

General index

| | |
|--|-----------|
| Abstract..... | 2 |
| Chapter 1 | 4 |
| Introduction..... | 4 |
| 1.1. Phenolic compounds | 4 |
| 1.1.1. Overview..... | 4 |
| 1.1.2. Biosynthesis | 5 |
| 1.1.3. Classification..... | 7 |
| 1.1.4. Metabolism | 12 |
| 1.1.5. Biological effects | 14 |
| 1.2. Currants and gooseberries (<i>Ribes</i> spp.)..... | 17 |
| 1.3. <i>In vitro</i> methodologies for the evaluation of the digestion process | 25 |
| 1.3.1. Overview..... | 25 |
| 1.3.2. Simulated gastric and intestinal digestion models | 27 |
| 1.3.3. Simulated colonic fermentation models..... | 30 |
| 1.4. Analysis of phenolic compounds | 32 |
| 1.4.1. Extraction..... | 32 |
| 1.4.2. Samples pre-treatment..... | 33 |
| 1.4.3. Phenolic profiling by HPLC-DAD and HPLC-ESI-MS/MS | 34 |
| 1.5. <i>In vitro</i> methods for biological activity evaluation | 35 |
| 1.5.1. Antioxidant activity..... | 35 |
| 1.5.2. Inhibition of metabolic syndrome-related enzymes | 36 |
| 1.5.3. Anti-inflammatory activity | 37 |
| 1.5.4. Prebiotic activity | 38 |
| Hypothesis..... | 40 |
| Objectives..... | 41 |
| 1. General objective | 41 |
| 2. Specific objectives | 41 |
| Chapter 2 | 43 |
| Materials and Methods..... | 43 |

| | |
|---|----|
| 2.1. Reagents and solvents | 43 |
| 2.2. Samples collection | 45 |
| 2.3. Sample processing | 46 |
| 2.3.1. Fruits extraction | 46 |
| 2.3.2. Phenolic-enriched extract (PEE) obtention..... | 47 |
| 2.4. <i>In vitro</i> gastrointestinal tract experiments..... | 47 |
| 2.4.1. Simulated gastrointestinal digestion (GID)..... | 47 |
| 2.4.2. Simulated colonic fermentation conditions..... | 48 |
| 2.5. Chromatographic analyses | 50 |
| 2.5.1. HPLC-DAD analyses of GID samples..... | 50 |
| 2.5.2. HPLC-DAD analyses of fermented samples..... | 51 |
| 2.5.3. Identification by HPLC-ESI-MS/MS ⁿ | 51 |
| 2.6. Total flavonoid (TF) and total phenolic (TP) content..... | 52 |
| 2.7. Antioxidant activity | 53 |
| 2.7.1. Discoloration of the DPPH radical..... | 53 |
| 2.7.2. Ferric reducing antioxidant power (FRAP)..... | 53 |
| 2.7.3. Trolox equivalent antioxidant capacity (TEAC)..... | 54 |
| 2.7.4. Cupric-reducing antioxidant power (CUPRAC) | 54 |
| 2.7.5. Superoxide anion scavenging capacity | 54 |
| 2.7.6. Oxygen radical absorbance capacity (ORAC) | 55 |
| 2.7.7. Cell-based assays | 55 |
| 2.8. Inhibitory effect on metabolic syndrome-associated enzymes | 57 |
| 2.8.1. α -amylase inhibition assay | 57 |
| 2.8.2. α -glucosidase inhibition assay | 57 |
| 2.8.3. Lipase inhibition assay..... | 58 |
| 2.9. Anti-inflammatory activity | 58 |
| 2.9.1. Cell-based assays | 58 |
| 2.9.2. COX-1 and COX-2 enzymes inhibition assay | 61 |
| 2.10. Prebiotic effect measurement..... | 61 |
| 2.10.1. Pre-reduced sterile bacterial growth medium | 61 |
| 2.10.2. <i>In vitro</i> batch-culture incubations of GID-extracts with human microbiota | 61 |
| 2.10.3. Determination of pH, and ammonia content | 63 |

| | |
|--|------------|
| 2.10.4. Short-chain fatty acids and branched-chain fatty acids..... | 63 |
| 2.10.5. Bacterial groups quantification | 64 |
| 2.11. Statistical analysis | 66 |
| Chapter 3 | 68 |
| Qualitative and quantitative changes in polyphenol composition and bioactivity of <i>Ribes magellanicum</i> and <i>R. punctatum</i> after <i>in vitro</i> gastrointestinal digestion..... | 68 |
| 3.1. Introduction..... | 68 |
| 3.2. Results and discussion | 71 |
| 3.2.1. Tentative identification of polyphenols from <i>R. magellanicum</i> and <i>R. punctatum</i> by HPLC-DAD-MS/MS ⁿ | 71 |
| 3.2.2. Effect of simulated gastrointestinal digestion (GID) on phenolic content and composition | 80 |
| 3.2.3. Changes in total phenolics (TP) and total flavonoid (TF) content before and after simulated digestion process | 84 |
| 3.2.4. Changes in antioxidant activity after simulated digestion process | 87 |
| 3.2.5. Inhibition of metabolic syndrome-associated enzymes before and after the simulated digestion process | 91 |
| 3.2.6. Cytoprotective effect of Chilean currants PEEs against oxidative and dicarbonyl stress | 94 |
| 3.2.7. Statistical correlations | 98 |
| Chapter 4 | 100 |
| Colonic fermentation of polyphenols from Chilean currants (<i>Ribes spp.</i>) and its effect on antioxidant capacity and metabolic syndrome-associated enzymes | 100 |
| 4.1. Introduction..... | 100 |
| 4.2. Results and discussion | 102 |
| 4.2.1. Chemical characterization of native phenolics in the PEE and their metabolites after <i>in vitro</i> colonic fermentation | 102 |
| 4.2.2. Quantitative changes in main precursors by colonic fermentation | 121 |
| 4.2.3. Effect of simulated colonic fermentation on the antioxidant capacity | 125 |
| 4.2.4. Effect of simulated colonic fermentation on the inhibition of metabolic syndrome-associated enzymes | 126 |
| Chapter 5 | 130 |
| Anti-inflammatory effect of polyphenols from Chilean currants (<i>Ribes magellanicum</i> and <i>R. punctatum</i>) after <i>in vitro</i> gastrointestinal digestion on Caco-2 cells | 130 |
| 5.1. Introduction..... | 130 |

| | |
|--|------------|
| 5.2. Results and discussion | 133 |
| 5.2.1. Cytotoxicity of the intestinal digested polyphenol-enriched extract (ID-PEE) from Chilean currants on intestinal Caco-2 cells..... | 134 |
| 5.2.2. Effect of ID-PEE from Chilean <i>Ribes</i> species on IL-8, IL-6, and TNF- α release | 134 |
| 5.2.3. Effect of the digested PEEs on COX-2 and iNOS expression in Caco-2 cells | 140 |
| 5.2.4. Inhibitory activity of Chilean currants digested extracts towards COX-1 and COX-2.. | 142 |
| Chapter 6 | 145 |
| Prebiotic effect of <i>in vitro</i> gastrointestinal digested polyphenolic enriched extracts of Chilean currants (<i>Ribes magellanicum</i> and <i>Ribes punctatum</i>) | 145 |
| 6.1. Introduction..... | 145 |
| 6.2. Results and discussion | 147 |
| 6.2.1. pH and ammonia variations throughout <i>in vitro</i> fermentation..... | 147 |
| 6.2.2. Branched-chain fatty acids (BCFA) production | 149 |
| 6.2.3. Short chain fatty acids (SCFA) analyses..... | 152 |
| 6.2.4. Changes in bacterial populations by Chilean currants digested extracts..... | 156 |
| Chapter 7 | 163 |
| Conclusions..... | 163 |
| References..... | 166 |
| Annexes | 202 |
| 1. Publications..... | 202 |
| 1.1 Thesis-related publications | 202 |
| 1.2 Other publications | 203 |
| 2. Conference presentations | 204 |
| 3. Negative controls of <i>in vitro</i> colonic fermentation | 205 |
| 3.1. HPLC-DAD traces of fermented samples (Donor 1) without extracts at 280, 330 and 520 nm after 8 h (A) and 24 h (B) of incubation. | 205 |
| 3.3. HPLC-DAD traces of fermented samples (Donor 2) without extracts at 280, 330 and 520 nm after 8 h (A) and 24 h (B) of incubation. | 206 |
| 4. Citotoxicity of the extracts in cell-based assays | 207 |
| 4.1. Cytotoxic effect of PEE (A), GD-PEE (B), and ID-PEE (C) of <i>R. magellanicum</i> and <i>R. punctatum</i> on gastric adenocarcinoma cell line (AGS)..... | 207 |
| 4.2. Cytotoxicity of <i>R. magellanicum</i> and <i>R. punctatum</i> ID-PEEs on Caco-2 clone C2BBe1 cells. | 208 |

Figures index

| | |
|---|-----|
| Figure 1. Main biosynthetic pathways of the most common phenolic compounds occurring in plants..... | 6 |
| Figure 2. Basic structural skeletons of common non-flavonoids..... | 8 |
| Figure 3. Basic structural skeletons of flavonoids..... | 11 |
| Figure 4. Overview of the metabolism of phenolic compounds..... | 14 |
| Figure 5. Ripe fruits of <i>R. magellanicum</i> (A), and <i>R. punctatum</i> (B)..... | 19 |
| Figure 6. Scheme of physiological processes emulated with <i>in vitro</i> digestión..... | 26 |
| Figure 7. Map of Chile showing the collection places of <i>Ribes</i> spp. fruits..... | 46 |
| Figure 8. HPLC-DAD chromatograms at 280 nm (black) and total ion chromatograms in the negative ionization mode (pink) of <i>R. magellanicum</i> polyphenolic-enriched extract (PEE), after gastric digestion (GD-PEE) and after intestinal (ID-PEE) digestion..... | 73 |
| Figure 9. HPLC-DAD chromatograms at 280 nm (black) and total ion chromatograms in the negative ionization mode (pink) of <i>R. punctatum</i> polyphenolic-enriched extract (PEE), after gastric digestion (GD-PEE) and after intestinal (ID-PEE) digestion | 74 |
| Figure 10. Protective effect of <i>Ribes magellanicum</i> PEEs, throughout <i>in vitro</i> digestion, on human AGS cells against the stress induced by H ₂ O ₂ and MGO, respectively..... | 96 |
| Figure 11. Protective effect of <i>Ribes punctatum</i> PEEs, before and after <i>in vitro</i> digestion, on human AGS cells against stress induced by H ₂ O ₂ and MGO | 97 |
| Figure 12. HPLC-DAD chromatograms at 280 nm (black) and total ion chromatograms in the negative ionization mode (red) of <i>Ribes magellanicum</i> after colonic fermentation with donor 1 (D1) and donor 2 (D2) samples, at time-points 0h, 8h and 24h. | 104 |
| Figure 13. HPLC-DAD chromatograms at 280 nm (black) and total ion chromatograms in the negative ionization mode (red) of <i>Ribes punctatum</i> after colonic fermentation with donor 1 (D1) and donor 2 (D2) samples, at time-points 0h, 8h and 24h. | 105 |
| Figure 14. Effect of intestinal-digested polyphenol-enriched extracts (ID-PEE) from <i>R. magellanicum</i> and <i>R. punctatum</i> on the secretion of cytokines and chemokines A) IL-8; B) TNF- α ; and C) IL-6 in intestinal Caco-2 cells stimulated with interleukin-1 β (IL-1 β)..... | 139 |
| Figure 15. Effect of the intestinal-digested polyphenol-enriched extracts (ID-PEE) from <i>R. magellanicum</i> and <i>R. punctatum</i> on the relative mRNA expression of A) COX-2; and B) iNOS in intestinal Caco-2 cells stimulated with interleukin 1 β (IL-1 β). | 142 |
| Figure 16. pH and ammonia variations during <i>in vitro</i> colonic fermentation of <i>Ribes punctatum</i> (Rp) and <i>R. magellanicum</i> (Rm) at 40, 80 and 160 μ g/mL..... | 149 |
| Figure 17. Branched-chain fatty acids (BCFA) formation during simulated colonic fermentation with the intestinal digested polyphenols from <i>R. punctatum</i> (Rp) and <i>R. magellanicum</i> (Rm) at 40, 80 and 160 μ g/mL..... | 151 |

| | |
|---|-----|
| Figure 18. Production of short chain fatty acids (SCFA), after simulated colonic fermentation with the intestinal digested polyphenols from <i>R. punctatum</i> (Rp) and <i>R. magellanicum</i> (Rm) at 40, 80 and 160 µg/mL..... | 155 |
| Figure 19. Influence of polyphenols from <i>R. punctatum</i> on the human bacterial composition during the simulated colonic fermentation..... | 161 |
| Figure 20. Changes in abundance of human colonic bacteria elicited by <i>R. magellanicum</i> polyphenols along time, during the <i>in vitro</i> fermentation..... | 162 |

Table Index

| | |
|---|-----|
| Table 1. Anthocyanins reported in the literature for Patagonian <i>Ribes</i> species..... | 21 |
| Table 2. Hydroxycinnamic acids and other flavonoids informed in the literature for Patagonian currants (<i>Ribes spp.</i>)..... | 22 |
| Table 3. Flavonoids reported in Patagonian currants (<i>Ribes spp.</i>)..... | 23 |
| Table 4. Primers employed for quantitative PCR analysis. | 60 |
| Table 5. Mass of the target and qualifier ions employed for the identification and quantification of short-chain fatty acids (SCFA). | 64 |
| Table 6. Sequence, annealing temperature, and concentration of primers..... | 66 |
| Table 7. Tentative identification of anthocyanins in undigested and digested <i>R. magellanicum</i> and <i>R. punctatum</i> PEE by HPLC-ESI-MS/MS ⁺ | 75 |
| Table 8. Tentative identification of flavonoids and hydroxycinamic acids in non-digested and digested <i>R. magellanicum</i> and <i>R. punctatum</i> by HPLC-ESI-MS/MS ⁻ | 78 |
| Table 9. Concentrations of main anthocyanins and hydroxycinnamic acids, expressed as mg of anthocyanin or 3-caffeoquinic acid equivalents, in Chilean <i>Ribes magellanicum</i> and <i>R. punctatum</i> PEEs, before and after <i>in vitro</i> gastric digestion (GD-PEE) and intestinal digestion (ID-PEE), per g of sample..... | 82 |
| Table 10. Yields of extraction, total phenolic (TP) and total flavonoid (TF) content and their recovery percentages (% rec.) from Chilean <i>R. magellanicum</i> and <i>R. punctatum</i> phenolic-enriched extracts (PEE), before and after <i>in vitro</i> gastric (GD-PEE) and intestinal digestion (ID-PEE)..... | 86 |
| Table 11. Antioxidant activity of Chilean <i>R. magellanicum</i> and <i>R. punctatum</i> PEEs, before and after <i>in vitro</i> gastric (GD-PEE) and intestinal digestion (ID-PEE). | 90 |
| Table 12. Effect of Chilean <i>R. magellanicum</i> and <i>R. punctatum</i> PEEs, before and after <i>in vitro</i> gastric digestion (GD-PEE) and intestinal digestion (ID-PEE) towards metabolic syndrome-associated enzymes. | 93 |
| Table 13. Pearson's correlation coefficient values among the content of the main phenolic groups occurring in Chilean currants and the antioxidant activity. | 99 |
| Table 14. Flavonoids and HCAs detected in <i>R. magellanicum</i> and <i>R. punctatum</i> PEEs and fermented samples by means of HPLC-ESI-MS/MS..... | 111 |
| Table 15. Native polyphenols and metabolites detected after <i>in vitro</i> colonic fermentation of Chilean currants (<i>R. magellanicum</i> and <i>R. punctatum</i>)..... | 118 |
| Table 16. Time-related quantitative modifications in main phenolic compounds (mg of compound/g PEE) of Chilean currants during <i>in vitro</i> fermentation. | 124 |

| | |
|---|-----|
| Table 17. Oxygen radical absorbance capacity and effect of Chilean <i>R. magellanicum</i> and <i>R. punctatum</i> PEEs, before and after <i>in vitro</i> fermentation towards metabolic syndrome-associated enzymes | 129 |
| Table 18. Percentage of inhibition of the PEEs from <i>Ribes magellanicum</i> and <i>R. punctatum</i> towards human COX-1 and COX-2 | 144 |

Glossary of abbreviations

| | | | |
|---------------|---|------------------------|--|
| ACN | Acetonitrile | L | Liter |
| AGS | Human gastric epithelial cells | LC | Liquid chromatography |
| amu | Atomic mass unit | M | Molar |
| APCI | Atmospheric pressure chemical ionization | MeOH | Methanol |
| APPI | Atmospheric pressure photoionization | µg | Microgram |
| BCFA | Branched-chain fatty acids | mg | Milligram |
| CH | Caffeoyl hexoside | min | Minute |
| 3-CQA | 3-caffeoquinic acid | µL | Microliter |
| 5-CQA | 5-caffeoquinic acid | mL | Milliliter |
| CE | Catechin equivalents | mM | Milimolar |
| 3-CoQA | 3-coumaroylquinic acid | MS | Mass spectrometry |
| CD | Crohn's disease | m/z | Mass/charge |
| COX | Cyclooxygenase | ng | Nanogram |
| CUPRAC | Cupric reducing antioxidant capacity | nm | Nanometer |
| DAD | Diode array detector | NO | Nitric oxide |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl | NSAIDs | Non-steroidal anti-inflammatory drugs |
| ESI | Electrospray ionization | ORAC | Oxygen radical absorbance capacity |
| FAO | Food and Agriculture Organization of the United Nations | PEE | Polyphenolic-enriched extract |
| FBS | Fetal bovine serum | PG | Prostaglandins |
| FOS | Fructooligosaccharides | Rt | Retention time |
| FQA | Feruloylquinic acid | rpm | Revolutions per minute |
| FRAP | Ferric reducing antioxidant capacity | SC₅₀ | Scavenging capacity 50 % |
| GAE | Gallic acid equivalents | SCFA | Short-chain fatty acids |
| GD-PEE | Gastric digested polyphenol-enriched extract | SEM | Standard error of the mean |
| GID | Gastrointestinal digestion | SET | Single electron transfer |
| g | Gram | SD | Standard deviation |
| GSH | Reduced glutathione | SPE | Solid phase extraction |
| HAT | Hydrogen atom transfer | TEAC | Trolox equivalent antioxidant capacity |
| HCA | Hydroxycinnamic acids | TE | Trolox equivalents |
| h | hours | TF | Total flavonoid content |
| HPLC | High performance liquid chromatography | TNF-α | Tumor necrosis factor alpha |
| IBD | Inflammatory bowel diseases | TP | Total phenolic content |
| ID-PEE | Intestinal digested polyphenol-enriched extract | UC | Ulcerative colitis |
| IL | Interleukin | UV | Ultraviolet |
| iNOS | Inducible nitric oxide synthase | WHO | World health organization |
| | | µM | Micromolar |

