

CONTENTS

ACKNOWLEDGEMENTS	4
JORNAL PUBLICATIONS AND POSTER PRESENTATIONS	6
SUMMARY	7
ZUSAMMENFASSUNG	9
CONTENTS	11
ABBREVIATIONS	14
1. INTRODUCTION	1
2. BACKGROUND	5
2.1. Food colorants	5
2.2. Anthocyanins	9
2.2.1. Factors affecting the stability of anthocyanins	12
2.2.1.1. pH	12
2.2.1.2. Copigmentation	13
2.2.1.3. Temperature	16
2.2.1.4. Oxygen, enzymes, and light	17
2.3. Origin of pyranoanthocyanins	18
2.3.1. Occurrence of pyranoanthocyanins	20
2.3.1.1. Origin of hydroxyphenyl-pyranoanthocyanins	22
2.3.2. Stability properties of pyranoanthocyanins	23
2.3.3. Pyranoanthocyanins as food colorants	25
2.4. Hydroxycinnamic acids	25
2.5. Genus <i>Lactobacillus</i>	28
2.5.1. Transformation of phenolic acid by <i>Lactobacilli</i>	29
2.6. Black carrot juice	33
3. AIM OF THE STUDY	35
4. MATERIALS AND METHODS	36
4.1. Material	36
4.1.1. Plant material	36
4.1.2. Microbiological material	36
4.1.3. Chemicals	37
4.1.4. Machines and equipment	38

4.2. Preparative methods	42
4.2.1. Preparation of culture media and buffer solutions	42
4.2.1.1. Media	42
4.2.1.2. Buffers	43
4.2.2. Processing of blackberry juice	44
4.2.3. Fractionation of anthocyanins and anthocyanin adducts by membrane adsorber	44
4.2.4. Preparation of chlorogenic acid extracts	45
4.2.5. Chlorogenic acid isolation by HPLCC	46
4.2.6. Separation of 4-CQA by semi-preparative HPLC	46
4.2.7. Identification of black carrot juice anthocyanins	47
4.3. Microbiological methods	48
4.3.1. Decarboxylation of free hydroxycinnamic acids by Lactobacilli	48
4.3.2. Metabolism of phenylpropanoid derivatives involved in the synthesis of <i>p</i> -CA	48
4.3.3. Effect of <i>p</i> -coumaric acid concentration on the production of volatile derivatives in MRS media	49
4.3.4. Decarboxylation of <i>p</i> -coumaric acid in minimal PMM7 media	49
4.3.5. TAL activity of <i>S. espanaensis</i> in GYM media	50
4.3.6. Metabolism of 5-CQA during fermentation by LAB	50
4.3.7. Evaluation of cinnamoyl esterase activity of <i>L. reuteri</i> , <i>L. helveticus</i> and <i>L. fermentum</i>	51
4.3.8. Synthesis of hydroxyphenyl-pyranoanthocyanins in buffer and MRS media	51
4.3.9. Stability of Cy-3-glu and pyranoanthocyanins derivatives at fermentation conditions	52
4.3.10. Color measurement	52
4.3.11. Lactic acid fermentation of BCJ	53
4.3.12. Decarboxylation activities of <i>L. plantarum</i> , <i>L. mali</i> , and <i>L. sakei</i> in BCJ	54
4.3.13. Evaluation of cinnamoyl esterase activities combined with phenolic decarboxylase activities of Lactobacilli	54
4.3.14. Co-fermentation of BCJ to produce pyranoanthocyanins	54
4.4. Analytical methods	56
4.4.1. Determination of the concentration	56

4.4.2. Determination of products from Lactobacilli activities at fermentation condition	56
4.4.3. Determination of anthocyanins and anthocyanins adducts	57
4.4.4. Determination of specific cinnamoyl esterase activity by UHPLC-ESI-MS ⁿ	58
4.4.5. Determination of pyranoanthocyanins generated during fermentation in BCJ by UHPLC-ESI-MS ⁿ	59
4.5. Statistical analysis	60
5. RESULTS AND DISCUSSION	61
5.1. Factors influencing cinnamate decarboxylase activity in lactobacilli	61
5.1.1. Effect of the pH value on the metabolism of free HCA.....	61
5.1.2. Metabolism of <i>p</i> -CA, FA, and CA in MRS media and in buffer by LAB	66
5.1.3. Effect of phenolic acid concentration on Lactobacilli metabolism.....	69
5.2. Potential presence of TAL or PAL activities.....	73
5.2.1. Enzymatic activities involve in the metabolism of <i>p</i> -CA	74
5.2.2. Production of <i>p</i> -CA by TAL activities displayed by <i>S. espanaensis</i>	79
5.2.3. PDC activities in PMM7 minimal media	79
5.2.4. PDC activities in phosphate buffer.....	82
5.3. Determination of cinnamoyl esterase activity.....	84
5.3.1. Determination of cinnamoyl esterase activity on 5-CQA by LAB.....	84
5.3.2. Specific cinnamoyl esterase activity from monochlorogenic acids and dichlorogenic acids.....	87
5.4. Synthesis of pyranoanthocyanins	98
5.4.1. Formation of pyranoanthocyanins in MRS media	98
5.4.2. Effect of the media on the stability of pyranoanthocyanins	104
5.4.3. Effect of the pH value in the decarboxylation activity by LAB in black carrot juice.....	108
5.4.4. Synthesis of pyranoanthocyanins through co-fermentation with LAB.....	114
6. CONCLUSION.....	121
7. REFERENCES.....	123