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**MAXIMIZING VIABILITY OF *LACTOBACILLUS PARACASEI*
SUBSP. *PARACASEI* L. *CASEI* 431 DURING PROCESSING
AND AMBIENT STORAGE**



**A THESIS PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY**

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ABSTRACT

In the present study, fluidized bed drying has been examined as a low-energy alternative to more expensive freeze-drying of the probiotic commercial strain *Lactobacillus paracasei* subsp. *paracasei* L. *casei* 431. The aim of this study was to maximize the viability of *L. casei* 431 during laboratory and industrial scale processing and storage. The study proceeded in three stages: a) Optimizing the growth conditions and medium composition for maximizing cell growth and desiccation tolerance b) Standardizing the harvesting conditions (harvesting time and techniques) and mixing conditions (mixing of cells with protective carrier) to minimize the mixing and drying loss c) Investigating the effect of various parameters during fluidized bed (FB) drying (initial moisture content, drying time and drying temperature) through Plackett-Burman (PB) and factorial design with the objective of identifying the ideal combination for maximizing the viability of *L. casei* 431 during drying and ambient storage.

The preliminary experiments were performed to optimize growth medium composition under controlled pH in a bioreactor. The effect of supplementing de Man, Rogosa and Sharpe (MRS) media with glucose and yeast extract on viable cell count during batch and fed-batch fermentation was compared. The pH controlled fed-batch fermentation resulted in a 5 fold increase in the viable cell count when compared to batch fermentation. But when the cells obtained from this pH controlled media showed huge drying and storage losses as compared to the uncontrolled pH media, a sequential PB design followed by central composite design matrix was used to screen and optimize the factors that could maximize cell growth under uncontrolled pH conditions. The supplementation of yeast extract and meat extract at a high concentration of 0.6-0.8% nitrogen in MRS media increased the viable cells of *L. casei* 431 by more than 2 fold and biomass by more than 1.5 fold as compared to control (MRS media). The cells from uncontrolled pH fermentations were then harvested by high speed centrifugation and collected cells were mixed with protective carrier (whole milk powder) of different water activity under different mixing conditions. Once growth and mixing conditions were standardized, another PB design followed by factorial design was used to illustrate the effect of various parameters such as harvesting at different growth phases, total solids of harvested cells, initial moisture content, and drying conditions such as drying temperature and drying time, on residual moisture content in combination with their effect on drying and storage stability of *L. casei* 431 under ambient and accelerated storage (37 °C) conditions.

The results showed that the pH during growth, the growth phase, the harvested cell solids/moisture content, the mixing techniques, the drying temperature and the final moisture content were important factors affecting cell stability during drying and storage. Fermentation with acid stress and controlled fluidized bed drying were able to keep the *L. casei* 431 cells relatively stable for 3 months at 25 °C in heat sealed aluminum bags (with inner polyethylene layer) during laboratory and pilot/industrial scale preservation. It was further observed that the shelf-life of FB dried *L. casei* 431 cells at 25 °C was 6 times longer than at 37 °C.

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TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xiii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	4
2.1 Probiotics	4
2.2 Lactic acid bacteria.....	6
2.2.1 The genus Bifidobacterium	6
2.2.2 The genus <i>Lactobacillus</i>	6
2.2.3 <i>Lactobacillus casei</i> group	7
2.3 Carbohydrate metabolism in LAB	8
2.4 Preservation of LAB.....	11
2.5 Drying methods.....	13
2.5.1 Freeze drying.....	13
2.5.2 Spray Drying	13
2.5.3 Fluidized bed drying.....	16
2.5.4 Vacuum drying	19
2.6 Factors affecting viability during convective drying	20
2.6.1 Resistance to stress.....	20
2.6.1.1 Osmotic Stress resistance.....	20
2.6.1.2 Oxidative Stress resistance.....	21
2.6.1.3 Heat Shock resistance.....	23

2.6.1.4 Acid stress response.....	24
2.6.1.5 Starvation response	26
2.6.1.6 Cold Stress.....	28
2.6.2 Factors affecting stress tolerance.....	29
2.6.2.1 Growth phase and harvesting time.....	29
2.6.2.2 Growth media composition	30
2.6.2.3 Pre- Adaptation Process.....	31
2.6.2.3 Growth medium pH	32
2.6.3 Initial moisture content/water activity and drying rate	32
2.6.4 Protective Carriers.....	34
2.7 Rehydration	36
2.8 Storage and Packaging.....	37
2.9 Commercial production of <i>Lactobacillus</i>	38
2.10 Concluding Remarks	40
CHAPTER 3 MATERIALS AND METHODS	43
3.1 Materials.....	43
3.2 Strain and inoculum preparation	43
3.3 Growth media composition.....	43
3.4 Fermentation experiments.....	44
3.4.1 Uncontrolled pH fermentation	44
3.4.2 Controlled pH batch fermentation	44
3.4.3 pH Feedback-Controlled Fed-Batch fermentation	44
3.5 Harvesting and mixing of cells with protective carrier (whole milk powder)	45
3.7 Drying.....	45
3.8 Packaging and storage.....	46
3.9 Powder Rehydration.....	46
3.10 Experimental design	46

3.10.1 Experimental design for uncontrolled pH fermentation experiments	47
3.10.2 Experimental design for making FB dried powders	48
3.11 Statistical data analyses	48
3.12 Analytical determination	49
3.12.1 Viable cell counts	49
3.12.2 Biomass	49
3.12.3 Moisture content and water activity	49
3.12.3.1 Comparison of moisture content obtained after conventional oven drying (at 105 °C) and vacuum oven drying (at 80 °C)	49
3.12.4 Optical Density (OD).....	50
3.12.5 Glucose and lactic acid	50
3.12.6 pH	51
3.12.7 X-ray diffraction (XRD)	51
CHAPTER 4	52
TO ESTABLISH AN EFFICIENT CULTURE METHOD FOR <i>L. CASEI</i> 431 TO OBTAIN HIGH DENSITY VIABLE CELLS IN GROWTH MEDIA	52
4.1 Introduction	52
4.2 Batch fermentation of <i>L. casei</i> 431 in MRS media at controlled pH 6.5.....	52
4.3 Batch fermentation of <i>L. casei</i> 431 in supplemented MRS media.....	54
4.4 Fed Batch Fermentation: pH feedback-controlled fed-batch fermentation	55
4.5 Comparison of different glucose and ammonium hydroxide ratio in the feeding solution during pH feedback-controlled fed-batch fermentation	56
4.6 Conclusions:	57
CHAPTER 5	58
OPTIMISING THE MIXING AND DRYING CONDITIONS TO GET THE MAXIMUM VIABLE COUNT OF <i>L. CASEI</i> 431 DURING DRYING AND STORAGE	58
5.1 Introduction	58

5.2 Powders made from pH-based feedback-controlled fed batch media	58
5.2.1 Results and Discussion.....	59
5.3 Comparing the mixing, drying and storage losses in FB dried probiotic powders made from (1) WMP of initial a_w 0.3 and 0.7 under different mixing conditions (hand mixing and Kenwood planetary mixer) (2) cells obtained from pH controlled (@6.5) and uncontrolled media (end pH 4.0 ± 0.1).....	60
5.3.1. Effect of mixing harvested cells with WMP of different water activity (a_w) through various mixing techniques.....	61
5.3.2 Effect of growth media pH on <i>L. Casei</i> 431 stability during drying and storage	63
5.4 Effect of maintaining growth media pH at 5.3 instead of 6.6 in the fermentor	64
5.5 Conclusions.....	65
CHAPTER 6.....	66
ENHANCEMENT OF GROWTH OF <i>L. CASEI</i> 431 DURING BATCH CULTIVATION IN UNCONTROLLED PH GROWTH MEDIA THROUGH RESPONSE SURFACE METHODOLOGY (RSM)66	
6.1 Introduction.....	66
6.2. Growth curve of <i>L. casei</i> 431 in MRS media under uncontrolled pH conditions	66
6.3 Initial screening through Plackett -Burman (PB) Design	68
6.3.1 Experimental Design.....	68
6.3.2 Screening of nitrogen sources	68
6.3.3 Results and discussion	69
6.3.3.1 Effect of sodium chloride (NaCl) - osmotic stress	71
6.3.3.2 Effect of initial pH.....	72
6.3.3.3 Effect of glucose.....	72
6.3.3.4 Effect of various nitrogen sources, vitamins and ammonium salts	73
6.4 Central composite design (CCD) to optimize the growth medium composition	73
6.4.1 Results and discussion	74
6.5 PB design to identify the important nitrogen sources effecting <i>L. casei</i> 431 viable cell count75	
6.5.1 Results and Discussion.....	75

6.6 Effect of % nitrogen present in growth media on viable cell count of <i>L. casei</i> 431.....	78
6.7 Conclusions	79
CHAPTER 7.....	80
COMPARING STABILIZATION OF <i>L.CASEI</i> 431 HARVESTED FROM PH UNCONTROLLED GROWTH MEDIA DURING LABORATORY AND INDUSTRIAL SCALE PRESERVATION PROCESSES AND STORAGE.....	80
7.1 Introduction	80
7.2 Viability of <i>L. casei</i> during laboratory scale and pilot scale preservation processes and storage	80
7.3 Results and Discussion	81
7.3.1 Effect of harvesting time/ growth phase	85
7.3.2 Effects of cell concentrate solids	85
7.3.3 Effect of block	86
7.3.4 Effect of storage.....	87
7.4 Conclusions	88
CHAPTER 8.....	90
OPTIMIZING THE DRYING CONDITIONS TO GET PROBIOTIC POWDERS WITH LOWER RESIDUAL MOISTURE CONTENT AND HIGH STORAGE STABILITY UNDER LABORATORY AND INDUSTRIAL PROCESSING CONITIONS THROUGH RESPONSE SURFACE METHODOLOGY	90
8.1 Introduction	90
8.2 Effect of drying time and temperature of FB drier on <i>L. Casei</i> 431 stability during laboratory scale preservation.....	90
8.2.1 Results and discussion	91
8.3 Comparison of cell viability in powders dried in presence and absence of dehumidifier.....	95
8.4 Effect of initial moisture content (before drying) and drying time at 50 °C on <i>L. Casei</i> 431 stability during industrial preservation.....	96
8.4.1 Results and discussion	97
8.5 Conclusions	100

CHAPTER 9	101
OVERALL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS	101
9.1 Overall discussion	101
9.2 Conclusions	102
9.3 Recommendations	104
REFERENCES	105
APPENDICES	119

LIST OF TABLES

Table No.	Title	Page
Table 2.4.1	Costs of drying processes referenced to that of freeze drying (adopted from Santivarangkna et al., 2007)	11
Table 3.12.3.1	Comparison of moisture content determined by oven drying and vacuum oven drying	50
Table 4.2	Viable cell count, OD biomass yield and other properties of <i>L. Casei</i> 431 during batch fermentation at controlled pH 6.5 in MRS media	53
Table 4.3	Viable cell count and OD of <i>L. Casei</i> 431 in supplemented MRS broth (with glucose 30 g l ⁻¹ and yeast extract 40 g l ⁻¹) at controlled pH 6.5 in batch culture	54
Table 4.4	Viable cell count, OD and biomass yield of <i>L. Casei</i> 431 in pH feedback-controlled fed-batch fermentation in MRS media supplemented with 40 g L ⁻¹ yeast extract	56
Table 4.5	Comparison of different glucose and ammonium hydroxide ratio in the feeding solution during pH feedback-controlled fed-batch fermentation	56
Table 5.2.1	Viability data of FB dried powder made from controlled pH fed-batch media after drying and 30 days of storage at 25 °C	59
Table 5.3	Viability of FB dried powder containing <i>L. casei</i> 431 made under different conditions	61
Table 5.3.1	Student's t-test results for comparing FB dried powders containing <i>L. casei</i> 431 made with WMP of different initial a _w	62
Table 5.3.2	Student's t-test results for comparing FB dried powders containing <i>L. casei</i> 431 made from pH controlled and uncontrolled growth media	64
Table 5.4.1	Effect of controlling growth media pH at 5.3 instead of 6.6 in the fermentor on <i>L. casei</i> 431 cell count after drying and storage	65

Table 6.2.1 - Viable cell count, OD and biomass yield of <i>L. Casei</i> 431 in uncontrolled pH MRS media.....	67
Table 6.3.1 - PB design matrix for increasing <i>L. casei</i> 431 cells count in pH uncontrolled growth media.....	70
Table 6.3.2 - Estimated effects and coefficients for <i>L. casei</i> 431 viable cell count (log cfu ml ⁻¹ media).....	71
Table 6.4.1 – Regression coefficients table for <i>L. casei</i> 431 Biomass yield (g kg ⁻¹ media)	75
Table 6.5.1 - PB design to study the effects of concentration of yeast extract, meat extract, peptone, glucose and harvesting time on viable cell count.....	77
Table 6.5.2 - Estimated Effects and Coefficients for <i>L. casei</i> 431 viable cell count (log cfu ml ⁻¹ media).....	78
Table 6.5.3 - Estimated Effects and Coefficients for <i>L. casei</i> 431 biomass yield (g kg ⁻¹ media)...	78
Table 7.3.1 – Results from PB design used for comparing powders made under laboratory and industrial processing.....	83
Table 7.3.2 - Main factor effects for PB design used for comparing powders made under laboratory and industrial processing conditions	84
Table 8.2.1 – The viability data of powders dried for different time-temperature combinations in FB drier	91
Table 8.2.2 Analysis of variance table for the experiment.....	94
Table 8.3 – Students T-test comparison of powders FB dried (at 50 °C) in presence and absence of dehumidifier	96
Table 9.1.1- Various parameters studied at each processing step to maximize cell viability during drying and ambient storage	101

LIST OF FIGURES

Figure No.	Title	Page
Figure 2.3.1	Major fermentation pathways of glucose: (A) Homolactic fermentation (glycolysis, Embden-Meyerhof-Parnas pathway); (B) Heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway). Selected enzymes are numbered: 1. Glucokinase; 2. Fructose-1,6-diphosphate aldolase; 3. Glyceraldehyde-3-phosphate dehydrogenase; 4. Pyruvate kinase; 5. Lactate dehydrogenase; 6. Glucose-6-phosphate dehydrogenase; 7. 6-Phosphogluconate dehydrogenase; 8. Phosphoketolase; 9. acetaldehyde dehydrogenase; 10. Alcohol dehydrogenase (Axelsson, 1998)	10
Figure 2.3.2	Galactose metabolism in lactic acid bacteria: (A) Galactose-6-phosphate pathway; (B) Leloir pathway (Axelsson, 1998)	11
Figure 2.4.1	Major processing steps during the dehydration of microorganisms and important extrinsic factors in each step. Dotted rectangles represent optional steps and extrinsic factors are shown in round-cornered rectangles (adopted from Fu and Chen, 2011)	12
Figure 2.5.3.1	Mechanism of drying in a Glatt Uni-Glatt laboratory fluid bed dryer	17
Figure 2.6.4	Schematic of the potential phase transition of cellular membrane upon dehydration and rehydration in the presence and absence of trehalose (Crowe et al., 1992)	35
Figure 3.7.1	Glatt Uni-Glatt laboratory fluid bed dryer	46
Fig 4.2	Relation between OD and Biomass of <i>L. Casei</i> 431 during batch fermentation at controlled pH 6.5 in MRS media	53
Fig 5.3.1	XRD analysis of FB dried powders containing <i>L. casei</i> 431 at day 30 and day 60	63
Fig 6.2.1	Growth curve of <i>L. casei</i> 431 in pH uncontrolled MRS media	67
Fig 6.6	Effect of supplemented nitrogen on viable cell count of <i>L. casei</i> 431	79
Figure 8.2.1.1	Normal probability plots of effects ($\alpha=0.05$) with <i>L. casei</i> 431 viable cell count as response	92

Figure 8.2.1.2 – Main effect plot of factors studied with response as *L. casei* 431 cell count.... 93

Figure 8.2.1.3 – Interaction plot of factors studied with response as *L. casei* 431 cell count 94

Figure 8.4.1 – Response contour plot with day 0 viable cell count as response..... 100