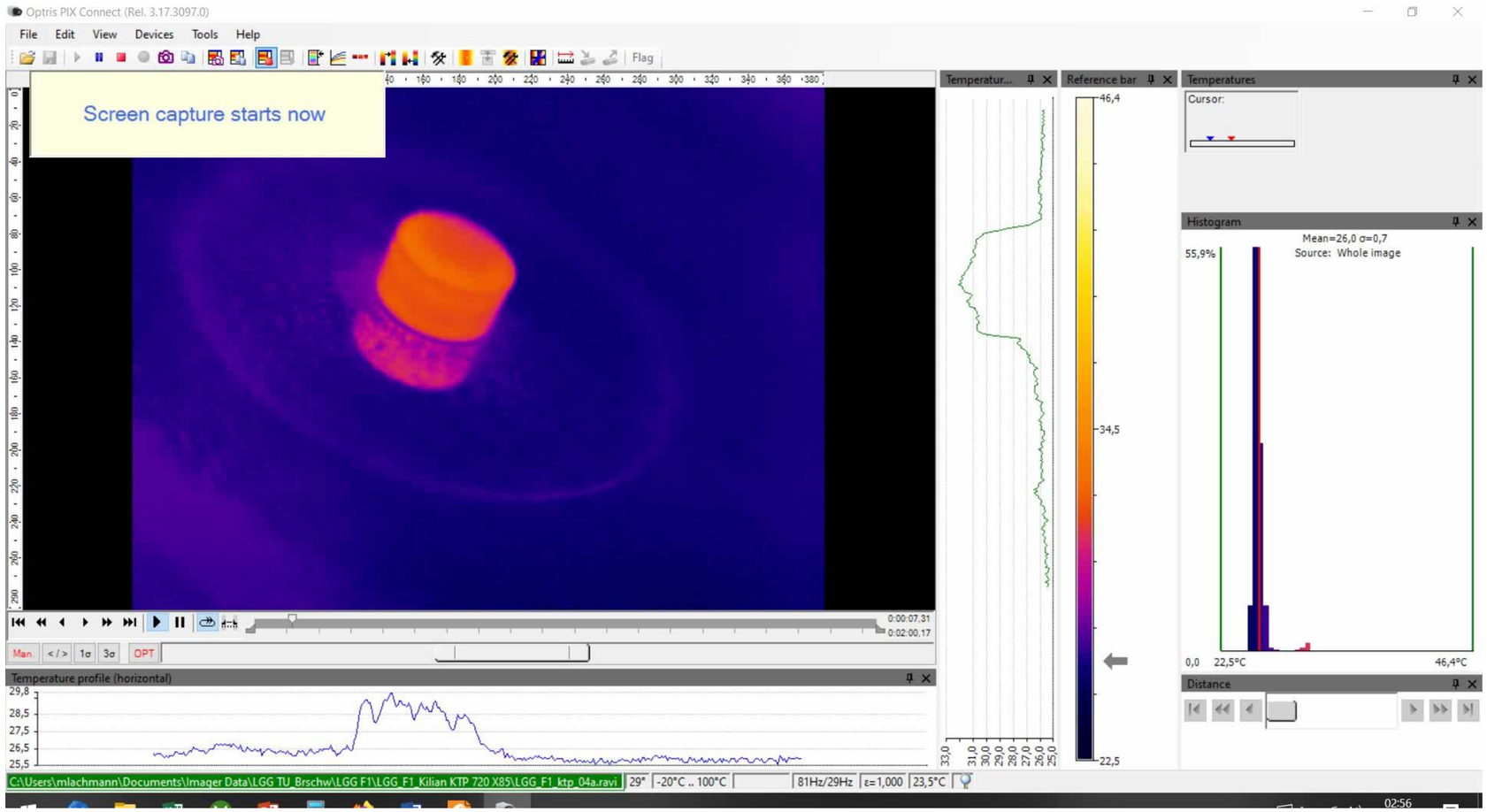
Compaction Simulation

- Video Capture of slow profile tableting on a Medelphamr StylOne compaction simulator



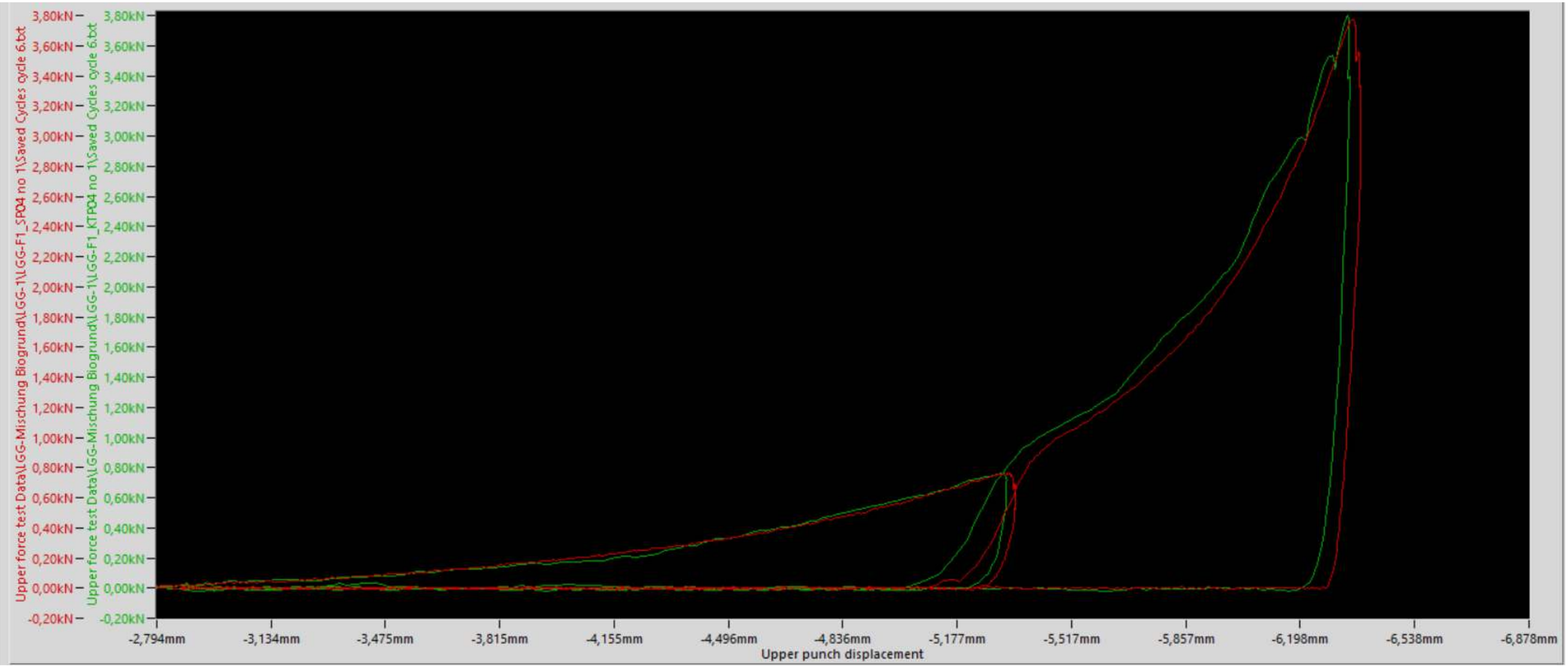
Compaction Simulation

- Video Capture of the simulation of a KTP 720X high speed press



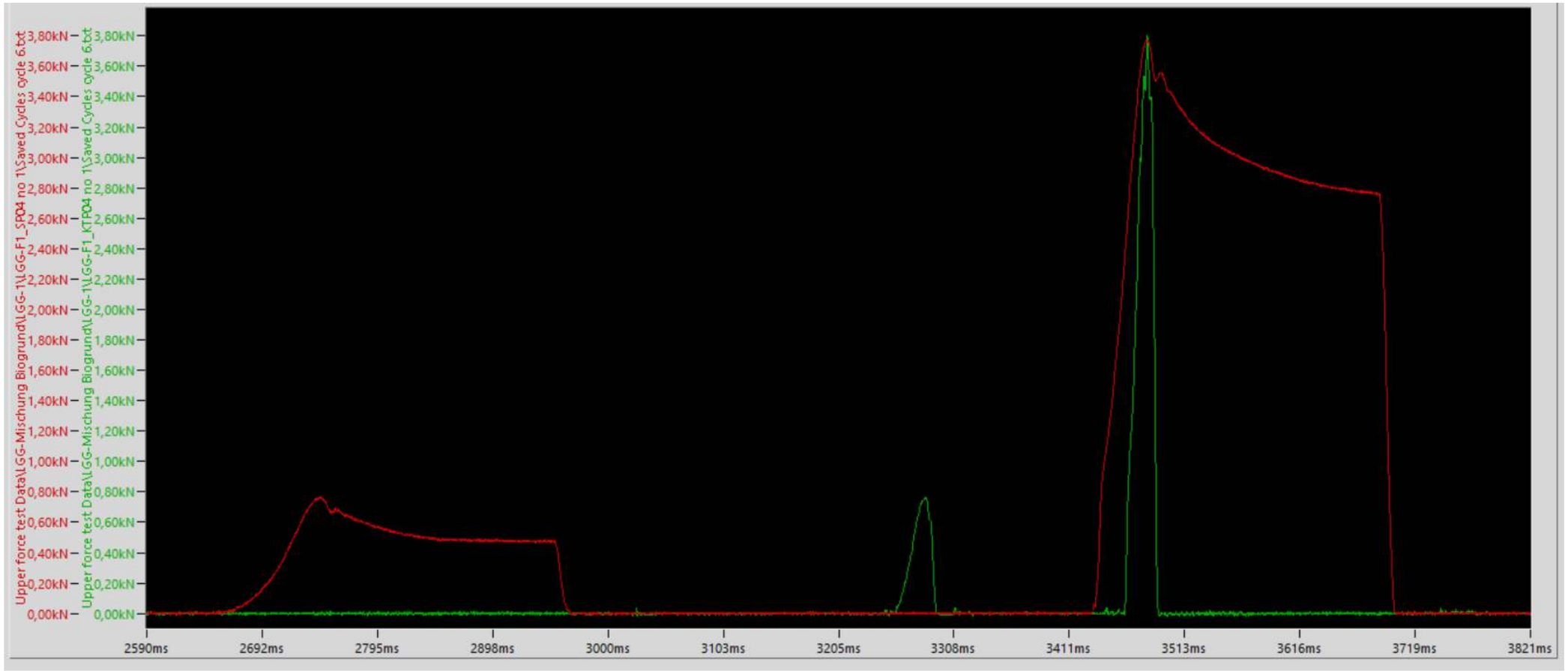
Compaction Simulation

- Force – Distance profiles for a slow and fast compaction process at 4 kN MCF



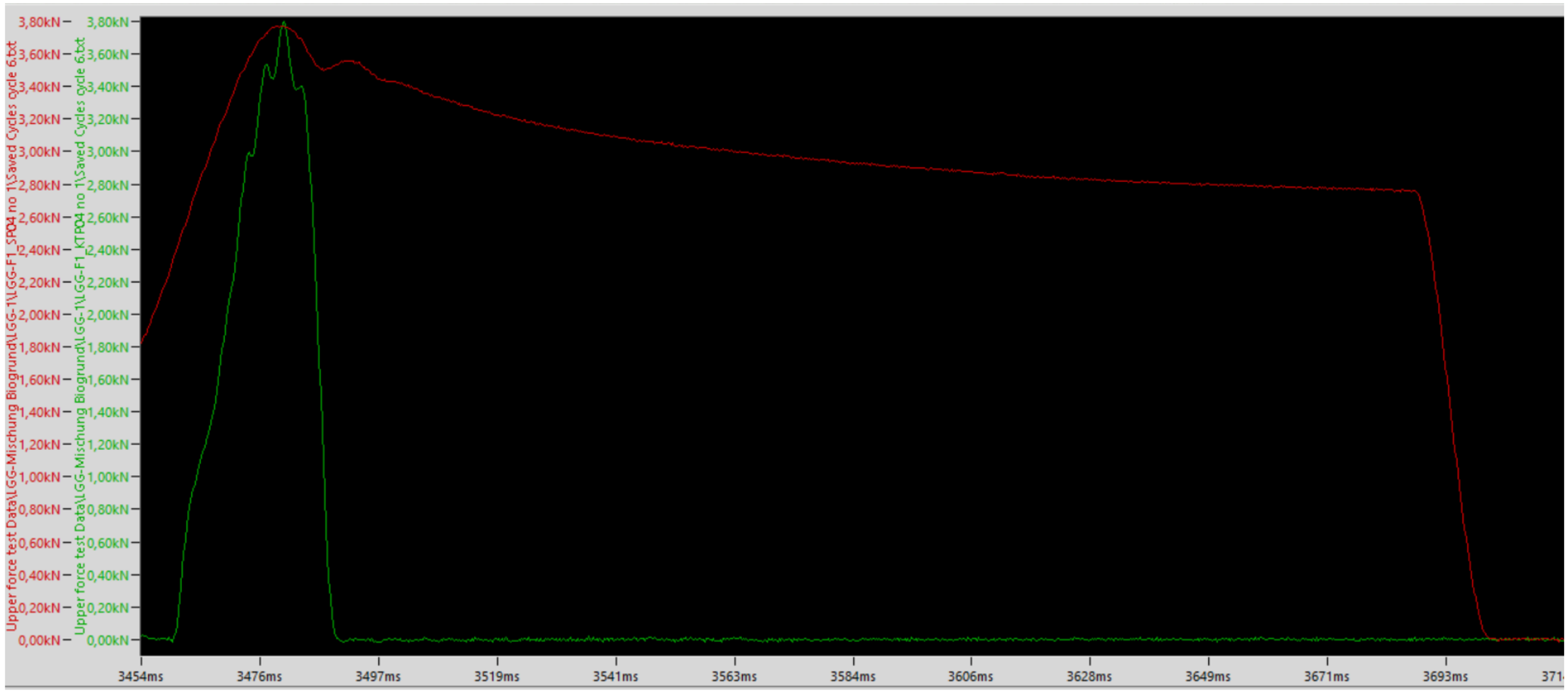
Compaction Simulation

- Force – Time curve for a slow and fast compaction process at 4 kN MCF

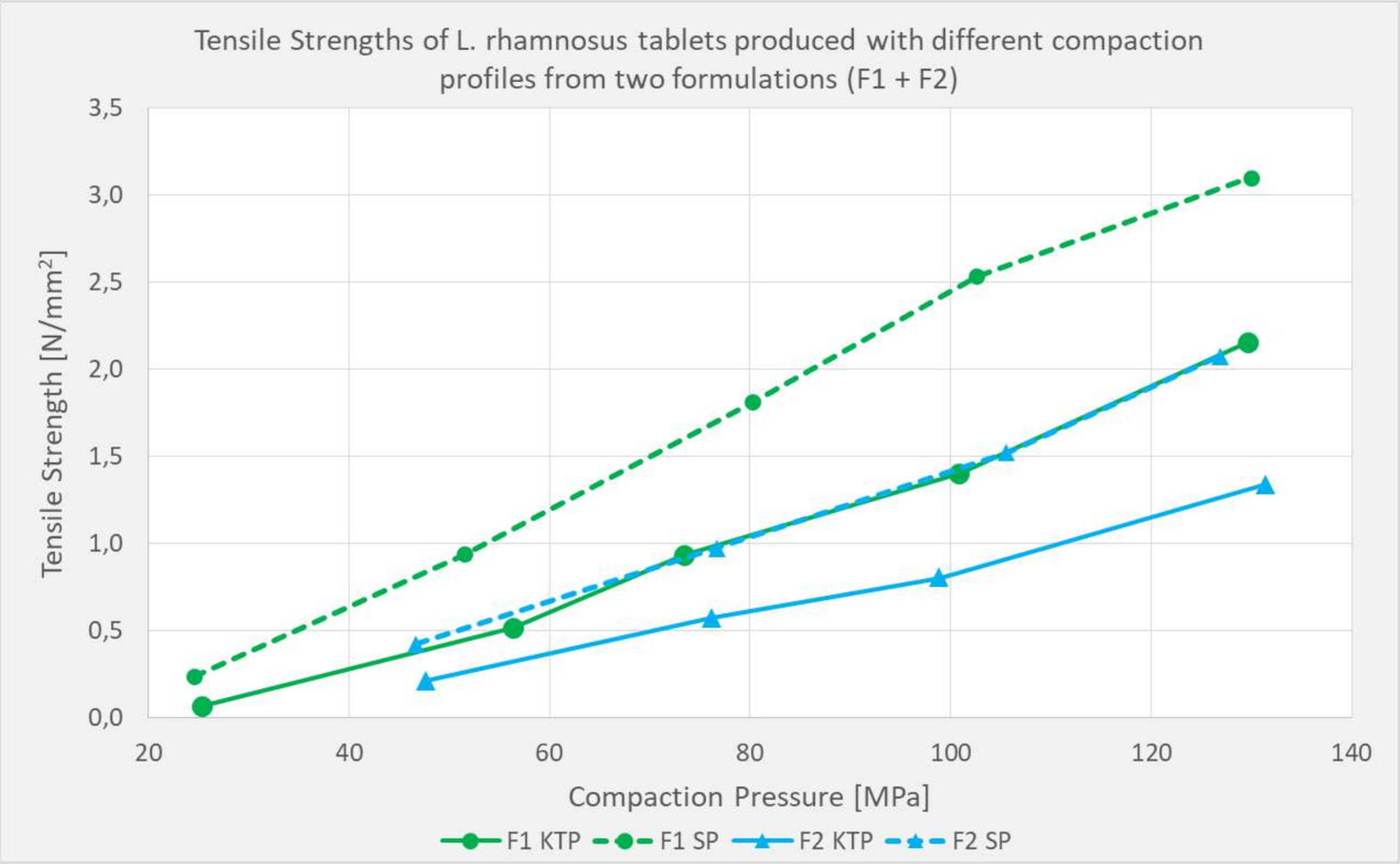


Compaction Simulation

- Dwell time from 10 ms to 200 ms



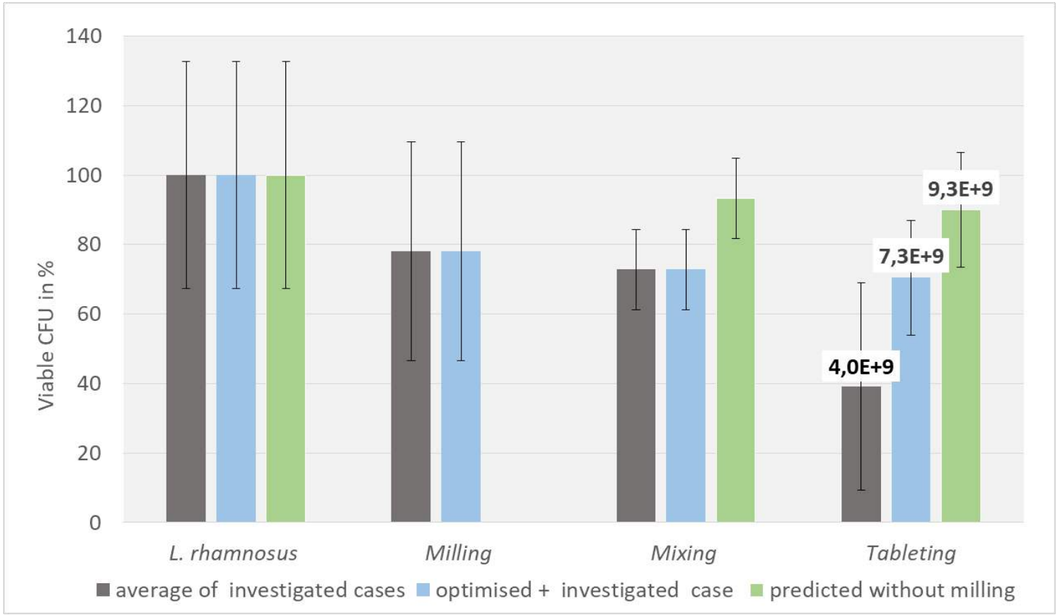
Compaction Simulation



New BonuTab® for Probiotics

NEW BIOGRUND BonuTab® XXX.xx

- A new BonuTab® type for tableting of probiotic bacteria
- The new BonuTab® offers compaction at high speed and low compaction forces
- The mixture was optimized to enhance survival of bacteria
- So far the mixture was only tested with L. rhamnosus but we are confident that it should work with other probiotic strains too
- The mixture also contains the prebiotic inulin
- According to our concurrent investigation and data modelling loss of bacteria can be reduced to roughly 20 % even during high speed tableting

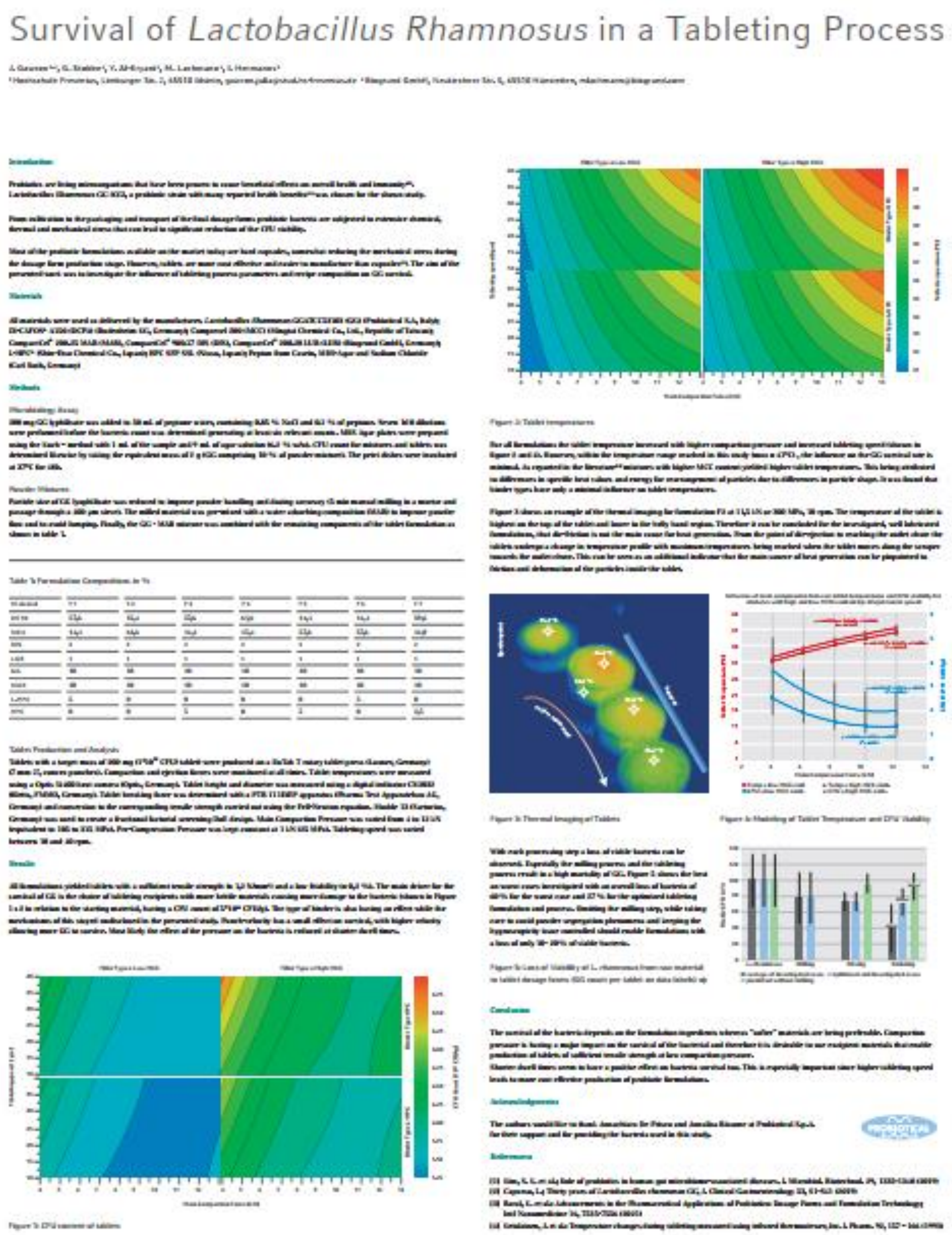


MCC	Oat Fibre
Magnesium Carbonate	Inulin powder
Isomalt	LGG
preg Starch Lycatab	Mg-Stearate
Psylium Husk	HPC

Publication

Publication

- Presented at:
- 4th European Conference on Pharmaceutics
- Please contact us for a copy



BIOGRUND

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