CONTENTS

Abstract i–ii
List of abbreviations iii–vii
List of tables viii–ix
List of figures x–xiii

Chapter 1. General Introduction 1–12
  1.1. Food colours, market and production 1–7
  1.2. Rivina humilis L. berries as alternative source of betalains 7–11
  1.3. Antioxidant and biological activities of betalains 11–12

Chapter 2. Review of Literature 13–40
  2.1. Mutual exclusiveness of betalains and anthocyanins 13–14
  2.2. Occurrence 14–17
  2.3. Structure identification 17–26
  2.4. Biosynthesis of betalains 26
    2.4.1. Biosynthesis pathway 26–27
    2.4.2. Tyrosinase 27–28
    2.4.3. Regulation of betalains biosynthesis 28–29
  2.5. Ecophysiological factors influencing betalains accumulation 29
    2.5.1. Physical and stress factors 29–31
    2.5.2. Elicitors 31–32
  2.6. Stability 32
    2.6.1. Factors affecting betalains stability 33
      2.6.1.1. Structure, concentration and composition 33
      2.6.1.2. pH 33
      2.6.1.3. Water activity (aw) 33
      2.6.1.4. Light 33–34
      2.6.1.5. Oxygen and other oxidants 34
      2.6.1.6. Antioxidants 34
      2.6.1.7. Temperature 34–35
      2.6.1.8. Metals 35
      2.6.1.9. Decolourising enzymes 35
  2.7. Stabilisation 35
    2.7.1. Complex formation 35–36
    2.7.2. Copigmentation 37
Chapter 3. Characterisation of pigments from fruits of *Rivina humilis* L. 41–56

3.1. Summary 41
3.2. Introduction 42
3.3. Materials and methods
   3.3.1. Source of berries 42
   3.3.2. Chemicals 42–43
   3.3.3. Pigment extraction and quantification 43
   3.3.4. Partial purification of betalains 43–44
   3.3.5. HPLC-MS analysis for pigment identification 44–45
   3.3.6. Sample preparation for in vitro antioxidant activity and bioactivity assays 45
   3.3.7. DPPH·-scavenging assay 45
   3.3.8. Reducing power assay 45
   3.3.9. In vitro cancer cell cytotoxicity - MTT assay 46
   3.3.10. Statistical analysis 46
3.4. Results and discussion 46
   3.4.1. Pigments of *R. humilis* berries 46–52
   3.4.2. Antioxidant activity of pigment rich extracts from *R. humilis* berries 52–54
   3.4.3. In vitro cancer cell cytotoxicity 54–56

Chapter 4. Studies on biochemical profile and selective enrichment of pigment in fruits 57–82

4.1. Summary 57
4.2. Introduction 58–59
4.3. Materials and methods
   4.3.1. Source of plant materials for ontogeny studies 59
   4.3.2. Chemicals 59
   4.3.3. Colour measurement 59–60
   4.3.4. Betalains quantification and diphenol/dopa oxidase activity assay 60–61
   4.3.5. Gene expression studies 61
   4.3.5.1. Isolation of total RNA 61
   4.3.5.2. Agarose gel electrophoresis of RNA 61
   4.3.5.3. Preparation of DNA-free RNA (DNase treatment) 61–62
4.3.4. Quantification of RNA

4.3.5. Preparation of first strand cDNA (Reverse transcriptase reaction)

4.3.6. PCR amplification of 18S rRNA, SOD, CAT, RhBGT and RhBGT2

4.3.6. Estimation of other biochemical components

4.3.6.1. Reducing sugar (Dinitrosalicylic acid, DNS, reagent method)

4.3.6.2 Cellulose (Anthrone reagent method)

4.3.6.3. Total carbohydrates

4.3.6.4. Total proteins content

4.3.6.5. Total phenols content

4.3.6.6. Total soluble solids (TSS)

4.3.6.7. Moisture content

4.3.6.8. Oil content

4.3.6.9. Crude fibre estimation

4.3.6.10. Energetic value

4.3.6.11. Fatty acid methyl esters (FAMEs) preparation

4.3.6.12. FAMEs analysis

4.3.6.13. Iodine value

4.3.6.14. Atherogenicity and thrombogenicity indices

4.3.6.15. Niacin

4.3.6.16. HPLC analysis of ascorbic acid

4.3.6.17. HPLC analysis of tocopherols

4.3.6.18. Organic acids estimation by HPLC

4.3.6.19. Elemental composition analysis

4.3.7. Elicitor treatment for enhancement of betalains in berries

4.3.7.1. Elicitor treatment

4.3.8. Statistical analysis

4.4. Results and discussion

4.4.1. Physical characteristics of green, pink and red berries

4.4.2. Pigment content, biosynthetic and antioxidant enzymes relative expression in green, pink and red berries

4.4.3. Other biochemical/nutritional components of R. humilis berries
4.4.4. Elicitor-mediated betalains enrichment 78–82

Chapter 5. Effect of physicochemical treatments on extracted pigment 83–102

5.1. Summary 83
5.2. Introduction 84–85
5.3. Materials and methods 85
  5.3.1. Preliminary stability studies 85
  5.3.2. Chemicals, source of berry and juice extraction 85–86
  5.3.3. Analysis of physicochemical properties of R. humilis berry juice 86
  5.3.4. Sample preparation 86
  5.3.5. Thermal stability study of betalains in presence of metals 86–87
  5.3.6. Storage stability study of betalains 87
  5.3.7. Stability study of purified betacyanins 87
  5.3.8. Regeneration studies of betalains 87–88
  5.3.9. HPLC analysis of betalains 88
  5.3.10. Statistical analysis 88

5.4. Results and discussion 88
  5.4.1. Preliminary stability studies 88–90
  5.4.2. Physicochemical properties of Rivina berry juice 90–91
  5.4.3. Effect of metals on betacyanins stability in presence or absence of ascorbic acid 91–93
  5.4.4. Stability of betalains in presence of selenium and ascorbic acid: effect of temperature 93–95
  5.4.5. Stability of purified betacyanins in presence of selenium and ascorbic acid 95–96
  5.4.6. Effect of ascorbic acid and selenium on colourant properties of betalains 97–98
  5.4.7. Regeneration of betacyanins 98–100
  5.4.8. Purified betacyanins profile as affected by thermal treatment in presence of selenium and ascorbic acid 100–102

Chapter 6. To assess food safety in animal models 103–121

6.1. Summary 103
6.2. Introduction 104–105
6.3. Materials and methods 105
  6.3.1. Source of berries and extraction of berry juice 105–106
  6.3.2. Animals and care 106
6.3.3. Acute toxicity study

6.3.4. Subacute (35 days) toxicity study

6.3.5. Subchronic (90 days) toxicity study

6.3.6. Preparation of ready to serve Rivina banana spread

6.3.7. Analysis of physicochemical characteristics in Rivina-banana spread

6.3.8. Microbiological assays in Rivina-banana spread

6.3.9. Sensory evaluation of Rivina-banana spread after Storage

6.3.10. Statistical analysis

6.4. Results and discussion

6.4.1. General condition, symptoms and mortality

6.4.2. Acute toxicity study

6.4.3. Feed intake and body weight of rats: subacute and subchronic toxicity studies

6.4.4. Organ weight and histopathology of rats: subacute and subchronic toxicity studies

6.4.5. Haematological profile of rats: subacute and subchronic toxicity studies

6.4.6. Serum biochemistry of rats: subacute and subchronic toxicity studies

6.4.7. Biochemical parameters of rat livers: subacute and subchronic toxicity studies

6.4.8. Physicochemical parameters of Rivina-banana spread before and after storage for 6 months at 5 °C

6.4.9. Microbial profile of Rivina banana spread after Storage

6.4.10. Sensory profile of Rivina banana spread after 6 months storage at 5 °C

Chapter 7. Summary and Conclusions

7.1. Summary

7.2. Conclusions

Appendix

Appendix I

Appendix II

Publications

References