UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

ISABELA MATEUS MARTINS

# ENZYMATIC BIOTRANSFORMATION OF RED AND WHITE GRAPE POMACE AND THEIR *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS

# BIOTRANSFORMAÇÃO ENZIMÁTICA DE RESÍDUOS DE VINIFICAÇÃO DE UVAS TINTA E BRANCA E SEUS EFEITOS ANTIOXIDANTE E ANTI-INFLAMATÓRIO *IN VITRO*

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutora em Ciência de Alimentos.

Supervisor/Orientadora: Dra. GABRIELA ALVES MACEDO
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* A ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica da aluna.

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**RESUMO**

Resíduos de vinificação de uvas têm sido considerados fontes potenciais de compostos fenólicos poliméricos e glicosídeos, conhecidos por terem menores biodisponibilidade e bioatividade do que fenólicos monoméricos e agliconas. Hidrólises enzimáticas são bioprocessos que podem liberar compostos fenólicos de seus conjugados, levando a um possível aumento em sua funcionalidade. O objetivo deste estudo foi avaliar mudanças no teor e perfil de polifenóis, atividades antioxidante e anti-inflamatória de resíduos de vinificação de uvas tintas (RUT), brancas (RUB) e de uma mistura de uvas (RMU). Biotransformações enzimáticas usando as enzimas tanase (T), combinação de pectinase e celulase (PC) e as três enzimas juntas (TPC) foram realizadas durante 5h a 40°C (pH 5.0). Além disso, foram avaliadas ações anti-inflamatórias do RUT e do resíduo após tratamento com tanase (RUTT) em células Caco-2. As biotransformações enzimáticas, em especial com tanase, foram capazes de modificar a forma glicosilada dos polifenóis nos resíduos e aumentaram as quantidades de ácidos fenólicos, em especial ácido gálico, quercetina e *trans*-resveratrol, consequentemente aumentando sua atividade antioxidante. Os tratamentos contendo tanase foram os mais eficientes em aumentar a atividade antioxidante dos resíduos, analisadas pelos métodos de ORAC, DPPH e FRAP. A quantidade de catequina foi aumentada especialmente no RUT após tratamento com PC e procianidina B2 teve seus valores aumentados especialmente após TPC no RUB. RUT e RUTT, nas concentrações 100 e 200 µg/mL de extrato seco (m/v) atuaram com a mesma eficácia na redução da produção de espécies reativas de oxigênio (ERO) em células Caco-2. Após 24 h de pré-tratamento com os extratos, RUTT 200 µg/mL inibiu a produção de prostaglandina 2 ($PGE_2$) e interleucina 8 (IL-8) em 107% e 83%, respectivamente, e reduziu a ativação do fator de transcrição kappa B (NF-κB) em 68% em células Caco-2 após indução de inflamação por interleucina 1-beta (IL-1β). Em geral, RUTT foi mais eficiente que RUT na atenuação de marcadores de inflamação nas células. Estes resultados demonstram benefícios no aproveitamento desses resíduos e do uso de bioprocessos na modificação de produtos naturais, aumentando a extração, biodisponibilidade e bioatividade de compostos fenólicos.

**Palavras-chave:** resíduo, uva, tanase, pectinase, celulase, polifenóis, atividade antioxidante, células Caco-2, inflamação.

## ABSTRACT

Grape pomace (GP) has been considered a potential source of polymeric phenolic compounds and glycosides, known to have lower bioefficacy than monomers and aglycones, which can compromise their bioavailability and properties. Enzymatic hydrolyzes may release the compounds from their conjugates, improving its functional activity. The aim of this study was to determine the release of phenolic compounds and the improvement of the antioxidant and anti-inflammatory activity of red (RGP), white (WGP) and a mixture of grape pomaces (MGP) from Brazilian wine industries. Tannase enzyme (T), the combination of pectinase and cellulase (PC) and the 3 enzymes together (TPC) were applied for 5h at 40°C (pH 5.0). Also, it was evaluated if RGP treated with tannase (RGPT) would improve anti-inflammatory actions in Caco-2 cells. Tannase treatment (GPT) was the most significant treatment on enhancing total phenols in GP samples. Enzymatic hydrolysis, notably tannase, modified the galloylated form of polyphenols and released phenolic acids, especially gallic acid, quercetin and *trans*-resveratrol, thus enhancing the antioxidant activity. The tannase-containing treatments were the most effective on enhancing the antioxidant capacity of residues, assessed by ORAC, DPPH and FRAP assays. Catechin amounts increased in RGP after PC treatment and procyanidin B2 was increased especially after TPC treatment in WGP. RGP and RGPT at 100 and 200 µg/mL of dry extract (wt/v) displayed comparable efficacy in the reduction of ROS production in Caco-2 cells. After 24-h pretreatment, GPT at 200 µg/mL decreased IL-1β-induced $PGE_2$ production by 107% and IL-8 by 83% and down-regulated NF-κB activation by 68%, as compared to control. Overall, GPT exhibited more potent efficacy than GP in the amelioration of IL-1β-induced inflammation in Caco-2 cells. These results demonstrate the benefits of the exploitation of this agroindustrial by-product and the biotechnological modification of natural products in order to enrich bioavailable and bioactive polyphenols in foods.

**Keywords:** residue, grape, tannase, pectinase, cellulase, polyphenols, antioxidant activity, Caco-2 cells, inflammation.

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| AA | Arachidonic acid |
| AAPH | 2,2'-Azobis(2-amidinopropane) dihydrochloride |
| ACN | Acetonitrile |
| ALP | Alkaline phosphatase |
| ANOVA | Analysis of variance |
| AP-1 | Activator protein-1 |
| ATCC | American type culture collection |
| AUC | Area under curve |
| BCA | Bicinchoninic acid |
| BSA | Bovine serum albumin |
| C | Cellulase |
| CCL3 | Chemokine (C-C motif) ligand 3 |
| CE | Catechin equivalent |
| CM-H2DCFDA | 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate |
| COX | Cyclooxygenase |
| COX-2 | Cyclooxygenase 2 |
| CRP | C reactive protein |
| CXCL10 | C-X-C motif chemokine 10 |
| DAD | Diode array detection |
| DE | Dry extract |
| DM | Dry matter |
| DMEM | Dulbecco's Modified Eagle's medium |
| DMSO | Dimethyl sulphoxyde |
| DNA | Deoxyribonucleic acid |
| DPPH | 2,2-difenil-1-picrilhidrazil |
| DSS | Dextran sulfate sodium |
| ECD | Eletrochemical detector |
| ELISA | Enzyme Linked Immune Sorbent Assay |
| ERK | Extracellular-signal-regulated kinases |
| ERO | Espécies reativas de oxigênio |
| FAS | Ferric ammonium sulphate |
| FBS | Fetal bovine serum |
| FL | Fluorescein |
| FRAP | Ferric-reducing antioxidant power |
| GA | Gallic acid |
| GAE | Gallic acid equivalent |
| GP | Grape pomace |
| GPE | Grape pomace extract |
| GPT | Grape pomace + tannase |
| GSe | Grape seed |
| GSE | Grape seed extract |
| GSk | Grape skin |
| HBSS | Hank's Balanced Salt Solution |
| HCl | Hydrochloric acid |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HOMA-IR | Homeostatic model assessment - insulin resistance |
| HPLC | High performance liquid chromatography |
| HSD | Honest significant difference |
| HT | Hydrolysable tannins |
| iba-1 | macrophage/microglia-specific calcium-binding protein |
| ibc-1 | Inflammatory breast cancer 1 |
| IBDs | Inflammatory bowel diseases |

| | |
|---|---|
| IFN-Ɣ | Interferon-Ɣ |
| IKb | IKb kinase |
| IKK | Kinase complex |
| IL-10 | Interleukin-10 |
| IL-17A | Interleukin-17 A |
| IL-1β | Interleucina-1β/interleukin-1β |
| IL-6 | Interleukin-6 |
| IL-8 | Interleucina 8/interleukin-8 |
| iNOS | Inducible nitric oxide synthase |
| JNK | c-Jun N-terminal kinase |
| LDL | Low density lipoprotein |
| LPS | Lipopolysaccharide |
| MAPK | Mitogen-activated protein kinase |
| MCP-1 | Monocyte chemotactic protein-1 |
| MGP | Mixed grape pomace |
| mRNA | Messenger ribonucleic acid |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| NF-kB | Factor nuclear kappa B |
| NLRP-3 | Gene - inflammasome |
| NO | Nitric oxide |
| NOx-1 | NADPH oxidase -1 |
| ORAC | Oxygen Radical Absorbance Capacity |
| P | Pectinase |
| PAI-1 | Plasminogen activator inhibitor-1 |
| PBS | Phosphate saline buffer |
| PC | Pectinase + celulase |
| PG | Prostaglandina/prostaglandin |
| $PGE_2$ | Prostaglandina E2/prostaglandin E2 |
| PT | Pretreatment |
| CT | Cotreatment |
| RGP | Red grape pomace |
| RIPA | Radio immunoprecipitation assay |
| RMU | Resíduo de mistura de uvas |
| RNA | Ribonucleic acid |
| ROS | Reactive oxigen species |
| RUB | Resíduo de uva branca |
| RUT | Resíduo de uva tinta |
| RUTT | Resíduo de uva tinta + tanase |
| SD | Standard deviation |
| STATs | Signal transducer and activator of transcription |
| T | Tanase/tannase |
| TAE | Tannic acid equivalent |
| TBARS | Thiobarbituric acid reactive substances |
| TE | Trolox equivalent |
| Th1 | Lymphocyte Th1 |
| Th17 | Lymphocyte Th17 |
| TLR-4 | Toll like receptor 4 |
| TNF-α | Tumor necrosis factor α |
| TPC | Tanase + pectinase + celulase/ tannase + pectinase + cellulase |
| TPTZ | 4,6-tripryridyl-s-triazine. |
| UV/VIS | Ultraviolet/visible |
| WAT | White adipocyte tissue |
| WGP | White grape pomace |

## SUMÁRIO

## INTRODUÇÃO GERAL

Estudos epidemiológicos mostram uma associação entre elevado consumo de alimentos vegetais com promoção de saúde e prevenção de doenças (JOHNSTON; TAYLOR; HAMPL, 2000). Além de seus constituintes promoverem efeitos benéficos à saúde (como fibras, vitaminas e minerais), os vegetais são ricos em polifenóis, compostos que são muito estudados por serem associados a efeitos positivos à saúde, mas que ainda necessitam ser totalmente caracterizados (CARDONA et al., 2013). Esta extensa família de compostos está amplamente distribuída em diversos alimentos e bebidas, especialmente em vinhos, café, chás, frutas, vegetais e cereais (CROZIER; JAGANATH; CLIFFORD, 2009). Sua distribuição na célula vegetal não é uniforme, podendo estar dentro de vacúolos (solúveis) ou ligados à parece celular (fenólicos insolúveis) e, desta forma, muitas vezes permanecem nos resíduos de alimentos após seu processamento (PINELO; ARNOUS; MEYER, 2006).

Resíduos agroindustriais, como sementes e cascas de frutos, são gerados e desperdiçados em grandes quantidades, sendo pouco aproveitados e muitas vezes deixados deteriorar no ambiente, podendo gerar diversos problemas ambientais. Recentemente, há um aumento no interesse e na valorização destas matérias-primas por possuírem quantidades interessantes de fibras dietéticas antioxidantes e compostos fenólicos bioativos, além de serem materiais abundantes e de baixo custo para aplicações promissoras, como produção de enzimas, ácidos orgânicos e compostos bioativos (PANDEY, 2003; MARTINS et al., 2011). Resíduos provenientes da produção de vinhos e sucos de uva são caracterizados por terem elevada quantidade de compostos fenólicos devido a ineficiente extração durante seu processamento, sendo considerados fontes potenciais de compostos fenólicos bioativos, com propriedades antioxidantes interessantes para indústrias de alimentos, de cosméticos e farmacêutica, com possíveis aplicações tecnológicas como aditivos funcionais alimentares (GONZÁLEZ-PARAMÁS et al., 2004; FONTANA; ANTONIOLLI; BOTTINI, 2013). Porém, os compostos fenólicos presentes nos alimentos estão muitas vezes ligados a carboidratos, proteínas, polimerizados e formando estruturas complexas que podem comprometer sua absorção, biodisponibilidade e, consequentemente, sua bioatividade (DEL RIO et al., 2010). Desta forma, estratégias foram desenvolvidas a fim de enriquecer os

alimentos com polifenóis mais biodisponíveis, como por exemplo a fermentação por micro-organismos ou a realização de hidrólises enzimáticas, o que pode levar à biotransformação e liberação dos compostos a partir de seus conjugados, melhorando sua atividade funcional, além de aumentar o rendimento de sua extração a partir da matriz alimentar (GEORGETTI et al., 2009). Enzimas comumente utilizadas para esse fim são as chamadas enzimas de maceração, como as celulases, pectinases e glucanases. Já a tanase é uma enzima que age sobre taninos hidrolisáveis e complexos, liberando compostos ligados à parede celular, além de remover compostos mais simples e de menor peso molecular a partir de seus conjugados, comumente liberando ácido gálico e transformando glicosídeos, que são menos biodisponíveis, em agliconas (BATTESTIN; MATSUDA; MACEDO, 2004).

Os polifenóis podem exercer diversos mecanismos de proteção celular, como proteção antioxidativa, proteção contra danos ao DNA, estímulos ao sistema imunológico e modulação hormonal. Devido a sua relação com mecanismos fisiológicos mais complexos, os compostos fenólicos podem agir na prevenção de diversas patologias (SCALBERT et al., 2005), doenças degenerativas como o câncer e a diabetes (YOU *et al*., 2012; SUN *et al*., 2012), podem reduzir fatores de risco de doenças cardiovasculares (ROUANET *et al*., 2010; OBOH; ADEMOSUN, 2012), além de possuírem efeitos antimutagênico, anti-inflamatório, antioxidante e antimicrobiano (BAO *et al*., 2004; TABASCO *et al*., 2011; LIM *et al*., 2012; ANASTASIADI *et al*., 2012).

A inflamação é o primeiro sistema fisiológico de defesa do corpo humano, que o protege de injúrias físicas, de compostos irritantes e que elimina micro-organismos infecciosos, mantendo nossas funções fisiológicas normais. No entanto, processos inflamatórios longos e excessivos podem causar disfunções fisiológicas, sendo considerados fatores críticos para muitas doenças humanas, como câncer, obesidade, diabetes tipo II, doenças cardiovasculares, neurodegenerativas e doenças inflamatórias intestinais como a colite ulcerativa e a doença de Crohn (SANTANGELO et al., 2007; LEE et al., 2010). A luz intestinal é revestida com uma camada de células epiteliais, que não são apenas responsáveis pela absorção e transporte de nutrientes, mas participam ativamente da resposta imunológica local, aumentando a proteção contra antígenos e agentes microbianos (SNOECK; GODDEERIS; COX, 2005). Alterações desse sistema de defesa levam a problemas

de natureza imunológica e a enfermidades inflamatórias crônicas (GEREMIA et al., 2014). Os efeitos dos polifenóis e seus metabólitos na resposta imunológica e inflamatória do epitélio intestinal ainda não foram totalmente elucidados. Muitos mecanismos podem estar relacionados entre si, como a modulação de fatores de transcrição, do estresse oxidativo e da sinalização celular. Os polifenóis podem modular a expressão gênica da ciclooxigenase, lipoxigenase e óxido nítrico sintase, além de diversas citocinas e quimiocinas, principalmente agindo através do fator nuclear kappa B (NF-κB) e de proteíno-quinase ativada por mitógenos (MAPK) (SANTANGELO et al., 2007). Alguns compostos fenólicos presentes em uvas possuem atividade anti-inflamatória, especialmente flavonoides e procianidinas (XIA et al., 2010).

A demanda por produtos que contribuam com a melhoria da qualidade de vida, especialmente os provenientes de fontes naturais, está em constante crescimento. Juntamente com a preocupação do setor industrial em atender essa exigência, há um aumento no número de pesquisas na busca de novas tecnologias, visando a promoção de saúde dos consumidores e, ao mesmo tempo, a diminuição de perdas econômicas e do impacto da atividade industrial ao meio ambiente (MELO, 2011). O conhecimento dessas relações é importante para uma melhor compreensão dos benefícios à saúde relacionados ao consumo de alimentos de origem vegetal.

Os objetivos deste estudo foram analisar diferentes ações de tratamentos enzimáticos utilizando tanase (produzida por *Paecylomyces variotii*), pectinase e celulase (comerciais), aplicados em resíduos de vinificação de uvas brancas e tintas, na hidrólise de polifenóis presentes nos resíduos, assim como avaliar as atividades antioxidantes e anti-inflamatórias do extratos resultantes sobre marcadores moleculares de inflamação [espécies reativas de oxigênio (ERO), interleucina 8 (IL-8), prostaglandina $E_2$ ($PGE_2$), ciclooxigenase 2 (COX-2) e fator nuclear kappa B (NF-κB)], através de um modelo *in vitro* intestinal utilizando células de adenocarcinoma humano (Caco-2).

# ARTIGO I

# REVISÃO DE LITERATURA

## Phenolic compounds in grape pomace: occurrence, enzymatic biotransformation, anti-inflammatory activity and possible use as functional ingredients

## Statistics of grape and wine production in Brazil

Grapes (*Vitis* sp.) are considered one of the main fruit crops in the world, reaching a global production of 75 million of tonnes in 2013 (FAOSTAT, 2015). Brazil was the 13$^{th}$ in the world rank production, reaching 1,439,535 tonnes in 2013 (FAOSTAT, 2015), being 679,79 millions of kg destined for process (e.g., wine, juice and derivatives), representing 48,11% of the national production, and the other 51,89% was for raw consumption (MELLO, 2014). Brazilian grape crops are found mainly in the South, Southeast and Northeast areas, especially in Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais e Pernambuco States, notably Rio Grande do Sul, which holds approximately 90% of the total production. Winemaking is important for the assurance and sustainability of small farm producers in Brazil, besides being essential for employments in companies of table and processed grapes (MELLO, 2012).

Every year, the world wine and juice production generates around 10 millions of tonnes of waste (MAIER et al., 2009), which are frequently used for few agronomical applications, composting and/or discarded in open areas potentially causing environmental problems (ARNOUS; MEYER, 2008). In the Brazilian wine production, 40% of processed grapes becomes a residue (EMBRAPA, 2007), and near 59.4 million kg of the pomace formed, considering 18 kg pomace/100 L wine, is treated as residue with low profitable uses such as animal feed and manure (DE CAMPOS et al., 2008; ROCKENBACH et al., 2011).

## Chemical characterization of grape pomace

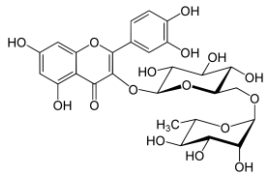
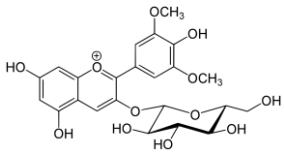
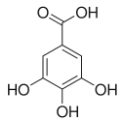
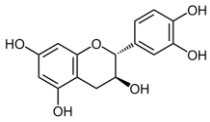
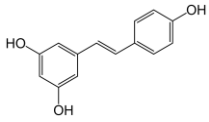
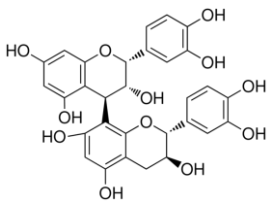
Recently, there is a great interest in the exploitation of industrial residues. Fruits and vegetables, olive oil, dairy, meat and seafood industrial wastes can be reused for different purposes, e.g., production of enzymes, aromatic compounds, ethanol, organic acids, polysaccharides, pigments, and antioxidants (KOSSEVA, 2011). In particular, the wastes of wine and grape juice industries may be reused as soil conditioner, for production of fertilizers, or as an alternative source of natural antioxidants, considered completely safe in comparison with synthetic antioxidants (ARVANITOYANNIS; LADAS; MAVROMATIS, 2006).

Grape pomace is the pressed residue after the wine production, which consists in skins, seed, some residual pulp, and stems (MEYER; JEPSEN; SØRENSEN, 1998). The chemical composition of grape pomaces normally changes as result of the plant variety. The crude protein can vary from 5 to 12%, fat from 1 to 11%, ash from 2 to 7%. The soluble sugars content is around 3%, moisture 5% and fiber 60% (DENG; PENNER; ZHAO, 2011; TSENG; ZHAO, 2013). The composition of the grape skin cell wall is complex, with 30% of neutral polysaccharides (e.g., cellulose, xyloglucan), 20% of acidic pectin substances, ~15% insoluble proanthocyanidins, and <5% structural proteins, lignin and polyphenols (PINELO; ARNOUS; MEYER, 2006). Grape pomace is characterized as being a high phenolic content residue because of the low extraction during winemaking (~60%) (HORNSEY, 2007). The main phenolic compounds present in grape and wine are flavonoids (anthocyanins, flavonols and flavanols), stilbenes and phenolic acids, compounds with proven biological properties (MONAGAS; BARTOLOMÉ; GÓMEZ-CORDOVÉS, 2005). The concentration of phenolic compounds in grapes is related to the variety of grapevine, also being influenced by viticulture and environmental factors (MONTEALEGRE et al., 2006; OBREQUE-SLIER et al., 2010). The phenolic groups normally present in grapes are presented in **Table 1.**

In grape berries, phenolic compounds are present mainly in seeds and skins, having large amounts of tannins, phenolics with higher polymerization status. The skin phenolics represent ~30% of total phenols in the fruit. They can be associated to cell wall polysaccharides, linked by hydrophobic interactions and hydrogen bonds, and also present confined in the vacuoles of plant cells and associated with the cell nucleus (PINELO; ARNOUS; MEYER, 2006). Also, the skin represents 15% of the total grape mass and it has the main aromatic compounds and flavor precursors. Catechin, epicatechin and epicatechin-gallate are the main constituents, although gallocatechin and epigallocatechin are also present in lower amounts (SOUQUET et al., 1996). Phenolic compounds are accumulated during the ripeness of the fruit, being important for the characterization of grapes and wines, being responsible for the grape pigmentation and astringency (HORNSEY, 2007). When ripped, grape skins have benzoic and cinnamic acids derivatives, flavonols and tannins, in both red and white grapes. Besides, anthocyanins are present in the red grape skins, including delphynidin, cyanidin, petunidin, peonidin and malvidin-3-glucosides, 3-(6-acetyl)-glucosides and 3-(6-p-coumaroil)-glucosides, peonidin and

malvidin 3-(6-cafeoil)-glucosides and piruvates, responsible for the wine color (MONAGAS; BARTOLOMÉ; GÓMEZ-CORDOVÉS, 2005). Grape seeds are a rich source of unsaturated fatty acids and contain flavonoids and non-flavonoids, including gallic acid, catechin, epicatechin, and procyanidins, besides large amounts of tannins, mostly monomers. They represent 4% of the fruit total mass and 38-52% of the pomace. Their phenolics represent ~60% of total phenolics in the fruit (HORNSEY, 2007; MAIER et al., 2009).

**Table 1.** Phenolic compounds normally found in grape seed and skins.

| | | |
|---|---|---|
| Skin | Flavonols (e.g., rutin) |  |
| | Anthocyanins (e.g., malvidin-3-glucoside) |  |
| Seed and skin | Phenolic acids (e.g., gallic acid) |  |
| | Flavan-3-ols (e.g., catechin) |  |
| | Stilbenes (e.g., resveratrol) |  |
| | Proanthocyanidins and condensed tannins (e.g., procyanidin B2) |  |

**Enzymatic biotransformation of phenolic compounds**

Phenolic compounds are abundant in our diet, present in a wide variety of plants, but their chemical structure and interaction with other compounds can affect their bioavailability and functionality (SCALBERT; WILLIAMSON, 2000; MESKIN et al., 2003; MANACH et al., 2004). The term bioavailability was introduced to quantify the percentage of a compound that is absorbed, distributed to the tissues, metabolized and eventually excreted. It is affected by diverse factors, as complexity of matrix, chemical structures, co-ingestion with other compounds, intestinal transit time, metabolism and gastric emptying (HOLST; WILLIAMSON, 2008). The glycosylated phenolic compounds are more soluble in water, but less reactive against free radicals due to unavailability of hydroxyl groups (VATTEM; SHETTY, 2003). There are evidences that monomeric phenolic and some oligomers are absorbable (SHOJI et al., 2006), but the polymeric forms have a low absorption rate (DONOVAN et al., 2002; GONTHIER et al., 2003). The hydrolysis of polysaccharides and polyphenols to simpler and smaller compounds may increase the bioavailability of bioactive compounds in a product.

The interest in the exploitation of functional compounds from agroindustrial residues, especially flavonoids and phenolic acids from plants, has been stimulating the studies of cell wall enzymatic hydrolysis by the use of pectinase, cellulase, hemicellulase and glucanase (MEYER; JEPSEN; SØRENSEN, 1998; ZHENG; HWANG; CHUNG, 2009; ARNOUS; MEYER, 2010; CHAMORRO et al., 2012). These enzymes have the ability of catalyzing the hydrolysis of cell wall polysaccharides linkages, being able to break the structure and separate polyphenols from other compounds attached to the cell wall. The degradation of the membrane polysaccharides can increase the extraction yields of phenolic compounds from the grape skin during wine production and also for the use of winemaking waste (PINELO; ARNOUS; MEYER, 2006).

Tannase (tannin acyl hydrolase E.C.3.1.1.20) catalyzes the hydrolysis reaction of ester bonds present in the gallotannins, complex tannins, and gallic acid esters, e.g., release of gallic acid from epicatechin-gallate, tannic acid transformation in glucose and gallic acid. Tannase may play an important role in plant cell-wall degradation by cleaving some of the cross-links existing between cell-wall polymers (GARCÍA-CONESA et al., 2001). Condensed tannins are not hydrolyzed by tannases

(CONTRERAS-DOMÍNGUEZ et al., 2006), but it is known this hydrolysis is possible by the initial degradation steps carried out by oxygenases. However, further studies are needed to characterize the degradation of condensed tannins (AGUILAR et al., 2007). Tannases are used in food and beverage processing, as clarifying agent in wines, beers, fruit juices, and in refreshing drinks with coffee flavor, but practical uses of this enzyme are still limited due to insufficient knowledge about its properties, optimal expression, and large-scale application (LEKHA; LONSANE, 1997). The capacity of *Paecilomyces variotti* tannase on hydrolyze epigallocatechin-gallate of green tea into gallic acid was previously demonstrated (BATTESTIN; MACEDO; DE FREITAS, 2008), resulting in an increase of tea antioxidant capacity after hydrolysis (BATTESTIN; MACEDO; DE FREITAS, 2008; MACEDO et al., 2011). Tannase also biotransformed the phenolic composition of orange juice, transforming glycosides in aglycones (acting on hesperidin and naringin), generating a product with higher functional activity, demonstrated by increases in antioxidant and antiproliferative actions on human cells (FERREIRA *et al*., 2013). Studies have evaluated the action of different enzymes on grape residues and also during the production of wine and grape juice (**Table 2**), showing bioprocesses like fermentation and enzymatic hydrolysis are clean technologies with great potential in the exploitation of biologically active compounds from natural sources (GEORGETTI et al., 2009). **Figure 1** shows some possible actions of tannase, cellulase and pectinase on polyphenols and food fiber matrix.

**Anti-inflammatory effects of phenolic compounds**

Changes in lifestyle in industrialized and developing countries brought significant changes in people's dietary habits, which may be associated with an increase in chronic diseases (WHO, 2002). The growing interest on polyphenols emerged from different studies connecting phenolic-rich diets with beneficial human health effects, like decrease in the incidence of degenerative diseases as cancer and diabetes (SUN et al., 2012; YOU et al., 2012), reduction of cardiovascular risk factors (OBOH; ADEMOSUN, 2012), anti-mutagenic, anti-inflammatory, antioxidative, and antimicrobial effects (BAO et al., 2004; TABASCO et al., 2011; ANASTASIADI et al., 2012; LIM; HWANG; SHIN, 2012).

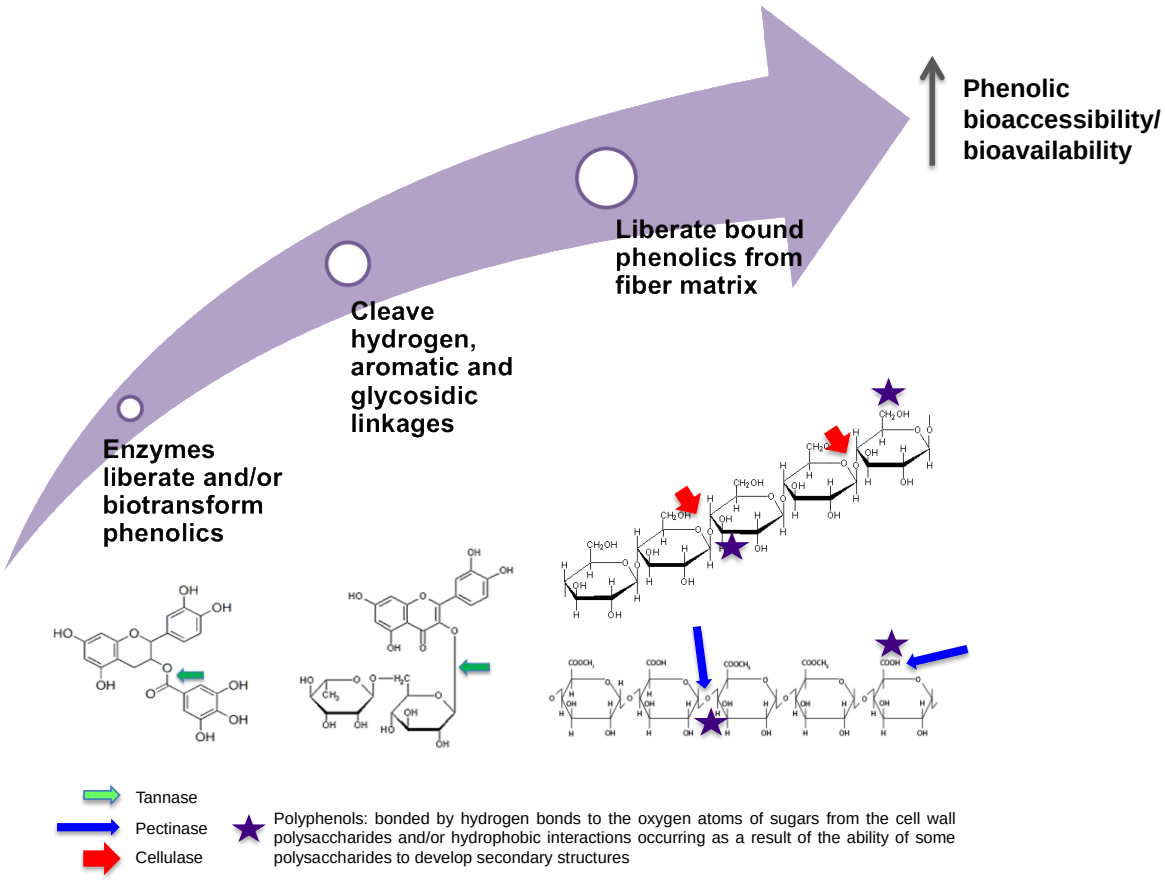



**Figure 1.** Enzymatic actions and the release and/or biotransformation of polyphenols linked to food fiber matrix.

Recent data suggest phenolic compounds can be important modulator metabolites due to their capacity to influence cellular pathways and molecules (SANTANGELO et al., 2007). The oxidative damage to cellular components (DNA, proteins and lipids), if accumulated with time, contributes to somatic cell degeneration, leading to the development of diseases. The antioxidant compounds present in food can offer antioxidative protection to these molecules, by accepting electrons and producing relatively stable radicals, breaking the oxidation chain of cell components (SCALBERT et al., 2005). They can also act stimulating endogenous defense systems, enhancing the immunological system, hormonal modulation and helping to limit cell injuries. These characteristics make these compounds important therapeutical tools for acute and chronic diseases (SANTANGELO et al., 2007).

**Table 2**. Enzymatic biotransformation in grape residues and during the production of grape by-products.

| Grape by-product | Treatment | Results | Study |
|---|---|---|---|
| Grape juice | • commercial pectinase<br>• 60°C, 3 mL/100 kg$^{-1}$, 1h | • ↑ extraction yield without pressing the grapes<br>• ↑ PROB1 and B2, ↑ anthocyanins, ↑ almost all organic acids<br>• ↑ AA<br>• ↓ C, ↓ acetic acid<br>• ↓ turbidity | LIMA et al., 2015 |
| Wine | • pectolytic enzyme before fermentation (winemaking)<br>• 65°C, 3 g/h l, 8 h | wines:<br>• ↑ GA<br>• ↓ monomeric flavan-3-ols<br>• ↓ phenolic acid compared with control | BORAZAN; BOZAN, 2013 |
| Grape skin | • mix: 70.3% pectin lyase, 22.2% polygalacturonase and 7.5% cellulase<br>• 25°C, pH 3.2, 2 and 5 g/100 kg, 8 days | • ↑ extraction yield of anthocyanins<br>• ↓ maceration time<br>• prevent the loss of the anthocyanins | RÍO SEGADE et al., 2015 |
| Grape skin | • polygalacturonase/pectin lyase and cellulase<br>• 45-60°C, pH 3.5-6.0, 0-24h | • ↑ anthocyanins (early phases), degraded during further enzymatic treatment<br>• transformation from glycosylated (rutin) to deglycosylated (quercetin)<br>↑ hydroxybenzoic acids and hydroxycinnamic acids, p-coumaric acid<br>• ↓ TP | ARNOUS; MEYER, 2010 |
| Grape skin and grape seed | • cellulase, pectinase, ß-glucosidase<br>• 50°C, pH 4.8, 1, 4, 8, 24 h | • ↓ TP yield compared with solvent extraction<br>• 1-h pectinase hydrolysis, ↑ TP<br>• ↓ extraction time<br>• modified galloylated form, ↑ low molecular weight phenolics<br>• ↑ phenolic acids (especially GA), ↑ AA | XU et al., 2014 |

**Table 2.** (cont.)

| | | | |
|---|---|---|---|
| | | tannase treatment grape seed:<br>• ↑ TP, ↑ GA, ↑EC, ↑ PROB2, C – no change<br>• ↑ AA | |
| Grape pomace and grape seed | • pectinase, cellulase and tannase<br>• 35°C, pH 5.5, 24 h | grape pomace:<br>• cellulase alone - no difference<br>• pectinase alone: ↑ GA , ↑ AA<br>• tannase alone: ↑ GA, ↑ AA<br>• 3 enzymes together - ↑ GA , ↑ PROB2<br>• C and EC – no change<br>• changed the galloylated form of C to its free form | CHAMORRO et al., 2012 |
| Grape pomace | • endoproteases mixture (trypsin- and chymotrypsin-like)<br>• bioreactor (60°C), pH 8<br>• pH-stat method | • no control analysis<br>• enzymatic extract with antioxidant and protective vascular properties | RODRIGUEZ-RODRIGUEZ et al., 2012 |
| Grape pomace | • endoprotease mixture<br>• 60°C, pH 8.0, 0,3%v/v<br>• pH-stat method | • peptides, carbohydrates, lipids and polyphenols in soluble form<br>• anti-inflammatory effects (N13 cells)<br>• no control (without enzymes) analysis | RODRÍGUEZ-MORGADO et al., 2015 |
| Grape pomace | • pectinase and cellulase (ratio 2:1)<br>• lab and pilot-plant scales<br>• 40°C, pH 4.0, 4,500 mg/kg, 2 h<br>• | • ↑ extraction yields for phenolic acids, non-anthocyanin flavonoids and anthocyanins<br>• ↑ phenolic compounds after 2-h treatment | MAIER et al., 2008 |
| Grape pomace | • cellulolytic enzyme preparation<br>• 50°C , pH 4.6, 5000 ppm and 2500 ppm, 0-240 min | • ↑ recovery rates<br>• ↑ ρ-coumaric, ↑ FA, ↑ C ↑ EC, ↑ PROB2, ↑ resveratrol ↑ quercetin, ↓ GA, ↓ CA<br>• ↑ anthocyanins, ↑ TP | KAMMERER et al., 2005 |
| Grape pomace | • pectinase and cellulase (0, 5, 10%)<br>• 40°C, pH 5.0, 1h, 24.5h, 48h | • phenols were degraded during the long enzyme treatment (48h)<br>• pectinase - degradation of polysaccharides<br>• ↓ human low-density lipoprotein oxidation *in vitro* | MEYER; JEPSEN; SØRENSEN, 1998 |

Abbreviations: TP – total polyphenolics, GA - gallic acid, FA - ferulic acid, C - catechin, EC -epicatchin, PRO - procyanidin, CA - caffeic acid, AA - antioxidant activity.

Inflammation is the first defense physiological system of the human body, a complex biological response to harmful stimuli, e.g., pathogens, infectious microorganisms, damaged cells, irritating agents and physical injuries. Together with inflammation, the tissue cure process associated with neutrophils and macrophages migration to the inflamed local, works to maintain the normal physiological functions in the body. However, long and excessive inflammatory processes may generate physiological dysfunctions, considered critical factors for human diseases, like cancer, obesity, type II diabetes, cardiovascular and neurodegenerative diseases (SANTANGELO et al., 2007; LEE et al., 2010).

The intestinal mucosa is constantly exposed to a vast array of microbes and oxidant nutritional compounds from the diet. For intestinal protection, the mucosa presents an organized immunological system that maintains the homeostasis between gut and food constituents. The immunological components not only induce protection against pathogenic microorganisms, but also have the capacity to
ns, like nutrients and commensal bacteria (LAMICHHANE; KIYONO; KUNISAWA, 2013). Intestinal inflammation causes loss of intercellular junctions, elevated production of pro-inflammatory mediators as cytokines, chemokines, adhesion molecules, associated with dysfunction of the immune system (SONIER et al., 2009), and reactive oxygen species (ROS) by activated leukocytes, that can contribute to abnormal or prolonged inflammatory responses (BIASI et al., 2013). Some mechanisms of action have been proposed and include inhibition of transcription factors nuclear factor kappa B (NF-κB), activating protein 1 (AP-1) pathways, mitogen-activated protein kinases (MAPK), signal transducers and activators of transcription (STATs). The inflammation mediators nitric oxide (NO), leukotrienes, adhesion molecules, cytokines and chemokines have been identified and studied in diverse inflammatory processes (SANTANGELO et al., 2007; ROMIER et al., 2009).

The production of pro-inflammatory cytokines and chemokines is a physiological process, with the purpose of activating the immune and intestinal epithelial cells in acute situations. However, this procedure can become chronic, as happens during inflammatory bowel diseases (IBDs), like ulcerative colitis and Crohn's disease (LAM et al., 2015). The ROS production and oxidative stress are known to be important components in inflammation due to the capacity of perpetuate and amplify inflammatory pathways (MATIAS et al., 2014). The expression of interleukin-8 (IL-8), the major human inflammatory mediator and neutrophil chemo

attractant in the gut, is known to be dependent on interleukin-1 beta (IL-1β) activation of NF-κB pathway, and its expression level is increased during inflammatory processes (MCCORMACK et al., 2001). Besides cytokines and chemokines, other mediators are released under inflammatory conditions. Prostaglandins are lipid autacoids generated from arachidonate by the action of cyclooxygenase (COX) isoenzymes, being mediators of many biological functions. COX-1 plays a role in homeostasis and is expressed in diverse tissues. COX-2 is induced by inflammatory stimuli and is highly expressed in inflammatory cells. In inflammation, prostaglandin $E_2$ ($PGE_2$) is involved in the generation of redness, swelling and pain, and its biosynthesis is significantly increased in the inflamed tissue (LEGLER et al., 2010; RICCIOTTI; FITZGERALD, 2011)

Multiple evidences indicate that NF-κB is related to the progress of inflammation and cancer. This transcription factor controls the expression of genes related to several mediators of inflammation. The inactive form of NF-κB is bound to its inhibitory protein (IκB) in the cytoplasm. After stimulation, IκB is phosphorylated by the IKK complex, leading to its degradation and dissociation from the NF-κB-complex. Free NF-κB translocates to the nucleus, where it binds to DNA and starts the transcription of various target inflammatory and immunoregulatory genes. Blocking NF-κB activation, anywhere in the cascade, will repress the transcription of these genes and reduce inflammation (KAULMANN; BOHN, 2014).

The effect of polyphenols and their derived metabolites on immunological and inflammatory responses in the intestinal epithelium still remains to be elucidated. Studies show an association between polyphenols and plant food intake with gut health promotion, reduction of inflammation and disease prevention (GONZÁLEZ-GALLEGO et al., 2010; RUSSELL; DUTHIE, 2011; CARDONA et al., 2013). Some phenolic compounds present in grapes e.g., quercetin, rutin, catechin, caffeic acid, gallic acid, epigallocatechin-3-gallate, procyanidin B2, epicatechin and resveratrol, were characterized for presenting some kind of anti-inflammatory action (MACKENZIE et al., 2009; VAZQUEZ-PRIETO et al., 2012; CHENG et al., 2013; CHUA, 2013; LIN et al., 2014). Recent data suggest beneficial actions of polyphenols are mediated by modulating signal transductions and the expression of coding genes for COX, lipoxygenase, NO syntase, and cytokines, operating especially on NF-κB and protein kinase signaling, inducing their beneficial actions (SANTANGELO et al., 2007; FRONTELA; CANALI; VIRGILI, 2010).

The anti-inflammatory effects are normally connected to other effects and to different diseases. *In vitro* (**Table 3**) and *in vivo* (**Table 4**) studies and clinical trials (**Table 5**) have demonstrated anti-inflammatory effects of grape products and residues normally connected to different diseases and biological effects, like adipogenesis and lipogenesis, vasodilator effect, hypoglycemic activity, antitumoral, antimetastic, cardioprotector effect, decrease of oxidative stress in skeletal muscle, prevention of oxidative damage in the liver, neuroprotection and anticonvulsant effects (LEIFERT; ABEYWARDENA, 2008; JEONG et al., 2012; RODRIGUES et al., 2012; ROOPCHAND et al., 2012; SOARES DE MOURA et al., 2012; SUN et al., 2012; DING et al., 2013).

Nutritional components derived from the diet or synthesized after metabolized are essential environmental factors for the development, maintenance, and regulation of gut immune responses. Besides, a poor or inappropriate nutrient intake may be related to the increased risk of IBDs development (LAMICHHANE; KIYONO; KUNISAWA, 2013; GEREMIA et al., 2014). Further explanations about how nutrients can regulate mucosal immunity by nutrition will increase the development of functional nutritional materials for the intestinal immune system control and thus prevent or treat intestinal diseases by alimentation.

**Potential uses of grape by-products as functional ingredients**

Residues from winemaking and grape juice industries are potential sources of antioxidant bioactive phenolic compounds of great value for food, cosmetic and pharmaceutical industries (GONZÁLEZ-PARAMÁS et al., 2004). Recently, reducing the environmental impact generated by industrial wastes has become a growing concern. Although these residues are not intrinsically harmful, its disposal and concentration on a determined place may release high levels of organic compounds, leading to environmental problems e.g., plant germination inhibition by immobilization of soil nutrients, or the increase of chemical and biological oxygen local requirements (SPIGNO; DE FAVERI, 2007; MOLDES et al., 2008). Grape residues also can be important sources of ethanol, tartrates and malates, citric acid, grape seed oil, hydrocolloids and dietary fiber, making its use remarkable and supporting a sustainable agricultural production (KAMMERER et al., 2004).

**Table 3**. Anti-inflammatory effects of grapes and derivatives (*in vitro* cell culture studies*).

| Intervention | Design | Inflammatory induction | Results | Study |
|---|---|---|---|---|
| Sardinian wine extracts and phenolic standards | • differentiated Caco-2 cells<br>• 1-h PT | Oxysterols: 4h or 24h | • Wine extracts, epicatechin and caffeic acid: ↓ MAPK p38 activation, but not JNK<br>• Cannonau red wine, epicatechin and caffeic acid: ↓ NF-κB activation<br>• epicatechin and caffeic acid: ↓ IL-8, ↓ NOx1 | GUINA et al., 2015 |
| Sardinian wine extracts and phenolic standards | • Caco-2 cells<br>• differentiated (21 days)<br>• 1-h PT | Oxysterols: 4h or 24h | • wine extracts: ↓ IL-8<br>• gallic acid, caffeic acid: more effective ↓ IL-8<br>• catechin, epicatechin and quercetin also ↓ IL-8 production | BIASI et al., 2013 |
| Anthocyanin-rich grape extract | • differentiated Caco-2 cells (100%) or Caco-2 + HT29-B6 cells (upper compartment) - transwell<br>• endothelial cells (lower compartment)<br>• 4h or 20h incubation (upper compartment) | TNF-α: 3h (lower compartment) | • ↓ IL-8, IL-6<br>• ↓ NF-κB mRNA<br>• ↓ leukocyte adhesion, expression of adhesion molecules<br>• only in 20h incubation on Caco-2 (100%) | KUNTZ et al., 2015 |
| Grape pomace enzymatic extract | • N13 microglia cells<br>• 1, 4 and 6-h CT | LPS: 1, 4 and 6h | • ↓ mRNA of TNF-α, TLR-4, IL-1β, iba-1, iNOS (dose-dependent manner) | RODRÍGUEZ-MORGADO et al., 2015 |
| Grape polyphenolic extract (GPoE) and GPoE + propolis (PR) | • mononuclear leukocytes from blood samples<br>• 48-h CT | LPS: 48h | • GPoE: ↓ TNF-α, IL-1β, IL-6 and IL-8<br>• did not affect IL-10, IFN-γ, PGE2, NOx<br>• GPoE + PR: inhibition of all mediators | MOSSALAYI et al., 2014 |
| *V. vinifera* leaves water extract | • Caco-2 cells (48h seeded)<br>• 6-h CT | TNF-α or IL-1β: 6h | • ↓ IL-8 secretion and expression<br>• ↓ NF-κB | SANGIOVANNI et al., 2015 |
| Raisin methanolic extract | • HT29 cells<br>• 24, 48, 72 and 96 h treatments | TNF-α: time not informed | • ↓ IL-8<br>• ↓ COX-2<br>• ↓ NF-κB | KOUNTOURI et al., 2012 |

**Table 3.** (cont.)

| | | | | |
|---|---|---|---|---|
| *Vitis thunbergii* | • primary human chondrocytes (PHCs)<br>• 18-h CT | LPS: 18h | • ↓ PGE2 | TSAI et al., 2014 |
| Grape powder extract | • adipocytes<br>• 1-h PT | TNF-α: 3h | • ↓ IL-8<br>• ↓ COX-2<br>• ↓ NF-κB | CHUANG et al., 2011 |
| Grape polyphenols | • human retinal pigmented epithelium (ARPE-19) cells, differentiated (21 days), transwell system<br>• PT (time not provided) | TNF-α: time not provided | • ↓ IL-1β<br>• ↓ IL-6<br>• ↓ NF-κB<br>• ↓ MCP-1 | HA et al., 2014 |
| Grape seed procyanidins | • human mature adipocytes (SGBS)<br>• macrophage-like (THP-1)<br>• 16-h PT + 8-h CT | LPS and TNF-α: 8h | • ↓ NF-κB in both cell lines<br>• ↓ IL-6, MCP-1 expression | CHACÓN et al., 2009 |
| Grape seed proanthocyanidins | • RAW 264.7 cells (macrophages)<br>• 24-h PT | LPS: 1h-24h | • ↓ TNF-α, IL-1β, COX-2 mRNA<br>• ↑ IL-10 mRNA<br>• ↓ expression of phosphorylated ERK, JNK, IKK, NF-κB p65 | CHU et al., 2016 |
| Grape seed extract | • Caco-2 cells<br>5 days confluent<br>• 4-h PT and 48-h CT | IL-1β: 48h | • ↓ IL-8 | ROMIER-CROUZET et al., 2009 |
| Muscadine grape seed oil | • primary human adipose-derived stem cells (hASCs) – differentiated (12 days)<br>• 24-h PT | LPS: 6 or 24h | • ↓ IL-6 and IL-8<br>• ↓ triglyceride accumulation | ZHAO et al., 2015 |
| Commercial feed additive and grape seed meal + grape marc meal | • Caco-2 cells – differentiated for 6 days<br>• 24-h CT | TNF-α: 24h | • ↓ mRNA of IL-1β, IL-8, MCP-1, CXCL10<br>• ↓ NF-κB | GESSNER et al., 2012 |

*Data from 2009 to 2016. Abbreviations - PT: pretreatment, CT: cotreatment, GPoE: grape polyphenolic extract, PR: propolis, LPS: lipopolysaccharide, TNF-α: tumor necrosis factor α, IL-1β: interleukin 1β, COX-2: cyclooxygenase 2, mRNA: messenger ribonucleic acid, IL-10: interleukin 10, ERK: extracellular-signal-regulated kinases, JNK: c-Jun N-terminal kinase, IKK: kinase complex, NF-κB: factor nuclear kappa B, MAPK: mitogen-activated protein kinase, IL-8: interleukin 8, NOx1: NADPH oxidase-1, IL-6: interleukin 6, TLR-4: toll like receptor 4, iba-1: macrophage/microglia-specific calcium-binding protein, iNOS: inducible nitric oxide synthase, MCP-1: monocyte chemotactic protein-1, IFN-γ: interferon γ, PGE2: prostaglandin 2, CXCL10: C-X-C motif chemokine 10.

**Table 4**. Anti-inflammatory effects of grapes and derivatives (*in vivo* animal studies*).

| Intervention | Design | Results | Study |
|---|---|---|---|
| • Grape seed extract (GSE)<br>• 0 or 0.1% GSE (w/v) in drinking water<br>• 12 weeks treatment | • F IL-10-deficient mice | • ↑ villus length, ↑ mucosal thickness, ↑ goblet cell density, ↑ expression of villin<br>• ↑ nutrient transporters in jejunum (alanyl aminopeptidase and glucose transporter-2)<br>• ↓ TNF-α, ↓ INF-γ, ↓NF-κB<br>• ↓ alkaline phosphatase activity, ↑ alkaline phosphatase protein | BIBI et al., 2016 |
| • HF diet + GSE or vehicle<br>• ND | • F rats during pregnancy and lactation<br>• 30-day-old male offspring were analyzed | GSE diet:<br>• ↑ adiposity<br>• ↑ plasma MCP-1 and gene expression<br>• more favorable adipose tissue than the HFD animals | DEL BAS et al., 2015 |
| • GSE gavaged daily<br>• 10 days | • M Sprague–Dawley rats<br>• ulcerative colitis induced by DSS (2% w/v) drinking water (days 5–10) | • did not prevent damage to the colonic crypts<br>• ↓ ileal villus height and mucosal thickness<br>• ↓ histological severity score | CHEAH et al., 2013 |
| • Grape seed procyanidin extract (GSPE)<br>• controlled oral intake (syringe)<br>• 10 weeks | • F obese Zucker fa/fa rats (genetically obese)<br>• chronic inflammation related to obesity | • ↓ CRP (after 5 weeks)<br>• did not decrease MCP-1, TNF-α, CRP (10 weeks)<br>• ↓ iNOS, IL-6, adiponectin in the adipocytes | PALLARÈS et al., 2013 |
| • LF diet<br>• HF diet<br>• HF diet + GSPE (HFGSPE)<br>• 19 weeks treatment | • M Zucker fa/fa rats<br>• HF diet: low-grade inflammation | HFGSPE diet:<br>• ↓ plasma CRP, but not IL-6<br>• ↓ TNF-α and IL-6 in the mesenteric WAT<br>• ↑ anti-inflammatory adiponectin | TERRA et al., 2009 |

**Table 4.** (cont.)

| | | | |
|---|---|---|---|
| GSPE:<br>• preventive: GSPE for 19 weeks<br>• corrective: GSPE for 10 and 30 days | • F Wistar rats<br>• preventive effects: rats fed a 60% kcal fat diet for 19 weeks<br>• corrective effects: cafeteria diet obesity (13 weeks) | Preventive model:<br>• ↓ body weigh, ↓ plasmatic TNF-α and CRP, ↓ NF-κB in liver<br>• ↑ adiponectin, ↓ TNF-α, ↓ IL-6 and CRP in mesenteric WAT<br>Corrective model: ↓ CRP plasma, no change TNF-α | TERRA et al., 2011 |
| • grape seed procyanidin B2 (GSPB2)<br>• intragastric administration<br>• 10 weeks<br>• | • M db/db (diabetic) mice | • ↓ body weight, ↓food intake, ↓ advanced glycation end-product<br>• no significant effect on glucose levels<br>• ↑ islet sizes, ↑ insulin levels, ↑ HOMA-IR<br>• ↓ protein level<br>• ↓ IL-1β, ↓ NLRP3 | YIN et al., 2015 |
| • grape seed proanthocyanidins extract<br>• administered per-oral (1h before carrageenan injection) | • F adult Balb/c mice<br>• carrageenan-induced lung inflammation (4h and 24h) | • ↓ IL-17A and GITR expressing cells<br>• ↓ cytokine levels in Th1/Th17 cells | AHMAD et al., 2014 |
| • proanthocyanidins from grape seeds<br>• intragastrically (for 7 days) after colitis was induced | • M Wistar rats<br>• TNBS-induced ulcerative colitis | • ↓ expression of TNF-α, p-IKKα/β, p-IκBα and NF-κB in the colon mucosa<br>• ↑ activity of GSH-Px and SOD | WANG et al., 2011 |
| • Muscadine grape polyphenols<br>• gavaged for 7 days | • C57BL/6 mice<br>• ocular inflammation - ocular LPS injection | • ↓ acute ocular inflammation, ↓ leukocyte infiltration<br>• ↓ inflammation-mediated loss of tight junctions and retinal permeability | HA et al., 2014 |
| • grape polyphenols + propolis<br>• 40–50 days | • F Lewis rats<br>• chronic induced arthritis<br>• subcutaneous injection: inactivated *Mycobacterium butyricum* | • ↓ arthritis scores and cachexia (loss of weight, muscle atrophy, fatigue, weakness, and significant loss of appetite)<br>• more significant effects in animals receiving continuous low doses compared to high doses | MOSSALAYI et al., 2014 |
| • grape juice (1 and 2%)<br>• Pretreatment and Post-treatment<br>• 22 weeks | • M Wistar rats<br>• colon carcinogenesis induced by AOM | 2% grape juice pretreatment:<br>• ↓ crypt multiplicity, ↓ carcinogenesis<br>• ↓ NF-kB | CAMPANHOLO et al., 2015 |

**Table 4.** (cont.)

| | | | |
|---|---|---|---|
| • GPow or GPowE<br>• HF diet + 3% GPow or 0.02% GPowE<br>18 weeks | • mice fed HF diets | GPow supplementation:<br>• ↑ glucose tolerance (at 5 weeks)<br>• ↓ markers of inflammation in serum and adipose tissue (at 18 weeks)<br>• no effect on body fat levels | CHUANG et al., 2012 |
| • HF diet<br>• HF + 0.5% grape pomace extract (GPE)<br>• HF + 0.5% GPE + 0.05% OFE<br>• 12 weeks<br>• | • M diet-Induced obese C57BL/6J mice | GPE + OFE:<br>• ↓ body weight gain,<br>• ↓ white adipose tissue weight, adipocyte size<br>• ↓ plasma free fatty acid<br>• ↓ adipokines (leptin, PAI-1, IL-6, and MCP-1)<br>↓ lipogenic and inflammatory genes in white adipose tissue | CHO et al., 2013 |
| • ND group<br>• HF diet group<br>• HFGPE group – HF + GPE<br>• 12 weeks | • M DIO mice | HFGPE:<br>• ↓ plasma CRP<br>GPE did not improve oxidative stress in DIO mice | HOGAN et al., 2010 |
| • Petit Verdot grape pomace:<br>- PVE<br>- EAF | • M Balb/c albino mice<br>• carrageenan-induced paw edema | • both extracts ↓ paw edema, ↓ neutrophil migration<br>• PVE: ↓ TNF-α, ↓ IL1-β in the peritoneal fluid<br>• EAF did not reduce cytokines significantly | DENNY et al., 2014 |
| • methanolic extracts from red and white GP (orally given)<br>• AIN93 M-based diet + 5% red GP<br>• 7 days | • Sprague−Dawley rats<br>• Chronic hepatic inflammation induced by LPS and GalN | • ↓ NF-κB (red GP better than white GP)<br>• ↓ COX-2<br>• ↓ iNOS | NISHIUMI et al., 2012 |

*Data from 2009 to 2016. Abbreviations – F: female, M: male, GP: grape pomace, GPow: grape powder, GPowE: grape powder extract, GSE: grape seed extract, GPE: grape pomace extract, GSPE: grape seed procyanidin extract, OFE: omija fruit extract, PVE: petit verdot extract, EAF: ethil acetate extracted fraction, LF: low-fat, HF: high-fat, ND: normal diet, DIO: diet-induced obese, CRP: C-reactive protein, WAT: white adipose tissue, LPS: lipopolysaccharide, GalN: galactosamine, AOM: azoxymethane, DSS: dextran sulfate sodium, TNF-α: tumor necrosis factor α, IL-1β: interleukin 1β, COX-2: cyclooxygenase 2, NF-κB: factor nuclear kappa B, IFN-γ: interferon γ, GITR: glucocorticoid-induced tumour necrosis factor receptor, HOMA-IR: homeostatic model assessment - insulin resistance, MCP-1: monocyte chemotactic protein 1, p-IKK α/β: kinase subunits, p-IKb α: kinase B subunit, GSH-px: glutathione peroxidase, SOD: superoxide dismutase, iNOS: inducible nitric oxide synthase, IL-6: interleukin 6, PAI-1: plasminogen activator inhibitor 1, Th1/Th17: lymphocytes, IL-17A: interleukin 17A, NLRP3: inflammasome gene.

**Table 5**. Clinical trials* with grape and grape-by products interventions.

| Intervention | Design | Results | Study |
|---|---|---|---|
| • red grape juice (Bordo/Isabel)<br>• organic red grape juice (Bordo)<br>• water (control)<br>• 400 mL | • crossover<br>• 30 days<br>• 24 healthy individuals (19 F, 5 M, 20–55 years) | • ↓ lipid peroxides in serum<br>• ↓ TBARS levels in plasma | TOALDO et al., 2015 |
| • purple grape juice (~170 g twice/day)<br>• clear apple juice (similar in calories but lower in flavonoid) | • crossover<br>• 4 weeks<br>• 24 cancer survivors (10–21 years) | • no changes in vascular function - endothelial function, plasma oxidized-LDL, myeloperoxidase, high sensitivity CRP | BLAIR et al., 2014 |
| • 330 mL ACN-rich beverage:<br>- placebo (8.9 mg/L ACN)<br>- juice (983.7 mg/L ACN)<br>- smoothie (840.9 mg/L ACN) | • crossover<br>• 14 days<br>• 30 healthy F | • ACN-rich beverage: ↑ Plasma SOD, ↑ catalase ↑Trolox equivalent antioxidant capacity ↓ malondialdehyde<br>• Plasma GSH-px and erythrocyte SOD activities: no change<br>• IL-2, -6, -8 and -10, CRP, TNF-α, MCP-1 and soluble cell adhesion molecules: no change<br>• placebo juice: no change | KUNTZ et al., 2014 |
| • 360 mL of Concord grape juice/day<br>• placebo juice | • parallel<br>• 9 weeks<br>• 85 healthy individuals<br>• M (n=26) and F (n=59) - (50-75 years) | • ↑ circulating gd T cells and ↑ serum vitamin C<br>• placebo: ↓ serum antioxidant activity, ↓gd T-cell proliferation, and ↑ DNA strand breaks when challenged with H2O2<br>• grape juice consumption - 61% of the variance in biological functions (at 9 weeks) | ROWE et al., 2011 |
| • grape powder (500 mg of polyphenols/day) (n = 16, 9 M, 7 F, 53.0 ± 9.8 years)<br>• placebo (n = 16, 9 M, 7 F, 52.7 ± 13.7 years) | • crossover<br>• 5 weeks<br>• 32 non-diabetic hemodialysis patients | • GPow: ↑ GSH-Px activity, CRP – no change<br>• Placebo: ↑ CRP | JANIQUES et al., 2014 |

**Table 5.** (cont.)

| | | | |
|---|---|---|---|
| • 46 g grape powder<br>• 46 g placebo<br>• twice/day | • crossover<br>• 9 weeks<br>• 24 obese subjects (20-60 years), BMI 30- 45 kg/m2 | • ↓ plasma LDL<br>• No difference on T-cell cytokines | ZUNINO et al., 2014 |
| • 46 g grape powder/day, equivalent to 252 g fresh grapes<br>• placebo (identical macronutrient composition and caloric value) | • crossover<br>• 4 weeks<br>• M with MetS (n = 24), 11 with high triglycerides and low HDL and 13 with no dyslipidemia | • ↑ plasma adiponectin, IL-10 and mRNA expression of (iNOS) - only in individuals without dyslipidemia | BARONA et al., 2012 |
| • low-GO (grape pomace extract [342.5 mg/day] + omija fruit extract [57.5 mg/day] *n* = 26)<br>• high-GO (GP extract [685 mg/day] + omija fruit extract [115 mg/day], *n* = 26)<br>• control (starch, 4 g/day, *n* = 24) | • parallel<br>• 10 weeks<br>• 76 overweight and obese subjects (30–70 years) | • high-GO: ↓ IL-1*β*, ↓ TNF-*α*<br>• greater effect than low-GO | HAN et al., 2016 |
| • red wine grape pomace flour prepared from red wine grapes (Cabernet Sauvignon variety)<br>• 20 g per day, (10 g of dietary fiber, 822 mg of polyphenols 7258 ORAC units) | • longitudinal<br>• 16 weeks<br>• 38 M, (30–65 years) at leat one component of metabolic syndrome<br>• intervention group (n = 25) vs. control group (n = 13) | • ↓ systolic and diastolic blood pressure<br>• ↓ fasting glucose levels.<br>• ↑ plasma γ-tocopherol and δ-tocopherol<br>• ↓ carbonyl group in plasma protein<br>• no difference - waist circumference, HDL cholesterol, triglycerides, total antioxidant capacity and vitamin C | URQUIAGA et al., 2015 |
| • whole grape extract: gelatin capsule + 350 mg WGE (60–70% proanthocyanidins)<br>• placebo: gelatin capsule + microcrystalline cellulose<br>• 1 capsule/day | • parallel<br>• 6 weeks<br>• 26 pre-hypertensive, overweight, and/or pre-diabetic subjects (18–65 years)<br>• 14 (6 M and 8 F) grape treatment<br>• 12 (6 M and 6 F) placebo | • WGE: ↓ superoxide dismutase, ↓ total cholesterol/HDL ratios , ↑ HDL levels , ↓ 8-isoprostane, ↓ oxidized LDL<br>• placebo: ↑ 8-isoprostane, ↑ oxidized LDL | EVANS et al., 2014 |

**Table 5.** (cont.)

| | | | |
|---|---|---|---|
| • GE (conventional grape extract lacking resveratrol) (n=13)<br>• GE-RES (grape extract containing resveratrol) (n=13)<br>• placebo (n=9) | • parallel<br>• one year<br>• n = 35; 9–13 per group<br>• 35 hypertensive M T2DM (60 ± 11 years) | • ↓ ALP ↓ IL-6<br>• ↓ CCL3, ↓ IL-1β ↓ TNF-α<br>• modifies the expression of a modulatory network of microRNAs<br>• beneficial immunomodulatory effect | TOMÉ-CARNEIRO et al., 2013 |
| • Muscadine grape seed supplementation (1300 mg daily)<br>• placebo | • crossover<br>• 4 weeks<br>• 50 adults with coronary disease or ≥1 cardiac risk | • no flow-mediated dilation improvement,<br>• ↑ baseline artery diameter (mm)<br>• no change in biomarkers of inflammation, lipid peroxidation, and antioxidant capacity | MELLEN et al., 2010 |
| • grape seed extract (600 mg/day)<br>• placebo | • crossover<br>• 4 weeks<br>• 32 T2DM patients (16 M, 16 F, 61.8 ± 6 years) | • ↑ fructosamine, ↑ whole blood GSH , ↓ hsCRP, ↓ total cholesterol<br>• endothelial function, HOMA–IR or TAOS - no significant changes | KAR et al., 2009 |

*Data from 2009 to 2016. Abbreviations – M: male, F: female, T2DM: type 2 diabetes Mellitus, GP: grape pomace, GO: GP+omija fruit, WGE: whole grape extract, GPow : grape powder, GE: grape extract, GE-RES: grape extract+resveratrol, GS: grape seed, GSE: grape seed extract, LDL: low density lipoprotein, HDL: high density lipoprotein, GSH-px: glutathione peroxidase, CRP: C-reactive protein, ACN: acetonitrile, SOD: superoxide dismutase, IL: interleukin, TNF-α: tumor necrosis factor α, IL-1β: interleukin 1β, MCP-1: monocyte chemotactic protein 1, ALP: alkaline phosphatase, CCL3: chemokine (C-C motif) ligand 3, mRNA: messenger ribonucleic acid, iNOS: inducible nitric oxide synthase, DNA: deoxyribonucleic acid, gd T-cell: gamma delta T-cell, , hs-CRP: high sensitivity CRP, HOMA-IR: homeostatic model assessment - insulin resistance, TAOS: total antioxidant status.

There is an increase in the interest of using processed fruit wastes as functional ingredients because they are rich in dietary fiber and bioactive compounds (BALASUNDRAM; SUNDRAM; SAMMAN, 2006). Besides the health benefits, grape pomace has proved antioxidant and antimicrobial actions, interesting for the extension of food products shelf life. White grape pomace was used as an antioxidant dietary fiber additive in restructured fish products, preventing lipid oxidation during ice storage (SÁNCHEZ-ALONSO et al., 2008). The pomace from *Pinot Noir* grape was used to fortify yogurt and salad dressing, enhancing dietary fiber, total phenols and delaying lipid oxidation during refrigeration storage (TSENG; ZHAO, 2013). A polyphenol extract from grape pomace also exhibited antimicrobial, antifungal, and antiradical actions when incorporated to juices and meat hamburgers (SAGDIC et al., 2011). A red grape flour with high antioxidant activity was used as an ingredient to the elaboration of cereal bars, resulting in an integral product with high dietary fiber content (BALESTRO; SANDRI; FONTANA, 2011). Grape seeds and skins were also incorporated to infusions and teas, enhancing antiviral and anticancer properties (MORRÉ; MORRÉ, 2006; BEKHIT et al., 2011), added to wheat flour to produce wheat biscuits (MILDNER-SZKUDLARZ et al., 2013) and also incorporated to pancakes and pasta (ROSALES SOTO; BROWN; ROSS, 2012). In addition of being used as food additives, grape pomace can also be used for technological purposes, e.g. development of a nutritional culture medium for *Lactobacillus rhamnosus* (BUSTOS et al., 2004, 2005), for the isolation and extraction of cellulose from the skin (LU; HSIEH, 2012) and to enhance the color and phenolic profile of red wine before being bottled (PEDROZA et al., 2013).

The rational and sustainable use of grape pomace to the development of new functional ingredients would be of great value, not only to diminish environmental impacts but also to generate high-value products (LU; HSIEH, 2012).

## Conclusions

Grape and other fruits wastes are rich in bioactive compounds, which can be related to antioxidant and anti-inflammatory activities. Inflammation is a complex process in response to harmful stimuli to our body, and when is long and excessive may generate physiological dysfunctions, considered critical factors for the development of human diseases. Bioactive compounds from food can be great

instruments for disease prevention, and the knowledge of the population about the importance of a healthy diet are primordial to reduce statistics of chronic diseases. Further, the use of biotechnological approaches to better explore food and its residues is necessary, and more studies are needed to understand the best ways to extract, modify bioactive compounds and improve the sustainable use of food and food wastes. Also, further research in food technology is necessary to increase the exploitation of these by-products, improving the incorporation of wastes into food products, developing palatable, safe products and acceptable by consumers. Efforts from the government are also necessary to make these residues interesting for the industries, leading to a transformation of our agriculture, making it sustainable and decreasing the environmental impacts caused by waste disposal.

## References

AGUILAR, C. N.; RODRÍGUEZ, R.; GUTIÉRREZ-SÁNCHEZ, G.; AUGUR, C.; FAVELA-TORRES, E.; PRADO-BARRAGAN, L. A.; RAMÍREZ-CORONEL, A.; CONTRERAS-ESQUIVEL, J. C. Microbial tannases: advances and perspectives. **Applied Microbiology and Biotechnology**, v. 76, n. 1, p. 47–59, 2007.

AHMAD, S. F.; ZOHEIR, K. M. A.; ABDEL-HAMIED, H. E.; ATTIA, S. M.; BAKHEET, S. A.; ASHOUR, A. E.; ABD-ALLAH, A. R. A. Grape seed proanthocyanidin extract protects against carrageenan-induced lung inflammation in mice through reduction of pro-inflammatory markers and chemokine expressions. **Inflammation**, v. 37, n. 2, p. 500–511, 2014.

ANASTASIADI, M.; PRATSINIS, H.; KLETSAS, D.; SKALTSOUNIS, A.-L.; HAROUTOUNIAN, S. A. Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. **LWT - Food Science and Technology**, v. 48, n. 2, p. 316–322, 2012.

ARNOUS, A.; MEYER, A. S. Comparison of methods for compositional characterization of grape (Vitis vinifera L.) and apple (Malus domestica) skins. **Food and Bioproducts Processing**, v. 86, n. 2, p. 79–86, 2008.

ARNOUS, A.; MEYER, A. S. Discriminated release of phenolic substances from red wine grape skins (Vitis vinifera L.) by multicomponent enzymes treatment. **Biochemical Engineering Journal**, v. 49, n. 1, p. 68–77, 2010.

ARVANITOYANNIS, I. S.; LADAS, D.; MAVROMATIS, A. Potential uses and applications of treated wine waste: a review. **International Journal of Food Science and Technology**, v. 41, n. 5, p. 475–487, 2006.

BALASUNDRAM, N.; SUNDRAM, K.; SAMMAN, S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. **Food Chemistry**, v. 99, n. 1, p. 191–203, 2006.

BALESTRO, E.; SANDRI, I.; FONTANA, R. Utilização de bagaço de uva com atividade antioxidante na formulação de barra de cereais. **Revista Brasileira de Produtos Agroindustriais**, v. 13, n. 2, p. 203–209, 2011.

BAO, H.; REN, H.; ENDO, H.; TAKAGI, Y.; HAYASHI, T. Effects of heating and the addition of seasonings on the anti-mutagenic and anti-oxidative activities of polyphenols. **Food Chemistry**, v. 86, n. 4, p. 517–524, 2004.

BARONA, J.; BLESSO, C. N.; ANDERSEN, C. J.; PARK, Y.; LEE, J.; FERNANDEZ, M. L. Grape Consumption Increases Anti-Inflammatory Markers and Upregulates Peripheral Nitric Oxide Synthase in the Absence of Dyslipidemias in Men with Metabolic Syndrome. **Nutrients**, v. 4, p. 1945-1957, 2012.

BATTESTIN, V.; MACEDO, G. A.; DE FREITAS, V. A. P. Hydrolysis of epigallocatechin gallate using a tannase from Paecilomyces variotii. **Food Chemistry**, v. 108, n. 1, p. 228–233, 2008.

BEKHIT, A. E.-D. A.; CHENG, V. J.; MCCONNELL, M.; ZHAO, J. H.; SEDCOLE, R.; HARRISON, R. Antioxidant activities, sensory and anti-influenza activity of grape skin tea infusion. **Food Chemistry**, v. 129, n. 3, p. 837–845, 2011.

BIASI, F.; GUINA, T.; MAINA, M.; CABBOI, B.; DEIANA, M.; TUBEROSO, C. I.; CALFAPIETRA, S.; CHIARPOTTO, E.; SOTTERO, B.; GAMBA, P.; GARGIULO, S.; BRUNETTO, V.; TESTA, G.; DESSI, M. A.; POLI, G.; LEONARDUZZI, G. Phenolic compounds present in Sardinian wine extracts protect against the production of inflammatory cytokines induced by oxysterols in CaCo-2 human enterocyte-like cells. **Biochemical Pharmacology**, v. 86, n. 1, p. 138–145, 2013.

BIBI, S.; KANG, Y.; YANG, G.; ZHU, M. J. Grape seed extract improves small intestinal health through suppressing inflammation and regulating alkaline phosphatase in IL-10-deficient mice. **Journal of Functional Foods**, v. 20, p. 245–252, 2016.

BLAIR, C. K.; KELLY, A. S.; STEINBERGER, J.; ELBERLY, L. E.; NAPURSKI, C.; ROBIEN, K.; NEGLIA, J. P.; MULROONEY, D. A.; ROSS, J. A. Feasibility and Preliminary Efficacy of the Effects of Flavanoid-Rich Purple Grape Juice on the Vascular Health of Childhood Cancer Survivors: A Randomized, Controlled Crossover Trial. **Pediatric Blood & Cancer**, v. 61, p. 2290–2296, 2014.

BORAZAN, A. A.; BOZAN, B. The influence of pectolytic enzyme addition and prefermentative mash heating during the winemaking process on the phenolic composition of Okuzgozu red wine. **Food Chemistry**, v. 138, n. 1, p. 389–95, 2013.

BUSTOS, G.; MOLDES, A. B.; CRUZ, J. M.; DOMINGUEZ, J. M. Evaluation of vinification lees as a general medium for Lactobacillus strains. **Journal of Agriculture and Food Chemistry**, v. 52, n. 16, p. 5233–5239, 2004.

BUSTOS, G.; MOLDES, A. B.; CRUZ, J. M.; DOMINGUEZ, J. M. Production of lactic acid from vine-trimming wastes and viticulture lees using a simultaneous saccharification fermentation method. **Journal of the Science of Food and**

**Agriculture**, v. 85, n. 3, p. 466–472, 2005.

CAMPANHOLO, V. M. de L. P.; SILVA, R. M.; SILVA, T. D.; NETO, R. A.; PAIOTTI, A. P. R.; RIBEIRO, D. A.; FORONES, N. M. Oral Concentrated Grape Juice Suppresses Expression of NF-kappa B , TNF-α and iNOS in Experimentally Induced Colorectal Carcinogenesis in Wistar Rats. **Asian Pacific Journal of Cancer Prevention**, v. 16, n. 3, p. 947–952, 2015.

CARDONA, F.; ANDRÉS-LACUEVA, C.; TULIPANI, S.; TINAHONES, F. J.; QUEIPO-ORTUÑO, M. I. Benefits of polyphenols on gut microbiota and implications in human health. **The Journal of Nutritional Biochemistry**, v. 24, n. 8, p. 1415–22, 2013.

CHACÓN, M. R.; CEPERUELO-MALLAFRÉ, V.; MAYMÓ-MASIP, E.; MATEO-SANZ, J. M.; AROLA, L.; GUITIÉRREZ, C.; FERNANDEZ-REAL, J. M.; ARDÈVOL, A.; SIMÓN, I.; VENDRELL, J. Grape-seed procyanidins modulate inflammation on human differentiated adipocytes in vitro. **Cytokine**, v. 47, n. 2, p. 137–42, 2009.

CHAMORRO, S.; VIVEROS, A.; ALVAREZ, I.; VEGA, E.; BRENES, A. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. **Food Chemistry**, v. 133, n. 2, p. 308–314, 2012.

CHEAH, K. Y.; BASTIAN, S. E. P.; ACOTT, T. M. V; ABIMOSLEH, S. M.; LYMN, K. A.; HOWARTH, G. S. Grape seed extract reduces the severity of selected disease markers in the proximal colon of dextran sulphate sodium-induced colitis in rats. **Digestive Diseases and Sciences**, v. 58, n. 4, p. 970–7, 2013.

CHENG, Y.-T.; WU, C.-H.; HO, C.-Y.; YEN, G.-C. Catechin protects against ketoprofen-induced oxidative damage of the gastric mucosa by up-regulating Nrf2 in vitro and in vivo. **The Journal of Nutritional Biochemistry**, v. 24, n. 2, p. 475–83, 2013.

CHO, S.-J.; JUNG, U. J.; PARK, H.-J.; KIM, H.-J.; PARK, Y. B.; KIM, S. R.; CHOI, M.-S. Combined ethanol extract of grape pomace and omija fruit ameliorates adipogenesis, hepatic steatosis, and inflammation in diet-induced obese mice. **Evidence-based Complementary and Alternative Medicine**, v. 2013, n. ID 212139, p. 1–11, 2013.

CHU, H.; TANG, Q.; HUANG, H.; HAO, W.; WEI, X. Grape-seed proanthocyanidins inhibit the lipopolysaccharide-induced inflammatory mediator expression in RAW264.7 macrophages by suppressing MAPK and NF-κb signal pathways. **Environmental Toxicology and Pharmacology**, v. 41, p. 159–166, 2016.

CHUA, L. S. A review on plant-based rutin extraction methods and its pharmacological activities. **Journal of Ethnopharmacology**, v. 150, n. 3, p. 805–17, 2013.

CHUANG, C.; SHEN, W.; CHEN, H.; XIE, G.; JIA, W.; CHUNG, S.; MCINTOSH, M. K. Differential effects of grape powder and Its extract on glucose tolerance and chronic inflammation in high-fat-fed obese mice. **Journal of Agricultural and Food Chemistry**, v. 60, p. 12458–12468, 2012.

CHUANG, C.-C.; BUMRUNGPERT, A.; KENNEDY, A.; OVERMAN, A.; WEST, T.; DAWSON, B.; MCINTOSH, M. K. Grape powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin resistance in primary cultures of human adipocytes. **The Journal of Nutritional Biochemistry**, v. 22, n. 1, p. 89–94, 2011.

CONTRERAS-DOMÍNGUEZ, M.; GUYOT, S.; MARNET, N.; LE PETIT, J.; PERRAUD-GAIME, I.; ROUSSOS, S.; AUGUR, C. Degradation of procyanidins by Aspergillus fumigatus: identification of a novel aromatic ring cleavage product. **Biochimie**, v. 88, n. 12, p. 1899–908, 2006.

DE CAMPOS, L. M. A. S.; LEIMANN, F. V.; PEDROSA, R. C.; FERREIRA, S. R. S. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (Vitis vinifera). **Bioresource Technology**, v. 99, n. 17, p. 8413–8420, 2008.

DEL BAS, J. M.; CRESCENTI, A.; AROLA-ARNAL, A.; OMS-OLIU, G.; AROLA, L.; CAIMARI, A. Grape seed procyanidin supplementation to rats fed a high-fat diet during pregnancy and lactation increases the body fat content and modulates the inflammatory response and the adipose tissue metabolism of the male offspring in youth. **International Journal of Obesity**, v. 39, p. 7–15, 2015.

DENG, Q.; PENNER, M. H.; ZHAO, Y. Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. **Food Research International**, v. 44, n. 9, p. 2712–2720, 2011.

DENNY, C.; LAZARINI, J. G.; FRANCHIN, M.; MELO, P. S.; PEREIRA, G. E.; MASSARIOLI, A. P.; MORENO, I. A. M.; PASCHOAL, J. A. R.; ALENCAR, S. M.; ROSALEN, P. L. Bioprospection of Petit Verdot grape pomace as a source of anti-inflammatory compounds. **Journal of Functional Foods**, v. 8, n. 1, p. 292–300, 2014.

DING, Y.; DAI, X.; JIANG, Y.; ZHANG, Z.; BAO, L.; LI, Y.; ZHANG, F.; MA, X.; CAI, X.; JING, L.; GU, J.; LI, Y. Grape seed proanthocyanidin extracts alleviate oxidative stress and ER stress in skeletal muscle of low-dose streptozotocin- and high-

carbohydrate/high-fat diet-induced diabetic rats. **Molecular Nutrition & Food Research**, v. 57, n. 2, p. 365–9, 2013.

DONOVAN, J. L.; LEE, A.; MANACH, C.; RIOS, L.; MORAND, C.; SCALBERT, A.; RÉMÉSY, C. Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. **British Journal of Nutrition**, v. 87, n. 04, p. 299, 2002.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Resíduo da uva processada para vinho é transformado em produto forrageiro**, 2007. Available at: http://www.renorbio.org.br/portal/noticias/residuo-da-uva-processada-para-vinho-e-transformado-em-produto-forrageiro.htm Acceced on: 10.01.16

EVANS, M.; WILSON, D.; GUTHRIE, N. A randomized, double-blind, placebo-controlled, pilot study to evaluate the effect of whole grape extract on antioxidant status and lipid profile. **Journal of Functional Foods**, v. 7, p. 680-691, 2014.

FAOSTAT. Statistics Division, Food and Agriculture Organization of the United Nations. Production, 2015. Available at: <http://faostat3.fao.org/home/E> Accessed on: 05.01.16.

FERREIRA, L. R.; MACEDO, J. A.; RIBEIRO, M. L.; MACEDO, G. A. Improving the chemopreventive potential of orange juice by enzymatic biotransformation. **Food Research International**, v. 51, n. 2, p. 526–535, 2013.

FRONTELA, C.; CANALI, R.; VIRGILI, F. Use of dietary phenols to modulate inflammatory bowel response. **Gastroenterología y Hepatología**, v. 33, n. 4, p. 307–12, 2010.

GARCÍA-CONESA, M.-T.; ØSTERGAARD, P.; KAUPPINEN, S.; WILLIAMSON, G. Hydrolysis of diethyl diferulates by a tannase from Aspergillus oryzae. **Carbohydrate Polymers**, v. 44, p. 319–324, 2001.

GEORGETTI, S. R.; VICENTINI, F. T. M. C.; YOKOYAMA, C. Y.; BORIN, M. F.; SPADARO, A. C. C.; FONSECA, M. J. V. Enhanced in vitro and in vivo antioxidant activity and mobilization of free phenolic compounds of soybean flour fermented with different beta-glucosidase-producing fungi. **Journal of Applied Microbiology**, v. 106, n. 2, p. 459–66, 2009.

GEREMIA, A.; BIANCHERI, P.; ALLAN, P.; CORAZZA, G. R.; DI SABATINO, A. Innate and adaptive immunity in inflammatory bowel disease. **Autoimmunity Reviews**, v. 13, n. 1, p. 3–10, 2014.

GESSNER, D. K.; RINGSEIS, R.; SIEBERS, M.; KELLER, J.; KLOSTER, J.; WEN,

G.; EDER, K. Inhibition of the pro-inflammatory NF-??B pathway by a grape seed and grape marc meal extract in intestinal epithelial cells. **Journal of Animal Physiology and Animal Nutrition**, v. 96, n. 6, p. 1074–1083, 2012.

GONTHIER, M.-P.; DONOVAN, J. L.; TEXIER, O.; FELGINES, C.; REMESY, C.; SCALBERT, A. Metabolism of dietary procyanidins in rats. **Free Radical Biology and Medicine**, v. 35, n. 8, p. 837–844, 2003.

GONZÁLEZ-GALLEGO, J.; GARCÍA-MEDIAVILLA, M. V.; SÁNCHEZ-CAMPOS, S.; TUÑÓN, M. J. Fruit polyphenols, immunity and inflammation. **The British Journal of Nutrition**, v. 104 Suppl , p. S15–S27, 2010.

GONZÁLEZ-PARAMÁS, A. M.; ESTEBAN-RUANO, S.; SANTOS-BUELGA, C.; DE PASCUAL-TERESA, S.; RIVAS-GONZALO, J. C. Flavanol content and antioxidant activity in winery byproducts. **Journal of Agricultural and Food Chemistry**, v. 52, n. 2, p. 234–8, 28 2004.

GUINA, T.; DEIANA, M.; CALFAPIETRA, S.; CABBOI, B.; MAINA, M.; TUBEROSO, C. I.; LEONARDUZZI, G.; GAMBA, P.; GARGIULO, S.; TESTA, G.; POLI, G.; BIASI, F. The role of p38 MAPK in the induction of intestinal inflammation by dietary oxysterols: modulation by wine phenolics. **Food & Function**, v. 6, n. 4, p. 1218–28, 2015.

HA, J.-H.; SHIL, P. K.; ZHU, P.; GU, L.; LI, Q.; CHUNG, S. Ocular Inflammation and Endoplasmic Reticulum Stress Are Attenuated by Supplementation with Grape Polyphenols in Human Retinal Pigmented Epithelium Cells and in C57BL/6 Mice. **The Journal of Nutrition**, v. 144, p. 799–806, 2014.

HAN, H.; JUNG, U.; KIM, H.; CHO, S.; KIM, A.; HAN, Y.; CHOI, M. Combined Supplementation with Grape Pomace and Omija Fruit Ethanol Extracts Dose-Dependently Improves Body Composition, Plasma Lipid Profiles, Inflammatory Status, and Antioxidant Capacity in Overweight and Obese Subjects. **Journal of Medicinal Food**, v. 19, n. 2, p. 170–180, 2016.

HOGAN, S.; CANNING, C.; SUN, S.; SUN, X.; ZHOU, K. Effects of Grape Pomace Antioxidant Extract on Oxidative Stress and Inflammation in Diet Induced Obese Mice. **Journal of Agricultural and Food Chemistry**, v. 58, p. 11250–11256, 2010.

HOLST, B.; WILLIAMSON, G. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. **Current Opinion in Biotechnology**, v. 19, n. 2, p. 73–82, 2008.

HORNSEY, I. **The Chemistry and Biology of Winemaking.** 1st ed. The Royal

Society of Chemistry: Cambridge, UK, 2007.

JANIQUES, A.; LEAL, V.; STOCKLER-PINTO, M.; MAFRA, D. Effects of grape powder supplementation on inflammatory and antioxidant markers in hemodialysis patients: A randomized double-blind study. **Jornal Brasileiro de Nefrologia**, v. 36, n. 4, p. 496–501, 2014.

JEONG, Y. S.; HONG, J. H.; CHO, K. H.; JUNG, H. K. Grape skin extract reduces adipogenesis- and lipogenesis-related gene expression in 3T3-L1 adipocytes through the peroxisome proliferator-activated receptor-γ signaling pathway. **Nutrition Research**, v. 32, n. 7, p. 514–21, 2012.

KAMMERER, D.; CLAUS, A.; CARLE, R.; SCHIEBER, A. Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. **Journal of Agricultural and Food Chemistry**, v. 52, n. 14, p. 4360–7, 2004.

KAMMERER, D.; CLAUS, A.; SCHIEBER, A.; CARLE, R. A Novel Process for the Recovery of Polyphenols from Grape ( Vitis vinifera L .) Pomace. **Food Chemistry and Toxicology**, v. 70, n. 2, p. 157–163, 2005.

KAR, P.; LAIGHT, D.; ROOPRAI, H. K.; SHAW, K. M.; CUMMINGS, M. Effects of grape seed extract in Type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. **Diabetic Medicine**, v. 26, p. 526–531, 2009.

KAULMANN, A.; BOHN, T. Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention. **Nutrition Research**, 2014.

KOSSEVA, M. R. **Management and Processing of Food Wastes**. In: Compreensive Biotechnology, Second Ed. Academic Press, p. 557-593, 2011.

KOUNTOURI, A. M.; GIOXARI, A.; KARVELA, E.; KALIORA, A. C.; KARVELAS, M.; KARATHANOS, V. T. Chemopreventive properties of raisins originating from Greece in colon cancer cells. **Food & Function**, v. 4, n. 3, p. 366–372, 2012.

KUNTZ, S.; ASSEBURG, H.; DOLD, S.; RÖMPP, A.; FRÖHLING, B.; KUNZ, C.; RUDLOFF, S. Inhibition of low-grade inflammation by anthocyanins from grape extract in an in vitro epithelial-endothelial co-culture model. **Food & Function**, v. 6, n. 4, p. 1136–49, 2015.

KUNTZ, S.; KUNZ, C.; HERRMANN, J.; BORSCH, C. H.; ABEL, G.; FROHLING, B.; DIETRICH, H.; RUDLOFF, S. Anthocyanins from fruit juices improve the antioxidant

status of healthy young female volunteers without affecting anti-inflammatory parameters: results from the randomised, double-blind, placebo-controlled, cross-over ANTHONIA (ANTHOcyanins in Nutrition Investigation Alliance) study. **British Journal of Nutrition,** v. 112, p. 925-936, 2014.

LAM, G.; APOSTOPOULOS, V.; ZULLI, A.; NURGALI, K. NADPH Oxidases and Inflammatory Bowel Disease. **Current Medicinal Chemistry**, v. 22, n. 17, p. 2100–2109, 2015.

LAMICHHANE, A.; KIYONO, H.; KUNISAWA, J. Nutritional components regulate the gut immune system and its association with intestinal immune disease development. **Journal of Gastroenterology and Hepatology**, v. 28 Suppl 4, p. 18–24, 2013.

LEE, C.-J.; CHEN, L.-G.; LIANG, W.-L.; WANG, C.-C. Anti-inflammatory effects of Punica granatum Linne in vitro and in vivo. **Food Chemistry**, v. 118, n. 2, p. 315–322, 2010.

LEGLER, D. F.; BRUCKNER, M.; UETZ-VON ALLMEN, E.; KRAUSE, P. Prostaglandin E2 at new glance: Novel insights in functional diversity offer therapeutic chances. **The International Journal of Biochemistry & Cell Biology**, v. 42, n. 2, p. 198–201, 2010.

LEIFERT, W. R.; ABEYWARDENA, M. Y. Cardioprotective actions of grape polyphenols. **Nutrition Research**, v. 28, n. 11, p. 729–37, 2008.

LEKHA, P. K.; LONSANE, B. K. Production and application of tannin acyl hydrolase: state of the art. **Advanced Applied Microbiology**, v. 44, p. 215–260, 1997.

LIM, J. W.; HWANG, H. J.; SHIN, C. S. Polyphenol compounds and anti-inflammatory activities of Korean black raspberry ( Rubus coreanus Miquel) wines produced from juice supplemented with pulp and seed. **Journal of Agricultural and Food Chemistry**, v. 60, n. 20, p. 5121–7, 2012.

LIMA, M. dos S.; DA CONCEIÇÃO PRUDÊNCIO DUTRA, M.; TOALDO, I. M.; CORRÊA, L. C.; PEREIRA, G. E.; DE OLIVEIRA, D.; BORDIGNON-LUIZ, M. T.; NINOW, J. L. Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration. **Food Chemistry**, v. 188, p. 384–392, 2015.

LIN, C.-C.; LI, C.-W.; SHIH, Y.-T.; CHUANG, L.-T. Antioxidant and Anti-Inflammatory Properties of Lower-Polymerized Polyphenols in Oolong Tea. **International Journal of Food Properties**, v. 17, n. 4, p. 752–764, 2014.

LU, P.; HSIEH, Y.-L. Cellulose isolation and core–shell nanostructures of cellulose

nanocrystals from chardonnay grape skins. **Carbohydrate Polymers**, v. 87, n. 4, p. 2546–2553, 2012.

MACEDO, J. A.; BATTESTIN, V.; RIBEIRO, M. L.; MACEDO, G. A. Increasing the antioxidant power of tea extracts by biotransformation of polyphenols. **Food Chemistry**, v. 126, n. 2, p. 491–497, 2011.

MACKENZIE, G. G.; DELFINO, J. M.; KEEN, C. L.; FRAGA, C. G.; OTEIZA, P. I. Dimeric procyanidins are inhibitors of NF-kappaB-DNA binding. **Biochemical Pharmacology**, v. 78, n. 9, p. 1252–62, 2009.

MAIER, T.; GÖPPERT, A.; KAMMERER, D. R.; SCHIEBER, A.; CARLE, R. Optimization of a process for enzyme-assisted pigment extraction from grape (Vitis vinifera L.) pomace. **European Food Research and Technology**, v. 227, n. 1, p. 267–275, 2008.

MAIER, T.; SCHIEBER, A.; KAMMERER, D. R.; CARLE, R. Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants. **Food Chemistry**, v. 112, n. 3, p. 551–559, 2009.

MANACH, C.; SCALBERT, A.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Polyphenols: food sources and bioavailability. **The American Journal of Clinical Nutrition**, v. 79, n. 5, p. 727–47, 2004.

MATIAS, A.; NUNES, S. L.; POEJO, J.; MECHA, E.; SERRA, A. T.; MADEIRA, P. J. A.; BRONZE, M. R.; DUARTE, C. M. M. Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate recovered from Opuntia ficus-indica juice. **Food & Function**, v. 5, n. 12, p. 3269–3280, 2014.

MCCORMACK, G.; MORIARTY, D.; O'DONOGHUE, D. P.; MCCORMICK, P. A.; SHEAHAN, K.; BAIRD, A. W. Tissue cytokine and chemokine expression in inflammatory bowel disease. **Inflammation Research**, v. 50, n. 10, p. 491–495, 2001.

MELLEN, P. B.; DANIEL, K. R.; BROSNIHAN, B.; HANSEN, K. J.; HERRINGTON, D. M. Effect of Muscadine grape seed supplementation on vascular function in subjects with or at risk of cardiovascular disease: a randomized crossover trial. **The Journal of the American College of Nutrition**, v. 29, n. 5, p. 469-475, 2010.

MELLO, L. M. R. Vitivinicultura brasileira: Panorama 2011. **Embrapa: Comunicado Técnico 115**, p. 1–4, 2012.

MELLO, L. M. R. Vitivinicultura Brasileira: Panorama 2013. **Embrapa: Comunicado Técnico 156**, v. Julho, p. 1–6, 2014.

MESKIN, M.; BIDLACK, W.; DAVIES, A.; LEWIS, D.; RANDOLPH, R. **Phytochemicals: mechanisms of action.** CRC Press, 2004.

MEYER, A. S.; JEPSEN, S. M.; SØRENSEN, N. S. Enzymatic Release of Antioxidants for Human Low-Density Lipoprotein from Grape Pomace. **Journal of Agricultural and Food Chemistry**, v. 46, n. 7, p. 2439–2446, 1998.

MILDNER-SZKUDLARZ, S.; BAJERSKA, J.; ZAWIRSKA-WOJTASIAK, R.; GÓRECKA, D. White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. **Journal of the Science of Food and Agriculture**, v. 93, n. 2, p. 389–95, 2013.

MOLDES, A. B.; VÁZQUEZ, M.; DOMÍNGUEZ, J. M.; DÍAZ-FIERROS, F.; BARRAL, M. T. Negative effect of discharging vinification lees on soils. **Bioresource Technology**, v. 99, n. 13, p. 5991–6, 2008.

MONAGAS, M.; BARTOLOMÉ, B.; GÓMEZ-CORDOVÉS, C. Updated knowledge about the presence of phenolic compounds in wine. **Critical Reviews in Food Science and Nutrition**, v. 45, n. 2, p. 85–118, 2005.

MONTEALEGRE, R.; PECES, R.; VOZMEDIANO, J. L.; GASCUEÑA, J.; ROMERO, E. Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. **Journal of Food Composition and Analysis**, v. 19, n. 6-7, p. 687–693, 2006.

MORRÉ, D. M.; MORRÉ, D. J. Anticancer activity of grape and grape skin extracts alone and combined with green tea infusions. **Cancer Letters**, v. 238, n. 2, p. 202–9, 2006.

MOSSALAYI, M. D.; RAMBERT, J.; RENOUF, E.; MICOULEAU, M.; MÉRILLON, J. M. Grape polyphenols and propolis mixture inhibits inflammatory mediator release from human leukocytes and reduces clinical scores in experimental arthritis. **Phytomedicine**, v. 21, n. 3, p. 290–297, 2014.

NISHIUMI, S.; MUKAI, R.; ICHIYANAGI, T.; ASHIDA, H. Suppression of lipopolysaccharide and galactosamine-induced hepatic inflammation by red grape pomace. **Journal of Agricultural and Food Chemistry**, v. 60, n. 36, p. 9315–20, 2012.

OBOH, G.; ADEMOSUN, A. O. Shaddock peels (Citrus maxima) phenolic extracts inhibit α-amylase, α-glucosidase and angiotensin I-converting enzyme activities: a nutraceutical approach to diabetes management. **Diabetes & Metabolic Syndrome**, v. 5, n. 3, p. 148–52, 2012.

OBREQUE-SLIER, E.; PEÑA-NEIRA, A.; LÓPEZ-SOLÍS, R.; ZAMORA-MARÍN, F.; RICARDO-DA SILVA, J. M.; LAUREANO, O. Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (Vitis vinifera L.) during ripening. **Journal of Agricultural and Food Chemistry**, v. 58, n. 6, p. 3591–9, 2010.

PALLARÈS, V.; CEDÓ, L.; CASTELL-AUVÍ, A.; PINENT, M.; ARDÉVOL, A.; AROLA, L.; BLAY, M. Effects of grape seed procyanidin extract over low-grade chronic inflammation of obese Zucker fa/fa rats. **Food Research International**, v. 53, n. 1, p. 319–324, 2013.

PEDROZA, M. A.; CARMONA, M.; ALONSO, G. L.; SALINAS, M. R.; ZALACAIN, A. Pre-bottling use of dehydrated waste grape skins to improve colour, phenolic and aroma composition of red wines. **Food Chemistry**, v. 136, n. 1, p. 224–36, 2013.

PINELO, M.; ARNOUS, A.; MEYER, A. S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. **Trends in Food Science & Technology**, v. 17, n. 11, p. 579–590, 2006.

RICCIOTTI, E.; FITZGERALD, G. A. Prostaglandins and Inflammation. **Arteriosclerosis, Thrombosis and Vascular Biology**, v. 31, n. 5, p. 986–1000, 2011.

RÍO SEGADE, S.; PACE, C.; TORCHIO, F.; GIACOSA, S.; GERBI, V.; ROLLE, L. Impact of maceration enzymes on skin softening and relationship with anthocyanin extraction in wine grapes with different anthocyanin profiles. **Food Research International**, v. 71, p. 50–57, 2015.

ROCKENBACH, I. I.; RODRIGUES, E.; GONZAGA, L. V.; CALIARI, V.; GENOVESE, M. I.; GONÇALVES, A. E. D. S. S.; FETT, R. Phenolic compounds content and antioxidant activity in pomace from selected red grapes (Vitis vinifera L. and Vitis labrusca L.) widely produced in Brazil. **Food Chemistry**, v. 127, n. 1, p. 174–179, 2011.

RODRIGUES, A. D.; SCHEFFEL, T. B.; SCOLA, G.; SANTOS, M. T. Dos; FANK, B.; DE FREITAS, S. C. V.; DANI, C.; VANDERLINDE, R.; HENRIQUES, J. A. P.; COITINHO, A. S.; SALVADOR, M. Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole. **Neurochemistry international**, v. 60, n. 8, p. 799–805, 2012.

RODRÍGUEZ-MORGADO, B.; CANDIRACCI, M.; SANTA-MARÍA, C.; REVILLA, E.; GORDILLO, B.; PARRADO, J.; CASTAÑO, A. Obtaining from Grape Pomace an

Enzymatic Extract with Anti-inflammatory Properties. **Plant Foods for Human Nutrition**, v. 70, n. 1, p. 42–49, 2015.

RODRIGUEZ-RODRIGUEZ, R.; JUSTO, M. L.; CLARO, C. M.; VILA, E.; PARRADO, J.; HERRERA, M. D.; ALVAREZ DE SOTOMAYOR, M. Endothelium-dependent vasodilator and antioxidant properties of a novel enzymatic extract of grape pomace from wine industrial waste. **Food Chemistry**, v. 135, n. 3, p. 1044–51, 2012.

ROMIER-CROUZET, B.; VAN DE WALLE, J.; DURING, A.; JOLY, A.; ROUSSEAU, C.; HENRY, O.; LARONDELLE, Y.; SCHNEIDER, Y.-J. Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. **Food and Chemical Toxicology**, v. 47, n. 6, p. 1221–30, 2009.

ROMIER, B.; SCHNEIDER, Y. J.; LARONDELLE, Y.; DURING, A. Dietary polyphenols can modulate the intestinal inflammatory response. **Nutrition Reviews**, v. 67, n. 7, p. 363–378, 2009.

ROOPCHAND, D. E.; KUHN, P.; POULEV, A.; OREN, A.; LILA, M. A.; FRIDLENDER, B.; RASKIN, I. Biochemical Analysis and in Vivo Hypoglycemic Activity of a Grape Polyphenol−Soybean Flour Complex. **Journal of Agricultural and Food Chemistry**, v. 60, p. 8860–8865, 2012.

ROSALES SOTO, M. U.; BROWN, K.; ROSS, C. F. Antioxidant activity and consumer acceptance of grape seed flour-containing food products. **International Journal of Food Science & Technology**, v. 47, n. 3, p. 592–602, 2012.

ROWE, C. A.; NANTZ, M. P.; NIEVES, C.; WEST, R. L.; PERCIVAL, S. S. Regular consumption of Concord grape juice benefits human immunity. **Journal of Medicinal Food,** v. 14, p. 69-78, 2011.

RUSSELL, W.; DUTHIE, G. Plant secondary metabolites and gut health: the case for phenolic acids. **The Proceedings of the Nutrition Society**, v. 70, n. 3, p. 389–396, 2011.

SAGDIC, O.; OZTURK, I.; OZKAN, G.; YETIM, H.; EKICI, L.; YILMAZ, M. T. RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices. **Food Chemistry**, v. 126, n. 4, p. 1749–1758, 2011.

SÁNCHEZ-ALONSO, I.; JIMÉNEZ-ESCRIG, A.; SAURA-CALIXTO, F.; BORDERÍAS, A. J. Antioxidant protection of white grape pomace on restructured fish products during frozen storage. **LWT - Food Science and Technology**, v. 41, n. 1, p. 42–50, 2008.

SANGIOVANNI, E.; DI LORENZO, C.; COLOMBO, E.; COLOMBO, F.; FUMAGALLI, M.; FRIGERIO, G.; RESTANI, P.; DELL'AGLI, M. The effect of in vitro gastrointestinal digestion on the anti-inflammatory activity of Vitis vinifera L. leaves. **Food & Function**, v. 6, n. 8, p. 2453–63, 2015.

SANTANGELO, C.; VARÌ, R.; SCAZZOCCHIO, B.; DI BENEDETTO, R.; FILESI, C.; MASELLA, R. Polyphenols, intracellular signalling and inflammation. **Annali dell'Istituto Superiore di Sanità**, v. 43, n. 4, p. 394–405, 2007.

SCALBERT, A.; MANACH, C.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Dietary polyphenols and the prevention of diseases. **Critical Reviews in Food Science and Nutrition**, v. 45, n. 4, p. 287–306, 2005.

SCALBERT, A.; WILLIAMSON, G. Dietary Intake and Bioavailability of Polyphenols. **The Journal of Nutrition**, n. 2074S, p. 2073–2085, 2000.

SHOJI, T.; MASUMOTO, S.; MORIICHI, N.; AKIYAMA, H.; KANDA, T.; OHTAKE, Y.; GODA, Y. Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the porter method and high-performance liquid chromatography/tandem mass spectrometry. **Journal of Agricultural and Food Chemistry**, v. 54, n. 3, p. 884–92, 2006.

SOARES DE MOURA, R.; DA COSTA, G. F.; MOREIRA, A. S. B.; QUEIROZ, E. F.; MOREIRA, D. D. C.; GARCIA-SOUZA, E. P.; RESENDE, A. C.; MOURA, A. S.; TEIXEIRA, M. T. Vitis vinifera L. grape skin extract activates the insulin-signalling cascade and reduces hyperglycaemia in alloxan-induced diabetic mice. **The Journal of Pharmacy and Pharmacology**, v. 64, n. 2, p. 268–76, 2012.

SONIER, B.; PATRICK, C.; AJJIKUTTIRA, P.; SCOTT, F. W. Intestinal immune regulation as a potential diet-modifiable feature of gut inflammation and autoimmunity. **International Reviews of Immunology**, v. 28, n. 6, p. 414–45, 2009.

SOUQUET, J.; CHEYNER, V. I. R.; BROSSAUD, F.; MOUTOUNET, M. Polymeric proanthocyanidins from grape skins. **Phytochemistry**, v. 43, n. 2, p. 509–512, 1996.

SPIGNO, G.; DE FAVERI, D. M. Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. **Journal of Food Engineering**, v. 78, n. 3, p. 793–801, 2007.

SUN, T.; CHEN, Q. Y.; WU, L. J.; YAO, X. M.; SUN, X. J. Antitumor and antimetastatic activities of grape skin polyphenols in a murine model of breast cancer. **Food and Chemical Toxicology**, v. 50, n. 10, p. 3462–7, 2012.

TABASCO, R.; SÁNCHEZ-PATÁN, F.; MONAGAS, M.; BARTOLOMÉ, B.; VICTORIA

MORENO-ARRIBAS, M.; PELÁEZ, C.; REQUENA, T. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. **Food Microbiology**, v. 28, n. 7, p. 1345–52, 2011.

TERRA, X.; MONTAGUT, G.; BUSTOS, M.; LLOPIZ, N.; ARDÈVOL, A.; BLADÉ, C.; FERNÁNDEZ-LARREA, J.; PUJADAS, G.; SALVADÓ, J.; AROLA, L.; BLAY, M. Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. **The Journal of Nutritional Biochemistry**, v. 20, n. 3, p. 210–8, 2009.

TERRA, X.; PALLARÉS, V.; ARDÈVOL, A.; BLADÉ, C.; FERNÁNDEZ-LARREA, J.; PUJADAS, G.; SALVADÓ, J.; AROLA, L.; BLAY, M. Modulatory effect of grape-seed procyanidins on local and systemic inflammation in diet-induced obesity rats. **The Journal of Nutritional Biochemistry**, v. 22, n. 4, p. 380–7, 2011.

TOALDO, I. M.; CRUZ, F. A.; ALVES, T. de L.; DE GOIS, J. S.; BORGES, D. L. G.; CUNHA, H. P.; DA SILVA, E. L.; BORDIGNON-LUIZ, M. T. Bioactive potential of Vitis labrusca L. grape juices from the Southern Region of Brazil: Phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. **Food Chemistry**, v. 173, p. 527–35, 2015.

TOMÉ-CARNEIRO, J.; LARROSA, M.; YÁÑEZ-GASCÓN, M. J.; DÁVALOS, A.; GIL-ZAMORANO, J.; GONZÁLVEZ, M.; GARCÍA-ALMAGRO, F. J.; RUIZ ROS, J. A; TOMÁS-BARBERÁN, F. A; ESPÍN, J. C.; GARCÍA-CONESA, M.-T. One-year supplementation with a grape extract containing resveratrol modulates inflammatory-related microRNAs and cytokines expression in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease. **Pharmacological Research** , v. 72, p. 69–82, 2013.

TSAI, C.; WANG, K.; CHEN, L.; LEE, C.; TSENG, S.; WANG, C. Anti-Inflammatory Effects of Vitis thunbergii var. taiwaniana on Knee Damage Associated with Arthritis. **Journal of Medicinal Food**, v. 17, n. 4, p. 479–486, 2014.

TSENG, A.; ZHAO, Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. **Food Chemistry**, v. 138, n. 1, p. 356–65, 2013.

URQUIAGA, I.; D'ACUÑA, S.; PÉREZ, D.; DICENTA, S.; ECHEVERRÍA, G.; RIGOTTI, A.; LEIGHTON, F. Wine grape pomace flour improves blood pressure, fasting glucose and protein damage in humans: a randomized controlled trial. **Biological Research**, v. 48, n. 1, p. 49, 2015.

VATTEM, D. A; SHETTY, K. Ellagic acid production and phenolic antioxidant activity in cranberry pomace (Vaccinium macrocarpon) mediated by Lentinus edodes using a solid-state system. **Process Biochemistry**, v. 39, n. 3, p. 367–379, 2003.

VAZQUEZ-PRIETO, M. A.; BETTAIEB, A.; HAJ, F. G.; FRAGA, C. G.; OTEIZA, P. I. (-)-Epicatechin prevents TNFα-induced activation of signaling cascades involved in inflammation and insulin sensitivity in 3T3-L1 adipocytes. **Archives of Biochemistry and Biophysics**, v. 527, n. 2, p. 113–8, 2012.

WANG, Y.-H.; GE, B.; YANG, X.-L.; ZHAI, J.; YANG, L.-N.; WANG, X.-X.; LIU, X.; SHI, J.-C.; WU, Y.-J. Proanthocyanidins from grape seeds modulates the nuclear factor-kappa B signal transduction pathways in rats with TNBS-induced recurrent ulcerative colitis. **International immunopharmacology**, v. 11, n. 10, p. 1620–7, 2011.

WHO. The world health report 2002 - Reducing risks, promoting healthy life, 2002. Available at: http://www.who.int/whr/2002/en/. Accessed on: Oct. 2015.

XU, C.; YAGIZ, Y.; BOREJSZA-WYSOCKI, W.; LU, J.; GU, L.; RAMÍREZ-RODRIGUES, M. M.; MARSHALL, M. R. Enzyme release of phenolics from muscadine grape (Vitis rotundifolia Michx.) skins and seeds. **Food Chemistry**, v. 157, p. 20–9, 2014.

YIN, W.; LI, B.; LI, X.; YU, F.; CAI, Q.; ZHANG, Z.; CHENG, M. Function procyanidin B2 on a diabetic pancreas. p. 3065–3071, 2015.

YOU, Q.; CHEN, F.; WANG, X.; JIANG, Y.; LIN, S. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. **LWT - Food Science and Technology**, v. 46, n. 1, p. 164–168, 2012.

ZHAO, L.; YAGIZ, Y.; XU, C.; LU, J.; MARSHALL, M. R. Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammatio. **Food & Function**, v. 6, n. 7, p. 2293–2302, 2015.

ZHENG, H.; HWANG, I.-W.; CHUNG, S.-K. Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. **Journal of Zhejiang University,** v. 10, n. 12, p. 912–9, 2009.

ZUNINO, S. J.; PEERSON, J. M.; FREYTAG, T. L.; BREKSA, A. P.; BONNEL, E. L.; WOODHOUSE, L. R.; STORMS, D. H. Dietary grape powder increases IL-1b and IL-6 production by lipopolysaccharide-activated monocytes and reduces plasma concentrations of large LDL and large LDL-cholesterol particles in obese humans British Journal of Nutrition. **British Journal of Nutrition**, v. 112, p. 369–380, 2014.

## ARTIGO II

# Enzymatic biotransformation of phenolic compounds and improvement of antioxidant activity in red and white grape pomace

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# Enzymatic Biotransformation of Phenolic Compounds and Improvement of Antioxidant Activity in Red and White Grape Pomace

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## ABSTRACT

Grape pomace (GP) has been considered a potential source of phenolic compounds, although these compounds have a chemical structure that can compromise their properties and bioavailability. Enzymatic hydrolysis may release the compounds from their conjugates, improving its functional activity. The aim of this study was to determine the release of phenolic compounds and the improvement of the antioxidant activity of red, white and a mixture of grape pomaces from Brazilian wine industries. Tannase enzyme (T), the combination of pectinase and cellulase (PC) and the 3 enzymes together (TPC) were applied for 5h at 40°C (pH 5.0). The tannase treatment was the most significant treatment on enhancing total phenols in GP samples. Enzymatic hydrolysis, notably tannase, modified the galloylated form of polyphenols, releasing gallic acid, caffeic acid, quercetin and *trans*-resveratrol, thus enhancing the antioxidant activity. Catechin amounts increased on red GP after PC treatment and procyanidin B2 was increased especially after TPC treatment in the white GP. These results demonstrate the benefits of the usage of agroindustrial by-products and the biotechnological modification of natural products in order to enrich bioavailable and bioactive polyphenols in foods, nutraceuticals and cosmetics.


**KEYWORDS:** tannase, pectinase, cellulase, grape pomace, polyphenols, antioxidant activity.

## INTRODUCTION

Agroindustrial wastes derived from food productions, e.g., almond skins generated from almond blanching and grape pomace (GP) from wine production, have become a value added product mainly due to their rich content of beneficial phytochemicals for development of neutraceuticals and functional foods. Historically, these wastes have been used as feed for domestic animals, soil fertilizer or fuel. Thus, the newly value added applications of these wastes can be an environmentally friendly alternative for their disposal, supporting a sustainable agriculture[1–4].

Grape (*Vitis* sp.) is one of the world's largest fruit crops and mainly used for wine production. Brazil produced 1,439,535 tons of grapes in 2013, ranked the 13$^{th}$ in the world[5]. Of these grapes, around 48% were used in the grape juice and wine production. Thus, approximately 20-25% of the grape weight becomes a waste and is not fully utilized[6,7] and even when they were used for certain applications, e.g., animal feeds or soil fertilizer, their full values have not been fully realized[8,9]. Considering their chemical and biological properties, constituents in GP have a potential for diverse applications, e.g., as antioxidant additives, natural colorants and food preservatives or formulated into functional foods, nutraceuticals and cosmetics[10,11]. These potential applications are derived from the literature showing GP protected susceptible lipids in restructured fish products from rancidity[12] and enhanced antimicrobial, antifungal, and antioxidant properties of juices and hamburgers[13]. Further, when formulated into yogurt and salad dressing, it significantly increased their dietary fiber and total phenol contents[14]. In addition, grape residues had been added in the production of cereal bars[15], rye bread[16], pancakes, and pastas[17].

Bioactive compounds have received great attention due to their beneficial effects in health promotion and prevention[2]. Thus, the interest in the exploitation of these compounds derived from the residues of processed food productions are increasing. However, their health efficacy may not have reached their maximum potential because they are tightly bound to cellulose matrices. Thus, enzymatic treatments, e.g., pectinases, cellulases and glucanases, have been employed to liberate them from the matrices[18–21]. Besides the abovementioned enzymes, tannin acyl hydrolases (tannases) can hydrolyze depside bonds and ester linkages of hydrolysable tannins or galloil esters[22], a reaction that potentially increases the

antioxidant capacity of final products[23,24]. In addition, this enzymatic hydrolysis is expected to help release monomeric and oligomeric phenolic compounds from their conjugates and therefore facilitate their absorption in the small intestine[25,26]. For example, tannase enhanced the release of phenolics in GP[19,20,27]; tannase produced by *Paecilomyces variotti* hydrolyzed epigallocatechin-gallate in green and mate teas[23,24]; and tannase hydrolyzed glycosidic hesperidin and naringin present in orange juice to their corresponding aglycones[28].

Different varieties of grape residues, from different winemaking procedures were analyzed after treatment with commercial maceration enzymes and a specific *Paecilomyces variotii* tannase, produced by solid-state fermentation. As enzymes may differ in efficacy and produce distinctive composition profiles, the objective of this study was to examine the differential impact of tannase, pectinase, and cellulase on the hydrolysis of polymeric polyphenols, as well as antioxidant activity of the hydrolyzed products, in red, white, and a mixture of grape pomaces from the wine production in Brazil.

## MATERIAL AND METHODS

### Chemicals

Folin Ciocalteu's reagent and arabic gum were purchased from Dinâmica Química Contemporanea (Diadema, Brazil), vanillin, ferric chloride, ferric ammonium sulfate from Vetec Química (São Paulo, Brazi), fluorescein, ammonium sulfate, sodium acetate and sodium chloride from Ecibra (São Paulo, Brazil), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid from Acros Organics (Belgium), monobasic and dibasic sodium phosphate, sodium carbonate, hydrochloric acid, and formic acid from LabSynth (Diadema, Brazil), LC grade methanol from JT Baker (Center Valley, PA, USA), tannic acid from Ajinomoto OmniChem Division (Wetteren, Belgium), and gallic acid, catechin, caffeic acid, procyanidin B2, *trans*-resveratrol, quercetin, rutin, tannic acid, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-Azobis (2-amidinopropane) dihydrochloride, 4,6-tripyridyl-s-triazine from Sigma-Aldrich, (St. Louis, MO, USA). The water used was produced from an ultrapure Milli-Q system (Merck Millipore, Darmstadt, Germany).

## Samples

Three GP samples containing seeds and skins were obtained when wines were produced in São Paulo and Rio Grande do Sul states (Brazil) between January and March 2013. Red GP (RGP) was derived from "Maximo IAC 138-22" grapes, a hybrid grape variety of Syrah (75%) and Seibel (25%); white GP (WGP) from Moscato branco grapes, and mixed GP (MGP) from mixed red and white grapes of different varieties. As the obtained RGP was wet, it was first frozen at -20°C, followed by lyophilization. Both WGP and MGP were dried already when obtained, WGP at ~60°C and MGP at ~100°C. All GP samples were grounded, aliquoted, and then stored at -80°C until analyses.

## Production and pre-purification of tannase extract

*Paecilomyces variotii* tannase was produced according to our previously published procedure[29,30] with minor modifications in the scale and formulation of the culture medium. Briefly, the enzyme was produced by mixing 20 g wheat bran, 20 mL distilled water, and 10% tannic acid (w/w). After the fermentation, extraction, filtration, saturation with solid ammonium sulfate, and dialysis, the semi-purified tannase extract was lyophilized and stored at -80°C.

## Enzymatic treatments

Three enzyme treatments were administered on the GP samples: T (5% w/w of tannase), PC (2.5% of commercial pectinase and cellulase each) and TPC (1.66% of tannase, pectinase, and cellulase each), which corresponded to 5 U of tannase in the T treatment, 1.7 U (pectinase) and 1 U (cellulase) in the PC treatment and 1.66 U (T), 1.1 U (P) and 0.7 U (C) in the TPC treatment, respectively. Tannase activity was expressed as U/mg of protein, considering U as the necessary tannase amount to produce 1 µmol of gallic acid in one minute. The enzymatic activities of pectinase and cellulase were expressed following the manufacturer's information.

GP (100 mg) in 1 mL sodium acetate buffer (0.02 M, pH 5.0) was incubated with one of the enzymatic treatments for 5 h at 40°C. The incubation condition was optimal for the tannase reaction[30], as well as for pectinase and

cellulase, according to the manufacturers' instructions. At the end of the enzymatic hydrolysis, phenolics were extracted with 2 mL methanol for 60 min (shaking) at room temperature and for 10 min in an ultrasound bath. After centrifugation for 15 min at 9,000 g (Daigger model SD, Illinois, USA), the supernatants were collected, filtered using a 0.45 µm membrane, and then used for assays, except ORAC. For the ORAC assay, the supernatants were dried under $N_2$ air and then reconstituted in phosphate buffer saline (pH 7.4). Controls were produced without using the enzymes.

**Phenolic composition**

Total phenolic compounds

The total phenolic content was determined using the method of Singleton and Rossi[31]. Concentrations were calculated using a calibration curve constructed using the authenticated gallic acid standard. All results were expressed as mg gallic acid equivalents (GAE)/g dry matter (DM).

Condensed tannins

Condensed tannins were quantified using a modified vanillin method[32], an assay specific for dihydroxy phenolics and particularly sensitive for meta-substituted, di- and tri-hydroxy-benzene containing molecules. This assay also measures its oligomeric and monomeric components (proanthocyanidins and flavanols)[33,34]. The GP extracts (0.5 mL) were mixed with 2.5 mL vanillin solution (equal volumes of 1% vanillin in methanol and 8% HCl in methanol), followed by the incubation for 20 min at room temperature. Absorbance was measured at 500 nm. A blank was prepared by mixing 2.5 mL of 4% HCl in methanol with the extracts. All results were expressed as mg of catechin equivalents (CE)/g DM.

Hydrolysable tannins

The hydrolysable tannins were determined using a modified ferric ammonium sulfate (FAS) method[35], which measures galloyl groups. GP extracts (0.4

mL) were added to 1.6 mL FAS reagent comprising 89% acetate buffer (0.1 M, pH 4.4), 10% arabic gum (1% in distilled water), and 1% FAS solution (5% ferric ammonium sulfate dissolved in 1 M HCl)]. After incubation for 15 min, the absorbance was read at 578 nm. The hydrolysable tannins in the GP extracts were calculated using a standard curve constructed using the authenticated tannic acid standard. Results were expressed as mg of tannic acid equivalents (TAE)/g DM.

Quantification of polyphenols

Polyphenols in the GP extracts treated with and without enzymes were quantified using a Dionex UltiMate 3000 (Dreieich, Germany) liquid chromatography system equipped with a RP18 Waters X-Terra® (5 µm, 4.6 x 150 mm) column maintained at 30°C and a UV/VIS detector (DAD-3000). Analyte separation was achieved using mobile phase A (water/formic acid, 99.9:0.1 v/v) and mobile phase B (methanol/formic acid, 99.9:0.1 v/v) in a linear gradient mode (% solvent A): 92% (0–5 min), 92–85% (5–13 min), 85–75% (13–45 min), 75–57% (45–67 min), 57–50% (67-77 min), 50–35% (77-95 min), 35–20% (95-108 min), 20–92% (108-110 min), 92% (110-120 min). The flow rate was set at 0.5 mL/min. The identification of individual compounds was achieved by comparing their retention time and spectra. Gallic and caffeic acids, catechin, and procyanidin B2 were quantified at 280 nm, quercetin and rutin at 260 nm, and resveratrol at 310 nm. Their quantities in the GP extracts were calculated using standard curves constructed with standards.

**Antioxidant activity**

DPPH radical scavenging activity

DPPH radical scavenging activity of the GP extracts was assessed according to Brand-William et al.[36] and Macedo et al.[24]. The assay was performed on a NovoStar Microplate reader (BMG LABTECH, Germany). All results were expressed as µmol of trolox equivalents (TE)/g DM.

Oxygen Radical Absorbance Capacity (ORAC)

ORAC assay was performed using fluorescein (FL) as the fluorescent probe, according to Prior et al.[37] and Macedo et al.[24]. The ORAC values were calculated using the difference between the area under the FL decay curve and the blank (net AUC) and the standard curves constructed using trolox. The final values were expressed as µmol TE/g of DM.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain[38]. The absorbance of the mixture was measured at 593 nm at 37°C in a 4-min kinetics. The results were expressed as µmol TE/g DM.

**Statistical analysis**

All measurements were performed in triplicate. Results are presented as means ± standard deviation (SD). The significant effects of GP, enzyme and their interaction was assessed using a two-way ANOVA, followed by Tukey-Kramer HSD multi-comparison test. All statistical analyses were performed using JMP 10 (Cary, NC, USA). The Pearson's correlation test was used to analyze the correlation between variables.

**RESULTS AND DISCUSSION**

In this study, the capacity of 3 hydrolysis enzymes to biotransform phenolic compounds was assessed in red, white, and mixed GP using an array of assays, i.e., total phenols, total antioxidant capacity, and phenolic profile. The quantity and composition of phenolics in GP are influenced by a wide range of factors, e.g., grape variety, growing climate and location, harvest time, type of vinification process, and processing and storage conditions[39]. Consistent with this notion, we found the grape variety factor, the enzyme factor, and their interaction had a high significant impact on total phenols and condensed tannins ($P \leq 0.0001$). Without the enzyme treatments, we noted RGP contained the highest total phenols,

at least 36.5% larger than WGP and MGP (**Table 1**), in agreement with red grapes being richer in anthocyanins, flavonoids, flavanols and catechins as compared to white grapes[40]. MGP consists in a mix of red and white grapes, and was expected to be ranked in the middle, with WGP presenting lower amounts of total phenols. But contrarily, MGP presented the lowest total phenols amounts. Drying at 100°C might have affect the ranking of MGP because some factors, including high temperature and pH, have been reported to cause degradation of phenolics[41]. Among the 3 enzyme mixes evaluated, tannase was the most effective to hydrolyze polymeric phenolics as this enzyme increased total phenol content by 61.1% in RGP, 21.5% in WGP and 33.2% in MGP as compared to the respective untreated GP. We noted PC was not very effective in the hydrolysis. TPC increased total phenols, but the magnitude was significantly smaller than T, probably because it only contained 1.66% tannase. This finding is consistent to Chamorro et al.[19] study showing the efficacy of TPC was dependent greatly on tannase content in the enzyme mix. In addition, our results confirmed their finding that neither pectinase nor cellulase were capable of changing total phenol content. In contrast to our and Chamorro et al.[19] studies, Meyer et al.[20] and Xu et al.[41] demonstrated P and C were efficacious to release phenolics bound to cellulose matrices of grape skins and seeds. This discrepancy is most likely ascribed to the difference in enzyme type, treatment duration, pomace particle size, existing phenolics composition, presence of lignin, and extraction solvent. In agreement with Río Segade et al.[42] observation that anthocyanins in grapes inhibited the activity of maceration enzymes, we noted pectinolytic and cellulolytic enzymes demonstrated to enhance amounts of total polyphenolics in WGP, but not in RGP and MGP. In opposite to the anticipated increase in total phenol contents, several studies showed total phenol contents became lower after enzymatic treatments[20,41,43]. While the exact mechanism(s) for the reduction remains unknown, it was probably due to the degradation of phenolics during the 48-h enzymatic incubation at 40°C[20]. Therefore, studies specifically designed to examine the effect of the abovementioned factors on the change in total phenols of GP are warranted.

Condensed tannins are predominant in grapes and wine. As previously shown by Negro et al.[44] an important part of the extracted phenolic substances on red grape marcs had flavonoidic origin. The detection of condensed tannins varied widely by ~ 2-fold between 3 GP samples (**Table 1**), with the descending order of

RGP, MGP, and WGP, whose ranking order was different from total phenols. Similar percentages of condensed tannins (21–52%) were also detected in French grape pomaces[45]. A red grape variety previously showed to have the highest quantity of total phenols and flavanols[46]. But contrary, Deng et al.[4747] detected similar amounts of flavanols in white and red GP. The prevalence of flavonoidic compounds in GP samples was also demonstrated by the detection of catechin and procyanidin B2 as the major compounds between the phenolics analyzed by HPLC, in the 3 GP samples (**Table 2**). The enzymatic treatments resulted in condensed tannins reductions in all GP samples. The average reduction was 46.6% after T treatment, 24.2% after PC treatment and 46.1% after TPC treatment. Overall, these reductions were larger in the RGP and MGP. Similar results were detected after pectolytic enzyme addition in wine and grape juice, decreasing flavan-3-ols and anthocyanins content[43,48]. Condensed tannins are not recognized as hydrolysable by classical tannases, but there are still insufficient knowledge about tannases properties and optimal expression[49]. It was proved before that procyanidin B2 dimer underwent a biodegradation by *Aspergillus fumigatus* oxygenase[50]. Therefore, possible explanations about the decrease on condensed tannins could be oxidative degradation, polymerization, precipitation or hydrolysis of these compounds. However, studies are needed to further characterize the degradation of condensed tannins.

Hydrolysable tannins are formed through ester linkages with gallic acid (gallotannins) and ellagic acid (ellagitannins). They are degraded to smaller molecules at low pH or through non-enzymatic or enzymatic hydrolysis[49,51]. The presence of hydrolysable tannins remains to be fully characterized in GP, but their presence in wine after oak-aging process is well appreciated[52]. Gallic acid, galloyl-glucose, digalloyl-glucose, ellagic acid, epigallocatechin-*O*-gallate, gallocatechin-*O*-gallate, epicatechin-*O*-gallate and other oligomeric hydrolyzable tannins have been found in *V. vinifera*, *V. rotundifolia* (Muscadine grapes), and grape skin, seeds, and pomace[19,53,54]. In this study, hydrolysable tannins were found in the GP samples with their content being comparable in RGP and WGP, but >2-fold lower in MGP (**Table 1**). All 3 enzymes appeared not to affect their contents assessed using the FAS assay, a result conflicting with the HPLC data showing gallic acid content was increased after the 3 enzyme treatments. The discrepancy may be ascribed to the

difference in sensitivity and specificity between the 2 assays with the spectrophotometric one being inferior to the chromatographic one.

The hydrolysis of rutin (quercetin-rutinoside) and the increase of its aglycone form, quercetin, was demonstrated (**Table 2**). Initially, WGP had 80% larger rutin than RGP and MGP. After all the enzymatic treatments, rutin was only detected after PC treatment in WGP (no significant difference from control). In contrast to rutin, the quercetin initial values were larger in RGP and MGP than WGP (3.2-fold difference). The treatments containing tannase (T and TPC) were the most effective and equally significant, increasing quercetin levels in 3.8-fold in RGP, 5.1-fold in WGP and 3.4-fold in MGP. As found in previous study[18] and in our data, pectinase and cellulase alone also increased 2.2-fold quercetin amounts in MGP, but resulting in no significant difference in RGP and WGP. Despite the raise in quercetin level did not coincide to the depletion of rutin, other degradation steps may happen. These data are consistent with the results presented by Kammerer et al.[27] and Ferri et al.[11]. Quercetin is well known to have a strong free radical scavenging and anti-oxidative stress activities, being more efficient than rutin in protecting against mitochondrial dysfunction[55,56].

*Trans*-resveratrol was only detected in the MGP, and its content was significantly increased by ~10-fold after T and TPC and 8.5-fold after PC treatment ($P \leq 0.0001$) (**Table 2**). Our results are consistent with the observed hydrolysis capacity of pectinase, cellulase, β-glucosidase, and endoproteases in GP and wine[27,57,58]. While the underlying mechanism(s) for the increase is unknown, we speculate that resveratrol aglycone was produced when the enzymes hydrolyzed glycosylated and/or oligomeric forms (e.g., piceid) of resveratrol[11,59]. The conversion of this bioactive compound to its aglycone form is expected to have a great value because resveratrol aglycone displays an array of bioactions beneficial to health, e.g., antioxidant, anticancer, antimicrobial and cardioprotective activities[60]. Interestingly, resveratrol was not found RGP and WGP even after the enzyme treatments, possibly due to the genetic factor for its synthesis in different varieties of grapes or due to the absence of contamination by *Botrytis cinerea*[61], which can be related to the production of resveratrol in grapevines.

Phenolic acids are normally present in esterified forms or bound with dietary fiber in foods[62]. Thus, treatments with pectinolytic/cellulolytic enzymes are expected to increase content of phenolic acids[63]. In this study, the phenolic acids

detected were gallic and caffeic acids. Both grape pomace type and enzyme treatment affected the contents of these 2 phenolic acids ($P \leq 0.0001$). Prior to the enzyme treatments, RGP had >2.7-fold larger gallic acid content than MGP and WGP. All 3 enzymes increased gallic acid content in the GP with the magnitude depending on enzyme and GP. Tannase increased the content by 2.8-fold in RGP, 21-fold in WGP, and 4.4-fold in MGP, PC increased 1.3-2-fold in all samples, and TPC increased 2.5-fold in RGP, 17.9-fold in WGP, and 4.4–fold in MGP (**Table 2**). T and TPC appeared to have a comparable efficacy to liberate gallic acid even though TPC contained much less T, suggesting the amount of T sufficed to mediate the hydrolysis. Further, the efficacy of PC toward bound gallic acids was noted in RGP and MGP was ~50% smaller than that of T and TPC. While WGP contained an abundant amount of bound gallic acid, PC activity seemed to be inhibited by unknown constituent(s). The lower PC activity observed in our study was consistent with Chamorro et al.[19] study with red GP, in which pectinase and cellulase alone were less efficacious than T and TPC. The largest increase in gallic acid content after the enzyme treatments was found in WGP, suggesting there were differences in the cell wall structure of grape varieties and in the vinification procedure. Particularly, white grapes are normally crushed and the pomace is removed in the early stage of vinification[16,64]. While caffeic acid was not detected in the untreated GP, it was detected only in RGP and MGP after all of 3 enzyme treatments. The enzyme efficacy in the generation of caffeic acid appeared to be dependent on GP type and enzyme. Overall, RGP contained ~14% more intrinsic bound caffeic acid than MGP that were liberated during the enzymatic hydrolysis. The increase of this phenolic could have been observed after β-glucanase and feruloyl esterases treatments in agroindustrial wastes (apple pomace, apple marc, coffee pulp, and wheat straw)[21,65–67]. Interestingly, a differential effect of the enzymes on the release of bound caffeic acid was noted with TPC being more efficacious toward RGP and PC toward MGP.

The major phenolics found in GP samples were catechin and procyanidin B2. GP type had the highest effect on their contents in the GP ($P \leq 0.0001$), but enzyme and the grape-enzyme interaction also significantly influenced their values. Before the enzyme treatments, catechin content varied widely by ~2.2 fold between 3 GP samples (**Table 2**), with a descending order of RGP, WGP, and MGP. The ranking was the same in procyanidin B2 content, but the variation (~4-fold) was even larger than catechin (**Table 2**). The enzymes displayed a very modest effect on

catechin contents. Similar to the results of catechin, procyanidin B2 contents were affected by GP type, enzyme and their interaction, but the changes were very modest, except for WGP after the TPC treatment which led to ~47.4% increase in procyanidin B2 content as compared to the untreated. Our results were in agreement with Kammerer et al.[27] study, showing both pectinase and cellulase increased catechin, epicatechin, and procyanidin B2 contents in grape pomace. While our study could not reveal mechanism(s) by which the enzymes increased catechin and procyanidin B2 contents, we speculate that the enzymes may either release these 2 compounds bound tightly to polysaccharides in the cell walls or produce them through the hydrolysis of polymeric procyanidins.

Phenolic acids and monomeric flavanols are potent antioxidants[68] so that their generation through enzymatic actions is anticipated to increase GP antioxidant activity. For example, an enzyme mix containing pectinase, cellulase, and tannase was found to increase antioxidant activity of GP and grape seeds[19,41]. In addition, enzyme treatment is expected to enhance the total amount of absorbed phenolics and bioefficacy in target tissues[69]. The 2-way analysis of variance showed GP type, enzyme, and their interaction had a significant effect on antioxidant activity of GP samples, assessed using three assays, i.e., DDPH, ORAC, and FRAP ($P \leq 0.0001$). Before the enzyme treatments, the RGP displayed the largest antioxidant activities of all 3 assays, followed by WGP and MGP (**Figure 1**). This ranking appeared to be consistent with total phenol content, tested by Pearson's correlation coefficient (r) analysis, i.e., TP and DPPH ($r= 0.6437$ and $P \leq 0.0001$); TP and FRAP ($r =0.8241$ and $P \leq 0.0001$), TP and ORAC ($r =0.4954$ and $P =0.0021$). We noted T was the most effective to increase DPPH and ORAC values of all 3 GP samples. RGP antioxidant activity was enhanced by 1.8- and 3.7-fold in the DPPH and ORAC assays, respectively, whereas WGP was increased by 1.6- and 5.9-fold, and MGP by 1.8 and 5.1-fold. PC in general was ineffective to alter DPPH and ORAC values besides 1.2-fold increase in DPPH value of RGP. TPC led to an increase in DPPH and ORAC values of all 3 GP, but the magnitude of the increases (1.4-1.6-fold for DPPH and 1.5-4.2-fold for ORAC) was smaller than T alone, probably related to the lower concentration of tannase in the enzyme mix. There is a highly positive statistically correlation between DPPH and ORAC data ($r =0.9295$, $P \leq 0.0001$). DPPH and ORAC assays assess the hydrogen donating capacity of GP constituents and FRAP values are indicative of electron transferring capacity. The rank of FRAP value of the

3 untreated GP was the same as for DPPH and ORAC assays, but the enzyme mediated increase in its values was not remarked, with the average increase at 1.4-fold (**Figure 1 C**). As the antioxidant activity of the GP constituents is influenced by the stoichiometric theory, as well as the number of hydroxyl group in phenol ring(s)[70], the small-molecule phenolics generated from enzyme-mediated hydrolysis of polymeric phenolics and liberation from the cellulose matrices are expected to increase the antioxidant activity of GP. These results provide a support for use of enzymes to increase health values of agroindustrial wastes, including grape pomace derived from wine production.

Grape pomace derived from wine production contains appreciable amounts of phenolics, including flavonoids and phenolic acids. Their contents and antioxidant activity are influenced by grape pomace type and enzyme treatment. Among 3 GP, red grape pomace contained the highest amount of phenolics and antioxidant activity. Three different enzyme mixes, i.e., tannase, pectinase plus cellulase, and tannase, pectinase, and cellulase, were administered to hydrolyze polymeric phenolics and/or liberate phenolics tightly bound to cellulose matrices. WGP appeared to be more responsive to pectinolytic and cellulolytic enzymes on augment content of phenolics, especially gallic acid. As compared to the other 2 enzyme mixes, tannase displayed the largest efficacy. Therefore, grape pomace derived from wine production is a value-added waste due to at least the content of phenolics. Further studies are necessary to develop functional foods, nutraceuticals, or cosmetics containing high phenolics grape pomace.

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## ABBREVIATIONS USED

GP, grape pomace; RGP, red grape pomace; WGP, white grape pomace; MGP, mixed grape pomace; TE, trolox equivalent; CE, catechin equivalent; GAE, gallic acid equivalent; TAE, tannic acid equivalent; DM, dry matter; FRAP, Ferric-reducing antioxidant power; ORAC, Oxygen Radical Absorbance Capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HT, hydrolysable tannins; T, tannase; P, pectinase; C, cellulase, PC, pectinase+cellulase; TPC, tannase+pectinase+cellulase; FL, fluorescein; AUC, area under curve; AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; FAS, ferric ammonium sulfate; TPTZ ,4,6-tripyridyl-s-triazine.

## REFERENCES


(1) Kammerer, D.; Claus, A.; Carle, R.; Schieber, A. Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *J. Agric. Food Chem.* **2004**, *52* (14), 4360–4367.

(2) Martins, S.; Mussatto, S. I.; Martínez-Avila, G.; Montañez-Saenz, J.; Aguilar, C. N.; Teixeira, J. A. Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. *Biotechnol. Adv.* **2011**, *29* (3), 365–373.

(3) Crozier, A.; Jaganath, I. B.; Clifford, M. N. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* **2009**, *26* (8), 1001–1043.

(4) Pandey, A. Solid-state fermentation. *Biochem. Eng. J.* **2003**, *13*, 81–84.

(5) FAOSTAT. FAO Statistical Database, **2015**. http://www.fao.org. (accessed on January, 2016).

(6) Mello, L. M. R.; Silva, G. A. Disponibilidade e características de resíduos provenientes da agroindústria de processamento de uva do Rio Grande do Sul. *Embrapa Comun. Técnico 155.* **2014**, *Fevereiro*, 1–6.

(7) Mello, L. M. R. Vitivinicultura Brasileira: Panorama 2013. *Embrapa Comun. Técnico 156* **2014**, *Julho*, 1–6.

(8) Rockenbach, I. I.; Rodrigues, E.; Gonzaga, L. V.; Caliari, V.; Genovese, M. I.; Gonçalves, A. E. D. S. S.; Fett, R. Phenolic compounds content and antioxidant activity in pomace from selected red grapes (Vitis vinifera L. and Vitis labrusca L.) widely produced in Brazil. *Food Chem.* **2011**, *127* (1), 174–179.

(9) de Campos, L. M. A. S.; Leimann, F. V.; Pedrosa, R. C.; Ferreira, S. R. S. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (Vitis vinifera). *Bioresour. Technol.* **2008**, *99* (17), 8413–8420.

(10) Yu, J.; Ahmedna, M. Functional components of grape pomace: their composition, biological properties and potential applications. *Int. J. Food Sci. Technol.* **2013**, *48* (2), 221–237.

(11) Ferri, M.; Bin, S.; Vallini, V.; Fava, F.; Michelini, E.; Roda, A.; Minnucci, G.; Bucchi, G.; Tassoni, A. Recovery of polyphenols from red grape pomace and assessment of their antioxidant and anti-cholesterol activities. *N. Biotechnol.* **2016**, *33* (3), 338–344.

(12) Sánchez-Alonso, I.; Jiménez-Escrig, A.; Saura-Calixto, F.; Borderías, A. J. Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT - Food Sci. Technol.* **2008**, *41* (1), 42–50.

(13) Sagdic, O.; Ozturk, I.; Ozkan, G.; Yetim, H.; Ekici, L.; Yilmaz, M. T. RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices. *Food Chem.* **2011**, *126* (4), 1749–1758.

(14) Tseng, A.; Zhao, Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. *Food Chem.* **2013**, *138* (1), 356–365.

(15) Balestro, E.; Sandri, I.; Fontana, R. Utilização de bagaço de uva com atividade antioxidante na formulação de barra de cereais. *Rev. Bras. Prod. Agroindustriais* **2011**, *13* (2), 203–209.

(16) Mildner-Szkudlarz, S.; Bajerska, J.; Zawirska-Wojtasiak, R.; Górecka, D. White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *J. Sci. Food Agric.* **2013**, *93* (2), 389–395.

(17) Rosales Soto, M. U.; Brown, K.; Ross, C. F. Antioxidant activity and consumer acceptance of grape seed flour-containing food products. *Int. J. Food Sci. Technol.* **2012**, *47* (3), 592–602.

(18) Arnous, A.; Meyer, A. S. Discriminated release of phenolic substances from red wine grape skins (Vitis vinifera L.) by multicomponent enzymes treatment. *Biochem. Eng. J.* **2010**, *49* (1), 68–77.

(19) Chamorro, S.; Viveros, A.; Alvarez, I.; Vega, E.; Brenes, A. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. *Food Chem.* **2012**, *133* (2), 308–314.

(20) Meyer, A. S.; Jepsen, S. M.; Sørensen, N. S. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. *J. Agric. Food Chem.* **1998**, *46* (7), 2439–2446.

(21) Zheng, H.; Hwang, I.-W.; Chung, S.-K. Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. *J. Zhejiang Univ. Sci. B* **2009**, *10* (12), 912–919.

(22) LEKHA, P. K.; LONSANE, B. K. Production and application of tannin acyl hydrolase; state of the art. *Adv. Appl. Microbiol.* **1997**, *44*, 215–260.

(23) Battestin, V.; Macedo, G. A.; De Freitas, V. A. P. Hydrolysis of epigallocatechin gallate using a tannase from Paecilomyces variotii. *Food Chem.* **2008**, *108* (1), 228–233.

(24) Macedo, J. A.; Battestin, V.; Ribeiro, M. L.; Macedo, G. A. Increasing the antioxidant power of tea extracts by biotransformation of polyphenols. *Food Chem.* **2011**, *126* (2), 491–497.

(25) Shoji, T.; Masumoto, S.; Moriichi, N.; Akiyama, H.; Kanda, T.; Ohtake, Y.; Goda, Y. Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the porter method and high-performance liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem.* **2006**, *54* (3), 884–892.

(26) Georgetti, S. R.; Vicentini, F. T. M. C.; Yokoyama, C. Y.; Borin, M. F.; Spadaro, A. C. C.; Fonseca, M. J. V. Enhanced in vitro and in vivo antioxidant activity and mobilization of free phenolic compounds of soybean flour fermented with different beta-glucosidase-producing fungi. *J. Appl. Microbiol.* **2009**, *106* (2), 459–466.

(27) Kammerer, D.; Claus, A.; Schieber, A.; Carle, R. A Novel Process for the Recovery of Polyphenols from Grape ( Vitis vinifera L .) Pomace. *Food Chem. Toxicol.* **2005**, *70* (2), 157–163.

(28) Ferreira, L. R.; Macedo, J. A.; Ribeiro, M. L.; Macedo, G. A. Improving the chemopreventive potential of orange juice by enzymatic biotransformation. *Food Res. Int.* **2013**, *51* (2), 526–535.

(29) Battestin, V.; Macedo, G. A. Tannase production by Paecilomyces variotii. *Bioresour. Technol.* **2007**, *98* (9), 1832–1837.

(30) Battestin, V.; Macedo, G. A. Purification and Biochemical Characterization of Tannase from a Newly Isolated Strain of Paecilomyces Variotii. *Food Biotechnol.* **2007**, *21* (3), 207–216.

(31) Singleton, V. L.; Rossi, J. A.; Jr, J. Colorimetric of total phenolics with acid reagents. *Am. J. Enol. Vitic* **1965**, *16* (3), 144–158.

(32) Price, M. L.; Van Scoyoc, S.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26* (5), 1214–1218.

(33) Maier, T.; Schieber, A.; Kammerer, D. R.; Carle, R. Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants.

*Food Chem.* **2009**, *112* (3), 551–559.

(34) Obreque-Slier, E.; Peña-Neira, A.; López-Solís, R.; Zamora-Marín, F.; Ricardo-da Silva, J. M.; Laureano, O. Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (Vitis vinifera L.) during ripening. *J. Agric. Food Chem.* **2010**, *58* (6), 3591–3599.

(35) Brune, M.; Hallberg, L.; Skanberg, A.-B. Determination of iron-binding phenolic groups in foods. *J. Food Sci.* **1991**, *56* (1), 128–131.

(36) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **1995**, *28* (1), 25–30.

(37) Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL)) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51* (11), 3273–3279.

(38) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal. Biochem.* **1996**, *239* (1), 70–76.

(39) Lafka, T.-I.; Sinanoglou, V.; Lazos, E. S. On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chem.* **2007**, *104* (3), 1206–1214.

(40) Katalinić, V.; Možina, S. S.; Skroza, D.; Generalić, I.; Abramovič, H.; Miloš, M.; Ljubenkov, I.; Piskernik, S.; Pezo, I.; Terpinc, P. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). *Food Chem.* **2010**, *119* (2), 715–723.

(41) Xu, C.; Yagiz, Y.; Borejsza-Wysocki, W.; Lu, J.; Gu, L.; Ramírez-Rodrigues, M. M.; Marshall, M. R. Enzyme release of phenolics from muscadine grape (Vitis rotundifolia Michx.) skins and seeds. *Food Chem.* **2014**, *157*, 20–29.

(42) Río Segade, S.; Pace, C.; Torchio, F.; Giacosa, S.; Gerbi, V.; Rolle, L. Impact of maceration enzymes on skin softening and relationship with anthocyanin extraction in wine grapes with different anthocyanin profiles. *Food Res. Int.* **2015**, *71*, 50–57.

(43) Borazan, A. A.; Bozan, B. The influence of pectolytic enzyme addition and

prefermentative mash heating during the winemaking process on the phenolic composition of Okuzgozu red wine. *Food Chem.* **2013**, *138* (1), 389–395.

(44) Negro, C.; Tommasi, L.; Miceli, A. Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresour. Technol.* **2003**, *87* (1), 41–44.

(45) Rondeau, P.; Gambier, F.; Jolibert, F.; Brosse, N. Compositions and chemical variability of grape pomaces from French vineyard. *Ind. Crops Prod.* **2013**, *43*, 251–254.

(46) Brahim, M.; Gambier, F.; Brosse, N. Optimization of polyphenols extraction from grape residues in water medium. *Ind. Crops Prod.* **2014**, *52*, 18–22.

(47) Deng, Q.; Penner, M. H.; Zhao, Y. Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Res. Int.* **2011**, *44* (9), 2712–2720.

(48) Lima, M. dos S.; da Conceição Prudêncio Dutra, M.; Toaldo, I. M.; Corrêa, L. C.; Pereira, G. E.; de Oliveira, D.; Bordignon-Luiz, M. T.; Ninow, J. L. Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration. *Food Chem.* **2015**, *188*, 384–392.

(49) Aguilar, C. N.; Rodríguez, R.; Gutiérrez-Sánchez, G.; Augur, C.; Favela-Torres, E.; Prado-Barragan, L. A.; Ramírez-Coronel, A.; Contreras-Esquivel, J. C. Microbial tannases: advances and perspectives. *Appl. Microbiol. Biotechnol.* **2007**, *76* (1), 47–59.

(50) Contreras-Domínguez, M.; Guyot, S.; Marnet, N.; Le Petit, J.; Perraud-Gaime, I.; Roussos, S.; Augur, C. Degradation of procyanidins by Aspergillus fumigatus: identification of a novel aromatic ring cleavage product. *Biochimie* **2006**, *88* (12), 1899–1908.

(51) Garrido, J.; Borges, F. Wine and grape polyphenols — A chemical perspective. *Food Res. Int.* **2013**, *54* (2), 1844–1858.

(52) Harbertson, J. F.; Parpinello, G. P.; Heymann, H.; Downey, M. O. Impact of exogenous tannin additions on wine chemistry and wine sensory character. *Food Chem.* **2012**, *131* (3), 999–1008.

(53) Lee, J. H.; Johnson, J. V.; Talcott, S. T. Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *J. Agric. Food Chem.* **2005**, *53* (15), 6003–6010.

(54) Narduzzi, L.; Stanstrup, J.; Mattivi, F. Comparing Wild American Grapes with

*Vitis vinifera* : A Metabolomics Study of Grape Composition. *J. Agric. Food Chem.* **2015**, *63* (30), 6823–6834.

(55) Carrasco-Pozo, C.; Mizgier, M. L.; Speisky, H.; Gotteland, M. Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. *Chem. Biol. Interact.* **2012**, *195* (3), 199–205.

(56) Saw, C. L. L.; Guo, Y.; Yang, A. Y.; Paredes-Gonzalez, X.; Ramirez, C.; Pung, D.; Kong, A.-N. T. The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: Involvement of the Nrf2-ARE signaling pathway. *Food Chem. Toxicol.* **2014**, *72* (August), 303–311.

(57) La Torre, G. L.; Laganà, G.; Bellocco, E.; Vilasi, F.; Salvo, F.; Dugo, G. Improvement on enzymatic hydrolysis of resveratrol glucosides in wine. *Food Chem.* **2004**, *85*, 259–266.

(58) Rodriguez-Rodriguez, R.; Justo, M. L.; Claro, C. M.; Vila, E.; Parrado, J.; Herrera, M. D.; Alvarez de Sotomayor, M. Endothelium-dependent vasodilator and antioxidant properties of a novel enzymatic extract of grape pomace from wine industrial waste. *Food Chem.* **2012**, *135* (3), 1044–1051.

(59) Berzas Nevado, J. J.; Contento Salcedo, A. M.; Castañeda Peñalvo, G. Simultaneous determination of cis- and trans-resveratrol in wines by capillary zone electrophoresis. *Analyst* **1999**, *124* (1), 61–66.

(60) Xia, E.-Q.; Deng, G.-F.; Guo, Y.-J.; Li, H.-B. Biological Activities of Polyphenols from Grapes. *Int. J. Mol. Sci.* **2010**, *11* (2), 622–646.

(61) Langcake, P.; Mc Carthy, W.V. The relationship of resveratrol production to infections of grapevine leaves by Botrytis cinerea. *Vitis* **1979**, *18*, 244-253.

(62) Scalbert, A.; Williamson, G. Dietary Intake and Bioavailability of Polyphenols. *J. Nutr.* **2000**, No. 2074S, 2073–2085.

(63) Maier, T.; Göppert, A.; Kammerer, D. R.; Schieber, A.; Carle, R. Optimization of a process for enzyme-assisted pigment extraction from grape (Vitis vinifera L.) pomace. *Eur. Food Res. Technol.* **2008**, *227* (1), 267–275.

(64) Jara-Palacios, M. J.; Hernanz, D.; González-Manzano, S.; Santos-Buelga, C.; Escudero-Gilete, M. L.; Heredia, F. J. Detailed phenolic composition of white grape by-products by RRLC/MS and measurement of the antioxidant activity. *Talanta* **2014**, *125*, 51–57.

(65) Benoit, I.; Navarro, D.; Marnet, N.; Rakotomanomana, N.; Lesage-Meessen, L.; Sigoillot, J. C.; Asther, M.; Asther, M. Feruloyl esterases as a tool for the release of phenolic compounds from agro-industrial by-products. *Carbohydr. Res.* **2006**, *341* (11), 1820–1827.

(66) Li, Y.; Ma, R.; Xu, Z.; Wang, J.; Chen, T.; Chen, F.; Wang, Z. Identification and quantification of anthocyanins in Kyoho grape juice-making pomace, Cabernet Sauvignon grape winemaking pomace and their fresh skin. *J. Sci. Food Agric.* **2013**, 93 (6), 1401-1411

(67) Zheng, H.; Hye-Ryun, L.; Sang-Han, L.; Chang-Scob, K.; Shin-Kyo, C. Pectinase Assited Extraction of Polyphenols from Apple Pomace. *Chinese J. Anal. Chem.* **2008**, *36* (3), 306–310.

(68) Rice-Evans, C.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2* (4), 152–159.

(69) Ferruzzi, M. G.; Lobo, J. K.; Janle, E. M.; Whittaker, N.; Cooper, B.; Simon, J. E.; Wu, Q.; Welch, C.; Ho, L.; Weaver, C.; et al. Implications for treatment in Alzheimer ' s Disease. **2010**, *18* (1), 113–124.

(70) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* **1996**, *20* (7), 933–956.

**Table 1.** Total phenols, condensed tannins, and hydrolysable tannins in the extracts of red grape pomace (RGP), white grape pomace (WGP), and mixed grape pomace (WGP) treated with and without enzymes*

| | | Enzyme treatment | | | | P-value** | | |
|---|---|---|---|---|---|---|---|---|
| | **GP** | **Control** | **T** | **PC** | **TPC** | **GP** | **Enzyme** | **Interaction** |
| **Total phenols** | RGP | $50.9 \pm 1.0^{c}$ | $82.1 \pm 1.9^{a}$ | $51.8 \pm 1.0^{c}$ | $60.1 \pm 1.0^{b}$ | | | |
| (mg gallic acid eq./g | WGP | $37.3 \pm 0.5^{f}$ | $45.4 \pm 0.6^{d}$ | $42.8 \pm 0.7^{d,e}$ | $40.5 \pm 1.0^{e}$ | <0.0001 | <0.0001 | <0.0001 |
| DM) | MGP | $19.1 \pm 0.4^{i}$ | $25.5 \pm 0.4^{g}$ | $19.3 \pm 0.7^{i}$ | $22.7 \pm 0.5^{h}$ | | | |
| **Condensed tannins** | RGP | $28.6 \pm 0.9^{a}$ | $11.0 \pm 0.3^{d}$ | $21.5 \pm 0.8^{b}$ | $13.1 \pm 0.8^{c}$ | | | |
| (mg catechin eq./g | WGP | $9.6 \pm 0.5^{d}$ | $6.3 \pm 0.3^{f,g,h}$ | $7.7 \pm 0.3^{e,f}$ | $6.7 \pm 0.2^{e,f,g}$ | <0.0001 | <0.0001 | <0.0001 |
| DM) | MGP | $10.9 \pm 0.5^{d}$ | $6.0 \pm 0.3^{g,h}$ | $7.9 \pm 0.3^{e}$ | $5.0 \pm 0.2^{h}$ | | | |
| **Hydrolysable** | RGP | $4.8 \pm 0.1^{b}$ | $3.4 \pm 0.1^{b}$ | $4.0 \pm 0.1^{b}$ | $3.7 \pm 0.1^{b}$ | | | |
| **tannins** (mg tannic | WGP | $4.9 \pm 0.2^{a}$ | $3.7 \pm 0.2^{a}$ | $8.0 \pm 0.2^{a}$ | $5.3 \pm 0.2^{a}$ | <0.0001 | 0.17 | 0.12 |
| acid eq./g DM) | MGP | $1.4 \pm 0.1^{c}$ | $1.1 \pm 0.0^{c}$ | $1.4 \pm 0.0^{c}$ | $1.0 \pm 0.1^{c}$ | | | |

*Abbreviations: T (tannase), P (pectinase), C (cellulase)
** $^{a,b,c,d,e,f,g,h,i}$ Values (mean ± SD) within each analyte with different letters significantly differ ($P \leq 0.05$), tested using two-way ANOVA, followed by post-hoc Tukey HSD

**Table 2.** Content of phenolics (µg/g dry matter), measured using an HPLC assay, in the extracts of red grape pomace (RGP), white grape pomace (WGP), and mixed grape pomace (WGP) treated with and without enzymes*

| Compound | GP | Enzyme treatment | | | | P-value** | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | T | PC | TPC | GP | Enzyme | Interaction |
| **Rutin** | RGP | 84.6 ± 6.8 | n.d. | n.d. | n.d. | - | - | - |
| | WGP | 153.7 ± 9.6 | n.d. | 153.2 ± 13.0 | n.d. | | | |
| | MGP | 89.8 ± 4.4 | n.d. | n.d. | n.d. | | | |
| **Quercetin** | RGP | $35.0 \pm 2.2^{f}$ | $131.6 \pm 6.6^{c}$ | $38.7 \pm 2.1^{e,f}$ | $128.7 \pm 5.9^{c}$ | <0.0001 | <0.0001 | <0.0001 |
| | WGP | $9.4 \pm 0.9^{g}$ | $47.6 \pm 3.9^{e}$ | $18.8 \pm 0.8^{g}$ | $46.9 \pm 0.7^{e}$ | | | |
| | MGP | $44.1 \pm 2.9^{e,f}$ | $150.6 \pm 2.3^{b}$ | $98.3 \pm 1.4^{d}$ | $162.3 \pm 5.7^{a}$ | | | |
| ***Trans*-resveratrol** | RGP | n.d. | n.d. | n.d. | n.d. | - | - | - |
| | WGP | n.d. | n.d. | n.d. | n.d. | - | - | - |
| | MGP | $1.4 \pm 0.1^{c}$ | $13.9 \pm 0.4^{a}$ | $12.1 \pm 0.4^{b}$ | $14.3 \pm 0.4^{a}$ | - | <0.0001 | - |
| **Gallic acid** | RGP | $241.5 \pm 6.6^{g}$ | $668.4 \pm 3,3^{c}$ | $313.0 \pm 1.8^{e}$ | $607.0 \pm 9.0^{d}$ | <0.0001 | <0.0001 | <0.0001 |
| | WGP | $40.7 \pm 1.1^{j}$ | $853.9 \pm 6.2^{a}$ | $83.2 \pm 2.2^{i}$ | $729.2 \pm 13.4^{b}$ | | | |
| | MGP | $65.5 \pm 3.2^{i}$ | $290.7 \pm 9.5^{f}$ | $111.8 \pm 1.8^{h}$ | $285.0 \pm 5.5^{f}$ | | | |
| **Caffeic acid** | RGP | n.d. | $132.8 \pm 7.7^{b}$ | $122.2 \pm 8.7^{b}$ | $174.6 \pm 9.7^{a}$ | <0.0001 | <0.0001 | <0.0001 |
| | WGP | n.d. | n.d. | n.d. | n.d. | | | |
| | MGP | n.d. | $59.7 \pm 3.8^{d}$ | $108.8 \pm 6.8^{c}$ | $99.5 \pm 3.4^{c}$ | | | |

**Table 2.** (cont.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Catechin** | RGP | 1907.5 ± 34.6$^{b}$ | 1929.0 ± 52.5$^{b}$ | 2009.31 ± 21.19$^{a}$ | 1973.4 ± 17.6$^{a,b}$ | <0.0001 | 0.002 | 0.009 |
| | WGP | 801.7 ± 15.6$^{c}$ | 813.6 ± 10.1$^{c}$ | 804.9 ± 11.4$^{c}$ | 799.0 ± 15.5$^{c}$ | | | |
| | MGP | 589.0 ± 15.1$^{d}$ | 575.9 ± 12.0$^{d}$ | 616.0 ± 11.9$^{d}$ | 590.9 ± 15.6$^{d}$ | | | |
| **Procyanidin B2** | RGP | 1045.45 ± 33.3$^{a}$ | 1048.8 ± 54.9$^{a}$ | 1033.3 ± 39.5$^{a}$ | 1071.0 ± 17.7$^{a}$ | <0.0001 | 0.01 | 0.002 |
| | WGP | 243.7 ± 15.4$^{c,d}$ | 314.3 ± 26.6$^{b,c}$ | 338.0 ± 19.0$^{b}$ | 380.3 ± 19.1$^{b}$ | | | |
| | MGP | 209.4 ± 11.3$^{d,e}$ | 166.0 ± 5.8$^{e}$ | 177.1 ± 16.4$^{e}$ | 186.8 ± 12.4$^{d,e}$ | | | |

*Abbreviations: T (tannase), P (pectinase), C (cellulase)

** $^{a,b,c,d,e,f,g,h,I,j,k}$Values (mean ± SD) within each analyte with different letters significantly differ (P ≤0.05), tested using two-way ANOVA, followed by post-hoc Tukey HSD

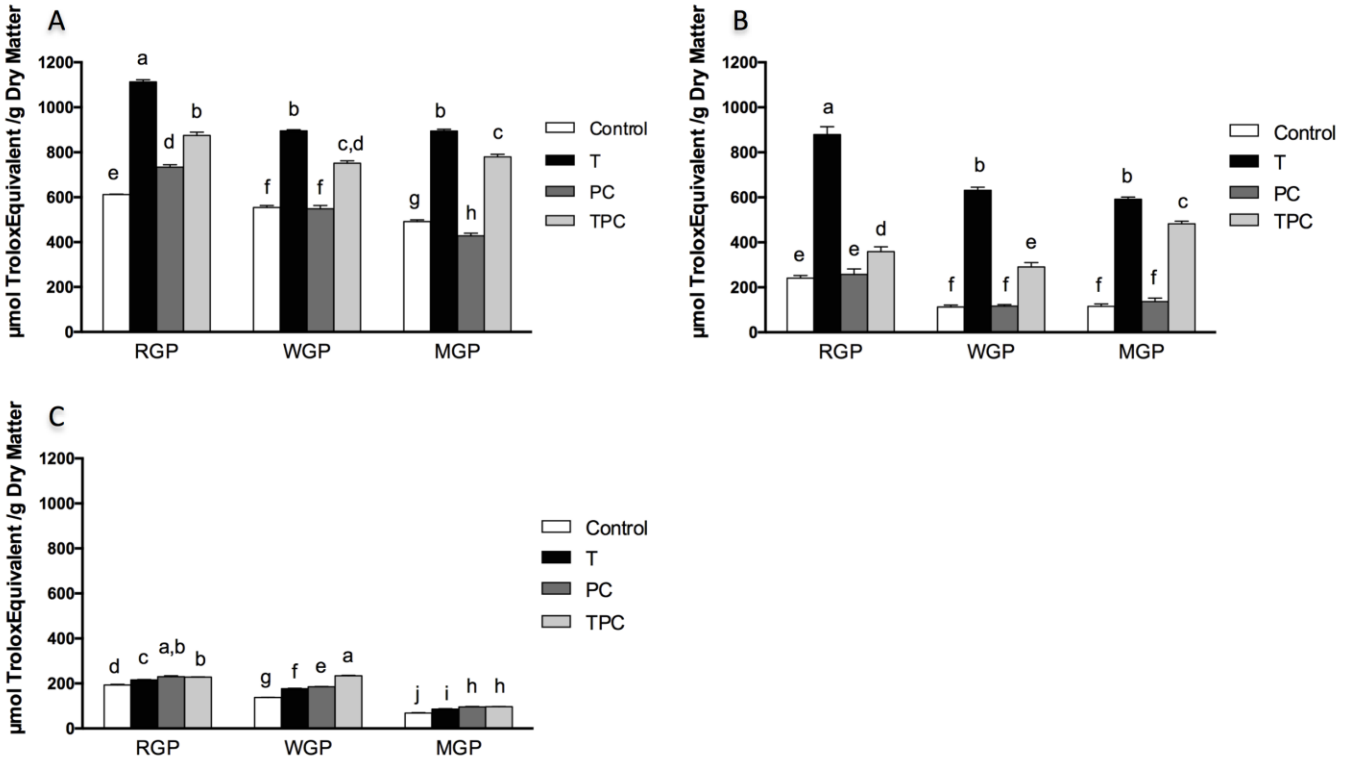


**Figure 1.** Antioxidant activity of the extracts of red grape pomace (RGP), white grape pomace (WGP), and mixed grape pomace (WGP) treated with and without enzymes, tested using DPPH assay (A), ORAC assay (B) and FRAP assay (C). Abbreviations: T (tannase), P (pectinase), C (cellulase), RGP (red grape pomace), WGP (white grape pomace) and MGP (mixed grape pomace). $^{a,b,c,d,e,f,g,h,i,j}$ Values (mean ± SD) within each analyte with different letters significantly differ (P ≤0.05), tested using two-way ANOVA, followed by post-hoc Tukey HSD. In the two-way ANOVA test, GP, enzyme, and their interaction were all statistically significant (P <0.0001).

## ARTIGO III

# Tannase treated grape pomace attenuates IL-1β induced inflammation in Caco-2 cells

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# Tannase treated grape pomace attenuates IL-1β induced inflammation in Caco-2 cells

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**Abstract**

Grape pomace (GP) is rich in polymeric phenolic compounds and glycosides, which are known to have lower bioefficacy than monomers and aglycones. The aim of this study was to evaluate if GP treated with tannin acyl hydrolase (tannase), would improve antioxidant and anti-inflammatory actions in Caco-2 cells. Grape pomace after tannase treatment (GPT) had 45% larger quercetin content and 71% less quercetin-3-O-rutinoside than GP. Also, the phenolic acids analyzed increased an average of 105% after biotransformation. Further, GPT had 51% more total phenols than GP and displayed 71, 249, and 12% larger DPPH, ORAC, and FRAP values, respectively. However, GP and GPT at 100 and 200 µg/mL (dry extract wt/v) displayed comparable efficacy in the reduction of ROS production in Caco-2 cells treated with or without 50 µM AAPH. After 24-h pretreatment, GPT at 200 µg/mL decreased IL-1β-induced $PGE_2$ production by 110% and IL-8 by 83% and down-regulated NF-κB activation by 68%, as compared to control. Overall, GPT exhibited more potent efficacy than GP in the amelioration of IL-1β-induced inflammation in Caco-2 cells. In conclusion, tannase treatment appears to enhance antioxidant and anti-inflammatory activities of grape pomace phenolics.

**Keywords:** Grape pomace; Tannase, Phenolic compounds; Caco-2, Gut inflammation.

## 1. Introduction

Food residues generated during production of processed foods, e.g, wines, orange and grape juices, vegetable oils, dairy, cereals, are historically underutilized. Recently, there is increasing appreciation of these food residues for being used as ingredients for biotechnological applications, i.e., production of enzymes, organic acids and bioactives. Besides using them for production of value added products, these utilizations reduce the detrimental impacts on environments caused by waste disposal, e.g., plant germination inhibition by immobilization of soil nutrients, the increase of chemical and biological oxygen local requirements, and impacts in the fauna and flora of discharge zones (Spigno; De Faveri, 2007; Galanakis, 2012).

Residues [grape pomace (GP)] generated during the production of wine and grape juice contain fiber and phenolic compounds (Kammerer, Claus, Carle, & Schieber, 2004; Mildner-Szkudlarz, Bajerska, Zawirska-Wojtasiak, & Górecka, 2013). Polyphenolics in GP have been reported to possess anti-inflammatory (Wang et al., 2011), anti-proliferative (Jara-Palacios et al., 2015), and antimicrobial activities (Friedman, 2014; Sagdic, Ozturk, & Kisi, 2012). These putatively beneficial attributes allow GP to be formulated into food products or dietary supplements for health promotion and prevention (Jara-Palacios et al., 2015; Mildner-Szkudlarz et al., 2013; Sánchez-Alonso et al., 2008; Walker et al., 2014; Wang et al., 2011).

Unfavorable lifestyle changes occurring in industrialized and developing countries, including dietary habit are associated with increased risk of chronic diseases (WHO, 2002), and cancer (Macdonald & Monteleone, 2005). Inflammatory Bowel diseases (IBD's) are common and incurable chronic inflammatory diseases that affects 0.5% the Western world general population (Molodecky et al., 2012). These intestinal inflammation generates elevated production of cytokines and reactive oxygen species (ROS) by activated leukocytes in the gut epithelium (Biasi et al., 2013), being regulated by the NF-κB transduction pathway (Romier, Schneider, Larondelle, & During, 2009; Romier-Crouzet et al., 2009). While clinical evidence remains lacking, preclinical evidence suggested polyphenols and other constituents might attenuate inflammation in the gut (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; González-Gallego, García-Mediavilla, Sánchez-Campos, & Tuñón, 2010; Russell & Duthie, 2011). For example, proanthocyanidins in

grape seed ameliorated colitis via modulation of the NF-κB signal pathway in rats (Wang et al., 2011), grape seed and red wine extracts decreased IL-8 secretion in Caco-2 cells (Biasi et al., 2013; Romier-Crouzet et al., 2009; Shin et al., 2015), and raisins and white grape pomace inhibited Caco-2 proliferation (Jara-Palacios et al., 2015; Kountouri et al., 2012). These results suggest polyphenols in plant foods and residues derived processed food productions may be protective against inflammatory conditions in the gut.

Polyphenols in nature are generally present in polymeric, glycosidic or esteric forms, which are appreciated to interfere their bioavailability and decrease bioefficacy (Holst & Williamson, 2008). Thus, approaches to transforming polyphenols, e.g., fermentation and enzymatic hydrolysis, to aglyconic form may help enhance their bioefficacy in the gut or whole body (Scalbert & Williamson, 2000). For example, *Paecilomyces variotti* tannin acyl hydrolase (tannase) was reported to cleave the ester and depside bonds between conjugates and polyphenol aglycones, such as tannic acid, epicatechin gallate, epigallocatechin gallate, and chlorogenic acid (Battestin, Macedo, & De Freitas, 2008; García-Conesa, Østergaard, Kauppinen, & Williamson, 2001; Macedo, Battestin, Ribeiro, & Macedo, 2011; Macedo et al., 2012). Furthermore, biotransformed polyphenols in tea infusions and orange juice mediated by this enzyme, resulting in significant increases in their antioxidant capacity and chemopreventive potential (Ferreira, Macedo, Ribeiro, & Macedo, 2013; Macedo et al., 2012). Thus, the aim of this study was to examine whether constituents in the GP treated with *Paecilomyces variotii* tannase (GPT) (Battestin & Macedo, 2007b) would be more potent to diminish IL-1β-induced inflammation in Caco-2 cells than untreated GP.

## 2. Material and Methods

### 2.1. Reagents

Gallic acid, catechin, epicatechin, caffeic acid, rutin (quercetin-3-O-rutinoside), quercetin, myricetin, ferulic acid, vanillic acid, ρ-coumaric acid, 3,4-dihydroxyphenil acetic acid, protocatechiuc acid, interleukin 1-beta (IL-1β), dimethyl sulfoxide (DMSO), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), bovine serum albumin (BSA), arachidonic acid (AA), 2,2-diphenyl-1-

picrylhydrazyl (DPPH), 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 4,6-tripyridyl-s-triazine (TPTZ), methanol and acetonitrile (ACN) were purchased from Sigma-Aldrich (St. Louis, MO, USA); ammonium sulfate, sodium acetate, and fluorescein from Ecibra (São Paulo, Brazil); tannic acid from Ajinomoto OmniChem Division (Wetteren, Belgium); monobasic and dibasic sodium phosphate from LabSynth (Diadema, Brazil); fetal bovine serum (FBS) from ATCC (Rockville, MD, USA), advanced Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, L-glutamine and HBSS from Life Technologies (Grand Island, NY, USA); 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-$H_2$DCFDA) probe (Life Technologies, Grand Island, NY, USA); Bicinchoninic acid (BCA) kit and sandwich human interleukin-8 (IL-8) ELISA Kit were purchased from Pierce Biotechnology (Rockford, IL, USA), cyclooxygenase 2 (COX-2) ELISA kit (EMD Millipore, Bedford, MA, USA), nucleic acid extraction kit, NF-κB p65 ELISA kit and prostaglandin $E_2$ ($PGE_2$) enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA); phosphate buffer saline (PBS) and radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific, Waltham, MA, USA).

### 2.2. Grape pomace

Red grape pomace (GP) was obtained from a winery in Valinhos, SP, Brazil during the wine production period between January and March 2013. GP comprised skins, seeds, and stems, as well as microbiota derived from the fermentation during wine production. The grape variety was "Maximo IAC 138-22" which is a hybrid of Syrah (75%) and Seibel (25%) varieties. After obtained, GP was frozen at -20°C, lyophilized, grounded, and then stored at -80°C.

### 2.3. Production of tannase extract

Tannase was produced by *Paecilomyces variotii* using a previously published procedure (Battestin & Macedo, 2007a; Battestin & Macedo, 2007b) with minor modifications in the culture medium, prepared by mixing 20 g wheat bran, 20 mL distilled water, and 10% tannic acid (w/w) in a 250-mL conical flask. After

sterilization of the medium, 5 x $10^7$ spores/mL of the pre-inoculum suspension (*Paecilomyces variotii*) were inoculated. After fermentation for 120 h at 30°C, 160 mL of 20 mM acetate buffer (pH 5.0) was added, following by shaking at 200 rpm for 1 h. Supernatant was generated after filtration and centrifugation at 9650 xg for 30 min at 4°C (Beckman J2-21 centrifuge, Beckman-Coulter, Inc. Fullerton, CA, USA). The supernatant was then treated with solid ammonium sulfate (80% saturation) at 4°C for overnight. After centrifugation (9650 xg for 30 min), resuspension, and dialysis with distilled water for 48 h, semi-purified tannase extract was produced after lyophilized.

### 2.4. Tannase treatment of grape pomace

Grape pomace (10 g) was incubated with 500 mg (1.2 U/mg) tannase extract in 100 mL of 0.02 M sodium acetate buffer (pH 5.0) for 5 h at 40 °C. The administered hydrolysis condition was optimized in our previous study (Battestin & Macedo, 2007a). At the end of the incubation, the mixture was chilled in an ice bath for 15 min to inhibit the enzyme activity. Subsequently, polyphenolics in the resulting mixture were incubated in 240 mL methanol for 60 min at room temperature, followed by 10-min sonication in a water-bath sonicator and centrifugation at 9000 xg for 15 min (Daigger model SD, Illinois, USA). The supernatant was collected, dried under $N_2$ air, lyophilized, and stored at -80 °C until use. The dry extract was reconstituted in PBS for tests.

### 2.5. Total polyphenolic content and antiradical activities

Total polyphenolic content was determined by the method of Singleton & Rossi (1965) and the results were expressed in mg gallic acid equivalent (GAE)/mg dried extract (DE). Antioxidant capacity was assessed using 3 assays, i.e., DPPH radical scavenging activity (DPPH), oxygen radical absorbance capacity (ORAC) and ferric-reducing antioxidant power (FRAP) assays (Benzie & Strain, 1996; Brand-Williams, Cuvelier, & Berset, 1995; Dávalos, Gómez-Cordovés, & Bartolomé, 2004; J. A. Macedo et al., 2011; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Peschel et al., 2006; Prior et al., 2003). The values are expressed as µmol Trolox equivalent (TE)/mg DE.

## 2.6. Detection and quantification of polyphenolic compounds (HPLC-ECD)

Polyphenolics were profiled using an high performance liquid chromatography (HPLC) system equipped with an electrochemical detector (ECD) (ESA Inc., Chelmsford, MA) and an Agilent ZORBAX SB-C18 column (5 µm, 4.6 mm × 250 mm, Santa Clara, CA), according to the method of Chen, Milbury, Lapsley, & Blumberg (2005). Analyte separation was achieved using 0.6 - 1.0 mL/min flow rate in a gradient mode. Mobile phase A comprised 75 mmol/L citric acid and 25 mmol/L ammonium acetate in 95% $H_2O$ and 5% acetonitrile (ACN) and mobile phase B comprised 75 mmol/L citric acid and 25 mmol/L ammonium acetate in 50% $H_2O$ and 50% ACN. The mobile phase gradient profile was (% solvent B): 1% (0–12 min), 1–10% (12–32 min), 10–30% (32–44 min), 30–40% (44–65 min), 40–50% (65-75 min), 50–60% (75-82 min), 60–80% (82-92 min), 80–0% (92-95 min), 0% (95-130 min). Detection was achieved with potentials applied from 60 to 720 mV with 60-mV increments. The identification of individual compounds (gallic acid, catechin, epicatechin, caffeic acid, rutin, quercetin, myricetin, ferulic acid, vanillic acid, ρ-coumaric acid, 3.4-dihydroxyphenilacetic acid, protocatechiuc acid) was achieved by comparing retention time and electrochemical response with authenticated standards. The quantity was calculated using standard curves constructed with authenticated standards.

## 2.7. Cell Culture

### 2.7.1. Caco-2 cells maintenance

Human colon adenocarcinoma-derived Caco-2 cells were obtained from the American Type Culture Collection (ATCC). The passages between 25 and 40 were used in the experiments. The cells were grown in 75 $cm^2$ flasks in DMEM supplemented with 10% FBS, 1% antibiotics (penicillin and streptomycin) (v/v), 1% L-glutamine and 10 mM of HEPES buffer at 37 °C in a humidified atmosphere with 5% $CO_2$. Culture medium was changed 3 times a week and cells were passaged at 75–85% confluent.

### 2.7.2. Cell viability

Cell viability was evaluated using the MTT assay, according to Mosmann (1983) method with minor modifications. Caco-2 cells (1 x $10^5$/well) were seeded in 24-well plates for 24 h. After the removal of the medium, the cells were treated with GP extracts in DMEM 0% FBS at selected concentrations (50-1000 µg/mL) for 48 h. At the end of the treatment, the medium was removed and replaced with 0.5 mg/mL MTT in DMEM without FBS. After 3-h incubation at 37 °C, formazan crystals were dissolved in 200 µL DMSO. Absorbance was measured at 570 nm using FLUOstar OPTIMA plate reader (BMG Labtech, Durham, NC, USA). Data were expressed as percentage of control.

### 2.7.3. Intracellular reactive oxygen species (ROS)

Intracellular ROS content was measured using an oxidant-sensitive fluorescent probe (Wang & Joseph 1999). Briefly, Caco-2 cells (3 x $10^4$/well) were seeded in 96-well black microplates. Once cells reached 80% confluence, the culture medium was replaced with HBSS containing 10 µmol/L CM-$H_2$DCFDA probe and incubated for 30 min at 37 °C. After washed with PBS to remove unabsorbed CM-H2DCFDA, the cells were treated with GP and GPT in HBSS at 2 concentrations (100 and 200 µg/mL) with and without (as control) 50 µmol/L AAPH as a ROS producer for 5 h. Fluorescence at 490ex and 520em were monitored at intervals of 60 min. Results were expressed as the percent increase in fluorescence from the respective baseline value.

### 2.7.4. IL-1β-induced inflammation

IL-1β is a cytokine known to play a major role in the initiation and amplification of inflammation in inflammatory bowel diseases (IBDs), and its exposure stimulates biomarkers and mediators of inflammation in Caco-2 cells (Martin & Wallace, 2006). Thus, inflammation was induced by treating Caco-2 cells with 1 ng/mL IL-1β in DMEM 0% FBS for 24 h. The IL-1β dose and treatment time were selected based on the data of a preliminary experiment (data was not shown).

The anti-inflammatory effect of GP extract at 100 or 200 µg/mL was evaluated in 2 conditions: pretreatment (prior to the IL-1β exposure) and pretreatment plus cotreatment. The detailed information on the study treatments is presented in **Fig. 1**. The post-treatment condition was also performed, but the preliminary results were not significant (data was not shown). The concentrations tested are physiologically relevant as we took the intestinal fluid volume into account (~100 mL) after the ingestion of 240 mL of water (Schiller et al., 2005; Mudie et al., 2014). Considering the average fasting and postprandial liquid volumes in the small and in the large intestine, 200 µg/mL GP was achievable when 20 mg of the extract was consumed, which was ~100 mg dried grape pomace.

Pre-confluent Caco-2 cells (2 x $10^5$ cells/well) were grown in 24-well plates for 24 h and then treated with GP for 6 or 24 h. Subsequently, the cells were exposed to IL-1β for 24 h. At the end of the treatments, media were collected for IL-8 quantification by the sandwich human IL-8 ELISA Kit, according to the manufacturer's instructions. Absorbance was measured at 450 nm. Values were calculated using a standard curve constructed with a recombinant human IL-8 standard. After the adjustment with protein content of cell lysates, results were expressed as percentage change from control, obtained using the following formula:

*% = [(sample value - negative control) - (positive control - negative control)/(positive control - negative control)]*100*

For $PGE_2$ measurement, Caco-2 cells (2 x $10^5$ cells/well) were grown in 24-well plates as the same way for the IL-8 measurement. At the end of the treatments, the cells were rinsed twice with HBSS, pH 7.4. Then, the cells were incubated in HBSS containing 0.1% (w/v) BSA and 10 µmol/L arachidonic acid for 30 min at 37°C. $PGE_2$ in the HBSS was determined using the $PGE_2$ enzyme immunoassay kit according to the manufacturer's instructions. Absorbance was measured at 420 nm. $PGE_2$ concentrations were calculated using a standard curve constructed with $PGE_2$ standard, followed by protein adjustment. The results are expressed as percentage change from control using the same formula for IL-8.

To assess COX-2 protein expression, Caco-2 cells (5 x $10^5$/well) were cultivated in 6-well plates for 24 h, followed by the GP and IL-1β treatments as the above-mentioned protocol. GP concentrations were the same but IL-1β was 20

ng/mL. IL-1β concentration was chosen after pre-tests and was found to be the best for COX-2 production. At the end of the treatments, cells were lysed using RIPA buffer in ice for 5 min in an orbital shaking platform. COX-2 protein in the cell lysate was determined using COX-2 ELISA kit, according to the manufacturer's instructions. Absorbance was measured at 450 nm. COX-2 concentrations were calculated using a standard curve constructed with a COX-2 standard, followed by protein adjustment. The results were expressed as percentage change from control using the same formula for IL-8.

To evaluate NF-κB activation, Caco-2 cells were grown and treated in the same manner for the COX-2 determination. At the end of the treatments, cells were scraped from the wells, followed by nucleic acids extraction using a nucleic acid extraction kit. The resulting nuclear fractions were used for the quantification of NF-κB p65 protein using NF-κB p65 ELISA kit, according to manufacturer's instructions. The final optical density at 450 nm is proportional to the quantity of the total NF-κB p65 protein. After protein adjustment, results were expressed as percentage change from control.

Protein concentration in cell lysates was determined using BCA protein assay and BSA used as the standard, according to the manufacturer's instructions.

### 2.8. Statistical analysis

Results were expressed as mean ± standard deviation (SD). One-way ANOVA was performed to examine statistical significance on the data of total polyphenols, antiradical activities, polyphenolic quantification, cell viability, and cellular ROS production. If significance was obtained, Tukey-Kramer HSD multi-comparison test was performed. For the inflammatory biomarkers detections, a three-way ANOVA with enzyme, time, and dose as the independent variables was performed to analyze significance, followed by Tukey-Kramer HSD multi-comparison test. P-value ≤0.05 was considered statistically significant. The Pearson's correlation test was used to analyze the correlation between variables.

## 3. Results

### 3.1. Tannase effect on GP total polyphenols, antioxidant capacity, and polyphenolic profile

The tannase treatment increased total polyphenols, DPPH, ORAC, and FRAP by 51, 71, 242 and 12%, respectively, as compared to GP (**Table 1**). Using the HPLC-ECD method, we quantified 6 phenolic acids and 5 flavonoids. Among the quantified flavonoids, catechin and epicatechin were predominant and the content of these 2 flavanols were not altered by tannase. In general, the phenolic acids analyzed had increased 105%. The enzyme treatment increased gallic, protocatechuic, 3,4-dihydroxyphenylacetic, and vanillic acids, myricetin, and quercetin by 124, 51, 221, 100, 84, and 45%, respectively, as compared to the untreated GP. Caffeic and ρ-coumaric acids became detectable after tannase treatment, and rutin was decreased by 71%.

### 3.2. Cell viability after GP and GPT treatments

Toxicity of GP extract was assessed using the MTT assay in Caco-2 cells treated with the concentrations ranging from 50 to 1000 µg/mL for 48 h. The vehicle used (PBS) did not affect cell viability. Both GP and GPT at concentrations ≤200 µg/mL did not change cell viability ($P > 0.05$) (**Fig. 2**). At 250 µg/mL, GP significantly reduced cell viability, contrary to GPT. At highest concentrations, GP and GPT significantly reduced Caco-2 cell viability by 65%. Thus, the concentrations of 100 and 200 µg/mL (less than 5% toxicity) of GP and GPT were selected to assess the beneficial effects in Caco-2 cells.

### 3.3. GP and GPT present cellular ROS scavenging activity

Using $H_2$DCFDA as a probe, the ROS level in Caco-2 cells cultured in HBSS for 5h was increased by 49% as compared to the baseline value. Both GP and GPT at concentrations of 100 and 200 µg/mL displayed similar efficacy in significantly decreasing endogenous ROS level by ~17% as compared to the negative control at 5h (**Fig. 3A**).

We observed Caco-2 cell treatment with IL-1β did not result in a highly significant increase in ROS production (data not shown). Thus, AAPH (50 µmol/L) was employed to increase ROS levels and test radical scavenging activity of GP constituents. AAPH (positive control) enhanced ROS production by 142% compared to negative control after 5h incubation (**Fig. 3B**). When Caco-2 cells were treated with AAPH and GP extracts simultaneously for up to 5h, the AAPH-induced ROS production was completely abolished.

### 3.4. Effects on inflammation biomarkers in IL-1β treated Caco-2 cells

The effects of GP extracts on the IL-1β-dependent activation of NF-κB were assessed by evaluating the nuclear translocation of the NF-κB p65 subunit. After the stimulus (IL-1β 20 ng/mL), the detection of NF-κB p65 in the nuclear extract increased 223% compared to negative control. IL-1β, as expected, caused activation and nuclear translocation of NF-κB p65. In pretreatment, enzyme and time were significant factors on reducing NF-κB p65 level. GPT was responsible for an average inhibition of 48% nuclear translocation of NF-κB p65 in comparison to GP (27%) and the 24-h pretreatments resulted in an average decrease of 49% the activation of the transcription factor, larger than 6-h pretreatments (26%) (**Fig. 7A**). Overall, the best inhibition was around 63% after pretreatment with GPT for 24h, independent on the dose. In pre/cotreatment, there was no significant difference in the effects of dose, time or enzyme on NF-κB p65 activation. All treatments in all conditions resulted in ~33% decrease of the transcription factor activation (**Fig. 7B**), but lower than pretreatments.

A preliminary experiment was first performed to optimize treatment duration and dose of IL-1β in the induction of IL-8 production in Caco-2 cells (data not shown). To assess the GP and GPT effect on the IL-8 production, Caco-2 cells were treated with 1 ng/mL IL-1β for 24 h, resulting in 3.1 ng IL-8/mg protein. Without the IL-1β treatment, IL-8 was below the limit of quantification of the kit. After pretreatment, we can observe that dose, enzyme, and time were considered significant factors on changing IL-1β-derived IL-8 levels (**Fig. 4**). The best inhibition was achieved after pretreatment with 200 µg/mL GPT for 24h, decreasing IL-8 detection by 83%, followed by pretreatment with 200 µg/mL GP for 24h (-59%) and pretreatment with 200 µg/mL GPT for 6h (-51%) (**Fig. 4A**). In all test conditions,

pretreatments with 100 µg/mL GP/GPT did not inhibit IL-1β-induced IL-8 production. The pre/cotreatment resulted in a homogeneus decrease in IL-8, independently of dose, being GPT treatment for 24h significantly more effective, decreasing IL-8 levels by 70%. The other conditions were not significantly different from each other, but inhibited IL-8 production in ~44% (**Fig. 4B**). NF-κB p65 activation had a significant and positive correlation (r=0.3590, p=0.0122) with IL-8 production, detected after performing the Pearson's correlation coefficient analysis.

The induction of $PGE_2$ production by Caco-2 cells was performed using 1 ng/mL of IL-1β in DMEM 0% FBS for 24h and subsequently, the cells were exposed to HBSS with AA. The transformation of AA into $PGE_2$ is related to the activity of COX-2 enzyme produced by Caco-2 cells. The basal $PGE_2$ level secreted by the unstimulated cells was 652.73 pg/mg of protein. In cells treated with IL-1β for 24h (positive control), the secretion of $PGE_2$ was increased in 214%. For the COX-2 protein evaluation, it was necessary a higher number of cells (6-well plates) and also higher IL-1β concentration (20 ng/mL) to promote a detectable increase of COX-2 in 24h. The baseline production of COX-2 was 3.77 ng/mg protein and after IL-1β challenge, this value increased 228%. In pretreatment, the dose and the interaction enzyme-dose were highly significant on reducing $PGE_2$ values in Caco-2 cells (**Fig. 5**). The $PGE_2$ detection shows the higher concentration was more effective on attenuating the biomarker (average of 95% decrease after 200 µg/mL *vs.* 40% decrease after 100 µg/mL), in agreement with IL-8 results (**Fig. 5A**). The best inhibition detected was after pretreatment with GPT 200 µg/mL (decrease of 107% $PGE_2$), followed by pretreatment with GP 200 µg/mL (reduction of 79% $PGE_2$ production). As the time factor was not statistically significant, we did not consider the differences between 6h and 24h treatments. In the pre/cotreatment, all different treatment conditions resulted in significant decreases in $PGE_2$ production (~88%), but no factor resulted in significant influence in the effects (**Fig. 5B**). IL-8 and $PGE_2$ had a strong positive correlation (r=0.8486, p<0.0001), detected after performing the Pearson's correlation coefficient analysis.

Contrary to expected, we could not note the same inhibitory action of GP extract treatments on COX-2 protein detection (**Fig. 6**). For both pretreatment and pre/cotreatment conditions, the significant factors influencing changes in COX-2 were the same: dose, time and interaction enzyme-dose-time. In both treatments, the higher concentration and 6-h treatments were responsible for the larger increases in

COX-2 (~64% and 47%), respectively. In general, GP and GPT extracts at 100 µg/mL maintained similar COX-2 protein levels as in positive control, while the higher concentration treatments enhanced this protein levels.

## 4. Discussion

Growing evidence suggests important roles of dietary factors in preserving health and even reversing the progression of chronic diseases, with anti-inflammatory effects as important underlying mechanisms. In the present study, we characterized polyphenolic compounds of GP before and after performing an enzymatic treatment using tannase, and also we evaluated their antioxidant activity. The tannase treatment increased total phenols amounts and antioxidant activity, also promoted the release of low molecular weight polyphenolics, especially gallic acid and vanillic acid, some of them being only detected after the enzymatic treatment, i.e., caffeic acid and ρ-coumaric acid. The enzyme also hydrolyzed glycoside polyphenolics, releasing aglycones, demonstrated by the decrease in rutin levels and increase in quercetin. Some of these compounds have higher antioxidant properties, possibly being more bioavailable and bioactive (Crozier, Del Rio, & Clifford, 2010; Rice-Evans, Miller, & Paganga, 1997). Accordingly, a previous tannase biotransformation showed an increase of 41% total polyphenols in grape seed extract (GSE) and 29% in grape pomace extract (GPE), also improving antiradical (DPPH) activity in 1.4% and 16.7% in GSE and GPE, respectively (Chamorro, Viveros, Alvarez, Vega, & Brenes, 2012). Similar effects were previously observed on grapes skins and seeds after β-glucosidase, pectinolytic and cellulolytic enzyme treatments (Arnous & Meyer, 2010; Xu et al., 2014).

After obtaining positive effects on GPT total polyphenolics and antioxidant properties, this study also aimed at determining if the GPT could, better than GP, inhibit anti-inflammatory biomarkers in an *in vitro* model of human inflamed intestinal epithelium using undifferentiated Caco-2 cells, that display a colonocytic phenotype (Sambuy et al., 2005), as the main bioactivities in the intestinal mucosa may occur in the colon due to low detection of post-prandial polyphenolic concentrations in plasma (Russell & Duthie, 2011). The intestinal production of pro-inflammatory cytokines and chemokines is a physiological process, with the purpose of activating the immune and intestinal epithelial cells in acute situations. However, this procedure can

become chronic, as happens during IBDs. Besides, the oxidative imbalance in the gastrointestinal tract is also considered a strong influence for IBDs development (Lam et al., 2015).

The imbalance in intracellular ROS is also recognized as an important component in inflammation, capable of perpetuate and amplify inflammatory pathways (Matias et al., 2014). The determination of intracellular ROS in Caco-2 cells can demonstrate the efficacy of food polyphenolics oxidative protection in the intestine. GP and GPT at both concentrations tested displayed comparable efficacy in the reduction of ROS production in Caco-2 cells treated with or without AAPH. AAPH (2,2'-azobis-(2-amidinopropane dihydrochloride)) is a water-soluble, heat-labile azo compound, which undergoes thermal decomposition to produce carbon-centred free radicals that may react with oxygen to produce alkoxyl and peroxyl radicals (Krishna et al., 1994). Without the AAPH, the extracts lead to lower ROS reductions, probably because only the polyphenolics compounds that could reach the intracellular compartment could prevented ROS formation. Using the ROS inducer, the effects of the polyphenolic compounds may occur in the intra and extracellular compartments, also preventing radicals to permeate through to Caco-2 membranes, or inhibit the formation of AAPH-induced radicals in the surrounding of intestinal epithelial cells, by removing precursors of free radicals or by inactivating catalysts, resulting in a bigger protection (Matias et al., 2014). These protective effects of polyphenolics are in agreement with previous results (Pinent et al., 2015; Ramos, Rodríguez-Ramiro, Martín, Goya, & Bravo, 2011), where proanthocyanidins from grape seeds and flavanols standards effectively decreased ROS detected in Caco-2 cells. The capacity of GP and GPT on decreasing ROS may be a possible key factor related to the inactivation of NF-κB transcription factor and the modulation of the secretion of inflammatory biomarkers.

Multiple evidences indicate NF-κB transcription factor is related to the progress of inflammation and cancer. The inactive form of NF-κB is the cytosolic p65/p50 heterotrimer, with the p65 subunit, containing the transcriptional activation domain, bound to the IκB inhibitory protein. After stimulation, IκB is phosphorylated leading to the entrance of NF-κB p65 subunit into the nucleus, and starting the transcription of various inflammatory and immunoregulatory genes (Karin, 1999). Blocking this activation lead to a repression of the transcription of these genes and reduce the inflammation (Kaulmann & Bohn, 2014). In our study, both GP and GPT

were able to suppress the translocation of the p65 subunit of NF- κB from the cytosol to the nucleus in IL-1β-stimulated intestinal cells, being the 24-h pretreatment with GPT the most effective treatment to decrease activated NF-κB p65 levels (63%), compared to control. In the 24-h pre/cotreatment the inactivation was similar to the 6-h treatments, suggesting a bigger influence of the compounds present in the sample and their bioavailability, not the time of exposure. Also, in this case, the cotreatment with IL-1β after the pretreatment with the extracts did not improve the inactivation of NF-κB p65. Other polyphenolic extracts previously inhibited NF-κB activation or expression, e.g., ACN-fraction of blueberries, hydrophilic pistachio extract, grape powder extract and grape juice reduced NF-κB expression in Caco-2 cells, adipocytes and *in vivo* (Campanholo et al., 2015; Chuang et al., 2011; Gentile et al., 2014; Taverniti et al., 2014).

Previously related to NF-κB inflammatory pathway, IL-8 is the most common chemokine studied in gut inflammation due to elevated production by neutrophils and recruited macrophages in IBDs, suggesting a mechanism for a continuing cycle of neutrophil attraction. In particular, Caco-2 cells have a high secretion level of IL-8, whose expression is known to be dependent on IL-1β activation of NF-κB (Garat, 2003). Our results illustrated that constituents in GP and GPT appeared to be capable of modulating this pathway. Accordingly, hydrophilic pistachio extract, chlorogenic acid, caffeic acid and anthocyanins from grape extracts previously demonstrated the same effect (Chuang et al., 2011; Gentile et al., 2014; Kuntz et al., 2015; Shin et al., 2015). The statistically significant correlation between IL-8 and NF-κB link the NF-κB inactivation to the decrease of IL-8, as also noted before (Hoffmann, Dittrich-Breiholz, Holtmann, & Kracht, 2002). These findings show, considerably in part, the anti-inflammatory capacity of GP extracts is mediated by the inhibition of the NF-κB pathway.

Besides pro-inflammatory cytokines and chemokines, other mediators are released under inflammatory conditions, i.e., prostaglandins, lipid autacoids generated from arachidonate by the action of COX isoenzymes, induced by inflammatory stimuli and mediators of many biological functions. $PGE_2$ is one of the prostaglandins that is significantly increased in the inflamed tissue and contributes to development of inflamed sign of redness, swelling, and pain (Ricciotti & FitzGerald, 2011). Our results show a decrease in $PGE_2$ levels after treatments with GP and GPT, but contrary to expectations, the COX-2 protein detection was not decreased,

and contrarily, after the higher dose treatments, it was increased, suggesting COX-2 expression and activity (as shown by $PGE_2$ production) could be regulated differently. The decrease in $PGE_2$ levels in Caco-2 cells were previously detected after treatments with grape seed extract and resveratrol (Cianciulli et al., 2012; Romier-Crouzet et al., 2009). Also, the inhibition of COX-2 expression was demonstrated by the action of hydrophilic pistachio extract in Caco-2 cells, grape powder extract in adipocytes, and raisins methanolic extract in HT29 cells (Chuang et al., 2011; Gentile et al., 2014; Kountouri et al., 2012). Furthermore, in an *in vivo* study, grape pomace fed rats had COX-2 protein levels decreased in liver (Nishiumi, Mukai, Ichiyanagi, & Ashida, 2012). In the present study, the action of GP and GPT could happen post-transcriptionally on COX-2, blocking part of its activity detected by the decrease of $PGE_2$ (107%), but not decreasing COX-2 protein in Caco-2 cells. Studies detected that some COX-2 inhibitory drugs also present this same property. In a murine model of metastatic breast cancer, despite the 95% inhibition of $PGE_2$ synthesis by indomethacin and NS398, the COX-2 enzyme levels (protein and mRNA) were increased in a dose-dependent manner by both drugs (Kundu, Yang, Dorsey, & Fulton, 2001). In a different study, although it was observed the tocopherols inhibited COX-2 activity, they had no effect on COX-2 expression in Caco-2 cells. Also, quercetin and quercetin-3-sulfate demonstrated to not affect COX-2 expression (O'Leary et al., 2004), and myricetin and epigallocatechin, did not suppress the transcriptional activity of COX-2 in a DLD-1 cell line (Mutoh et al., 2000), suggesting an influence of the compound chemical structure (flavonoids with 3', 4', and 5' hydroxyl groups in the B ring), which may explain these discrepancies. Another explanation to the decrease in $PGE_2$ levels without decreasing COX-2 after GP and GPT treatments could be the upregulation of the degradation of $PGE_2$ by 15-hydroxy prostaglandin dehydrogenase, or the suppression of cellular $PGE_2$ biosynthesis by inactivation of microsomal prostaglandin $E_2$ synthase-1, that catalyzes the transformation of $PGH_2$ to $PGE_2$ in the same AA cascade, as shown by the action of vitamin D and epigalocatechin-gallate, also failed to inhibit COX-2, but downregulated $PGE_2$ in cell culture models (Liu et al., 2014; Koeberle et al., 2009). Further studies are necessary to understand the action of phenolic compounds in AA cascade and confirm these inhibitory effects.

In general, both treatment conditions were effective on decreasing IL-8, $PGE_2$ and NF-κB. After pretreatment we observed a significantly larger anti-inflammatory effects by GPT at higher concentration. Further, after pre/cotreatment, mostly both concentrations of the extracts had similar actions, showing that a longer exposure to the polyphenolics, even concomitantly with IL-1β, have generated similar decreases in the biomarkers, independent on the treatment or dose applied. The study of the relationship and interaction between food dietary components and the gut mucosa will help to explain the details of the benefits from consuming plant foods and also, to show the importance of the valorization of agroindustrial residues rich in bioactive compounds for the production of new value-added products.

## 5. Conclusions

Tannase treatment improved GP total polyphenols, antioxidant activity, releasing monomeric polyphenolics and aglycones, which may be related with a larger bioavailability and bioefficacy of grape polyphenolic compounds on attenuating the biomarkers. In our model, the activation of Caco-2 cells by IL-1β resulted in a large increase in the secretion of IL-8 and $PGE_2$, effects remarkably inhibited by GP and/or GPT, with higher inhibitions after 24-h pretreatment with GPT. The anti-inflammatory effects seemed to be associated with decrease of ROS levels, reduced activation of NF-κB and downstream events, such as the release of inflammatory biomarkers IL-8 and $PGE_2$. Therefore, tannase treatment appears to enhance anti-inflammatory potential of grape pomace, proving this bioprocess can be beneficial and suggests a potential use and application of this residue in food, cosmetic and pharmaceutical industries. GP presented anti-inflammatory actions in both pretreatment and pre/cotreatment conditions, but in general, the extracts and doses seemed to generate similar results in the pre/cotreatment. The data can also indicate that a daily consumption of GP may protect against oxidative imbalance or prevent an inflammatory condition in the gastrointestinal tract.

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## References


Arnous, A., & Meyer, A. S. (2010). Discriminated release of phenolic substances from red wine grape skins (Vitis vinifera L.) by multicomponent enzymes treatment. *Biochemical Engineering Journal*, *49*(1), 68–77.

Battestin, V., & Macedo, G. A. (2007a). Purification and Biochemical Characterization of Tannase from a Newly Isolated Strain of Paecilomyces Variotii. *Food Biotechnology*, *21*(3), 207–216.

Battestin, V., & Macedo, G. A. (2007b). Tannase production by Paecilomyces variotii. *Bioresource Technology*, *98*(9), 1832–1837.

Battestin, V., Macedo, G. A., & De Freitas, V. A. P. (2008). Hydrolysis of epigallocatechin gallate using a tannase from Paecilomyces variotii. *Food Chemistry*, *108*(1), 228–233.

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, *239*(1), 70–76.

Biasi, F., Guina, T., Maina, M., Cabboi, B., Deiana, M., Tuberoso, C. I., Calfapietra, S., Chiarpotto, E., Sottero, B., Gamba, P., Gargiulo, S., Brunetto, V., Testa, G., Dessi, M. A., Poli, G., & Leonarduzzi, G. (2013). Phenolic compounds present in Sardinian wine extracts protect against the production of inflammatory cytokines induced by oxysterols in Caco-2 human enterocyte-like cells. *Biochemical Pharmacology*, *86*(1), 138–145.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30.

Campanholo, V. M. de L. P., Silva, R. M., Silva, T. D., Neto, R. A., Paiotti, A. P. R., Ribeiro, D. A., & Forones, N. M. (2015). Oral Concentrated Grape Juice Suppresses Expression of NF-kappa B , TNF-α and iNOS in Experimentally Induced Colorectal Carcinogenesis in Wistar Rats. *Asian Pacific Journal of Cancer Prevention*, *16*(3), 947–952.

Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J., & Queipo-Ortuño, M. I. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry*, *24*(8), 1415–1422.

Chamorro, S., Viveros, A., Alvarez, I., Vega, E., & Brenes, A. (2012). Changes in

polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. *Food Chemistry*, *133*(2), 308–314.

Chen, C., Milbury, P. E., Lapsley, K., & Blumberg, J. B. (2005). Flavonoids from Almond Skins Are Bioavailable and Act Synergistically with Vitamins C and E to Enhance Hamster and Human LDL Resistance to Oxidation. *The Journal of Nutrition*, *135*(January), 1366–1373.

Chuang, C.-C., Bumrungpert, A., Kennedy, A., Overman, A., West, T., Dawson, B., & McIntosh, M. K. (2011). Grape powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin resistance in primary cultures of human adipocytes. *The Journal of Nutritional Biochemistry*, *22*(1), 89–94.

Crozier, A., Del Rio, D., & Clifford, M. N. (2010). Bioavailability of dietary flavonoids and phenolic compounds. *Molecular Aspects of Medicine*, *31*(6), 446–467.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *Journal of Agricultural and Food Chemistry*, *52*(1), 48–54.

Dinicola, S., Mariggiò, M. A., Morabito, C., Guarnieri, S., Cucina, A., Pasqualato, A., D'Anselmi, F., Proietti, S., Coluccia, P. & Bizzarri, M. (2013). Grape seed extract triggers apoptosis in Caco-2 human colon cancer cells through reactive oxygen species and calcium increase: extracellular signal-regulated kinase involvement. *The British Journal of Nutrition*, *110*(5), 797–809.

Ferreira, L. R., Macedo, J. A., Ribeiro, M. L., & Macedo, G. A. (2013). Improving the chemopreventive potential of orange juice by enzymatic biotransformation. *Food Research International*, *51*(2), 526–535.

Friedman, M. (2014). Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *Journal of Agricultural and Food Chemistry*, *62*(26), 6025–6042.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology,* 26, 68-87.

Garat, C. (2003). Intracellular IL-1Ra type 1 inhibits IL-1-induced IL-6 and IL-8 production in Caco-2 intestinal epithelial cells through inhibition of p38 mitogen-activated protein kinase and NF-κB pathways. *Cytokine*, *23*(1-2), 31–40.

García-Conesa, M.-T., Østergaard, P., Kauppinen, S., & Williamson, G. (2001). Hydrolysis of diethyl diferulates by a tannase from Aspergillus oryzae.

*Carbohydrate Polymers*, *44*, 319–324.

Gentile, C., Perrone, A., Attanzio, A., Tesoriere, L., & Livrea, M. A. (2015). Sicilian pistachio (Pistacia vera L.) nut inhibits expression and release of inflammatory mediators and reverts the increase of paracellular permeability in IL-1β-exposed human intestinal epithelial cells. *European Journal of Nutrition*, *54*(5), 811-821.

González-Gallego, J., García-Mediavilla, M. V., Sánchez-Campos, S., & Tuñón, M. J. (2010). Fruit polyphenols, immunity and inflammation. *The British Journal of Nutrition*, *104 Suppl* , S15–S27.

Hoffmann, E., Dittrich-Breiholz, O., Holtmann, H., & Kracht, M. (2002). Multiple control of interleukin-8 gene expression. *Journal of Leukocyte Biology*, *72*(November), 847–855.

Holst, B., & Williamson, G. (2008). Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. *Current Opinion in Biotechnology*, *19*(2), 73–82.

Jara-Palacios, M. J., Hernanz, D., Cifuentes-Gomez, T., Escudero-Gilete, M. L., Heredia, F. J., & Spencer, J. P. E. (2015). Assessment of white grape pomace from winemaking as source of bioactive compounds, and its antiproliferative activity. *Food Chemistry*, *183*, 78–82.

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry*, *52*(14), 4360–4367.

Karin, M. (1999). How NF-kB is activated: the role of the IkB kinase (IKK) complex. *Oncogene*, *18*, 6867–6874.

Kaulmann, A., & Bohn, T. (2014). Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention. *Nutrition Research*, *34*(11), 907-929.

Koeberle, A., Bauer, J., Verhoff, M., Hoffmann, M., Northoff, H. & Werz, O. (2009). Green tea epigallocatechin-3-gallate inhibits microsomal prostaglandin $E_2$ synthase-1. *Biochemical and Biophysical Research Communications*, 388, 350–354.

Kountouri, A. M., Gioxari, A., Karvela, E., Kaliora, A. C., Karvelas, M., & Karathanos, V. T. (2012). Chemopreventive properties of raisins originating from Greece in colon cancer cells. *Food & Function*, *4*(3), 366–372.

Krishna, M. C., Dewhirst, M. W., Friedman, H. S., Cook, J. A., DeGraff, W., Samuni, A., Russo, A. & Mitchell, J. B. (1994). Hyperthermic sensitization by the radical initiator 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). I. In vitro studies. *International Journal of Hyperthermia*, *10*(2), 271-281.

Kundu, N., Yang, Q., Dorsey, R., & Fulton, A. M. (2001). Increased cyclooxygenase-2 (cox-2) expression and activity in a murine model of metastatic breast cancer. *International Journal of Cancer. Journal International Du Cancer*, *93*(5), 681–686.

Kuntz, S., Asseburg, H., Dold, S., Römpp, A., Fröhling, B., Kunz, C., & Rudloff, S. (2015). Inhibition of low-grade inflammation by anthocyanins from grape extract in an in vitro epithelial-endothelial co-culture model. *Food & Function*, *6*(4), 1136–1149.

Lam, G., Apostopoulos, V., Zulli, A., & Nurgali, K. (2015). NADPH Oxidases and Inflammatory Bowel Disease. *Current Medicinal Chemistry*, *22*(17), 2100–2109.

Legler, D. F., Bruckner, M., Uetz-von Allmen, E., & Krause, P. (2010). Prostaglandin E2 at new glance: Novel insights in functional diversity offer therapeutic chances. *The International Journal of Biochemistry & Cell Biology*, *42*(2), 198–201.

Li, Y.-M., Li, X.-M., Li, G.-M., Du, W.-C., Zhang, J., Li, W.-X., Xu, J., Ming, H. & Zhu, Z. (2008). In vivo pharmacokinetics of hesperidin are affected by treatment with glucosidase-like BglA protein isolated from yeasts. *Journal of Agricultural and Food Chemistry*, *56*(14), 5550–5557.

Liu, X., Nelson, A., Wang, X., Farid, M., Gunji, Y., Ikari, J., Iwasawa, S., Basma, H., Feghali-Bostwick, C. & Rennard, S. I. (2014). Vitamin D Modulates Prostaglandin $E_2$ Synthesis and Degradation in Human Lung Fibroblasts. *American Journal of Respiratory Cell and Molecular Biology, 50*(1), 40-50.

Macdonald, T. T., & Monteleone, G. (2005). Immunity, inflammation, and allergy in the gut. *Science (New York, N.Y.)*, *307*(5717), 1920–1925.

Macedo, J. A., Battestin, V., Ribeiro, M. L., & Macedo, G. A. (2011). Increasing the antioxidant power of tea extracts by biotransformation of polyphenols. *Food Chemistry*, *126*(2), 491–497.

Macedo, J., Ferreira, L., Camara, L., Santos, J., Gambero, A., Macedo, G., & Ribeiro, M. (2012). Chemopreventive potential of the tannase-mediated

biotransformation of green tea. *Food Chemistry*, *133*(2), 358–365.

Martin, G. R., & Wallace, J. L. (2006). Gastrointestinal inflammation: a central component of mucosal defense and repair. *Experimental Biology and Medicine (Maywood, N.J.)*, *231*(2), 130–137.

Matias, A., Nunes, S. L., Poejo, J., Mecha, E., Serra, A. T., Madeira, P. J. A., Bronze, M. R. & Duarte, C. M. M. (2014). Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate recovered from Opuntia ficus-indica juice. *Food & Function*, *5*(12), 3269–3280.

Mildner-Szkudlarz, S., Bajerska, J., Zawirska-Wojtasiak, R., & Górecka, D. (2013). White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *Journal of the Science of Food and Agriculture*, *93*(2), 389–395.

Molodecky, N. A., Soon, I. S., Rabi, D. M., Ghali, W. A., Ferris, M., Chernoff, G., Benchimol, E. I., Panaccione, R., Ghosh, S., Barkema, H. W., Kaplan, G. G. (2012). Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*, 142, 46–54.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1-2), 55–63.

Mudie, D., Murray, K., Hoad, C. L., Pritchard, S. E., Garnett, M., Amidon, G. L., Gowland, P. A., Spiller, R. C., Amidon, G. E. & Marciani, L. (2014). Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Molecular Pharmaceutics*, (11), 3039–3047.

Mutoh, M., Takahashi, M., Fukuda, K., Komatsu, H., Enya, T., Matsushima-Hibiya, Y., Mutoh, H., Sugimura, T. & Wakabayashi, K. (2000). Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. *Japanese Journal of Cancer Research*, *91*, 686–691.

Nishiumi, S., Mukai, R., Ichiyanagi, T., & Ashida, H. (2012). Suppression of lipopolysaccharide and galactosamine-induced hepatic inflammation by red grape pomace. *Journal of Agricultural and Food Chemistry*, *60*(36), 9315–9320.

O'Leary, K. A., Pascual-Tereasa, S., Needs, P. W., Bao, Y.-P., O'Brien, N. M., & Williamson, G. (2004). Effect of flavonoids and Vitamin E on cyclooxygenase-2

(COX-2) transcription. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *551*(1-2), 245–254.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A, & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, *50*(11), 3122–3128.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventós, R., Buxaderas, S. & Codina, C. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry*, *97*(1), 137–150.

Pinent, M., Castell-Auví, A., Genovese, M. I., Serrano, J., Casanova, A., Blay, M., & Ardévol, A. (2015). Antioxidant effects of proanthocyanidin-rich natural extracts from grape seed and cupuassu on gastrointestinal mucosa. *Journal of the Science of Food and Agriculture*, 96(1), 178-182

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, *51*(11), 3273–3279.

Ramos, S., Rodríguez-Ramiro, I., Martín, M. A., Goya, L., & Bravo, L. (2011). Dietary flavanols exert different effects on antioxidant defenses and apoptosis/proliferation in Caco-2 and SW480 colon cancer cells. *Toxicology in Vitro*, *25*(8), 1771–1781.

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and Inflammation. *Arteriosclerosis, Thrombosis and Vascular Biology*, *31*(5), 986–1000.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*(4), 152–159.

Rodriguez-Rodriguez, R., Justo, M. L., Claro, C. M., Vila, E., Parrado, J., Herrera, M. D., & Alvarez de Sotomayor, M. (2012). Endothelium-dependent vasodilator and antioxidant properties of a novel enzymatic extract of grape pomace from wine industrial waste. *Food Chemistry*, *135*(3), 1044–1051.

Romier, B., Schneider, Y. J., Larondelle, Y., & During, A. (2009). Dietary polyphenols can modulate the intestinal inflammatory response. *Nutrition Reviews*, *67*(7),

363–378.

Romier-Crouzet, B., Van De Walle, J., During, A., Joly, A., Rousseau, C., Henry, O., Larondele, Y. & Schneider, Y.-J. (2009). Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food and Chemical Toxicology*, *47*(6), 1221–1230.

Rosales Soto, M. U., Brown, K., & Ross, C. F. (2012). Antioxidant activity and consumer acceptance of grape seed flour-containing food products. *International Journal of Food Science & Technology*, *47*(3), 592–602.

Russell, W., & Duthie, G. (2011). Plant secondary metabolites and gut health: the case for phenolic acids. *The Proceedings of the Nutrition Society*, *70*(3), 389–396.

Sagdic, O., Ozturk, I., & Kisi, O. (2012). Modeling antimicrobial effect of different grape pomace and extracts on S. aureus and E. coli in vegetable soup using artificial neural network and fuzzy logic system. *Expert Systems with Applications*, *39*(8), 6792–6798.

Sambuy, Y., Angelis, I. De, Ranaldi, G., Scarino, M. L., Stammati, A., & Zucco, F. (2005). The Caco-2 cell line as a model of the intestinal barrier : influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*, 21, 1–26.

Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F., & Borderías, A. J. (2008). Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT - Food Science and Technology*, *41*(1), 42–50.

Sandoval-Acuña, C., Ferreira, J., & Speisky, H. (2014). Polyphenols and mitochondria: An update on their increasingly emerging ROS-scavenging independent actions. *Archives of Biochemistry and Biophysics*, *559*, 75–90.

Scalbert, A., & Williamson, G. (2000). Dietary Intake and Bioavailability of Polyphenols. *The Journal of Nutrition*, (2074S), 2073–2085.

Schiller, C., Fröhlich, C. P., Giessmann, T., Siegmund, W., Mönnikes, H., Hosten, N., & Weitschies, W. (2005). Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Alimentary Pharmacology and Therapeutics*, *22*(10), 971–979.

Shin, H. S., Satsu, H., Bae, M.-J., Zhao, Z., Ogiwara, H., Totsuka, M., & Shimizu, M. (2015). Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in

C57BL/6 mice. *Food Chemistry*, *168*, 167–175.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.

Spigno, G., De Faveri, D. M. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. *Journal of Food Engineering, 78*, 793–801.

Taverniti, V., Fracassetti, D., Bo, C. Del, Lanti, C., Minuzzo, M., Klimis-zacas, D., Riso, P. & Guglielmetti, S. (2014). Immunomodulatory E ff ect of a Wild Blueberry Anthocyanin-Rich Extract in Human Caco-2 Intestinal Cells. *Journal of Agricultural and Food Chemistry*, 62, 8346-8351.

Tesoriere, L., Attanzio, A., Allegra, M., Gentile, C., & Livrea, M. A. (2013). Indicaxanthin inhibits NADPH oxidase (NOX)-1 activation and NF-κB-dependent release of inflammatory mediators and prevents the increase of epithelial permeability in IL-1β-exposed Caco-2 cells. *The British Journal of Nutrition*, (15), 1–9.

Walker, R., Tseng, A., Cavender, G., Ross, A., & Zhao, Y. (2014). Physicochemical, Nutritional, and Sensory Qualities of Wine Grape Pomace Fortified Baked Goods. *Journal of Food Science*, *79*(9), S1811–S1822.

Wang, H., & Joseph, J. A. (1999). Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader11Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the United States Department of Agriculture and does not imp. *Free Radical Biology and Medicine*, *27*(5-6), 612–616.

Wang, Y.-H., Ge, B., Yang, X.-L., Zhai, J., Yang, L.-N., Wang, X.-X., Liu, X., Shi, J.-C. & Wu, Y.-J. (2011). Proanthocyanidins from grape seeds modulates the nuclear factor-kappa B signal transduction pathways in rats with TNBS-induced recurrent ulcerative colitis. *International Immunopharmacology*, *11*(10), 1620–1627.

WHO. (2002). The World Health Organization Report 2002: Reducing Risks, Promoting Healthy Life. Geneva: WHO. URL: http://www.who.int/whr/2002/en/ Accessed on: Dec 2015.

Xu, C., Yagiz, Y., Borejsza-Wysocki, W., Lu, J., Gu, L., Ramírez-Rodrigues, M. M., & Marshall, M. R. (2014). Enzyme release of phenolics from muscadine grape

(Vitis rotundifolia Michx.) skins and seeds. *Food Chemistry*, *157*, 20–29.

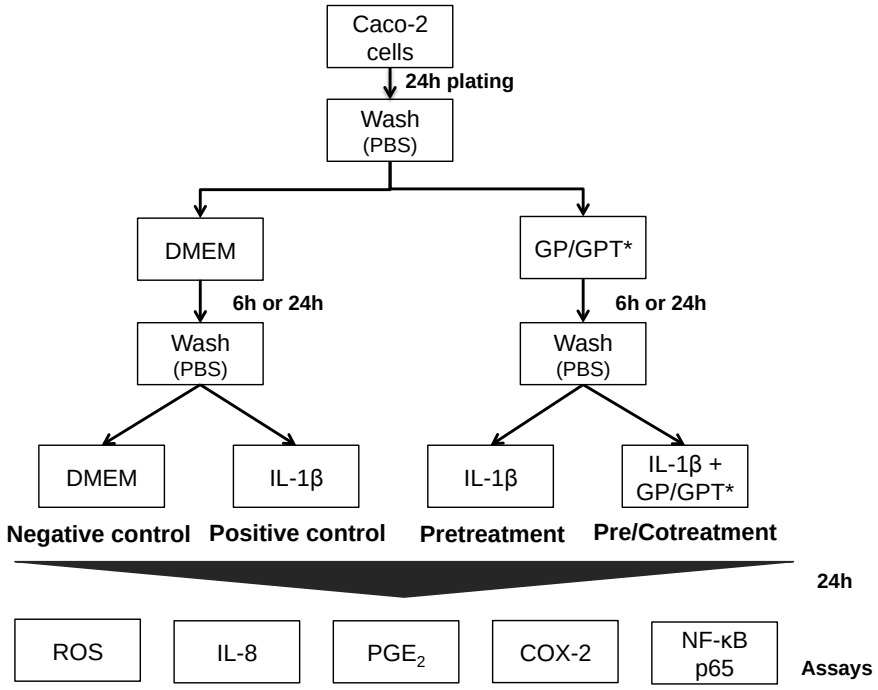



**Fig. 1.** Cell culture experimental treatments performed to evaluate the anti-inflammatory capacity of grape pomace extracts. Abbreviations: PBS: Phosphate Buffer Saline; GP: grape pomace; GPT: grape pomace + tannase; IL-1β: pro-inflammatory cytokine (1 ng/mL or 20 ng/mL in DMEM no FBS); DMEM: Dulbecco's Modified Eagle Medium no addition of FBS *Concentrations: 100 and 200 µg/mL in DMEM no FBS.

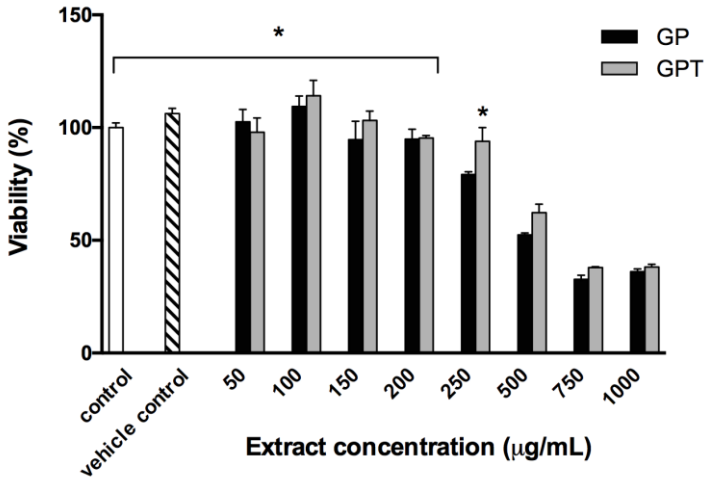



**Fig. 2.** Cell viability after 48h treatments with different concentrations of GP extracts. Abbreviations: GP (grape pomace); GPT (grape pomace + tannase). Control: cells + DMEM 0% FBS; vehicle control: PBS in DMEM 0% FBS. *Values (mean ± SD) are not significantly different (P ≤0.05), tested using one-way ANOVA, followed by post-hoc Tukey HSD.

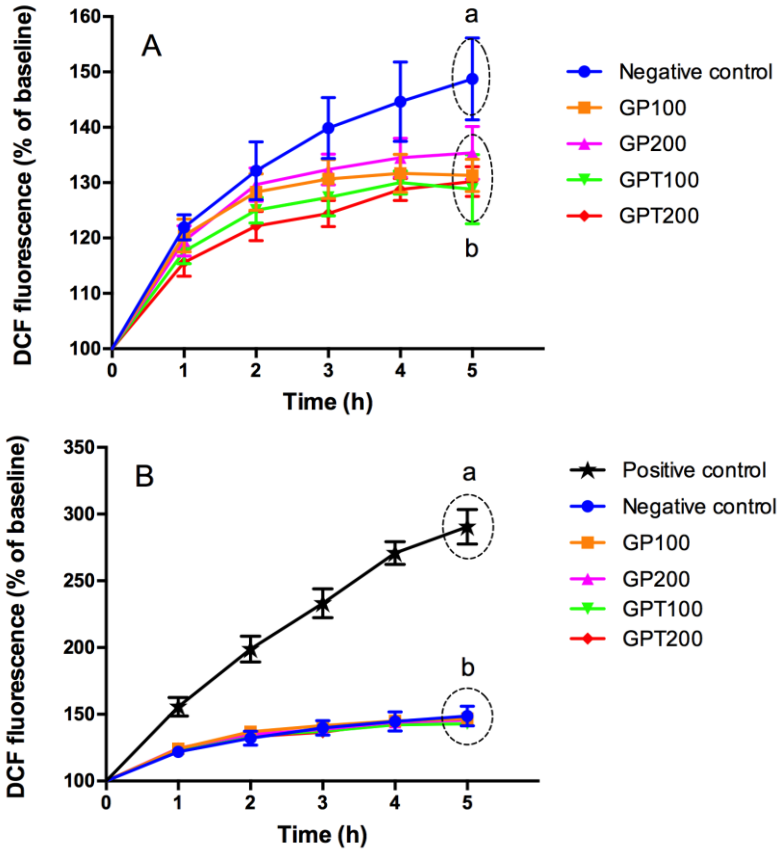



**Fig. 3**. Effects of GPE against intracellular ROS generation detected by DCF fluorescence. Abbreviations: GP: grape pomace; GPT: grape pomace + tannase; 100/200: concentration of extracts (µg/mL). Treatments: negative control: cells + HBSS; positive control: cells + AAPH 50 µM in HBSS; treatments: GP and GPT extracts in HBSS. (A) no AAPH added; (B) addition of AAPH. $^{a,b}$ Values (mean ± SD) with different superscript letters are significantly different ($P \leq 0.05$), tested using one-way ANOVA, followed by post-hoc Tukey HSD.

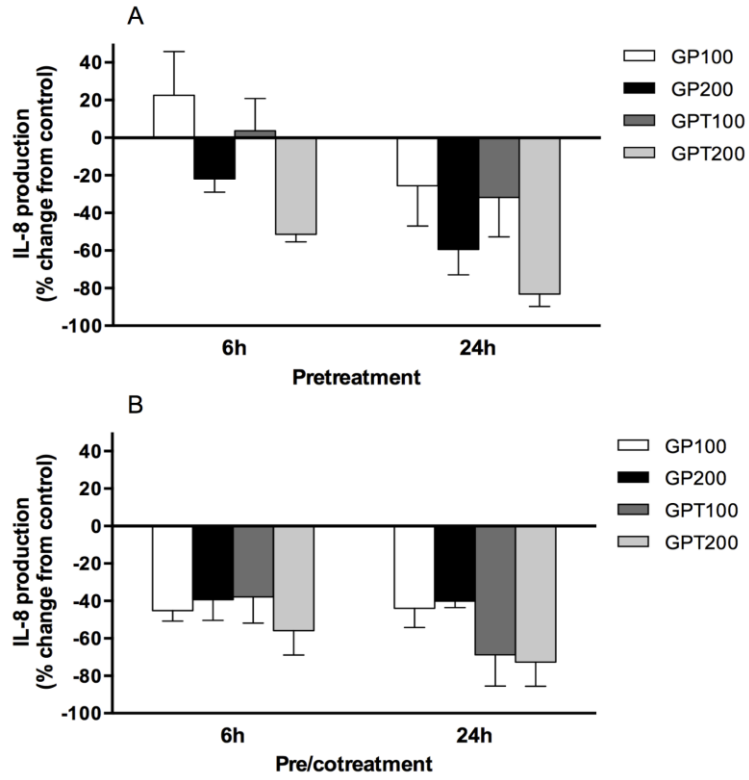



**Fig. 4.** Changes in IL-8 detection in Caco-2 cells culture medium after GP and GPT pretreatment (A) and pre/cotreatment (B). Abbreviations: GP: grape pomace; GPT: grape pomace + tannase; 100/200: concentration of extracts (µg/mL). Values (mean ± SD) were tested using three-way ANOVA, followed by post-hoc Tukey HSD ($P \leq 0.05$). Statistically significant factors pretreatment: enzyme ($P = 0.0083$), dose ($P < 0.0001$); time ($P < 0.0001$); pre/cotreatment: enzyme ($P = 0.0029$), time ($P = 0.02$), enzyme *vs*. time ($P = 0.02$).

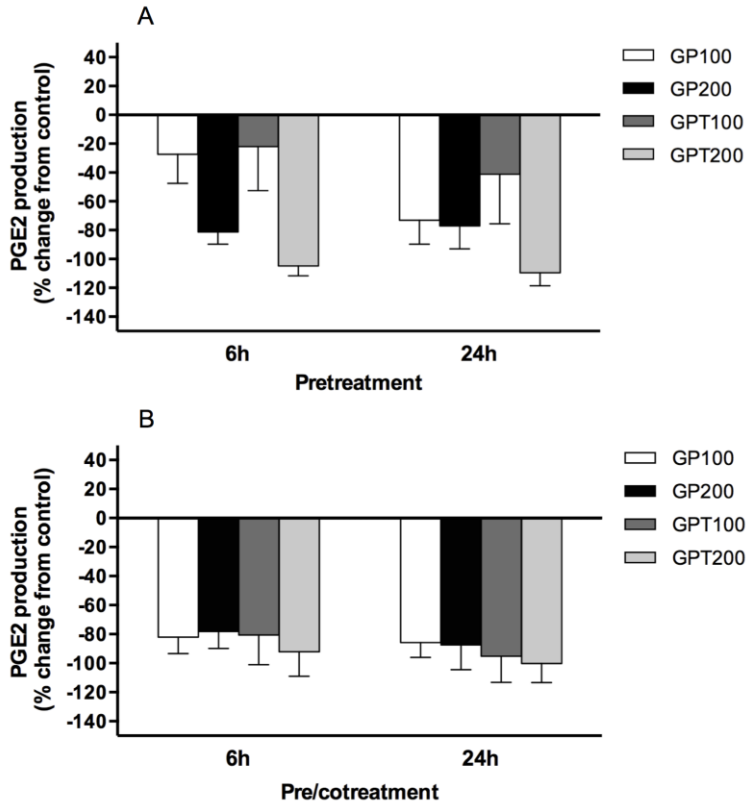



**Fig. 5.** Changes in $PGE_2$ detection in Caco-2 cell culture medium after GP and GPT pretreatment (A) and pre/cotreatment (B). Abbreviations: GP: grape pomace; GPT: grape pomace + tannase; 100 and 200: concentration of extracts (µg/mL). Values (mean ± SD) were tested using three-way ANOVA, followed by post-hoc Tukey HSD ($P \leq 0.05$). Statistically significant factors pretreatment: dose ($P = 0.0083$), enzyme *vs*. dose ($P < 0.0001$); pre/cotreatment: three-way ANOVA ($P = 0.63$).

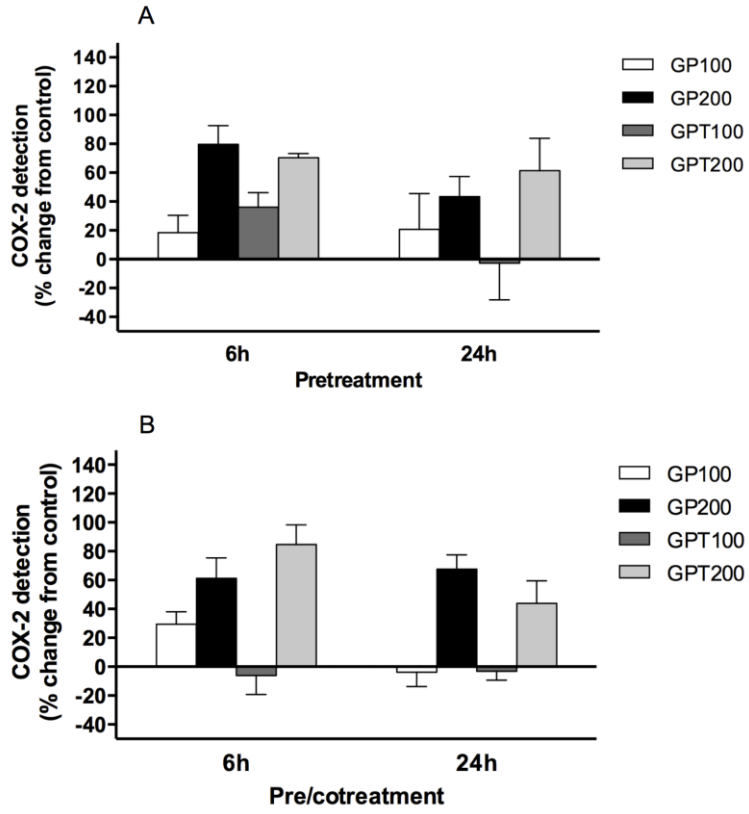



**Fig. 6.** Changes in COX-2 protein detection in Caco-2 cell lysate after GP and GPT pretreatment (A) and pre/cotreatment (B). Abbreviations: GP: grape pomace; GPT: grape pomace + tannase; 100/200: concentration of extracts (µg/mL). Values (mean ± SD) were tested using three-way ANOVA, followed by post-hoc Tukey HSD ($P \leq 0.05$). Statistically significant factors pretreatment: dose ($P < 0.0001$), time ($P = 0.01$); enzyme *vs.* dose *vs.* time ($P = 0.02$); pre/cotreatment: dose ($P < 0.0001$), time ($P = 0.0039$); enzyme *vs.* dose *vs.* time ($P = 0.0005$).

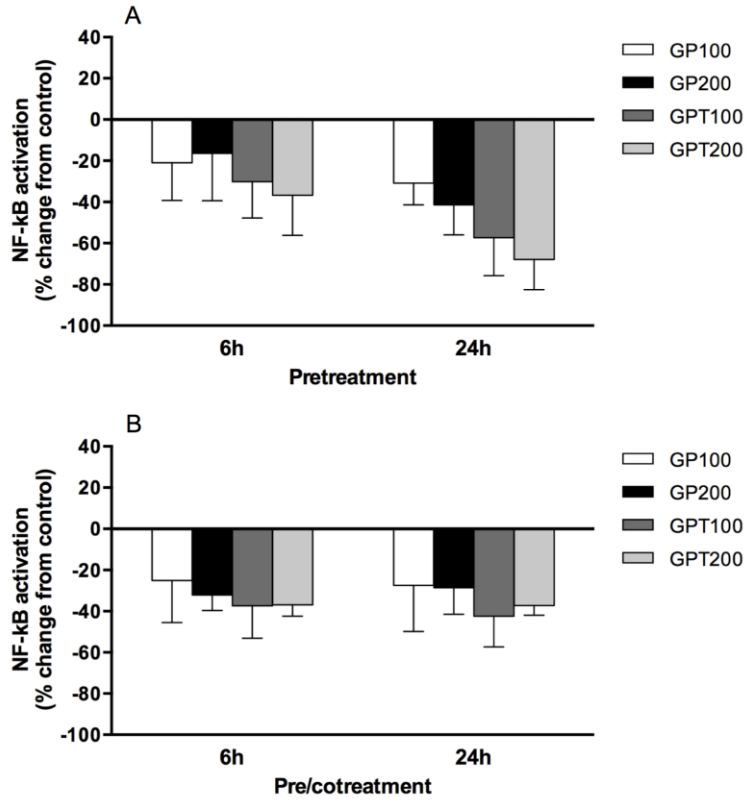



**Fig. 7.** Effects of GP extracts pretreatment (A) and pre/cotreatment (B) on IL-1β-induced activation of NF-κB transcription factor in Caco-2 cells. Abbreviations: GP: grape pomace, GPT: grape pomace + tannase; 100/200: concentration of extracts (μg/mL). Values (mean ± SD) were tested using three-way ANOVA, followed by post-hoc Tukey HSD ($P \leq 0.05$). Statistically significant factors pretreatment: enzyme ($p=0.01$), time ($p=0.0048$); pre/cotreatment: three-way ANOVA ($p=0.80$).

**Table 1.** Effect of tannase treatment on GPE total phenols [mg gallic acid equivalent (GAE)/g dry extract (DE)] and antiradical activities (µmol Trolox equivalent/g DE)

| | **Samples*** | |
|---|---|---|
| | **GP** | **GPT** |
| Total Phenols | 249.60 ± 4.99[a] | 377.57 ± 8.92[b] |
| DPPH | 2997.58 ± 11.36[a] | 5119.89 ± 45.49[b] |
| ORAC | 1181.58 ± 58.55[a] | 4045.24 ± 63.20[b] |
| FRAP | 948.01 ± 16.21[a] | 993.42 ± 12.88[b] |

*Abbreviations: GP (grape pomace); GPT (GP + tannase)
[a,b] Values (mean ± SD) within a row with different superscript letters are significantly different (P ≤0.05), tested using one-way ANOVA.

**Table 2.** Effect of tannase treatment on grape pomace flavonoids and phenolic acids profile (mg/g DE)

| **Phenolic compounds** | **GP*** | **GPT** |
|---|---|---|
| Gallic acid | 3.6 ± 0.21[a] | 8.07 ± 0.22[b] |
| Protocatechuic acid | 0.67 ± 0.00[a] | 1.01 ± 0.00[b] |
| 3,4-dihydroxyphenylacetic acid | 0.24 ± 0.02[a] | 0.77 ± 0.05[b] |
| Catechin | 13.55 ± 0.9[a] | 14.32 ± 0.46[a] |
| Vanillic acid | 0.09 ± 0.00[a] | 0.18 ± 0.01[b] |
| Caffeic acid | n.d. | 0.2 ± 0.02 |
| Epicatechin | 7.14 ± 0.56[a] | 7.76 ± 0.49[a] |
| ρ-coumaric acid | n.d. | 1.65 ± 0.04 |
| Rutin (quercetin-3-O-rutinoside) | 1.27 ± 0.11[a] | 0.36 ± 0.03[b] |
| Myricetin | 0.33 ± 0.03[a] | 0.61 ± 0.03[b] |
| Quercetin | 0.58 ± 0.04[a] | 0.84 ± 0.06[b] |

*Abbreviations: GP: grape pomace; GPT grape pomace tannase; n.d: not detected
[a,b] Values (mean ± SD) within a row with different superscript letters are significantly different (P ≤0.05), tested using one-way ANOVA.

## DISCUSSÃO GERAL

A quantidade e composição de fenólicos presentes nas uvas e em seus resíduos podem ser influenciadas por diversos fatores como a variedade, o clima e o local do cultivo, o tempo de colheita, o tipo de processo de vinificação, processamento, além de condições de armazenamento (LAFKA; SINANOGLOU; LAZOS, 2007). Os resultados obtidos nesse estudo foram consistentes com esta afirmação, pois a variedade, o tratamento enzimático aplicado e a interação entre esses dois fatores tiveram impactos significativos no teor de fenóis totais e taninos condensados nos resíduos de uvas.

O resíduo de uva tinta (RUT) apresentou maiores quantidades de fenóis totais do que as demais amostras, o que possivelmente ocorre devido a presença de maiores quantidades de antocianinas, flavonoides e catequinas nas variedades de uvas tintas em comparação com as brancas (KATALINIĆ et al., 2010). Como o resíduo de mistura de uvas (RMU) é formado por uma mistura de uvas brancas e tintas, esperava-se encontrar quantidades intermediárias de fenóis totais, com o resíduo de uva branca (RUB) apresentando as menores quantidades. Porém, devido ao tratamento térmico elevado sofrido por RMU, a amostra apresentou as menores quantidades de fenóis totais. Sabe-se que além da temperatura, o pH também pode causar degradação de compostos fenólicos (XU et al., 2014).

Após as diferentes hidrólises enzimáticas realizadas, o tratamento com tanase (T) demonstrou ser o mais eficiente em liberar compostos de menor peso molecular a partir de fenólicos poliméricos, levando a maiores aumentos em quantidade de fenóis totais nos três resíduos. Ao contrário, o tratamento com pectinase e celulase (PC) não foi um tratamento efetivo no aumento de fenóis totais nas amostras. O tratamento com a combinação das três enzimas (TPC) gerou aumentos menores nos fenóis totais, provavelmente por conter menor porcentagem de tanase na mistura de enzimas (1.66%) do que no tratamento apenas com tanase (5%). Esses resultados são similares aos encontrados por Chamorro et al. (2012), que demonstraram que a eficiência do tratamento TPC foi dependente especialmente da tanase presente na mistura enzimática. Além disso, em concordância com o presente estudo, pectinase ou celulase não foram capazes de modificar significativamente o conteúdo de fenólicos nos resíduos. Porém, outros

estudos demonstraram anteriormente a eficácia dessas enzimas em extrair compostos fenólicos a partir de cascas e sementes de uvas (MEYER; JEPSEN; SØRENSEN, 1998; XU et al., 2014). Estas discrepâncias podem ser referentes a ao grau de pureza das preparações enzimáticas, podendo haver contaminação com outras enzimas catalisadoras de diferentes reações. Além disso, podem estar relacionadas com diferenças no tipo e atuação das enzimas, a duração do tratamento enzimático, o solvente utilizado, o tamanho das partículas do resíduo, sua composição fenólica e presença de lignina.

Os taninos condensados foram detectados nos três resíduos, sendo as maiores quantidades encontradas no RUT. Estes dados foram confirmados pelas análises cromatográficas, onde catequina e procianidina B2 apareceram como compostos majoritários nas três amostras. Os tratamentos enzimáticos reduziram os teores de taninos condensados nas amostras. Mesmo estes não sendo reconhecidos como hidrolisáveis, foi demonstrado anteriormente que procianidina B2 pôde ser biodegradada por oxigenase de *Aspergillus fumigatus* (CONTRERAS-DOMÍNGUEZ et al., 2006). Outras possíveis explicações para o decréscimo de taninos condensados após tratamentos enzimáticos seriam através de degradação oxidativa, polimerização, precipitação ou hidrólise. Porém, mais estudos são necessários para caracterizar estas ações sobre taninos condensados.

Os três tratamentos enzimáticos não afetaram os taninos hidrolisáveis detectados pelo método espectrofotométrico, opostamente ao demonstrado pelos resultados de HPLC, onde detectou-se aumentos de ácido gálico e ácido caféico após os 3 diferentes tratamentos, demonstrando que ocorreram reações de hidrólise. Esta diferença de detecção entre os métodos pode ser explicada pela maior sensibilidade e especificidade do método cromatográfico. A hidrólise de rutina (quercetina-rutinosídeo) e o aumento de sua forma aglicona, quercetina, também foram detectados por cromatografia. Os tratamentos T e TPC foram aos mais efetivos em aumentar os níveis de quercetina nas amostras. Porém, P e C também podem agir da mesma forma, como demonstrado em estudos anteriores (KAMMERER et al., 2005; ARNOUS; MEYER, 2010; FERRI et al., 2016). A quercetina é um composto com maior potencial antioxidante e mais eficiente que a rutina em determinadas ações celulares, como por exemplo contra a disfunção mitocondrial (CARRASCO-POZO et al., 2012; SAW et al., 2014).

O *trans*-resveratrol foi apenas detectado no RMU e seu conteúdo foi aumentado após os tratamentos enzimáticos, ações que também já foram comprovadas em outros estudos, onde pectinase, celulase, β-glucosidase e endoproteases foram efetivas em liberar esse composto em resíduo de uva e vinhos. Apesar do mecanismo responsável por esse aumento não ser comprovado, possivelmente a forma aglicona de resveratrol foi produzida após hidrólise enzimática de seu respectivo glicosídeo ou a partir de formas oligoméricas (BERZAS NEVADO; CONTENTO SALCEDO; CASTAÑEDA PEÑALVO, 1999; FERRI et al., 2016).

Os ácidos fenólicos detectados nos resíduos de vinificação de uvas foram o ácido gálico e o ácido caféico. Pôde-se verificar que a variedade do resíduo e o tratamento enzimático foram fatores que afetaram seus conteúdos. T e TPC foram igualmente efetivos na liberação de ácido gálico de seus conjugados, concluindo-se que a quantidade de tanase presente no TPC (1.66%) já é o suficiente para mediar a máxima hidrólise para liberação do composto. Os maiores aumentos desse composto foram detectados no RUB, sugerindo que possíveis diferenças na estrutura de parede celular e no processo de vinificação podem influenciar na remoção de compostos fenólicos da matriz celular. Durante a produção de vinho branco, as uvas são prensadas e o resíduo é removido nas primeiras fases do processo de vinificação, não sendo fermentado (MAIER et al., 2008; MILDNER-SZKUDLARZ et al., 2013). O ácido caféico foi apenas detectado após a realização dos tratamentos enzimáticos, aparecendo apenas no RUT e RMU. Foram observados diferentes efeitos das enzimas na liberação desse composto, sendo TPC o tratamento mais eficaz no RUT e PC no RMU.

As enzimas atuaram de forma modesta sobre catequina e procianidina B2, com exceção do RUB, em que após tratamento com TPC, resultou em 47% de aumento em procianidina B2 comparado com o controle. Da mesma forma, Kammerer et al. (2005) demonstraram que pectinase e celulase aumentaram as quantidades de catequina, epicatequina e procianidina B2 em resíduos de uva. Embora no presente estudo não tenha sido possível determinar os mecanismos pelos quais as enzimas atuaram, possíveis explicações seriam de que as enzimas liberam esses compostos que estavam ligados a polissacarídeos de membrana, ou teriam sido produzidos a partir da hidrólise de compostos poliméricos, além da

ocorrência de uma possível contaminação com outras enzimas nas misturas enzimáticas utilizadas.

Os ácidos fenólicos e flavanóis monoméricos já foram caracterizados como antioxidantes potentes (RICE-EVANS; MILLER; PAGANGA, 1997). Nos resíduos de uva analisados, foram observados maiores valores de DPPH e ORAC após a biotransformação por tanase, provavelmente devido a suas ações hidrolíticas. Outros estudos também observaram que misturas de enzimas elevaram a capacidade antioxidante de bagaço de uva e sementes de uva (CHAMORRO et al., 2012; XU et al., 2014). Além de aumentarem o poder antioxidante, as transformações enzimáticas também são capazes de gerar mais fenólicos biodisponíveis e aumentar sua bioeficácia celular (FERRUZZI et al., 2010). Os resultados de FRAP foram semelhantes aos de DPPH e ORAC (que tiveram elevada correlação estatística), porém foram menos expressivos. O resíduo de uva tinta apresentou maiores atividades antioxidantes, especialmente após o tratamento com tanase, sendo RUT e RUTT os extratos selecionados para os ensaios anti-inflamatórios utilizando cultura celular. Estes extratos também foram analisados quanto a presença de diferentes compostos fenólicos utilizando outro método de separação cromatográfica e detecção eletroquímica (HPLC-ECD). RUTT apresentou maiores quantidades de fenólicos de baixo peso molecular, como ácido gálico e ácido vanílico, além de ácido caféico e ρ-cumárico, apenas detectados após tratamento enzimático. O tratamento com a tanase também transformou glicosídeos em agliconas, novamente demonstrados pelo aumento de quercetina e diminuição de rutina.

Após a comprovação dos efeitos positivos da biotransformação com tanase, seus possíveis efeitos biológicos *in vitro* também foram explorados. Como os componentes da dieta ficam em contato direto com a mucosa intestinal após serem ingeridos, este estudo teve como objetivo avaliar a atividade anti-inflamatória dos extratos utilizando um modelo intestinal com células Caco-2 não diferenciadas, que apresentam fenótipo de células do cólon (colonócitos) em seu estágio de pré-confluência *in vitro* (SAMBUY et al., 2005). Este fenótipo foi escolhido pois as possíveis bioatividades de compostos na mucosa intestinal possivelmente ocorrem no cólon devido a baixa absorção destes no intestino delgado, já que são detectados em baixas concentrações plasmáticas pós-prandiais (RUSSELL; DUTHIE, 2011). Foram realizadas duas formas de tratamento no modelo celular: pré-tratamentos

com os extratos antes da indução de inflamação e pré/co-tratamentos, adicionando-se extratos juntamente com o indutor de inflamação, após o pré-tratamento nas células Caco-2.

A produção de citocinas e quimiocinas na mucosa intestinal é um processo fisiológico que faz parte da ativação do sistema imunológico local em situações agudas. Entretanto, esse processo pode se tornar crônico, como acontece nas doenças inflamatórias intestinais. Além disso, o alto teor de radicais livres e estresse oxidativo no trato gastrointestinal são também considerados fortes influências para o desenvolvimento de doenças (LAM et al., 2015). RUT e RUTT foram eficientes em reduzir a formação de espécies reativas de oxigênio (ERO) em células Caco-2, estando de acordo com resultados prévios (RAMOS et al., 2011; PINENT et al., 2015). Este efeito pode gerar um equilíbrio no balanço local de radicais livres e consequentemente, ajudar a controlar um processo inflamatório excessivo. A capacidade dos extratos em reduzir ERO nas células Caco-2 pode também estar correlacionada com a inativação do fator de transcrição NF-kB e com a modulação da secreção de biomarcadores inflamatórios. Diversas evidências indicam que o fator NF-κB está relacionado a progressão de inflamação e câncer. Sua forma inativa fica no citosol, sendo o heterotrímero p65/p50, com a subunidade p65 contendo o sítio de ativação de sua transcrição, ligado a uma proteína inibitória IkB. Após estímulo, IkB é fosforilada levando à entrada de NF-κB p65 no núcleo celular e iniciando a transcrição de diversos genes imuno-regulatórios relacionados à inflamação (KARIN, 1999). O bloqueio desta ativação leva à redução da transcrição destes genes e ao efeito anti-inflamatório (KAULMANN; BOHN, 2014). RUTT apresentou os melhores resultados em inibir a ativação de NF-κB p65 neste estudo. Diversos extratos fenólicos também apresentaram efeitos inativadores de NF-κB p65 em outros estudos, como extrato de mirtilo, de pistache, extrato de pó de uva e suco de uva (CHUANG et al., 2011; GENTILE et al., 2015; TAVERNITI et al., 2014; CAMPANHOLO et al., 2015).

A interleucina 8 (IL-8) é a quimiocina mais comumente estudada em processos de inflamação intestinal devido a sua elevada produção por neutrófilos e macrófagos recrutados quando há doenças inflamatórias intestinais, gerando um mecanismo contínuo no ciclo de atração de neutrófilos. As células Caco-2 são reconhecidas por secretarem elevadas quantidades desta quimiocina, cuja expressão é dependente de IL-1β e da ativação de NF-κB (GARAT, 2003). Os

extratos avaliados demonstraram ter efeitos reguladores de IL-8. Da mesma maneira, extrato hidrofílico de pistache, ácido clorogênico, acido caféico e antocianinas de uvas modularam a produção de IL-8 anteriormente (CHUANG et al., 2011; GENTILE et al., 2015; KUNTZ et al., 2015; SHIN et al., 2015). Uma correlação significativa entre IL-8 e NF-κB foi encontrada nos dados obtidos neste estudo, estabelecendo uma relação entre a inativação de NF-κB e a diminuição da produção de IL-8, o que também foi notado em estudos prévios (HOFFMANN et al., 2002), nos mostrando que a capacidade anti-inflamatória de extrato de resíduo de uva pode ser considerada, pelo menos em parte, mediada pela inibição da cascata do NF-κB .

Além de citocinas e quimiocinas pró-inflamatórias, outros mediadores são liberados em condições inflamatórias, como as prostaglandinas (PG), lipídeos autacóides gerados a partir do araquidonato por ação de isozimas ciclooxigenases (COX), induzidos por estímulos de inflamação e mediadores de funções biológicas. $PGE_2$ é uma das prostaglandinas mais produzidas em tecidos inflamados, além de estar relacionada com a produção de vermelhidão, inchaço e dor (LEGLER et al., 2010; RICCIOTTI; FITZGERALD, 2011). Foram detectados menores níveis de $PGE_2$ no meio de cultivo celular, mas a expressão da proteína COX-2 no produto da lise celular não foi diminuída, e sim aumentada em alguns tratamentos. Sugere-se que a expressão e a atividade de COX-2 podem ser reguladas de maneiras diferentes, sendo que as ações dos extratos podem ter ocorrido após transcrição, bloqueando parte de sua atividade, detectada pela diminuição de $PGE_2$, mas não da expressão proteica de COX-2. O bloqueio da atividade de COX-2 foi demonstrado anteriormente pela ação de extrato de pistache em células Caco-2, extrato de uva em adipócitos e extratos de uva passa em células HT29 (CHUANG et al., 2011; KOUNTOURI et al., 2012; GENTILE et al., 2015). Há indícios de que algumas drogas inibidoras de atividade de COX-2 também apresentam esta característica de não diminuir sua expressão. Indometacina e NS398 inibiram a síntese de $PGE_2$ em um modelo celular de câncer mamário, porém os níveis de proteína e mRNA de COX-2 aumentaram (KUNDU et al., 2001). Diferenças na estrutura química dos polifenóis podem influenciar esta atividade, já que flavonoides com grupos hidroxila nas posições 3', 4', e 5' do anel B, como miricetina e epigalocatequina, não foram capazes de suprimir a transcrição de COX-2 em células DLD-1 (MUTOH et al., 2000), o que pode explicar estas diferenças. Além da ação direta sobre a COX-2, outros possíveis mecanismos em que os extratos podem ter atuado seria

estimulando a via de degradação da $PGE_2$ pela ação da 15-hidroxil prostaglandina desidrogenase ou pela supressão da biossíntese de $PGE_2$ através da inativação da prostaglandina $E_2$ sintase-1 microssomal, que catalisa a transformação de $PGH_2$ em $PGE_2$, na mesma via do ácido araquidônico, como demonstrado anteriormente, por ação de vitamina D e epigalocatequina-galato, que também não atuaram sobre a COX-2, mas diminuíram níveis de $PGE_2$ em modelos celulares (LIU et al., 2014; KOEBERLE et al., 2009).

No geral, ambos os tratamentos realizados foram efetivos. No pré-tratamento conseguimos observar efeitos anti-inflamatórios mais significativos do resíduo tratado com tanase e nas maiores concentrações. Porém, no pré/cotratamento, na maior parte dos resultados, ambas as concentrações testadas apresentaram resultados similares, demonstrando que um período mais longo de exposição ao extrato fenólico, mesmo concomitantemente com o indutor de inflamação, gerou efeitos anti-inflamatórios mais equilibrados, independente do tipo de extrato ou concentração aplicados.

O entendimento das relações e interações entre os compostos da dieta e a mucosa intestinal é muito importante para a explanação de detalhes sobre os benefícios do consumo de frutos e vegetais, além de mostrar a importância da valorização de resíduos agroindustriais para a produção de produtos funcionais, com elevado valor agregado, contribuindo também para o desenvolvimento ambiental sustentável.

## CONCLUSÕES GERAIS

Os resíduos de vinificação de uvas analisados apresentaram grandes quantidades de compostos fenólicos, especialmente flavonoides e ácidos fenólicos, influenciados pela variedade da uva e pelo tratamento enzimático utilizado. Entre as amostras analisadas, o RUT apresentou maior quantidade de fenóis totais e atividade antioxidante. O RUB foi mais responsivo à ação de enzimas celulolíticas e pectinolícas na liberação de compostos fenólicos, especialmente ácido gálico. MRU provavelmente apresentou índices mais baixos de fenóis totais e atividade antioxidante devido ao tratamento térmico sofrido, a temperaturas mais elevadas do que as demais amostras. Em comparação às misturas enzimáticas, o tratamento com tanase foi o mais eficaz, aumentando fenóis totais, atividade antioxidante e liberando fenólicos monoméricos e agliconas, o que pode estar relacionado a melhores bioatividades e biodisponibilidade de compostos. No modelo *in vitro* utilizando células Caco-2, ambos os extratos fenólicos testados (RUT e RUTT) foram efetivos na atenuação da produção de IL-8, $PGE_2$ e na inativação do NF-kB p65 após indução de inflamação nas células por IL-1β, além de reduzirem expressivamente a geração de ERO induzida por AAPH, sugerindo que um consumo frequente de uva e derivados pode proteger à mucosa intestinal de desequilíbrio oxidativo. O tratamento enzimático com tanase elevou a atividade anti-inflamatória do RUT, comprovando a eficiência na biotransformação e liberação de compostos mais funcionais. Ambos os tratamentos (pré-tratamento e pré/cotratamento) foram efetivos, porém no pré/cotratamento, os resultados foram mais homogêneos, independentemente da concentração de extrato utilizada. Resíduos de uvas provenientes da vinificação devem ser considerados resíduos de alto valor agregado. Futuros estudos são necessários para garantir o desenvolvimento de alimentos ou ingredientes funcionais, nutracêuticos e cosméticos a partir desses resíduos ricos em compostos fenólicos e pouco utilizados atualmente.

## REFERÊNCIAS BIBLIOGRÁFICAS

ANASTASIADI, M.; PRATSINIS, H.; KLETSAS, D.; SKALTSOUNIS, A.-L.; HAROUTOUNIAN, S. A. Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. **LWT - Food Science and Technology**, v. 48, n. 2, p. 316–322, 2012.

ARNOUS, A.; MEYER, A. S. Discriminated release of phenolic substances from red wine grape skins (Vitis vinifera L.) by multicomponent enzymes treatment. **Biochemical Engineering Journal**, v. 49, n. 1, p. 68–77, 2010.

BAO, H.; REN, H.; ENDO, H.; TAKAGI, Y.; HAYASHI, T. Effects of heating and the addition of seasonings on the anti-mutagenic and anti-oxidative activities of polyphenols. **Food Chemistry**, v. 86, n. 4, p. 517–524, 2004.

BATTESTIN, V.; MATSUDA, L. K.; MACEDO, G. A. Fontes e aplicações de taninos e tanases em alimentos. **Alimentos e Nutrição**, v. 15, n. 1, p. 63–72, 2004.

BERZAS NEVADO, J. J.; CONTENTO SALCEDO, A. M.; CASTAÑEDA PEÑALVO, G. Simultaneous determination of cis- and trans-resveratrol in wines by capillary zone electrophoresis. **The Analyst**, v. 124, n. 1, p. 61–66, 1999.

CAMPANHOLO, V. M. de L. P.; SILVA, R. M.; SILVA, T. D.; NETO, R. A.; PAIOTTI, A. P. R.; RIBEIRO, D. A.; FORONES, N. M. Oral Concentrated Grape Juice Suppresses Expression of NF-kappa B , TNF-α and iNOS in Experimentally Induced Colorectal Carcinogenesis in Wistar Rats. **Asian Pacific Journal of Cancer Prevention**, v. 16, n. 3, p. 947–952, 2015.

CARDONA, F.; ANDRÉS-LACUEVA, C.; TULIPANI, S.; TINAHONES, F. J.; QUEIPO-ORTUÑO, M. I. Benefits of polyphenols on gut microbiota and implications in human health. **The Journal of Nutritional Biochemistry**, v. 24, n. 8, p. 1415–22, 2013.

CARRASCO-POZO, C.; MIZGIER, M. L.; SPEISKY, H.; GOTTELAND, M. Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. **Chemico-biological Interactions**, v. 195, n. 3, p. 199–205, 2012.

CHAMORRO, S.; VIVEROS, A.; ALVAREZ, I.; VEGA, E.; BRENES, A. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. **Food Chemistry**, v. 133, n. 2, p. 308–314, 2012.

CHUANG, C.-C.; BUMRUNGPERT, A.; KENNEDY, A.; OVERMAN, A.; WEST, T.;

DAWSON, B.; MCINTOSH, M. K. Grape powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin resistance in primary cultures of human adipocytes. **The Journal of Nutritional Biochemistry**, v. 22, n. 1, p. 89–94, 2011.

CONTRERAS-DOMÍNGUEZ, M.; GUYOT, S.; MARNET, N.; LE PETIT, J.; PERRAUD-GAIME, I.; ROUSSOS, S.; AUGUR, C. Degradation of procyanidins by Aspergillus fumigatus: identification of a novel aromatic ring cleavage product. **Biochimie**, v. 88, n. 12, p. 1899–908, 2006.

CROZIER, A.; JAGANATH, I. B.; CLIFFORD, M. N. Dietary phenolics: chemistry, bioavailability and effects on health. **Natural Product Reports**, v. 26, n. 8, p. 1001–43, 2009.

DEL RIO, D.; COSTA, L. G.; LEAN, M. E. J.; CROZIER, A. Polyphenols and health: what compounds are involved? **Nutrition, Metabolism, and Cardiovascular Diseases : NMCD**, v. 20, n. 1, p. 1–6, 2010.

DINICOLA, S.; MARIGGIÒ, M. A.; MORABITO, C.; GUARNIERI, S.; CUCINA, A.; PASQUALATO, A.; D'ANSELMI, F.; PROIETTI, S.; COLUCCIA, P.; BIZZARRI, M. Grape seed extract triggers apoptosis in Caco-2 human colon cancer cells through reactive oxygen species and calcium increase: extracellular signal-regulated kinase involvement. **The British Journal of Nutrition**, v. 110, n. 5, p. 797–809, 2013.

FERRI, M.; BIN, S.; VALLINI, V.; FAVA, F.; MICHELINI, E.; RODA, A.; MINNUCCI, G.; BUCCHI, G.; TASSONI, A. Recovery of polyphenols from red grape pomace and assessment of their antioxidant and anti-cholesterol activities. **New Biotechnology**, v. 33, n. 3, p. 338–344, 2016.

FERRUZZI, M. G.; LOBO, J. K.; JANLE, E. M.; WHITTAKER, N.; COOPER, B.; SIMON, J. E.; WU, Q.; WELCH, C.; HO, L.; WEAVER, C.; GIULIO, M. Implications for treatment in Alzheimer ' s Disease. **Journal of Alzheimers Disease**, v. 18, n. 1, p. 113–124, 2010.

FONTANA, A. R.; ANTONIOLLI, A.; BOTTINI, R. Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics. **Journal of Agricultural and Food Chemistry**, v. 61, n. 38, p. 8987–9003, 2013.

GARAT, C. Intracellular IL-1Ra type 1 inhibits IL-1-induced IL-6 and IL-8 production in Caco-2 intestinal epithelial cells through inhibition of p38 mitogen-activated protein kinase and NF-κB pathways. **Cytokine**, v. 23, n. 1-2, p. 31–40, 2003.

GENTILE, C.; PERRONE, A.; ATTANZIO, A.; TESORIERE, L.; LIVREA, M. A.

Sicilian pistachio (Pistacia vera L.) nut inhibits expression and release of inflammatory mediators and reverts the increase of paracellular permeability in IL-1β-exposed human intestinal epithelial cells. **European Journal of Nutrition**, v. 54, n. 5, p. 811-821, 2015.

GEORGETTI, S. R.; VICENTINI, F. T. M. C.; YOKOYAMA, C. Y.; BORIN, M. F.; SPADARO, A. C. C.; FONSECA, M. J. V. Enhanced in vitro and in vivo antioxidant activity and mobilization of free phenolic compounds of soybean flour fermented with different beta-glucosidase-producing fungi. **Journal of Applied Microbiology**, v. 106, n. 2, p. 459–66, 2009.

GEREMIA, A.; BIANCHERI, P.; ALLAN, P.; CORAZZA, G. R.; DI SABATINO, A. Innate and adaptive immunity in inflammatory bowel disease. **Autoimmunity Reviews**, v. 13, n. 1, p. 3–10, 2014.

GONZÁLEZ-PARAMÁS, A. M.; ESTEBAN-RUANO, S.; SANTOS-BUELGA, C.; DE PASCUAL-TERESA, S.; RIVAS-GONZALO, J. C. Flavanol content and antioxidant activity in winery byproducts. **Journal of Agricultural and Food Chemistry**, v. 52, n. 2, p. 234–8, 28, 2004.

HOFFMANN, E.; DITTRICH-BREIHOLZ, O.; HOLTMANN, H.; KRACHT, M. Multiple control of interleukin-8 gene expression. **Journal of Leukocyte Biology**, v. 72, n. November, p. 847–855, 2002.

JOHNSTON, C. S.; TAYLOR, C. A.; HAMPL, J. S. More Americans are eating “5 a day” but intakes of dark green and cruciferous vegetables remain low. **The Journal of Nutrition**, v. 130, n. 12, p. 3063–7, 2000.

KAMMERER, D.; CLAUS, A.; SCHIEBER, A.; CARLE, R. A Novel Process for the Recovery of Polyphenols from Grape ( Vitis vinifera L .) Pomace. **Food Chemistry and Toxicology**, v. 70, n. 2, p. 157–163, 2005.

KARIN, M. How NF-kB is activated: the role of the IkB kinase (IKK) complex. **Oncogene**, v. 18, p. 6867–6874, 1999.

KATALINIĆ, V.; MOŽINA, S. S.; SKROZA, D.; GENERALIĆ, I.; ABRAMOVIČ, H.; MILOŠ, M.; LJUBENKOV, I.; PISKERNIK, S.; PEZO, I.; TERPINC, P. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). **Food Chemistry**, v. 119, n. 2, p. 715–723, 2010.

KAULMANN, A.; BOHN, T. Carotenoids, inflammation and oxidative stress – implications of cellular signaling pathways and relation to chronic disease prevention.

**Nutrition Research**, v. 34, n. 11, p. 907–929, 2014.

KOUNTOURI, A. M.; GIOXARI, A.; KARVELA, E.; KALIORA, A. C.; KARVELAS, M.; KARATHANOS, V. T. Chemopreventive properties of raisins originating from Greece in colon cancer cells. **Food & Function**, v. 4, n. 3, p. 366–372, 2012.

KOEBERLE, A.; BAUER, J.; VERHOFF, M.; HOFFMANN, M.; NORTHOFF, H.; WERZ, O. Green tea epigallocatechin-3-gallate inhibits microsomal prostaglandin $E_2$ synthase-1. **Biochemical and Biophysical Research Communications**, v. 388, p. 350–354, 2009.

KUNDU, N.; YANG, Q.; DORSEY, R.; FULTON, A. M. Increased cyclooxygenase-2 (cox-2) expression and activity in a murine model of metastatic breast cancer. **International Journal of Cancer**, v. 93, n. 5, p. 681–6, 2001.

KUNTZ, S.; ASSEBURG, H.; DOLD, S.; RÖMPP, A.; FRÖHLING, B.; KUNZ, C.; RUDLOFF, S. Inhibition of low-grade inflammation by anthocyanins from grape extract in an in vitro epithelial-endothelial co-culture model. **Food & Function**, v. 6, n. 4, p. 1136–49, 2015.

LAFKA, T.-I.; SINANOGLOU, V.; LAZOS, E. S. On the extraction and antioxidant activity of phenolic compounds from winery wastes. **Food Chemistry**, v. 104, n. 3, p. 1206–1214, 2007.

LAM, G.; APOSTOPOULOS, V.; ZULLI, A.; NURGALI, K. NADPH Oxidases and Inflammatory Bowel Disease. **Current Medicinal Chemistry**, v. 22, n. 17, p. 2100–2109, 2015.

LEE, C.-J.; CHEN, L.-G.; LIANG, W.-L.; WANG, C.-C. Anti-inflammatory effects of Punica granatum Linne in vitro and in vivo. **Food Chemistry**, v. 118, n. 2, p. 315–322, 2010.

LEGLER, D. F.; BRUCKNER, M.; UETZ-VON ALLMEN, E.; KRAUSE, P. Prostaglandin E2 at new glance: Novel insights in functional diversity offer therapeutic chances. **The International Journal of Biochemistry & Cell Biology**, v. 42, n. 2, p. 198–201, 2010.

LIM, J. W.; HWANG, H. J.; SHIN, C. S. Polyphenol compounds and anti-inflammatory activities of Korean black raspberry (Rubus coreanus Miquel) wines produced from juice supplemented with pulp and seed. **Journal of Agricultural and Food Chemistry**, v. 60, n. 20, p. 5121–7, 23, 2012.

LIU, X.; NELSO, A.; WANG, X.; FARID, M.; GUNJI, Y.; IKARI, J.; IWASAWA, S.; BASMA, H.; FEGHALI-BOSTWICK, C.; RENNARD, S. I. Vitamin D Modulates

Prostaglandin $E_2$ Synthesis and Degradation in Human Lung Fibroblasts. **American Journal of Respiratory Cell and Molecular Biology**, v. 50. n. 1, p. 40-50, 2014.

MAIER, T.; GÖPPERT, A.; KAMMERER, D. R.; SCHIEBER, A.; CARLE, R. Optimization of a process for enzyme-assisted pigment extraction from grape (Vitis vinifera L.) pomace. **European Food Research and Technology**, v. 227, n. 1, p. 267–275, 2008.

MARTINS, S.; MUSSATTO, S. I.; MARTÍNEZ-AVILA, G.; MONTAÑEZ-SAENZ, J.; AGUILAR, C. N.; TEIXEIRA, J. A. Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. **Biotechnology Advances**, v. 29, n. 3, p. 365–73, 2011.

MELO, P. S.; BERGAMASCHI, K. B.; TIVERON, A. P.; MASSARIOLI, A. P.; LUIZA, T.; OLDONI, C.; ZANUS, M. C.; PEREIRA, G. E.; ALENCAR, S. M. Composição fenólica e atividade antioxidante de resíduos agroindustriais. **Ciência Rural**, v. 41, n. 6, p. 1088–1093, 2011.

MEYER, A. S.; JEPSEN, S. M.; SØRENSEN, N. S. Enzymatic Release of Antioxidants for Human Low-Density Lipoprotein from Grape Pomace. **Journal of Agricultural and Food Chemistry**, v. 46, n. 7, p. 2439–2446, 1998.

MILDNER-SZKUDLARZ, S.; BAJERSKA, J.; ZAWIRSKA-WOJTASIAK, R.; GÓRECKA, D. White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. **Journal of the Science of Food and Agriculture**, v. 93, n. 2, p. 389–95, 2013.

MUTOH, M.; TAKAHASHI, M.; FUKUDA, K.; KOMATSU, H.; ENYA, T.; MATSUSHIMA-HIBIYA, Y.; MUTOH, H.; SUGIMURA, T.; WAKABAYASHI, K. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. **Japanese Journal of Cancer Research : Gann**, v. 91, n. July, p. 686–691, 2000.

OBOH, G.; ADEMOSUN, A. O. Shaddock peels (Citrus maxima) phenolic extracts inhibit α-amylase, α-glucosidase and angiotensin I-converting enzyme activities: a nutraceutical approach to diabetes management. **Diabetes & Metabolic Syndrome**, v. 5, n. 3, p. 148–52, 2012.

PANDEY, A. Solid-state fermentation. **Biochemical Engineering Journal**, v. 13, p. 81–84, 2003.

PINELO, M.; ARNOUS, A.; MEYER, A. S. Upgrading of grape skins: Significance of

plant cell-wall structural components and extraction techniques for phenol release. **Trends in Food Science & Technology**, v. 17, n. 11, p. 579–590, 2006.

PINENT, M.; CASTELL-AUVÍ, A.; GENOVESE, M. I.; SERRANO, J.; CASANOVA, A.; BLAY, M.; ARDÉVOL, A. Antioxidant effects of proanthocyanidin-rich natural extracts from grape seed and cupuassu on gastrointestinal mucosa. **Journal of the Science of Food and Agriculture**, v. 96, n. 1, p. 178-182, 2015.

RAMOS, S.; RODRÍGUEZ-RAMIRO, I.; MARTÍN, M. A.; GOYA, L.; BRAVO, L. Dietary flavanols exert different effects on antioxidant defenses and apoptosis/proliferation in Caco-2 and SW480 colon cancer cells. **Toxicology in Vitro**, v. 25, n. 8, p. 1771–1781, 2011.

RICCIOTTI, E.; FITZGERALD, G. A. Prostaglandins and Inflammation. **Arteriosclerosis, Thrombosis and Vascular Biology**, v. 31, n. 5, p. 986–1000, 2011.

RICE-EVANS, C.; MILLER, N.; PAGANGA, G. Antioxidant properties of phenolic compounds. **Trends in Plant Science**, v. 2, n. 4, p. 152–159, 1997.

ROUANET, J.-M.; DÉCORDÉ, K.; RIO, D. Del; AUGER, C.; BORGES, G.; CRISTOL, J.-P.; LEAN, M. E. J.; CROZIER, A. Berry juices, teas, antioxidants and the prevention of atherosclerosis in hamsters. **Food Chemistry**, v. 118, n. 2, p. 266–271, 2010.

RUSSELL, W.; DUTHIE, G. Plant secondary metabolites and gut health: the case for phenolic acids. **The Proceedings of the Nutrition Society**, v. 70, n. 3, p. 389–396, 2011.

SAMBUY, Y.; DE ANGELIS, I.; RANALDI, G.; SCARINO, M. L.; STAMMATI, A.; ZUCCO, F. The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on Caco-2 cell functional characteristics. **Cell Biology and Toxicology**, v. 21, n. 1, p. 1–26, 2005.

SANDOVAL-ACUÑA, C.; FERREIRA, J.; SPEISKY, H. Polyphenols and mitochondria: An update on their increasingly emerging ROS-scavenging independent actions. **Archives of Biochemistry and Biophysics**, v. 559, p. 75–90, 2014.

SANTANGELO, C.; VARÌ, R.; SCAZZOCCHIO, B.; DI BENEDETTO, R.; FILESI, C.; MASELLA, R. Polyphenols, intracellular signalling and inflammation. **Annali dell'Istituto Superiore di Sanità**, v. 43, n. 4, p. 394–405, 2007.

SAW, C. L. L.; GUO, Y.; YANG, A. Y.; PAREDES-GONZALEZ, X.; RAMIREZ, C.;

PUNG, D.; KONG, A.-N. T. The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: Involvement of the Nrf2-ARE signaling pathway. **Food and Chemical Toxicology**, v. 72, n. August, p. 303–311, 2014.

SCALBERT, A.; MANACH, C.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Dietary polyphenols and the prevention of diseases. **Critical Reviews in Food Science and Nutrition**, v. 45, n. 4, p. 287–306, 2005.

SHIN, H. S.; SATSU, H.; BAE, M.-J.; ZHAO, Z.; OGIWARA, H.; TOTSUKA, M.; SHIMIZU, M. Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in C57BL/6 mice. **Food chemistry**, v. 168, p. 167–75, 2015.

SNOECK, V.; GODDEERIS, B.; COX, E. The role of enterocytes in the intestinal barrier function and antigen uptake. **Microbes and Infection / Institut Pasteur**, v. 7, n. 7-8, p. 997–1004, 2005.

SUN, T.; CHEN, Q. Y.; WU, L. J.; YAO, X. M.; SUN, X. J. Antitumor and antimetastatic activities of grape skin polyphenols in a murine model of breast cancer. **Food and Chemical Toxicology**, v. 50, n. 10, p. 3462–7, 2012.

TABASCO, R.; SÁNCHEZ-PATÁN, F.; MONAGAS, M.; BARTOLOMÉ, B.; VICTORIA MORENO-ARRIBAS, M.; PELÁEZ, C.; REQUENA, T. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. **Food Microbiology**, v. 28, n. 7, p. 1345–52, 2011.

TAVERNITI, V.; FRACASSETTI, D.; BO, C. Del; LANTI, C.; MINUZZO, M.; KLIMIS-ZACAS, D.; RISO, P.; GUGLIELMETTI, S. Immunomodulatory E ff ect of a Wild Blueberry Anthocyanin-Rich Extract in Human Caco - 2 Intestinal Cells. **Journal of Agricultural and Food Chemistry**, v. 62, p. 8346–8351, 2014.

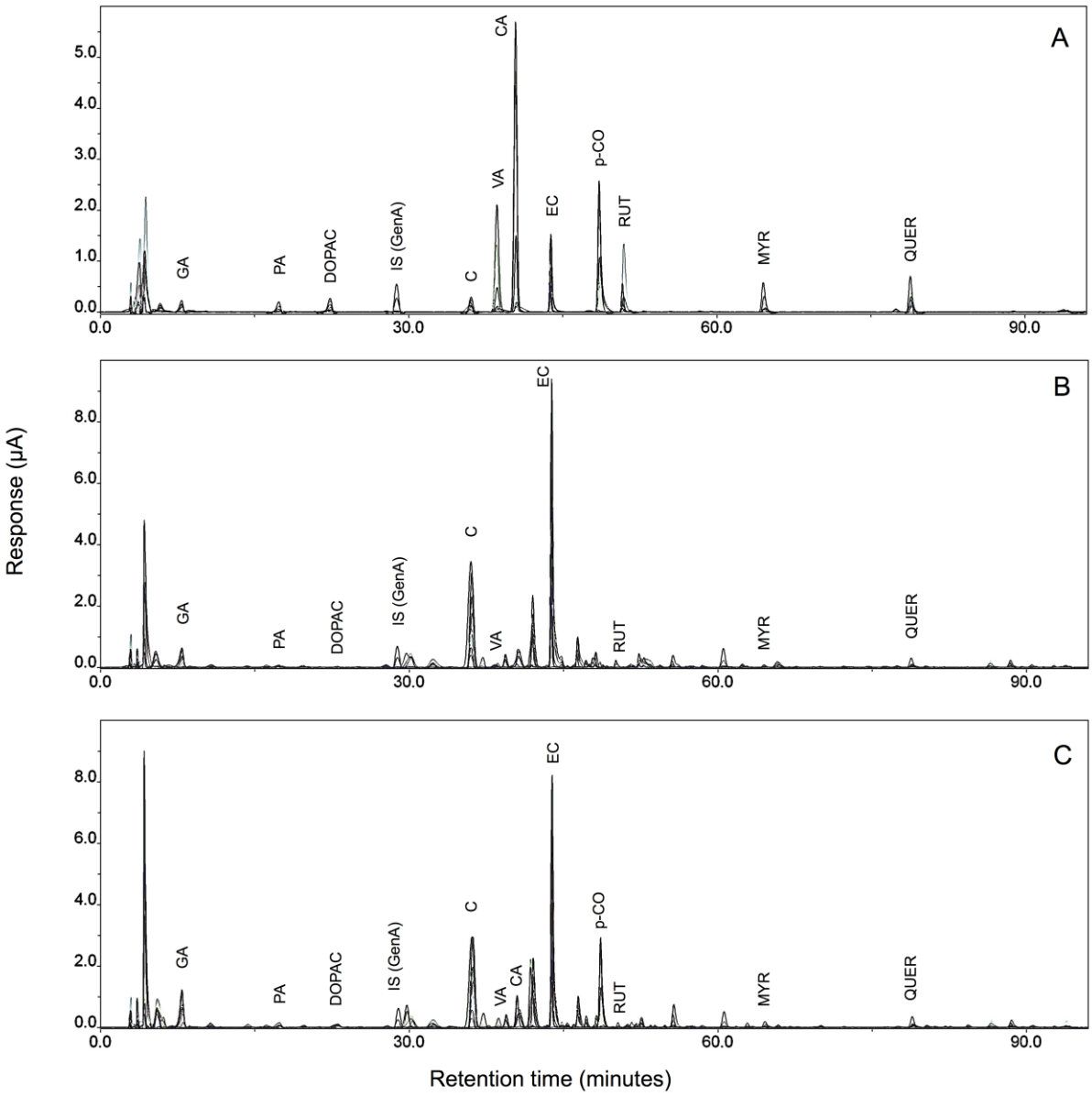
XIA, E.-Q.; DENG, G.-F.; GUO, Y.-J.; LI, H.-B. Biological Activities of Polyphenols from Grapes. **International Journal of Molecular Sciences**, v. 11, n. 2, p. 622–646, 2010.

XU, C.; YAGIZ, Y.; BOREJSZA-WYSOCKI, W.; LU, J.; GU, L.; RAMÍREZ-RODRIGUES, M. M.; MARSHALL, M. R. Enzyme release of phenolics from muscadine grape (Vitis rotundifolia Michx.) skins and seeds. **Food Chemistry**, v. 157, p. 20–9, 2014.

YOU, Q.; CHEN, F.; WANG, X.; JIANG, Y.; LIN, S. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. **LWT -**

**Food Science and Technology**, v. 46, n. 1, p. 164–168, 2012.

## APÊNDICE




HPLC-ECD chromatograms of polyphenolics analyzed in GP and GPT samples (Article III). Figure A: polyphenolic standards; Figure B: GP sample; Figure C: GPT sample. Abbreviations – GA: gallic acid, PA: protocatechuic acid, DOPAC: 3,4-dihydroxyphenylacetic acid, IS (GenA): internal standard (gentisic acid), C: catechin, VA: vanillic acid, CA: caffeic acid, EC: epicatechin, ρ-CO: ρ-coumaric acid, RUT: rutin, MYR: myricetin, QUER: quercetin.